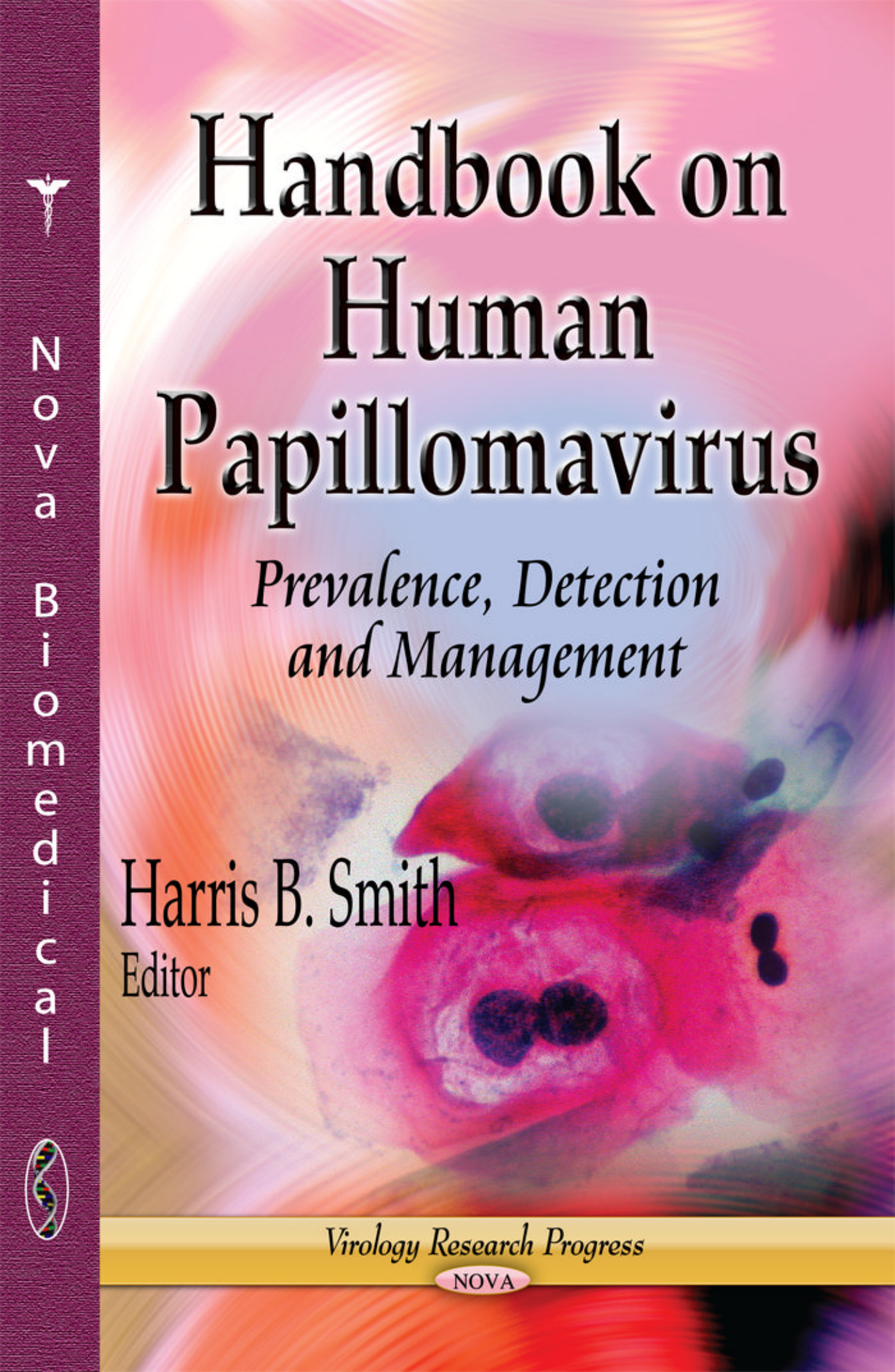
Nova Biomedical
Handbook on Human Papillomavirus
Prevalence, Detection and Management
Harris B. Smith
Editor
Virology Research Progress
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HANDBOOK ON HUMAN PAPILLOMAVIRUS

PREVALENCE, DETECTION AND MANAGEMENT

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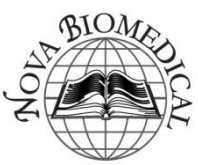
HANDBOOK ON HUMAN PAPILLOMAVIRUS

PREVALENCE, DETECTION AND MANAGEMENT

HARRIS B. SMITH
EDITOR



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PREFACE

Human Papillomavirus (HPV) is a common sexually transmitted infection (STI). It affects genital and oral mucosa and is associated with cellular lesions which can progress to cervical cancer and some head and neck cancers respectively. In this handbook, the authors present current research on the prevalence, detection and management of HPV. Topics include HPV in adolescents; clinical utility of HPV testing in cervical cancer screening; HPV epidemiology in females and risk for cervical cancer; HPV infection in pregnant women; immunogenicity of HPV vaccination; HPV in systemic rheumatic disorders; prevalence and distribution of HPV 16, 18 and 58 in Southeast Mexico; the triad of HPV-oral sex-and oral cancer; HPV related malignancies of the reproductive tract; in silico characterization of major capsid protein L1 of HPV type 16 by molecular dynamics and determination of linear B-cell consensus epitopes; the role of HPV in non-small cell lung cancer; and the statistical aspects of HPV modelling in a quantitative manner.

Chapter 1 - Human Papilloma Virus (HPV) is a virus belonging to the group of Papillomaviruses. HPV infections are very common and can cause disease of the skin and mucous membranes. There are about 100 types of Papillomaviruses, divided into 16 groups designated progressively with the letters from A to P, in accordance with the sequence homologies of deoxy-ribonucleic acid (DNA). It is also possible to classify Papillomaviruses in cutaneous and mucosal, according to the tissue for which they are specific. Most of the viruses in this family cause disease which are not serious, such as skin warts, though some can cause benign tumors such as genital condyloma as well as malignant cancer of the cervix, the mouth, anus, esophagus, and the larynx. HPV are bare (without pericapsid), possess an icosahedral capsid with a diameter around 50 nm, formed by 72 capsomeres which may be pentons or exons. Each capsomer gives rise to a protuberance which has a shape similar to a five-pointed star with a channel at the center. The capsid contains a genome consisting of double-stranded circular DNA 8 Kb long, which codes for eight early genes (early, E1 to E8) and two late genes (late, L1 and L2). Upstream of the early genes there is a regulatory region containing the origin of replication, some sequences regulating the transcription, and a N-terminal sequence common to all early proteins. The early proteins are used to modify the metabolism of the infected cell to make it available to HPV, while the late ones are the structural proteins that join together to form the structure of icosahedral viral capsid. All genes of HPV are placed on the positive strand. The functions of these proteins are: 1) L1 and L2 proteins encode capsid proteins, as we have seen; 2) E1 protein enables episomal replication, with helicase activity; 3) E2 protein participates in the transcription of the E6

promoter, actives E1, and has trans-activating ability: in particular, E2 inhibits transcription of E6 and E7; when the HPV DNA is integrated with the human genome, there is the breaking of the gene sequences of E2, with suppression of inhibition against E6 and E7; 4) E4 protein is expressed in the late stages of infection and is very important in viral maturation and proliferation: it is able to bind to cytoskeletal proteins, causing the deformation of infected cells (koilocytosis); 5) E5 protein locks the exposure of the histocompatibility complexes type I and II, avoiding the T-cell mediated response; E5 protein inhibits apoptosis and also alters the signals initiated by the binding of epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) with their respective receptors; 6) E6 protein binds to p53, interfering with DNA repair and with the triggering of apoptosis; and 7) E7 protein binds to the retinoblastoma (Rb) protein, preventing the blocking of the cell cycle. By virtue of their binding properties and inactivating tumor suppressor genes (TSG) such as p53 and Rb, proteins encoded by the viral genome promote intense cell replication that results in the formation of papillomas, condylomas, focal epithelial hyperplasia, warts, and cancers. However, not all Papillomaviruses are responsible of forms of carcinoma. In particular, carcinomas of the uterine cervix are triggered by genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, although the types 16 and 18 are the most frequent, while the simple warts and condylomas are generated by genotypes 2, 7 (common warts), 1, 2, 4 (plantar warts), and 3, 10 (flat warts). The E7 oncoprotein is a small protein of only 98 water-soluble aminoacids, poorly immunogenic. For this reason, it can not be used individually as a hapten and must be "merged" with the most immunogenic protein complexes. The stimulation of an immune response polarized by a vaccine containing fragments or proteins complexed with E6 and E7 may be able to eliminate the risk of cancer associated with HPV infection. All cancers of the cervix are caused by HPV. The types of HPV can be divided into low-risk HPV, which attack the skin (6, 11, 42, 43, 44) and high-risk HPV, which attack the mucous membranes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). It is estimated that over 70% of women contract genital infection with HPV in their lifetime, but the vast majority of these infections is likely to disappear spontaneously in the course of a few months due to their immune system. Only in case of persistence over time of infection by high-risk oncogenic HPV, it will be possible, in a minority of cases and in the course of several years, the development of a malignant cancer of the cervix. The early proteins of the virus have the aim of favoring the growth and division of the cell; HPV in fact can replicate only in cells in replication, as it does not code for its own DNA polymerase and needs the polymerase of the host cell, which is synthesized in cells that are dividing. The target cells of the virus are the skin and mucous membranes, two tissues that regenerate constantly. The virus induces growth of the basal and spinous layers of the epidermis (acanthosis) or of the superficial layer of the mucosa, giving rise, depending on the site of infection, to skin warts or papillomas of the mucous membranes; it also promotes the synthesis of keratins (hyperkeratosis). The expression of genes of HPV is correlated with the different types of keratin expressed in the layers of the epidermis. The basal and the spinous layers of the epidermis contain within the nucleus of their cells also the HPV DNA, while the late genes L1 and L2, which allow the formation of the mature virion, are expressed only in the stratum corneum of the epidermis. The virus replication occurs preferentially in the stratum granulosum. The virus is released along with the dead keratinocytes of the upper layers of the epidermis. Genital warts (GW), usually caused by HPV, are skin growths of verrucous type that affect by preference genital areas, both in the male (glans, less frequently under the foreskin, shaft of the penis, and scrotum) and in female

(perineum, vulva , vagina, and cervix). The dangerous types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) differ from the harmless ones (6, 11, 42, 43, 44) both in the site of action (the first attack the mucous membranes and the latter the skin) and on some mutations of E7 oncoprotein. This different dangerousness is due to the fact that mucous membranes are much more susceptible to HPV infections than the skin because the lower strength of the cellular membranes of the cells of the mucous membranes facilitates the entry of viruses within them, and in addition the E7 oncoproteins of high-risk Papillomaviruses have mutations that allow an aminoacid binding and inhibition of Rb protein better than the low-risk E7 types. For example, the difference between the primary E7 HPV 16 (dangerous) and 6 (not dangerous) aminoacid is an aspartic acid in position 21 present in the first instead of a glycine present in the second: this only difference causes the first protein to have a capacity ligand (and therefore to cause cancer) by 41% higher than the second. Once that HPV is entered into it, the cell is an infected cell that synthesizes two proteins called E6 and E7, which bind and inhibit the Rb protein (a protein that serves to regulate the cellular mitosis), which causes uncontrolled cell divisions. It is thought that these physiological changes are given to serve the virus-infected cells to spread better. HPV is contracted through direct contact (sexual, oral, and skin) or in dirty places (e.g. public toilets not cleaned to the standard). HPV is not present in body fluids such as blood or sperm. The risk of contracting an HPV infection increases with the number of sexual partners, and is highest among young adults (20-35 years). The virus is most frequently found among the promiscuous populations and in poor conditions of hygiene. The use of condoms does not seem to have a complete protective action, because the infection is often spread to the skin of the vulva and perineum. HPV infection is asymptomatic in most cases. In some cases, it may instead manifest as warts in the genital area (penis or vulva, perineum). HPV lesions of the cervix can be detected by a Papanicolaou (Pap) test, colposcopy, or techniques of molecular pathology, and lesions of the penis by penescopia. As in many viral infections, treatment of HPV is often problematic. However, since the majority of HPV infections regress spontaneously, only a minority of cases require treatment. In cases of persistent infection of the cervix, there are currently no highly effective non-invasive treatments. In case the infection is associated with changes to premalignant epithelium, it can be taken into consideration laser therapy or conization, i.e. resection of a small part of the uterine cervix to remove a lesion that may become malignant or that already is but of small size. For the removal of condylomata acuminata (CA) of the vulva, penis, or perineum, it can be used laser vaporization, elecro-coagulation, cryotherapy, or podophyllin applications. Every year, in Italy, there are around 3,500 women who get cervical cancer. Nearly half die. 400,000 women worldwide each year get sick and half of them die. It is estimated that 75% of the population comes into contact with the virus at least once during its life. It was recently discovered a molecule that allows the treatment of HPV infection, even in the external skin (wart). The drug product is called cidofovir, and is a potent broad-spectrum antiviral, even more effective than aciclovir. Cidofovir is used primarily in people with immune system problems, such as people living with Human Immunodeficiency Virus (HIV), in liquid formulation for intravenous use, but also in topical formulations for external forms (warts) which are unfortunately still widespread in Italy. It is possible that a woman contracts HPV infection in pregnancy, resulting in concern about the state of infection and transmission to the fetus. The risk of infection during pregnancy is not different from the non-pregnant woman, and the distribution of viral types with high- and low-risk is not different. The infection may appear for the first time in pregnancy and result in

genital lesions which may be of aberrant aspects, very large, giant, and brittle. Despite the maternal-fetal transmission is possible at the level of the birth canal and can cause recurrent juvenile papillomatosis and GW in the neonate, cesarean section is not indicated, unless very large lesions that obstruct the birth canal are present. In the case of HPV, there are two vaccination strategies: preventive and therapeutic. The first is intended to prevent the onset of infections, the second (still at an experimental level) to treat them when they are already in place. With regard to the first strategy, it is already on the market, and is distributed free of charge to girls under the age of 12 years (in some regions such as Basilicata, in other age groups), a vaccine containing two transgenic viral capsid proteins of HPV 16 and 18 (responsible for 70% of cervical cancers) and thus casing proteins (L1 and L2) such as virus-like particles (PLV). The vaccine is safe and real virus-free; it has almost completely efficacy in preventing the infection by HPV 16 and 18. It is only ineffective in treating any infection already in place by the aforementioned viruses, because L1 and L2 proteins are produced in only a small fraction of the time of infection (when the virus passes from one cell killed to another). The available vaccines are Cervarix® and Gardasil®. Instead a therapeutic vaccine, i.e. that it cures infections already in place, must produce proteins that are synthesized during the whole life cycle of the virus, such as E6 and E7. For this purpose, experiments with transgenic plants are ongoing which produce these two antigens (obviously with mutations that make them absolutely safe for human health). According to recent studies HPV, in addition to be the cause of many tumors of mucous membranes, could have a role in the pathogenesis of several chronic inflammatory and autoimmune diseases, including multiple sclerosis, Kawasaki syndrome, lupus erythematosus, and rheumatoid arthritis.

Chapter 2 - Human Papillomavirus (HPV) is the etiological agent of cervical cancer. Like other oncogenic viruses, HPV encode oncoproteins whose role in cellular immortalization and transformation has been extensively investigated. HPV E6 and E7 proteins disable tumor suppressors p53 and Rb and up-regulate telomerase, fundamental changes for cell immortalization. Another important step in the induction of cancer by oncogenic viruses seems to be the specific interaction of some viral proteins with mitochondria, an organelle that has been implicated for decades in carcinogenesis. The identification of non-coding RNAs (ncRNAs) has opened new research areas and there has been an explosive increase of reports showing that the expression of these RNAs is deregulated in many different human diseases, including cancer. The ncRNAs can be classified into two groups based on their length: small transcripts (20–200 nucleotides), such as microRNAs (miRs), piwi-interacting RNAs and long transcripts (higher than 200 nucleotides). While the function of small ncRNAs has been well documented, the role of long ncRNAs is still not completely understood. MicroRNAs are small 21–22 nt non-protein-coding RNAs that regulate mRNA translation and decay. It has become evident that miRs plays a pivotal role in the development of human cancer. Some miRs have been characterized as tumor suppressors and others as oncogenic (onco-miRs). MiR patterns are tissue specific, and the expression profile could allow to distinguish carcinomas from normal cells. Moreover, miR expression profiles of cervix, head and neck cancers have been carried out in different studies associated to HPV infection. In this scenario, the HPV E5, E6 and E7 proteins modulate the expression of several cellular miRs. Deregulation of miRs expression might be used to identify cancer progression and also as potential target for therapy against HPV infection and cervical cancer development. The differential expression of a family of long non-coding mitochondrial RNAs (ncmtRNAs) in response to HPV infection has been recently reported. The expression profile

of these transcripts allows distinguishing between normal, pre-tumoral and cancer cells. One of these transcripts, SncmtRNA-1, has been characterized as a regulator of cell cycle progression while two others, ASncmtRNA-1 and 2, has been suggested to act as tumor suppressors. HPV E2, E6 and E7 modulate the expression of this family of mitochondrial long ncRNAs. Further evidence suggests that other viruses such a Hepatitis B virus, Human T-cell lymphotropic Virus type 1 and Epstein Barr virus can also modulate the expression of these long ncRNAs in human cells. In this chapter the authors will discuss how the differential expression of both, microRNAs and long ncRNAs, in response to HPV infection, might serve as early biomarkers for progression of cervical dysplasia in PAP smears and biopsies allowing the detection of precursor lesion of cervical cancer.

Chapter 3 - Human papillomavirus (HPV) is one of the most common causes of sexually transmitted infection (STI) in both men and women around the world, with prevalence rates varying according to the studied population and geographical localization. It is estimated that over 80% of sexually active women are exposed to the virus and acquire the infection 3-4 years after sexual debut. Most HPV infections are transient and asymptomatic without causing any clinical manifestation. Young women are more vulnerable and often become infected by multiple types of the virus. The high prevalence of HPV in young women underscores the vulnerability of adolescents to HPV, while the low prevalence of cervical cancer in this group underscores the benign nature of initial HPV infections. Evidence indicates that there are risk behaviors and / or biological risks that make the adolescents vulnerable not only to HPV infection but also to the persistence of the HPV that is strongly linked to the development of high-grade squamous intra-epithelial lesions and invasive cancer. Among the biological factors that predispose an individual to HPV the authors can highlight the immaturity of both the epithelium and the immune system. It has been observed that the association between early age of first sexual intercourse and HPV infection was partially mediated by a set of risk behaviors and related conditions, including the number of sexual partners, history of STI, risk behaviors such as the consumption of alcohol and illicit drugs, and behavior of sexual partners. Anovulatory menstrual cycles and large glandular aversion of the cervix with active squamous metaplasia, common in adolescent girls after menarche, are associated with the lack of cyclic progesterone production, which may lead to the decreased production of cervical mucus, which is thought to serve as a protective barrier against infectious agents. In addition, the adolescent cervix is characterized by a large area of cervical ectopy, defined as the area of immature columnar and metaplastic cells on the ectocervix. HPV infects epithelial basal cells and its replication is dependent on the active cell division and differentiation, which occurs normally in pubertal adolescent girls. These cells may be particularly vulnerable to infection with HPV. There is rapid change in the cervical epithelium during puberty as squamous metaplasia occurs, the process by which the columnar epithelium is transformed into mature squamous epithelium. In this chapter the authors discuss the natural history of HPV infection in adolescents and the factors that may increase the vulnerability of this group of women to HPV.

Chapter 4 - Cervical cancer remains the second most common cause of cancer death among women around the world. It has been recognized as a rare outcome of Human Papillomavirus (HPV), a common sexually transmitted infection. Persistent infection with high-risk oncogenic HPV types is a known cause of cervical cancer. The benefits of cytological screening for cervical cancer are well known. However, the necessary resources, infrastructure and technological expertise, together with the need for repeated screening at

regular intervals, make cytological screening resource intense. HPV DNA testing is a viable alternative to cytological screening. With optimal testing systems, HPV DNA can be identified in nearly all specimens of invasive cervical cancer and in the vast majority (>95%) of the immediate cervical cancer precursors, namely high-grade squamous intraepithelial lesions (HSILs). HPV DNA testing has been shown to decrease cervical cancer mortality. In this chapter, the clinical utility of HPV DNA testing for cervical cancer will be reviewed with a focus on primary screening, combination with cytology (co-testing), triage for abnormal cytology, and post-treatment surveillance. The review focuses on cost and patient outcomes in a wide variety of resource settings, contrasting HPV testing to other screening strategies. Technology exists today, which could eradicate death from cervical cancer. It is time to act.

Chapter 5 - Human papillomaviruses (HPV) are small, double-stranded DNA viruses that primarily infect basal epithelial cells of skin and mucosal body membrane. Of 120 HPV genotypes which have been identified, at least 40 infect the genital region. Genital HPV can be classified as low-risk HPV (LR-HPV) and high-risk HPV (HR-HPV) based on their oncogenic potential. LR-HPV cause benign lesions, while HR-HPV cause precancerous and cancerous lesions of lower part of female genital tract.

HR-HPV DNA is detected in virtually all squamous cell carcinomas, which represent 90% of cervical cancer. In adenocarcinomas, the HPV prevalence is lower. HPV16 and HPV18 are the most frequent and the most aggressive HPV genotypes, contributing for at least 70% of all cervical cancer. The distribution of the other HPV genotypes varies by geographical regions and histological types of cervical cancer.

Genital HPV infection is the most common sexually transmitted disease and the main factor in cervical cancer development. The lifetime risk of genital HPV infection for sexually active women is more than 80%. Most of these infections clear spontaneously within a year or two. In 10–20% of women, infection remains persistent, giving the risk of progression to grade 2/3 cervical intraepithelial neoplasm (CIN). About 30–40% of CIN3 progress to invasive cervical cancer. The time period from the detection of HR-HPV to cervical cancer is 10–20 years, approximately.

Integration of HR-HPV genotypes in the DNA of host cell is the key event in progression of neoplastic lesions to cancer. It results in uncontrolled and higher expression of E6 and E7 virus oncoproteins. These oncoproteins inhibit the function of P53 and RB tumor-suppressor and other cellular proteins and lead to cell proliferation, disruption of DNA repair, differentiation, and apoptosis.

Cervical cancer is the third most common malignancy in females worldwide with more than 85% of cases in middle and low-resource settings of Eastern, Western, and Southern Africa, as well as South-Central Asia and South America. The HPV prevalence in women with normal cervical cytology is also higher in less-developed than more-developed region. These differences arise from socio-economical, cultural, lifestyle and biological factors. The meta-analysis of one million women with normal cytological findings was estimated that global HPV prevalence was 11.7%. Sub-Saharan Africa (24.0%) had the highest HPV prevalence, followed by Latin America and Caribbean (16.1%).

In developed countries, cervical screening programmes dramatically reduce cervical cancer incidence. HR-HPV testing is part of these programmes. HPV testing has higher sensitivity and higher negative predictive value, but lower specificity than cytology for detecting CIN2 or worse.

Effective tool for prevention of cervical cancer is also prophylactic HPV vaccination, using two approved HPV vaccine: quadrivalent (protect against HPV6, 11, 16, 18)-Gardasil® and bivalent vaccine (protect against HPV16, 18)- Cervarix®.

Implementation of HPV vaccination will not only reduce cervical cancer, but also the other HPV-related disease. Distribution of HPV genotypes will be certainly changed, raising the future necessity for more specific screening and preventive strategies.

Chapter 6 - Human Papillomavirus (HPV) is a common sexually transmitted infection (STI). It affects genital and oral mucosa and is associated to cellular lesions which can progress to cervical cancer and some head and neck cancers respectively. HPV prevalence, genotypes and associated risk factors varies from one region to another. Effectively, risk factors are linked to sexual behavior, health history, hygienic habits and some cultural considerations, that differ across geographical regions. Pregnancy is also considered, by some authors, as a risk factor of HPV infection. However, this infection in pregnant women and its outcomes are not largely studied, in spite of the risk of vertical transmission (during pregnancy or delivery) to newborns.

In this review, the authors tried to summarize pubmed available data on genital HPV infection in pregnant women. Data on HPV worldwide distribution, associated risk factors and rates of transmission to newborns were reported and compared according to geographical regions. Also, comparison between HPV prevalence in pregnant and non pregnant women was made to discuss the association of this event to HPV infection.

Pregnant women show high rate of infection comparing to others that can, certainly, be associated to the variation in the microenvironment of the female reproductive tract. The rate of HPV infection in pregnant women varies between 4.5% and 77.7% according to geographical regions and seems to be associated, among others, to primiparity. Therefore, rate of vertical transmission shows very large variability. As in non-pregnant women, the HPV infection prevalence remains relatively low in developed countries compared to developing countries.

This review underlines the association between HPV infection and pregnancy, but it shows some limits. These limits were linked to different used methodology in different included studies. A meta-analysis could be an alternative to verify the authors' conclusions.

Chapter 7 - Naturally induced humoral immunity after human papillomavirus (HPV) infection is relatively low and often inconclusive. The main reason could be the local character of HPV disease because the immune system obtains little or no information about the on-going infection. Conversely, this is altered by HPV vaccination because the robust immune response is observed in most vaccines compared to naturally infected individuals.

Although at present there is no immune correlate of protection, it is becoming evident that neutralizing IgG antibodies play one of the most crucial roles. Moreover, vaccine induced antibodies can transudate from the serum to the oral or vaginal mucosa where they take a part in virus elimination.

Seroconversion rates and antibody levels are limited by the different specificity of detection antigens, cut-offs and the type of method. Current various serological assays measure not only total type-specific IgG antibodies but also functional neutralizing ones. Only a part of the total or neutralizing antibodies most likely can contribute to real protection against HPV. Even less than 1% of vaccine-induced antibodies is eligible to cross-react with other human papillomaviruses highly related to vaccine ones and neutralize them.

While the antibody levels peak and subsequently decay within the first 6 months after vaccination, the matured antibody levels slightly but significantly increase to reach a plateau. Total type-specific IgG and neutralizing antibodies for all vaccine HPV types persist in lower levels related to their peak for at least 48 months following immunization regardless of the HPV vaccine used. Finally, long-lasting protection can be sustained with the vaccine generated immune memory independent of the presence or the absence of antibodies.

Antibody response inversely correlates with age and the highest was observed in subjects younger than 12 years old regardless of gender. Interestingly, Black men had higher titers of neutralizing antibodies than did both Caucasian and Asian men.

The post-vaccination response becomes stronger if the interval of the first two doses is extended over 6 months. Therefore, an alternative 2-dose regimen could eventually replace currently recommended 3-dose vaccination.

A higher antigen content formulation of vaccines or a combination more than one antigen in multivalent vaccines did not alter immune response compared to licensed vaccines or monovalent candidate vaccines, respectively.

The result of HPV vaccination is unaffected by concomitant immunization with other inactivated vaccines.

Maternal antibodies of immunized women undergo transplacental transport during pregnancy and they can protect new-born infants at least in the short-term. In spite of poor response to HPV vaccination, there is a humoral and specific cell-mediated immunity confirmed in immunocompromised individuals.

Chapter 8 - There is strong evidence that women with systemic rheumatic disorders, particularly systemic lupus erythematosus (SLE) have an increased risk for developing cervical cancer. Several epidemiological studies have shown causal relationship between human papilloma virus (HPV) infection and cervical neoplasia in general population, nevertheless; information about the impact of HPV infection in women with rheumatic disorders treated with immunosuppressive drugs or biologic therapy is still evolving. HPV infection is more frequently observed in some rheumatic systemic diseases mainly in SLE with relevant data available. Prevalence figures in SLE for HPV infection vary from 11.8% to 54%. Recently, the author's group reported in Mexican women with SLE, HPV infection is 14.7%, a figure that is important for Latin America where cervical cancer is highly prevalent. Longitudinal designs have been rarely performed; one study reported an increase in frequency of HPV infection from 12.5% to 25% after 3 years of follow-up. In SLE there is a high frequency of infection with several type of virus (around 17%), most of them considered as high-risk virus for cervical neoplasia. Data in African American women with SLE show that around 3% have high-grade squamous intraepithelial lesions (HSIL) and 1.2% cervical cancer. Epidemiological studies of HPV infection in other rheumatic diseases are infrequent; the author's group reported HPV infection in 31% of the rheumatoid arthritis (RA) patients, whereas others reported 3% in Sjögren syndrome. Although common risk factors such as age, occupation, lifetime sexual partners, other sexually transmitted co-infections, or early age at first intercourse, are related with HPV; in rheumatic disorders the association between HPV infection with utilization of immunosuppressive drugs or with longer duration of treatment with corticosteroids but with no disease activity is relevant. Some immune abnormalities induced by the treatment have been associated with an increased frequency of HPV, where lower levels of B lymphocytes and NK cells in peripheral blood are observed in SLE under treatment with immunosuppressive drugs. TNF-α is involved in signaling apoptosis in

infected cells, participating in the inhibition of viral replication, therefore TNF inhibitors may theoretically increase the risk for persistent infection but data supporting this hypothesis are still insufficient. HPV immunization with recombinant vaccine is useful to prevent precancerous cervical lesions, although; some autoimmune adverse events have been developed after the vaccine application including Guillain-Barré, transverse myelitis, optic neuritis, multiple sclerosis or myasthenia gravis, and there are some rare case-reports of RA, SLE, mixed connective tissue disease, Sjogren syndrome, dermatomyositis and scleroderma developed after the vaccination. On counterpart, vaccination is safe in patients with inactive RA or SLE. No significant differences in seroconversion rates have been observed between users and non users of immunosuppressive drugs except for mycophenolate mofetil. In summary, HPV infection in rheumatic disorders is an exciting area for research and a future task is to design a clinical guide for preventive measures in these patients, as well as a strategy for follow-up and treatment in those patients who have HPV infection; particularly for those who receive immunosuppressive therapy or anti-TNF agents.

Chapter 9 - Worldwide, cervical cancer is among the most common cancers in women. Human papillomavirus (HPV) has a strong association with cervical cancer. Since HPV plays an important etiological role in cervical cancer, it is logical to use HPV as a marker for early detection of cervical cancer and precancer. Recent advances in technology enable the development of high-throughput HPV assays of different formats, including DNA-based, mRNA-based, high-risk group-specific and type-specific methods. These assays are to improve the accuracy and cost-effectiveness of cervical cancer screening programs. While conventional Pap smear or liquid-based cytology is still the standard for care in many parts of the world, the intrinsic drawbacks of cytology-based screening call for replacement by HPV testing or the addition of adjunct markers. However, HPV test results are bound to have a low positive predictive value that may subject women to unnecessary follow-up investigations. A fine balance has to be established between the sensitivity and specificity of the HPV test to achieve a clinically useful predictive value, thus maximizing the efficacy of screening.

Chapter 10 - Human Papillomavirus is a double stranded DNA virus that is now well established as a causative agent in human cervical cancer. Although many women who contract high risk HPV strains have an immune response which successfully eliminates it, the virus is the root of the overwhelming majority of both squamous cell carcinoma and adenocarcinoma histologies. Though strains 16 and 18 are the root of up to 70% of cervical cancers, there are now at least 14 identified strains of HPV which are considered high risk including 33, 45, 51, and 52, with increasing evidence that the epidemiology of HPV infection may differ across women of different races. Recent advances have allowed for early detection of HPV concurrent with pap smears, prior to the onset of advanced cervical dysplasia, making its detection a dramatic step in prevention of cervical cancer. This is now becoming standard of care, allowing women to have less frequent screening with the more sensitive HPV DNA test in combination with cytology versus with cytology alone. Additionally, there are new developments in the detection of HPV DNA in which patients may self-swab, or undergo urine screening with potentially equal sensitivity, which may lead to better patient compliance with screening. This chapter will detail the various assays for HPV detection including the details of standard detection methods, new assays, and the potential for HPV testing to be used as primary cervical cancer screening.

Chapter 11 - Human papillomavirus (HPV) remains a risk factor for ano-genital and cervical cancers and for more than a decade, emphasis has also been placed on the links

between oral sex, HPV infection, and head and neck malignancies. However, the level of knowledge that dental professionals and lay people have in terms of HPV transmission through oral sex and oral cancer development remains unknown. In this chapter, the authors summarize the results from two major studies they conducted in the past five years in Vancouver, Canada: one in a format of a consensus-building community forum gathering researchers, clinicians, and the community to discuss these issues, and another in a format of a knowledge-based survey questionnaire distributed at not-for-profit health organizations and dental schools. Although recent attention has been given to the potential links between HPV infection and oral cancer, such links remain mostly unknown by the public, and HPV vaccines are yet to be proven efficacious in also preventing oral malignancies. Physicians and dentists could discuss oral sex practices to raise awareness with their patients.

Chapter 12 - HPV types 16 and 18 are considered responsible for 70% of cervical neoplasias worldwide. However, variations concerning the genotypes involved in cervical cancer and its precursor lesions in different geographic regions of the world have been reported. This fact could highly affect the impact of HPV vaccination, which mainly focuses on genotypes 16 and 18. Genotype 58 is the seventh most important in cervical cancer worldwide, but in the case of certain countries from Asia and Latin America, it is present with importantly higher frequencies. In Yucatán, southeast Mexico, the author's research group has found this genotype is highly prevalent in different populations, being as frequent as HPV16; contrary to this observation, HPV18 has been found with lower prevalence than reported worldwide.

This chapter describes the prevalence and distribution of genotypes 16, 18 and 58 in different populations, results from various projects carried out by the author's research group in Yucatan: women without cervical pathology attending for Pap smear, women with cervical lesions receiving attention in dysplasia clinics and anticancer center; obstetric patients attending for term pregnancies and for spontaneous abortion. In addition the authors have studied a population of incarcerated women from a social readaptation center prison. Finally, results from HPV58 and HPV16 genetic variability will be described, and perspectives for future research will be exposed.

Chapter 13 - The detection and treatment of pre-invasive cervical lesions are keys to the prevention of invasive cervical cancer (ICC) morbidity and mortality. The identification of human papillomaviruses (HPVs) as the main oncogens involved in anogenital as well as other HPV-induced cancers has revolutionized our understanding of the natural history of HPV-related pre-invasive and invasive cervical lesions. The availability of various methods to test HPV DNA has compelled us to reconsider the soundness of the traditional screening method based essentially on the practice of cytology.

Currently, a large number of screening algorithms based on cytology, HPV, and visual inspection (each alone or combined) have been and still are in the process of validation. Evidence is accumulating in favor of HPV testing as an essential first step. The traditional yearly Pap test follow-up is now challenged favoring HPV testing at longer time intervals (provided the last screening by cytology and/or HPV was negative).

The arguable inclusion of ICC amongst the AIDS-defining illnesses has added to uncertainties concerning screening algorithms for HIV-infected women. The developing world faces the double burden of high HIV and cervical cancer incidences. This is compounded by the difficult task of choosing between and prioritizing a range of other pressing medical conditions.

It is clear that cervical cancer primary prevention through vaccination and life-style changes is currently out of reach in resource-limited settings. Because of the lack of facilities, professionals, education, and high rates of women lost to follow-up, "first world style" secondary prevention is not yet feasible. Therefore, screening algorithms must be tailor-made to meet as best as is possible the need for ICC prevention in resource-limited settings. This may also require reconsidering some cancer prevention programs.

Chapter 14 - Over 120 sub-types of small, non-enveloped, DNA human papillomavirus (HPV) selectively infect keratinocyte stem cells on the surface layer of human skin and mucus. Most of them are easily cleared up by immune system. However, HPV subtypes with oncogenic potential can induce cell proliferation and they origin benign warts or malign tumors. Over 5% of worldwide newly diagnosed malignancies are attributable to HPV. Pelvic and perineal structures are exposed to the virus through direct skin contact during the sexual practices, and can be place of persistent warts known as condylomata acuminata, premalignant lesions, and malignancies. Many vulvar, vaginal, penile, and anal cancers are also origin by HPVs. The authors review the role of HPV in all those malignancies of the human reproductive tract.

Chapter 15 - This paper has a dual purpose. First, to determine the stable regions of the capsid human papillomavirus type HPV16 L1 from a molecular dynamics simulation up to 4 ns. This study introduces the concept of standard normalized deviation to confirm that the dynamics converge to a stationary value. The authors found that the region of greatest mobility is the area of interaction between the monomers to assemble into a pentameric structure. Secondly, it also evaluates a recent methodology for identifying computational linear B-cell consensus epitopes from the available data in the IEDB database that may trigger an immune response. Following this study, 13 potential epitopes were obtained that perfectly match with the capsid of the virus.

Chapter 16 - Over 120 sub-types of small, non-enveloped, DNA human papillomavirus (HPV) selectively infect keratinocyte stem cells on the surface layer of human skin and mucus. Most of them do not generate any symptom and can be easily cleared up by immune system. However, HPV subtypes with oncogenic potential induce cell proliferation and origin the development of benign warts or malign tumors. HPVs are the most important infectious cause of cancer. Over 5% of worldwide newly diagnosed malignancies are attributable to them. Pelvic and perineal structures are exposed to the virus through direct skin contact during the sexual practices, and can be place of persistent warts known as condylomata acuminata, premalignant lesions, and malignancies. Almost all cervical cancers, the most common female malignancy worldwide, and many vulvar, vaginal, penile, and anal cancers are caused by HPVs. Changes in sexual behavior are increasing the HPV presence in oral and upper respiratory track, and the incidence of HPV-related oropharyngeal cancers. The authors review current literature about epidemiology, and outcome of HPV-related non-small cell lung cancer.

Chapter 17 - This chapter details an overview of each of the statistical aspects of HPV modeling in a quantitative manner. The authors introduce practitioners to development of quantitative models for HPV epidemic dynamics in a generic Bayesian framework. This is done in two settings, when the population dynamics are deterministically described by a dynamical model or are treated as stochastic processes. In each case, under the Bayesian formulation the authors consider, the model parameters are treated as static random vectors to be estimated from the data along with the dynamics of the epidemic in a population. For these

generic classes of Bayesian models the authors specify how one can statistically formulate key inferential quantities for generic HPV model frameworks related to point estimation, interval estimators, model selection, prediction and forecasting. The authors then provide explicit details in the HPV epidemics context of popular approaches to constructing the model components underpinning the presented Bayesian framework.

Next the authors discuss sexual mixing matrices which describe sexual behaviour in a population. The authors present from first principles the components of such a matrix and then discuss to extend aspects of this model component to incorporate additional flexibility in the populations behavioural assumptions through introducing stochasticity to the mixing matrix.

Having formulated the models the authors then carefully detail for practitioners the sampling approaches that can be adopted to make inference from the Bayesian models developed, based on Markov chain Monte Carlo sampling algorithms. In particular the authors present for practitioners new to this field, an elementary discussion on Markov chain samplers. Then for the benefit of those familiar with the basic mechanisms involved with such techniques, the authors present details of state-of-the-art samplers which are adaptive in their Markov chain proposals. The authors provide algorithms for the Gibbs sampler, the Slice sampler, the Adaptive Metropolis sampler and the Riemann-Manifold Hamiltonian Monte Carlo sampler.

The authors then conclude the chapter by considering an example based on the actual data, which incorporates calibration as well as an analysis of the impact of vaccination.

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Chapter 1

HUMAN PAPILLOMAVIRUS INFECTION: OVERVIEW

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ABSTRACT

Human Papilloma Virus (HPV) is a virus belonging to the group of Papillomaviruses. HPV infections are very common and can cause disease of the skin and mucous membranes. We know about 100 types of Papillomaviruses, divided into 16 groups designated progressively with the letters from A to P, in accordance with the sequence homologies of deoxy-ribonucleic acid (DNA). It is also possible to classify Papillomaviruses in cutaneous and mucosal, according to the tissue for which they are specific. Most of the viruses in this family cause disease which are not serious, such as skin warts, though some can cause benign tumors such as genital condyloma as well as malignant cancer of the cervix, the mouth, anus, esophagus, and the larynx. HPV are bare (without pericapsid), possess an icosahedral capsid with a diameter around 50 nm, formed by 72 capsomeres which may be pentons or exons. Each capsomer gives rise to a protuberance which has a shape similar to a five-pointed star with a channel at the center. The capsid contains a genome consisting of double-stranded circular DNA 8 Kb long, which codes for eight early genes (early, E1 to E8) and two late genes (late, L1 and L2). Upstream of the early genes there is a regulatory region containing the origin of replication, some sequences regulating the transcription, and a N-terminal sequence common to all early proteins. The early proteins are used to modify the metabolism of the infected cell to make it available to HPV, while the late ones are the structural proteins that join together to form the structure of icosahedral viral capsid. All genes of HPV are placed on the positive strand. The functions of these proteins are: 1) L1 and L2 proteins encode capsid proteins, as we have seen; 2) E1 protein enables episomal replication, with helicase activity; 3) E2 protein participates in the transcription of the E6 promoter, actives E1, and has trans-activating ability: in particular, E2 inhibits transcription of E6 and E7; when the HPV DNA is integrated with the human genome, there is the breaking of the gene sequences of E2, with suppression of inhibition against E6 and E7; 4) E4 protein is expressed in the late stages of infection and is very important in viral

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maturation and proliferation: it is able to bind to cytoskeletal proteins, causing the deformation of infected cells (koilocytosis); 5) E5 protein locks the exposure of the histocompatibility complexes type I and II, avoiding the T-cell mediated response; E5 protein inhibits apoptosis and also alters the signals initiated by the binding of epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) with their respective receptors; 6) E6 protein binds to p53, interfering with DNA repair and with the triggering of apoptosis; and 7) E7 protein binds to the retinoblastoma (Rb) protein, preventing the blocking of the cell cycle. By virtue of their binding properties and inactivating tumor suppressor genes (TSG) such as p53 and Rb, proteins encoded by the viral genome promote intense cell replication that results in the formation of papillomas, condylomas, focal epithelial hyperplasia, warts, and cancers. However, not all Papillomaviruses are responsible of forms of carcinoma. In particular, carcinomas of the uterine cervix are triggered by genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, although the types 16 and 18 are the most frequent, while the simple warts and condylomas are generated by genotypes 2, 7 (common warts), 1, 2, 4 (plantar warts), and 3, 10 (flat warts). The E7 oncoprotein is a small protein of only 98 water-soluble aminoacids, poorly immunogenic. For this reason, it can not be used individually as a hapten and must be "merged" with the most immunogenic protein complexes. The stimulation of an immune response polarized by a vaccine containing fragments or proteins complexed with E6 and E7 may be able to eliminate the risk of cancer associated with HPV infection. All cancers of the cervix are caused by HPV. The types of HPV can be divided into low-risk HPV, which attack the skin (6, 11, 42, 43, 44) and high-risk HPV, which attack the mucous membranes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). It is estimated that over 70% of women contract genital infection with HPV in their lifetime, but the vast majority of these infections is likely to disappear spontaneously in the course of a few months due to their immune system. Only in case of persistence over time of infection by high-risk oncogenic HPV, it will be possible, in a minority of cases and in the course of several years, the development of a malignant cancer of the cervix. The early proteins of the virus have the aim of favoring the growth and division of the cell; HPV in fact can replicate only in cells in replication, as it does not code for its own DNA polymerase and needs the polymerase of the host cell, which is synthesized in cells that are dividing. The target cells of the virus are the skin and mucous membranes, two tissues that regenerate constantly. The virus induces growth of the basal and spinous layers of the epidermis (acanthosis) or of the superficial layer of the mucosa, giving rise, depending on the site of infection, to skin warts or papillomas of the mucous membranes; it also promotes the synthesis of keratins (hyperkeratosis). The expression of genes of HPV is correlated with the different types of keratin expressed in the layers of the epidermis. The basal and the spinous layers of the epidermis contain within the nucleus of their cells also the HPV DNA, while the late genes L1 and L2, which allow the formation of the mature virion, are expressed only in the stratum corneum of the epidermis. The virus replication occurs preferentially in the stratum granulosum. The virus is released along with the dead keratinocytes of the upper layers of the epidermis. Genital warts (GW), usually caused by HPV, are skin growths of verrucous type that affect by preference genital areas, both in the male (glans, less frequently under the foreskin, shaft of the penis, and scrotum) and in female (perineum, vulva , vagina, and cervix). The dangerous types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) differ from the harmless ones (6, 11, 42, 43, 44) both in the site of action (the first attack the mucous membranes and the latter the skin) and on some mutations of E7 oncoprotein. This different dangerousness is due to the fact that mucous membranes are much more susceptible to HPV infections than the skin because the lower strength of the cellular membranes of the cells of the mucous membranes facilitates the entry of viruses within them, and in addition the E7 oncoproteins of high-risk Papillomaviruses have mutations that allow an aminoacid binding and inhibition of Rb protein better than the low-risk E7 types. For example, the difference between the

primary E7 HPV 16 (dangerous) and 6 (not dangerous) aminoacid is an aspartic acid in position 21 present in the first instead of a glycine present in the second: this only difference causes the first protein to have a capacity ligand (and therefore to cause cancer) by 41% higher than the second. Once that HPV is entered into it, the cell is an infected cell that synthesizes two proteins called E6 and E7, which bind and inhibit the Rb protein (a protein that serves to regulate the cellular mitosis), which causes uncontrolled cell divisions. It is thought that these physiological changes are given to serve the virus-infected cells to spread better. HPV is contracted through direct contact (sexual, oral, and skin) or in dirty places (e.g. public toilets not cleaned to the standard). HPV is not present in body fluids such as blood or sperm. The risk of contracting an HPV infection increases with the number of sexual partners, and is highest among young adults (20-35 years). The virus is most frequently found among the promiscuous populations and in poor conditions of hygiene. The use of condoms does not seem to have a complete protective action, because the infection is often spread to the skin of the vulva and perineum. HPV infection is asymptomatic in most cases. In some cases, it may instead manifest as warts in the genital area (penis or vulva, perineum). HPV lesions of the cervix can be detected by a Papanicolaou (Pap) test, colposcopy, or techniques of molecular pathology, and lesions of the penis by penescopia. As in many viral infections, treatment of HPV is often problematic. However, since the majority of HPV infections regress spontaneously, only a minority of cases require treatment. In cases of persistent infection of the cervix, there are currently no highly effective non-invasive treatments. In case the infection is associated with changes to premalignant epithelium, it can be taken into consideration laser therapy or conization, i.e. resection of a small part of the uterine cervix to remove a lesion that may become malignant or that already is but of small size. For the removal of condylomata acuminata (CA) of the vulva, penis, or perineum, it can be used laser vaporization, elecro-coagulation, cryotherapy, or podophyllin applications. Every year, in Italy, there are around 3,500 women who get cervical cancer. Nearly half die. 400,000 women worldwide each year get sick and half of them die. It is estimated that 75% of the population comes into contact with the virus at least once during its life. It was recently discovered a molecule that allows the treatment of HPV infection, even in the external skin (wart). The drug product is called cidofovir, and is a potent broad-spectrum antiviral, even more effective than aciclovir. Cidofovir is used primarily in people with immune system problems, such as people living with Human Immunodeficiency Virus (HIV), in liquid formulation for intravenous use, but also in topical formulations for external forms (warts) which are unfortunately still widespread in Italy. It is possible that a woman contracts HPV infection in pregnancy, resulting in concern about the state of infection and transmission to the fetus. The risk of infection during pregnancy is not different from the non-pregnant woman, and the distribution of viral types with high- and low-risk is not different. The infection may appear for the first time in pregnancy and result in genital lesions which may be of aberrant aspects, very large, giant, and brittle. Despite the maternal-fetal transmission is possible at the level of the birth canal and can cause recurrent juvenile papillomatosis and GW in the neonate, cesarean section is not indicated, unless very large lesions that obstruct the birth canal are present. In the case of HPV, there are two vaccination strategies: preventive and therapeutic. The first is intended to prevent the onset of infections, the second (still at an experimental level) to treat them when they are already in place. With regard to the first strategy, it is already on the market, and is distributed free of charge to girls under the age of 12 years (in some regions such as Basilicata, in other age groups), a vaccine containing two transgenic viral capsid proteins of HPV 16 and 18 (responsible for 70% of cervical cancers) and thus casing proteins (L1 and L2) such as virus-like particles (PLV). The vaccine is safe and real virus-free; it has almost completely efficacy in preventing the infection by HPV 16 and 18. It is only ineffective in treating any infection already in place by the aforementioned viruses, because L1 and L2 proteins are produced in only a

small fraction of the time of infection (when the virus passes from one cell killed to another). The available vaccines are Cervarix® and Gardasil®. Instead a therapeutic vaccine, i.e. that it cures infections already in place, must produce proteins that are synthesized during the whole life cycle of the virus, such as E6 and E7. For this purpose, experiments with transgenic plants are ongoing which produce these two antigens (obviously with mutations that make them absolutely safe for human health). According to recent studies HPV, in addition to be the cause of many tumors of mucous membranes, could have a role in the pathogenesis of several chronic inflammatory and autoimmune diseases, including multiple sclerosis, Kawasaki syndrome, lupus erythematosus, and rheumatoid arthritis.

INTRODUCTION

Human Papillomaviruses (HPV) are simple, non-enveloped tumor viruses with a double stranded deoxy-ribonucleic acid (DNA) approximately 8 kilobases (kb) in length. The viral genome is enclosed by a spherical capsid with icosahedral symmetry and a diameter of about 55 nm [1]. HPV consist of a heterogeneous group of viruses known to induce a variety of squamous cell tumours (papillomas and warts) in the skin, and on mucous membranes of respiratory, gastrointestinal, and genitourinary tracts. HPV is one of the most common causes of sexually transmitted diseases (STD). In the past 30 years, biochemical and serological studies have shown the existence of more than 200 different types of HPV, of which approximately 50 infect the genital area [2]. These viruses show hardly no antigenic relationships. Their DNA differ by their sensitivity to restriction endonucleases, and show little, if any, sequence homology, as detected by molecular hybridization using complementary ribonucleic acid (RNA) transcribed in vitro [3].

Geographic differences in base composition of individual genotypes are generally small and suggest a low mutation rate, and thus an ancient origin of today's prototypes. The relatively small size of the genome permitted an analysis of individual gene functions and of interactions of viral proteins with host cell components. Proliferating cells contain the viral genome in a latent form, large scale viral DNA replication, as well as translation and functional activity of late viral proteins, and viral particle assembly are restricted to differentiating layers of skin and mucosa. In humans, HPV infections cause a variety of benign proliferations: warts, epithelial cysts, intraepithelial neoplasias, anogenital, oro-laryngeal, and -pharyngeal papillomas, keratoacanthomas, and other types of hyperkeratoses. Their involvement in the etiology of some major human cancers is of particular interest: persistent infection with oncogenic "high risk" (HR) HPV causes all cervical cancers, most anal cancers, and a subset of vulvar, vaginal, penile, and oropharyngeal cancers (OPCA). In recent years, cutaneous beta-HPV types have been associated with the pathogenesis of non-melanoma skin cancers (NMSC). Specific types (HPV 16, 18, and several others) have been identified on a global scale as causative agents of more than 95% of cancers of the cervix and are also linked to more than 50% of other infection-linked anogenital cancers in females, in males for barely 5%. These HPV types are considered as HR infections. Their "early" (E) E6/E7 oncoproteins stimulate cell proliferation by activating cyclins E and A, and interfere with the functions of the cellular proteins (p) 53 and retinoblastoma (Rb), respectively. The latter interaction appears to be responsible for their mutagenic and aneuploidizing activity as an underlying principle for the progression of these HPV-containing lesions and the role of

HR HPV types as solitary carcinogens. In non-transformed human keratinocytes transcription and function of viral oncoproteins is controlled by intercellular and intracellular signaling cascades, their interruption emerges as a precondition for immortalization and malignant growth. Recently, novel and known HPV types have also been identified in a high percentage of NMSC (basal and squamous cell carcinomas [SCC]). Similar to observations in patients with a rare hereditary condition, epidermodysplasia verruciformis (EV), characterized by an extensive verrucosis and development of skin cancer, basal and SCC develop preferentially in light-exposed sites. This could suggest an interaction between a physical carcinogen (ultraviolet [UV]-part of the sunlight) and a "low risk" (LR) (non-mutagenic) HPV infection. Reports on the presence of HPV infections in cancers of the oral cavity, the larynx, and the esophagus further emphasize the importance of this virus group as proven and suspected human carcinogens [4-6].

Cervical HPV infections and cervical intraepithelial neoplasias (CIN) are precursors to cervical cancer, the second most common cancer in women worldwide. HPV satisfies the epidemiologic criteria for causality. The role of other cofactors is under study. Natural history studies show that most low grade (LG) lesions (productive HPV infections) regress or persist, whereas high grade (HG) lesions (those with integrated HPV DNA) progress [7]. Progress in this field has been and remains hampered by the lack of cell culture systems allowing replication of these highly host- and tissue-specific viruses, and by the widely variable virus content of the different human lesions known to be associated with a HPV. Further studies have been warranted by the role of these widespread and epitheliotropic viruses in the origin of some carcinomas in man [3]. Knowledge of HPV infection is a rapidly changing area which presents a diagnostic and therapeutic dilemma. The clinical expression of HPV infection is highly variable and includes spontaneous regression and recurrence. A variety of molecular biologic methods are available for HPV detection and typing [8]. Immunobiologic studies demonstrate that infection peaks in the early 20s, leading to a 10- to 20-year period of persistent infection, before finally progressing to a preinvasive or invasive lesion. Papanicolaou (Pap) screening has lowered the morbidity and mortality from cervical cancer in every country in which screening programs have been introduced. The diagnostic strategy for an abnormal Pap smear includes colposcopy. The role of HPV DNA testing in screening or diagnosis is now clear. Detection techniques include Hybrid Capture (HC) (Digene, Silver Springs, MD, United States of America [USA]) and polymerase chain reaction (PCR) [7].

Progress in understanding the structure of HPV and its pathogenesis has led to a wide variety of possible new treatment modalities to combat HPV-related disease. Most HPV infections (whether HR or LR) resolve without any medical intervention. Several therapy options exist, and the choice of treatment depends on the physician and the circumstances and desires of the patient. Persistent or progressive disease, however, remains difficult to treat. Although currently available therapies have proved efficacious and tolerable in the treatment of nongenital and genital warts (GW), no single therapy is uniformly effective in eradicating persistent HPV infection. So, the management of the patient with HPV includes recognition that the disease process cannot necessarily be cured, nor can the viral infection be totally or reliably eradicated from the genital tract with current methods. Cytodestructive methods, such as cryotherapy, remain the primary treatment modality for non-GW. Immune response modifiers (IRM), such as imiquimod, currently show the greatest efficacy in treating HPV-induced anogenital lesions, both with respect to complete response and in preventing recurrence [9]. The study of the immune response against HPV has been hampered by the

lack of a cell culture system for the virus. A breakthrough was made by the discovery that a major capsid protein "late" (L) L1 self-assembles into virus-like particles (VLP) when expressed in eukaryotic systems. The E6 and E7 oncoproteins are attractive targets for cancer immunotherapy because their expression is required to maintain the oncogenicity of cervical cancer cells. Cancer immunotherapy for cervical cancer with vaccinations of E7 peptides or dendritic cell-based immunotherapy is now a reality [10]. The viral cause of cervical cancer is also its Achilles heel. Prophylactic vaccines to prevent HPV infection and therapeutic vaccines targeted at the HPV tumor antigens are now ready. A firm grasp of the molecular pathogenesis of HPV and the natural history of genital HPV infections, combined with greater understanding of how to trigger effective immune responses, offers hope for the elimination of HPV-associated diseases [11]. Two prophylactic HPV vaccines based on VLP are licensed. These are up to 100% effective in preventing HPV 16 and HPV 18 infections and associated genital lesions in women, who have not been previously infected with these types. One vaccine also prevents GW caused by HPV 6 and HPV 11 [1,12].

HISTORICAL NOTES

HPV infection of squamous epithelial tissues is very common, but its importance has only recently been recognized by the medical community. HPV are now realized to consist of many genotypes and are associated with a diverse spectrum of clinical manifestations. Within the genital tract, some diseases have been recognized since antiquity: for example, GW which are caused by HPV types distinct from those causing genital cancer.

However, others (such as cervical cancer), although recognized centuries ago as linked to sexual activity, have only been associated with oncogenic HPV relatively recently, with the tools of molecular biology [13]. Papillomas of man and animals were shown to contain an infectious agent at the beginning of this century. Since then, various attempts were made to isolate the papilloma inducing agent. Electron microscopic studies later revealed that the agent is a virus, however all attempts to culture it with conventional virological techniques failed. It took until the development of molecular cloning techniques before a detailed study of this virus could start. Today, it is clear that the papilloma-associated viral agent represents one of a very heterogeneous group of viruses, now referred to as Papillomaviruses. They induce various neoplastic lesions of the skin and mucosal epithelia. Most interestingly, it emerged that particular types are associated with human cancers [14]. So, though the existence of disease associated with HPV has been documented for centuries, it has been only within the past 4 decades that we have recognized the clinical diversity and significant morbidity and mortality associated with HPV infections. DNA of specific HPV types has since been found in almost all cervical cancer biopsies. HPV oncogenes that are expressed in these cells are involved in their transformation and immortalization, and are required for the progression towards malignancy. Epidemiological studies have underlined that HPV are the main etiological factor for cervical cancer [15].

The original lack of interest and nonavailability of in vitro culture systems has hampered research. However, with the advent of molecular diagnostic techniques, strong evidence suggests that HPV plays a major role in the development of specific anogenital cancers, including cervical, vaginal, vulvar, penile, and anal. It is this association between anogenital

cancers and HPV that results in treatment guidelines that eventually will eliminate these cancers.

Moreover, to the extent that this association has resulted in overdiagnosis and unnecessarily aggressive treatment, new and ongoing research may create more appropriate treatment options [16]. After the introduction of Pap test almost 50 years ago, in the middle of the 1970s the hypothesis that cervical cancer may arise from viruses was established and in the 1990s the relationship between HPV and cervical neoplasia was confirmed. The European Course on HPV Associated Pathology (ECHPV) was founded in 1990 by a group of clinicians, pathologists, and virologists to teach important principles for the practice and management of HPV disease to gynecologists, dermatologists, and other medical disciplines. These guidelines are intended to assist the practice of primary care physicians for diagnosis and treatment of ano-GW [17].

The most familiar HPV manifestation in the genital tract is the venereal wart (condyloma acuminatum [CA]) recognized since antiquity, and shown to be a STD. Tumorigenesis due to Papillomavirus infection was first demonstrated in rabbits and cattle early last century. Despite the evidence obtained in animals, the role of viruses in human cancer was dismissed as irrelevant. It took a paradigm shift in the late 1970s for some viruses to be recognized as "tumor viruses" in humans. In 1976, two other morphologically distinct HPV lesions were described in the uterine cervix, currently known as a flat and an inverted condyloma. Subsequently, these new HPV lesions were also shown to be an STD, and in addition, they frequently seem to occur concomitantly with CIN, carcinoma in situ (CIS), and occasionally with invasive cervical carcinomas as well.

These morphologic findings substantiated by the reports on malignant transformation of HPV lesions, as well as data from animal experiments and epidemiologic surveys, have lent support to the concept that HPV might be involved in the development of cervical (and other) human SCC. Further evidence has been provided by the discoveries of HPV structural proteins (viral antigens) and HPV 11 DNA in lesions of CIN, and HPV 16 and 18 DNA predominantly in invasive cervical carcinomas, and in 1995, more than 60 years after Rous's first demonstration of Cottontail Rabbit Papillomavirus (CRPV) oncogenicity, World Health Organization (WHO) officially declared that "HPV 16 and HPV 18 are carcinogenic to humans". As a consequence, cervical (and other) HPV lesions have been the subject of intense study utilizing epidemiologic, morphologic, immunohistochemical, biochemical, and molecular biologic methods (recombinant gene technology) to provide further evidence of the suggested causal relationship between HPV and cancer. Prospective follow-up studies have also been made to explore the natural history of cervical HPV lesions as well as the factors (e.g. immunologic, epidemiologic, synergistic actions) modifying them. Thanks to the rapid progress made in HPV research in the last few years, many important questions have been answered, leading to the final conclusions drawn as to the role of HPV in cervical carcinogenesis. In the light of present understanding, the factors linking HPV to cervical squamous cell carcinogenesis can be summarized as follows:

1) HPV infection in the uterine cervix is a STD;
2) HPV lesions in the uterine cervix seem to be equivalent to CIN in their clinical behavior, i.e. possess the potential to progress towards CIS;
3) malignant transformation seems to depend on HPV type, being conditioned by integration of HPV DNA with the host cell DNA;

4) malignant transformation most probably requires synergistic effects between the virus and chemical or physical carcinogens, or other infectious agents; and
5) genetic disposition (data available on animals only) significantly contributes to the process [18,19].

Infectious etiologies for certain human cancers have long been suggested by epidemiological studies and studies with experimental animals. Important support for this concept came from the discovery by Harald zur Hausen's group that human cervical carcinoma almost universally contains certain HR HPV types. Over the years, much has been learned about the carcinogenic activities of HR HPV. These studies have revealed that two viral proteins, E6 and E7, that are consistently expressed in HPV-associated carcinomas, are necessary for induction and maintenance of the transformed phenotype. Hence, HPV-associated tumors are unique amongst human solid tumors in that they are universally caused by exposure to the same, molecularly defined oncogenic agents, and the molecular signal transduction pathways subverted by these viral transforming agents are frequently disrupted in other, non-virus-associated human cancers [20]. Experimental studies with animal Papillomaviruses have been a determining factor in these findings. Animal Papillomaviruses have been studied both as agents of disease in animals and as models of HPV infection. In addition to the study of Papillomavirus infection in whole animals, in vitro studies with animal Papillomavirus proteins have contributed greatly to the understanding of the mechanisms of cell transformation. Animal Papillomaviruses cause distressing diseases in both farm and companion animals, such as teat papillomatosis in cattle, equine sarcoids, and canine oral papillomatosis and there is an urgent need to understand the pathogenesis of these problematic infections. Persistent and florid teat papillomatosis in cows can lead to mastitis, prevent the suckling of calves, and make milking impossible. Heavily affected animals are culled and so occasionally are whole herds. Equine sarcoids are often recurrent and untreatable and lead to loss of valuable animals. Canine oral papillomatosis can be very extensive and persistent and lead to great distress. Thus, the continuing research in the biology of animal Papillomaviruses is amply justified. Bovine Papillomaviruses (BPV) and CRPV have been for many years the model systems with which to study the biology of HPV. Induction of papillomas and their neoplastic progression has been experimentally demonstrated and reproduced in cattle and rabbits, and virus-cofactor interactions have been elucidated in these systems. With the advancements in molecular and cell culture techniques, the direct study of HPV has become less problematic and understandably research efforts have shifted in focus from animal to HPV. However, there are still areas in which studies on animal Papillomaviruses will continue to provide answers to questions pertaining to virus biology. One of these questions is the involvement of HPV in esophageal and bladder cancer in humans as is the case for BPV in cattle. Another is the site of viral latency. Lymphocytes have been proposed as a site of latency for both BPV and HPV but only experiments performed in animals could clarify this point. Animal Papillomaviruses have been instrumental in the development of vaccines as cattle, rabbit, and more recently dog all provide the opportunity to study vaccination in the natural host. Several anti-HPV vaccines, both prophylactic and therapeutic, based on those developed in animals, are now available with encouraging results. In vitro studies with two animal Papillomavirus E proteins, the transcriptional regulator E2 and the oncoprotein E5, among others, have contributed to the elucidation of viral gene control and cell transformation. BPV E2 was the first viral product to

be identified as a transcriptional regulator. More recently, its association with mitotic chromosomes has been suggested as a mechanism for the partition of viral genomes between daughter cells, and its L2-mediated localization in the sub-nuclear compartments promyelocytic leukemia (PML) oncogenic domains (POD) is believed to favor viral DNA encapsidation. E5 is the major transforming protein of several BPV. Many of the functions of E5 proteins have been first established for BPV E5 and later validated for HPV E5. E5 interacts with 16 kb ductin/subunit c and this interaction is deemed responsible for the down-regulation of gap junction intercellular communication and the inhibition of acidification of endomembranes. E5 activates growth factor receptors (GFR) and numerous kinases, including cyclin-dependent kinases (CDK), and down-regulates expression of major histocompatibility complex (MHC) class I. Thus, E5 would help the establishment of viral infection by promoting both cell proliferation and immune evasion. Despite the extensive studies on vaccination in animals, E5 has not been tried in animal models as a possible anti-Papillomavirus vaccine. A recent study has reported that vaccination of mice with HPV 16 E5 in a recombinant Adenovirus reduced the growth of tumors induced by E5-expressing cells. Perhaps this is an instance in which work on animal Papillomaviruses should follow HPV and the potential for E5 vaccination should be validated in naturally occurring animal models [21].

Twenty years have passed since the first studies using HPV testing began in clinical settings. At that time, controversy regarding the role of HPV in cervical carcinogenesis still divided the scientific world. Epidemiological and natural history studies on HPV and cervical cancer in the ensuing two decades secured the necessary role of HR (carcinogenic) HPV in the genesis of cervical cancer, providing the rationale for testing for its cause. Subsequently, cross-sectional studies and large randomized controlled trials (RCT) have provided clinical validation for HR HPV testing in triage of atypical squamous cells of undetermined significance (ASC-US), in postcolposcopy management of women referred for ASC-US, ASC “cannot rule out HG” (ASC-H), atypical glandular cells “not otherwise specified” (AGC NOS) and LG squamous intraepithelial lesion (L-SIL) and not found to have CIN 2+ or adenocarcinoma in situ (AIS) at initial colposcopy, in post-treatment of CIN 2+ surveillance, and in cotesting with the Pap test of women age 30 and over. This is the story of the road traveled that brought the clinical use of HPV testing from its genesis only a few years after Dr. zur Hausen’s discovery to its present eminent role in both primary cervical cancer screening and abnormal Pap management [22].

In 2003, the WHO convened a gathering of experts, including scientists, national regulatory authorities, industry representatives, epidemiologists, and government officials from both developed and developing countries to discuss appropriate endpoint measurements for HPV vaccine efficacy and effectiveness trials. The consultation also considered the regulatory requirements and public health issues that vaccine candidates should address before deployment, particularly in developing countries.

The general consensus of the consultation was that it would be desirable to have a globally-agreed, measurable efficacy endpoint for considering deployment of HPV vaccines in public health settings. After hearing from experts about virological and clinical endpoints to be considered, requirements of regulatory authorities of various countries and endpoints used to measure efficacy and effectiveness for another known cancer vaccine (hepatitis B), the experts agreed that ethical and time considerations make it necessary to use a surrogate endpoint, and not invasive cervical cancer, to define efficacy of HPV vaccines. While

regulatory authorities of each country ultimately will determine the endpoints required for licensure, the consultation recommended that the endpoint for efficacy in population-based studies be, based on current knowledge, histologically-classified CIN of moderate or HG, as well as cancer. Since persistent infection with the same HR type is considered a predictor for moderate or HG cervical dysplasias and cancer, they might represent a useful endpoint in future vaccine efficacy studies. Indeed, if vaccines prove to be effective against transient or persistent HPV infections, it is likely that they will protect women against cervical cancer. The consultation recognized that in the context of many developing countries, efficacy alone might not provide enough information for countries to decide whether or not to adopt HPV vaccines as a public health prevention tool against cervical cancer. The consultation unanimously agreed that additional clinical bridging studies as well as studies to clarify local epidemiology should be conducted in certain developing countries to determine the potential impact of vaccination. Such countries should also undertake targeted interventions to ensure acceptability and programmatic feasibility of the vaccination. Recognizing that upon vaccine introduction it will be some years before a reduction in cervical cancer is detectable at the population level, the consultation stressed the importance of maintaining existing cervical screening programmes while such long-term studies are conducted [23].

Over the past decade, several vaccines that target common HPV types have entered clinical trials. Both GlaxoSmithKline (GSK, Brentford, Middlesex, United Kingdom [UK]) and Merck and Co., Inc. (Whitehouse Station, NJ, USA) companies have developed vaccines for the prevention of HPV infection [24]. Two HPV L1 VLP vaccines have been developed, a quadrivalent HPV 6/11/16/18 product and a bivalent HPV 16/18 product: both have been shown to be highly immunogenic with a good safety profile and 100% efficacy against HPV 16/18-related HG CIN 2/3, implying that they will be effective at preventing HPV 16/18-related cervical cancer [25]. The vaccine development story unfolded from an industrial perspective, since without the massive commitment shown by manufacturers, Merck and GSK, over the last decade, without any guarantee of success, there would be no such prospect [26].

In the Spring of 2006, over 100 experts in HPV, cervical cancer screening, and vaccination worked together to define how best to incorporate HPV DNA testing and the HPV vaccines into cervical cancer prevention efforts [27]. In June 2006, the Food and Drug Administration (FDA) approved the first HPV vaccine.

The vaccine was subsequently recommended by the Centers for Disease Control (CDC) and Prevention's Advisory Committee for Immunization Practices (ACIP) for routine vaccination of 11-12-year-old girls and catch-up vaccination of females 13-26 years of age [28]. In October 2009, the ACIP approved a newly licensed vaccine, Cervarix® (GSK), directed against HPV to prevent cervical cancer. The ACIP also expanded its recommendations against HPV by giving permission to physicians to vaccinate males aged 9 to 26 years with the previously licensed vaccine, Gardasil® (Sanofi Pasteur MSD, SNC, Lyon Cedex, France), to prevent GW, in addition to its previous recommendation for females aged 9 to 26 years to prevent cervical cancer and GW [29]. The American Cancer Society (ACS) has developed guidelines for the use of the prophylactic HPV vaccine for the prevention of CIN and cervical cancer. These recommendations are based on a formal review of the available evidence. They address the use of prophylactic HPV vaccines, including who should be vaccinated and at what age, as well as a summary of policy and implementation issues [30].

The terminology for HPV-associated squamous lesions of the lower anogenital tract has a long history marked by disparate diagnostic terms derived from multiple specialties. It often does not reflect current knowledge of HPV biology and pathogenesis. A consensus process was convened to recommend terminology unified across lower anogenital sites. The goal was to create a histopathologic nomenclature system that reflects current knowledge of HPV biology, optimally uses available biomarkers, and facilitates clear communication across different medical specialties.

The Lower Anogenital Squamous Terminology (LAST) Project was co-sponsored by the College of American Pathologists (CAP) and the American Society for Colposcopy and Cervical Pathology (ASCCP) and included 5 working groups: 3 work groups performed comprehensive literature reviews and developed draft recommendations, another work group provided the historical background, and the fifth will continue to foster implementation of the LAST recommendations. After an open comment period, the draft recommendations were presented at a consensus conference attended by LAST work group members, advisors, and representatives from 35 stakeholder organizations including professional societies and government agencies. Recommendations were finalized and voted on at the consensus meeting. The final, approved recommendations standardize biologically relevant histopathologic terminology for HPV-associated SIL and superficially invasive squamous carcinomas across all lower anogenital tract sites and detail the appropriate use of specific biomarkers to clarify histologic interpretations and enhance diagnostic accuracy. A plan for disseminating and monitoring recommendation implementation in the practicing community was also developed. The implemented recommendations will facilitate communication between pathologists and their clinical colleagues and improve accuracy of histologic diagnosis with the ultimate goal of providing optimal patient care [31, 32].

EPIDEMIOLOGY

Extensive laboratory and epidemiological evidence demonstrate that HPV is the major cause of SCC, its precursor lesions (CIN), and several other benign and malignant clinical manifestations including GW, CA, Bowenoid papulosis, verrucae, vaginal, vulvar, and anal intraepithelial neoplasia (VIN and AIN) and carcinoma, penile carcinoma, and other squamous neoplasias of the head and neck districts, like the oropharynx and the esophagus. Other cancers causally linked to HPV are NMSC and cancer of the conjunctiva. In addition, mother-to-child transmission is probably responsible for recurrent laryngeal and pulmonary papillomatosis in infants [33]. Both HPV infection and cervical cancer are associated with a substantial economic burden. Pharmacoeconomic data from the USA indicate that HPV and Human Immunodeficiency Virus (HIV) infections were associated with similar total direct medical costs, and HPV infection was more costly than genital herpes and hepatitis B combined in the 15-25 age group. Furthermore, false-negative Pap smears from women with precancerous lesions are among the most frequent reasons for medical malpractice litigation in the USA [34]. Since the identification of HPV as the necessary cause of cervical cancer, HPV-based technology has become the center of novel primary and secondary cervical cancer prevention strategies by the introduction of HPV testing in screening and of HPV vaccines in preadolescent girls and young women. If implemented widely and wisely, the deployment of

these protocols has the potential to complete Papanicolaou's goal of cervical cancer eradication by extending the benefits of prevention to the developing populations of the world [35].

In both the USA and the UK, CA is the most commonly diagnosed viral STD. Cancer of the cervix is the second most common cancer in women after cancer of the breast, being the second most common cancer at all after lung cancer, and the commonest in developing countries [36]. The frequency of HPV infections varies widely from one population to another. HPV infection is highly prevalent in sexually active adolescents and young adults. The highest incidence is among females 20-24 years of age. Epidemiological data from the USA National Health and Nutrition Examination Survey (NHANES) determined that the prevalence of HPV infection in a representative sample of women was highest in those aged 20-24 years (44.8%). Sexual activity is the most important risk factor for infection, with 64% to 82% of sexually active adolescent girls testing positive for HPV. In young women, the prevalence of HPV infections in Pap smears is 1.5% to 44%, and the annual incidence approximately 8%. The lifetime risk approaches 80% for women between 20 and 80 years of age. In the 1990s, 25% of women between 20 and 29 years of age in the USA were seropositive for HPV 16 [37]. The results of studies to examine the prevalence of HPV suggest a rate of 1-13%, with a positive association to the prevalence of CIN in that population. It is estimated that genital HPV infection has a 10% prevalence among men and women in the USA 15-49 years of age: 1% have CA, 2% have lesions visible only by magnification with the colposcope or hand lens, and 7% have clinically inapparent infections. The HPV genotypes linked to skin infections differ from those infecting the anogenital area. The majority of HPV-associated disease is caused by 4 HPV types: HPV 6 and 11 are responsible for LG genital lesions and more than 90% of GW, and HPV 16 and 18 both account for approximately 70% of all HG CIN or dysplasia and invasive cancer of the cervix, vagina, and anus and for about 30-40% of cancers of the vulva, penis, and oropharynx.. The HPV types that cause GW do not cause cervical cancer. Current evidence suggests a long latency between acquisition of genital HPV infection and the development of cervical malignancy, with cervical cancer generally not developing until the 5^{th} decade and beyond [38]. Geographical widespread data on HPV type-distribution are essential for estimating the impact of HPV 16/18 vaccines on cervical cancer and cervical screening programmes. Epidemiological studies employing a variety of HPV typing protocols have been collated in meta-analyses. As we have said, HPV 16/18 is estimated to account for 70% of all cervical cancers worldwide, although the estimated HPV 16/18 fraction is slightly higher in more developed (72-77%) than in less developed (65-72%) regions. About 41-67% of HG SIL (H-SIL), 16-32% of L-SIL, and 6-27% of ASC-US are also estimated to be HPV 16/18-positive, thus highlighting the increasing relative frequency of HPV 16/18 with increasing lesion severity. After HPV 16/18, the six most common HPV types are the same in all world regions, namely 31, 33, 35, 45, 52, and 58: these account for an additional 20% of cervical cancers worldwide [39].

Although tissues in the case series of anal, penile, vaginal, and vulvar neoplasms that have looked for evidence of HPV infection by probing for HPV DNA have been selected for convenience, they support the view that HPV, especially type 16, is associated with approximately 50% of these tumors. A higher percentage of the anal, vaginal, and vulvar tumors are associated with HPV 16 than are penile tumors. This discrepancy may be due to the low number of penile tumors studied or to a true difference in the proportion of penile

cancer cases related to HPV. HPV 6/11 and 18 are found less frequently at all anatomic sites. About 10% of tumors that are probed for these viruses are positive, although there are some notable exceptions such as a study that found 39% of penile tumors positive for type 18 and a study that found approximately two thirds of vulvar tumors positive for HPV 18 using Southern blot (SB) hybridization. For all of these tumous, there is likely to be a subset of the cases who develop their cancer through mechanisms that do not involve HPV.

The case-control studies found a strong association with GW, number of sexual partners and, with the exception of vaginal cancer, heavy smoking at the time of diagnosis of the disease. A history of GW, smoking at diagnosis, and seropositivity to Herpes Simplex Virus (HSV) 2 are exposures that have also been found to be associated with cervical cancer. A population-based case-control study in western Washington and Vancouver, British Columbia that studied all anogenital cancers found that a history of GW was stronger among patients with vulvar, anal, vaginal, and penile cancer than among those with cervical cancer. This was also true of smoking at diagnosis, with the exception of vaginal cancer, where there was little excess risk. This study and other supporting data indicate that these anogenital tumors share many of the same risk factors as cervical cancer [40].

Epidemiological studies have shown that only a small fraction of women infected with oncogenic HPV types will eventually progress to H-SIL and cervical cancer. In the majority of individuals, HPV infections are transient and asymptomatic with most new infections resolving within 2 years. Cervical cancer is actually a rare outcome of HPV infection [41]. Only 5% of women in developing countries have had a Pap smear in the past five years, and nearly 500,000 new cases of cervical cancer and 274,000 cervical cancer deaths are occurring worldwide each year. Approximately 80% of the 500,000 new cases occur in developing countries and this percentage is expected to increase to 90% by 2020. In developing countries, cervical cancer tends to affect relatively young poor women and is the single largest cause of years of life lost to cancer, since screening and treatment programs, and health care, in general, are relatively inaccessible to these women [42,43]. Although current screening methods have proven effective in reducing cervical cancer incidence and associated mortality, more than 10,000 women are diagnosed annually and 4000 USA women die from the disease each year [44].

Virus infection and viral gene expression emerge as necessary but obviously not sufficient factors for cancer induction. Additional modifications of host cell genes appear to be required for malignant progression of infected cells. The expression of viral oncoproteins in cells infected by HR types (e.g. HPV 16, HPV 18), in contrast to LR types (e.g. HPV 6, HPV 11), results in chromosomal instability and apparently in accumulation of mutational events. These “endogenous” modifications seem to be most important in the pathogenesis of premalignant lesions and tumor progression. Exogenous mutagens should act as additional cofactors [45,46]. Because infection by oncogenic HPV is a necessary but not a sufficient cause of cervical cancer, it has been assumed that other factors, acting in conjunction with HPV, influence the risk of progression from cervical HPV infection to cervical malignancy. Co-factors assessed include high parity (five or more full term pregnancies), use of oral contraceptives (OC) for five or more years, hormone replacement therapy (HRT), tobacco smoking, infection with other STD (e.g. Chlamydia Trachomatis and HSV 2), dietary and nutritional factors, immunosuppressive conditions and polymorphisms in the human leukocyte antigen (HLA) system. Women exposed to HIV are at HR for HPV infection, HPV DNA persistency, and progression of HPV lesions to cervical cancer [47-49]. Genetic and

immunological host factors and viral factors other than type, such as variants of type, viral load, and viral integration, are likely to be important but have not been clearly identified [50]. A number of cervical factors, such as infection by sexually transmitted pathogens other than HPV, chronic cervical inflammation, and antioxidant nutrients, may influence the natural history of HPV infection along the pathways of persistence and progression or resolution [51]. Based on the evidence provided by the largest epidemiological studies that using sensitive detection methods allowed for the effects of HPV, it can be concluded that, among HPV positive women, high parity, smoking, other genital infections, and less consistently long-term OC use, are the identified environmental cofactors likely to influence the risk of progression from cervical HPV infection to H-SIL and invasive cervical cancer. There is limited evidence for a role of dietary factors in HPV carcinogenesis. From a public health point of view, parity seems to be the behavioral cofactor explaining the highest proportion of cervical cancer cases among HPV-infected women. Smoking and long-term OC use may have a similar impact in populations that are heavily exposed to HPV and to these cofactors. On-going epidemiological studies will shed more light into the role of these and other cofactors, but if confirmed, these conclusions may imply that multiparous women, women who are smokers, and women on long-term OC use, might need a closer cytological and HPV surveillance than women in the general population [47, 48].

The inability to produce mature HPV virions in animal models has been a major limitation in fully elucidating the oncogenic potential and role of associated cofactors in promoting malignant transformation in HPV-infected cells [52]. Sexual behavioral patterns across age groups and populations are central to the description of the HPV circulation and of the risk of infection. The concept of group sexual behavior (in addition to individual sexual behavior) is important in exploring HPV transmission and has implications for defining and monitoring HPV and cancer prevention strategies. In natural history studies, the pattern of HPV DNA prevalence by age groups is similar to the patterns of HPV incidence. Rates of exposure in young women are high and often include multiple types. There is a spontaneous and rapid decrease of the HPV DNA detection rates in the middle-age groups followed by a second rise in the post-menopausal years [53]. The number of sexual partners during the past 2 years (relative risk [RR] > 9.0) and current smoking (RR > 5.0) proved to be the two most significant risk factors for clinical HPV infection in a case-control study. In a prospective follow-up study, clinical progression was significantly related to the grade of HPV lesion ($p < 0.0001$), and to HPV type, with the progression rate of HPV 16 lesions being more than five times greater than that of HPV 6 or 11 lesions. The detection rate of HPV in men is significantly lower (approximately 30%) than in women, and the concordance of HPV types in the couples having sexual relations is surprisingly low (5% to 10%) [37]. Although condoms most likely prevent HIV infection, evidence of their effectiveness against other STD is mixed. Among estimates from several studies, there was no consistent evidence that condom use reduces the risk of becoming HPV DNA-positive. However, risk for GW, CIN 2/3, and invasive cervical cancer was somewhat reduced. Available data are too inconsistent to provide precise estimates. However, they suggest that while condoms may not prevent HPV infection, they may protect against GW, CIN 2/3, and invasive cervical cancer [54].

Sexual transmission of HPV between women has been postulated on the basis of reports of abnormal Pap smears in women who reported no prior sex with men and by studies using amplified DNA technology for HPV detection. Several factors, including prior or concurrent sex with men and sexual behaviors between women, validate the possibility of HPV infection

among women who have sex with women, and data support that HPV transmission also occurs. Limited data indicate that the frequency of routine Pap smear screening among women who have sex with women may be suboptimal relative to heterosexual women. Education of women who have sex with women and the providers of their health care should counter any assumptions that sex between women confers no risk of HPV transmission. Women who have sex with women should receive Pap smear screening in accord with current guidelines [55].

The effect of smoking on HPV activity and subsequent dysplasia and neoplasia remains controversial. Several recent large studies demonstrated that smoking was associated with a greater incidence of cervical, vulvar, penile, anal, oral, and head and neck cancer in a dose-dependent fashion, while other studies did not show any correlation between smoking and cervical dysplasia after multivariate adjustment. Recent studies have also indicated that smoking may be more closely related to HG lesions of the cervix and vulva. These data provide evidence of an association between HPV, smoking, and cancer. Progression of dysplasia likewise seems to be associated with smoking. Several groups have attempted to discern whether the connection between smoking and cervical cancer is from local immunosuppression and/or from direct carcinogenic effects [56].

The role of inflammation in HPV infection and disease is complex since it involves responses capable of preventing initial infections, clearing those ongoing, as well as promoting persistence and progression of associated lesions. Avoiding the immune response has been considered a key aspect of HPV persistence which is the main factor leading to HPV-related neoplasia. HPV have evolved different ways of targeting immune signaling pathways. Moreover, host inflammatory response may promote lesion progression and affect tumor fate by diverse mechanisms including the direct participation of inflammatory cells [57].

HPV prevalence and type distribution in normal smears and in cancer specimens show relatively small international variation. State-of-the-art detection techniques have unequivocally shown that HPV DNA can be detected in 95% to 100% of adequate specimens of cervical cancer, supporting the claim that HPV is the necessary cause. The odds ratios (OR) for cervical cancer related to a cross-sectional detection of HPV DNA range from 50 to several hundred in all studies. The risk for any of 15 HR types is not statistically different from the risk reported for HPV 16. The estimates of the attributable fraction range from 90% to 98% [58]. HPV is highly persistent in the environment, on contaminated objects, linen, floors. Skin infections can occur through indirect or direct contact. Most anogenital infections are sexually transmitted. Most HPV infections are asymptomatic, latent or transient. Various factors, especially immunosuppression, increase the persistence and severity of infections, and can promote progression to cancer. Malignant transformation of lesions due to HPV seems to be facilitated by HPV persistence, a high HPV viral load in the cervix, and immunosuppression. However, HPV infection rarely leads to progression to cancer. Only a minority of infections persist for several years, and only about 10% of LG lesions progress to a higher grade. About 5% of HG lesions progress to invasive cancer. Other anogenital cancers, such as cancer of the anus, vulva, vagina, and penis, appear to be linked to HPV (usually genotype 16). Skin cancer due to HPV is rare. Some cancers of the respiratory and gastrointestinal tracts appear to be linked to HPV infection [59].

The identification of people at HR requires careful clinical examination supplemented by the use of a magnification system, histology, and cervical cytology. The identification of HPV

genotypes in genital epithelia does not seem to be useful in this context, because they are present in a proportion of normal epithelia [60]. The spectrum of genital HPV infections comprises clinical, subclinical, and latent disease in addition to HPV-associated neoplasia. Colposcopy, cytology, and histopathology play a central role in diagnosis of clinical HPV infections, whereas DNA hybridization techniques and DNA amplification with PCR are needed to detect the subclinical and latent HPV infections. The biologic behavior of genital HPV infections is a complex one: regression, persistence, progression, and fluctuation are recognized disease patterns [37]. The definition of subclinical and latent HPV infection is still incomplete and awaits clarification by highly sensitive HPV detection systems that preserve the morphology of the tissue. Genital HPV infect the human body mainly by sexual transmission, but other pathways of HPV transmission may be possible, as suggested by:

1) high prevalences of antibody reactivity in children;
2) lack of association of HPV seropositivity with sexual activity;
3) presence of HPV DNA in oral cavity scrapings of children and adults; and
4) development of recurrent respiratory papillomatosis (RRP) among children exposed to HPV 6 or 11 during birth.

Successful infection depends on the infection site and the immunological state of the host: susceptibility to genital HPV seems highest for the squamous epithelium of the lower genital tract. Compared with humoral response, cellular immune response is probably more important for regression of genital HPV infection: the immune response in HPV-infected tissues is characterized by depletion of T helper/inducer cells or Langerhans cells (LC) and an impaired immunological function of natural killer (NK) cells or the infected keratinocyte. Epidemiological studies indicate that individuals with cell-mediated immunodeficiencies are at increased risk for genital HPV infections. In condylomata, replication and transcription of viral nucleic acids and antigen production coincide with cellular differentiation. However, the interaction between HPV and the keratinocyte on a molecular level in subclinical and latent disease is not well understood. Regression or persistence of subclinical and latent genital HPV infections, as analyzed in longitudinal investigations, show a constant come-and-go of HPV presence. In an infected individual, complete clearing of the virus seems rather exceptional. With respect to progression, the biological potential of cervical HPV infections is characterized by an increased risk for development of HPV-associated neoplasia, especially in lesions infected with HR HPV types (e.g. HPV 16 and 18). Demographic data of genital HPV infections are very variable due to differences in the HPV detection assays used and in the populations examined, the prevalence of subclinical and latent genital HPV infections appears to be at least three times higher compared with clinical HPV infections. This rate increases by a further 3-5-fold when patients are examined several times. Seroreactivity against genital HPV types may be due to an active infection or the result of contact with HPV earlier in life. Antibodies against the HPV 16 E7 protein indicate an increased risk for cervical cancer [61,62].

Appropriately sophisticated statistical approaches are crucial for addressing the increasingly complex set of critical questions that follow from the recognition that HPV is a necessary causal factor for cervical cancer. Cervical cancer researchers have defined the major stages of cervical carcinogenesis, with HPV infection as the necessary cause. Focus of etiologic studies is shifting from establishing causality to determining risk factors for HPV

persistence and neoplastic progression using serially collected biomarkers. Prevention-oriented epidemiology and trials of new screening strategies and vaccines will rely on surrogate endpoints because we cannot let women develop cancer when it can be prevented. Future epidemiologic and prevention studies of HPV infection and cervical carcinogenesis will exploit subtle pathologic distinctions and will employ improved measurements of complex molecular biologic phenomena [63-72].

PATHOGENESIS

Papillomaviruses have been identified as the causative agents of benign proliferation of epithelia and subepidermal fibroblasts in many animals including man. Recent evidence has shown that each viral type will only infect a specific species and tissue. Furthermore, certain types have been found associated with lesions capable of malignant conversion, particularly in response to secondary physical or chemical factors. Many advances have been made in identifying particular HPV types inducing papillomas capable of progression and in the identification of the virally encoded functions involved in viral replication and cellular transformation [73]. During the viral life cycle, HPV genomes receive, process, and generate signals. Transcription factors binding to their enhancer carry information about tissue specificity and hormonal regulation, while other factors in the cornified layer of the epidermis activate capsid protein production. Furthermore, products of the viral E1 and E2 genes constitute feedback signals that modulate viral transcription and replication. Proteins derived from the genes E5, E6, and E7 modulate cellular homeostasis so as to induce neoplatic transformation [74].

There is clear evidence that certain types of HPV (HPV 16 and 18) are associated with human genital cancer. Other virus types, such as HPV 6 or HPV 11, are more regularly found in benign GW. Since all viruses can be present in putative precancerous lesions of the uterine cervix (dysplasia, CIN) it has been postulated that individual HPV types have different "oncogenic potential". Expression of parts of the E region of the HPV genome in cell lines established from genital cancer supports the hypothesis that HPV are involved in inducing and/or maintaining the transformed phenotype of cancer cells [75]. It is clear that the relation between HPV infection and cervical neoplasia is more complex than initially realized. Preliminary molecular virologic data suggest preferential distributions of LR and HR HPV types in CIN that tend to correlate with the morphologic appearance. Thus, mild and moderate dysplasias (CIN 1 and 2) contain a diverse distribution of HPV types, including a minority that have a HR of malignant potential. HPV, therefore, appears to play a major role as a promoter. Neoplastic transformation is probably determined by specific HPV types but, in addition, requires initiation by some other carcinogenic stimulus, e.g. HSV 2 and cigarette smoking. Despite numerous studies, performed during the past 30 years, the long-term behavior of dysplasia remains uncertain. The presence of associated viral changes can be considered and added to the diagnosis, e.g. "moderate dysplasia (CIN 2) with evidence of HPV infection". Treatment should be the same for all intraepithelial lesions, regardless of the presence of morphologic evidence of HPV [76].

The association of certain types primarily with normal tissues and benign lesions, as opposed to cancer-associated types, has led to the concept of low and high oncogenic risk

HPV, respectively. The latter express oncogenic proteins that interfere with cell growth control functions. As a consequence of the continuous expression of these viral genomes, chromosome instability may occur, leading to fully transformed cells. Studies indicate that persistence of HR HPV may determine progression to more severe stages of cervical disease, while the majority of HPV infections are transient and do not seem to be important in cervical carcinogenesis. The risk for disease progression seems also to be associated with viral burden [77]. DNA from HPV 16 and HPV 18, two types frequently found in cervical cancer tissue, can immortalize cells in laboratory cultures, unlike DNA from HPV types associated with benign genital lesions. Studies indicate that malignant transformation involves the viral E6 and E7 gene products, which exert their effect by interfering with the cellular proteins that regulate cell growth [78]. While the genome consists of 6 E genes and 2 L genes, the E6 and E7 genes have been most studied because they interact with p53 and Rb, respectively, thus contributing to the ability of HPV to mediate oncogenesis. This same HPV-related progression has also been observed in other anogenital malignancies including anal, penile, and vulvar carcinomas. Although the evidence is not as conclusive, HPV also likely plays a role in the development of a subset of SCC of the head and neck as well as other cutaneous malignancies. While HPV infection is common, the progression to malignancy is relatively rare indicating a potential role for immune protection against persistent infection. This is supported by the fact that HPV infection and related malignancies are common in the immunosuppressed population [79].

The combination of the malignant potential of HPV and its high prevalence of infection confers to it an importance of generalized clinical and virological significance. The natural history of HPV infection with or without treatment varies from spontaneous regression to persistence. The major steps in cervical carcinogenesis include HPV infection, HPV persistence over a certain period of time, progression to precancer, and invasion. Backward steps include clearance of HPV infection and regression of precancer [80]. The most important mechanism for wart regression appears to be cell-mediated immunity (CMI). Cytokines released by keratinocytes or cells of the immune system may play a part in the induction of an effective immune response against HPV infection and the subsequent regression of lesions [81].

The crucial role of genital oncogenic HPV in cervical carcinoma development is now well established. In contrast, the role of cutaneous HPV in skin cancer development remains a matter of debate. Cutaneous beta-HPV strains show an amazing ubiquity. The fact that a few oncogenic genotypes cause cancers in patients suffering from EV is in sharp contrast to the unapparent course of infection in the general population. Recent investigations revealed that a natural barrier exists in humans, which protects them against infection with these HPV. A central role in the function of this HPV-specific barrier is played by a complex of the zinc-transporting (Zn-T) proteins EVER1, EVER2, and ZnT-1, which maintain cellular zinc homeostasis. Apparently, the deregulation of the cellular Zn balance emerges as an important step in the life cycles not only of cutaneous but also of genital HPV, although the latter viruses have developed a mechanism by which they can break the barrier and impose a Zn imbalance [82]. The productive life cycle of HPV is linked to epithelial differentiation. HPV are thought to infect cells in the basal layer of stratified epithelia and establish their genomes as multicopy nuclear episomes. In these cells, viral DNA is replicated along with cellular chromosomes. Following cell division, one of the daughter cells migrates away from the basal layer and undergoes differentiation. In highly differentiated suprabasal cells, vegetative viral

replication and L-gene expression are activated, resulting in the generation of progeny virions. Since virion production is restricted to differentiated cells, infected basal cells can persist for up to several decades or until the immune system clears the infection. The E6 and E7 genes encode viral oncoproteins that target Rb and p53, respectively. During the viral life cycle, these proteins facilitate stable maintenance of episomes and stimulate differentiated cells to reenter the S phase. The E1 and E2 proteins act as origin recognition factors as well as regulators of E viral transcription. The functions of the E5 and E1-E4 proteins are still largely unknown, but these proteins have been implicated in modulating L viral functions. The L1 and L2 proteins form icosahedral capsids for progeny virion generation. The characterization of the cellular targets of these viral proteins and the mechanisms regulating the differentiation-dependent viral life cycle remain active areas for the study of these important human pathogens [83].

The pathogenesis of vulvar and cervical cancer are thought to be similar and to be related to a sexually transmitted agent, which, in recent years, has been demonstrated to be HPV. These two neoplasms differ dramatically in age distribution and relationship to precursor lesions, making a common etiology unlikely. The apparent discrepancies can be explained by a hypothesis that implicates HPV as an etiologic factor for the majority of cervical carcinomas but for only a small proportion of vulvar carcinomas. Most vulvar carcinomas occur in older women and are not related to HPV, whereas a subset of vulvar carcinomas occur in young women and are related to this virus. Characterization of two distinct types of vulvar carcinoma may clarify associated risk factors and may have important implications in the clinical management of this disease [84]. Vulvar SCC appears to arise via two distinct pathways: a significant minority are associated with oncogenic HPV infection and undifferentiated VIN, however, the majority arises in the absence of HPV, on a background of chronic inflammation. Until recently, it was assumed that lichen sclerosus was the underlying inflammatory condition in the majority of HPV-negative cancers. This pathway of carcinogenesis has been less well studied than the HPV pathway. Emerging evidence implicates differentiated VIN (DVIN), rather than lichen sclerosus, as the most likely precursor lesion in HPV-negative vulvar SCC. Clinical and molecular evidence implicates DVIN as a lesion with a high malignant potential. This lesion is probably underdiagnosed and may be undertreated. Better recognition of DVIN by gynecologists and pathologists may therefore offer an opportunity to prevent some vulvar cancers [85].

NATURAL HISTORY

During the past decades, much has been learned about the natural history of HPV. Pathologic and epidemiologic studies performed over the past three decades have provided evidence that the development of SCC of the cervix is a multistep process involving a precursor preinvasive stage. The results of recent molecular analyses now suggest that HPV plays a role in this process and is an important but insufficient factor in the development of invasive carcinoma. Infection by a variety of HPV types may result in active viral intranuclear replication without integration into the cellular genome. This episomal form of infection is manifested morphologically by the development of mild dysplasia, CIN 1 with koilocytosis, and acanthosis. Approximately 20 different HPV types have been associated

with CIN 1 lesions, whereas HG dysplasia and CIS (CIN 2 and 3) are associated with only a few viral types (mainly HPV 16, 31, 33, and 35). LG lesions are differentiated and have a LR of progression to cancer, whereas HG lesions are characterized by nearly complete or complete loss of squamous maturation and a higher risk of progression to invasive cancer [86]. HPV infection of metaplastic epithelium in the cervical transformation zone is relatively common, producing latent infection in susceptible persons. The epidemiological characteristics of subclinical Papillomavirus infection (SPI) and cervical cancer are essentially identical and there is a strong clinico-pathological association between condylomas and anogenital carcinoma. Tissue culture cells have been transformed from a normal to a neoplastic phenotype by animal Papillomaviruses, and there is extensive data reporting upon the successful identification of HPV genomic sequences in tumor cells. SPI commonly coexists with foci of CIN. Areas of apparent transition are seen, and these two lesions are linked by a discernible spectrum of morphologic change. Such circumstantial evidence gave biological plausibility to the suggestion that HPV could be a cervical carcinogen. It has been postulated that cervical neoplasia arises by progression from benign viral hyperplasia, through varying stages of koilocytotic atypia with associated dysplasia, to unremarkable CIS. Invasion was presumed to reflect the emergence of an aggressive heteroploid clone, an age-related decline in host immune surveillance, or an interaction of both factors [87].

HPV infection of the cervix affects the developing immature metaplastic cells of the transformation zone. Cervical neoplasia can be viewed as the interaction of HR HPV and immature metaplastic epithelium. Once maturity is reached, there is minimal risk of subsequent development of cervical squamous neoplasia. Exposure to HPV is an extremely common event, especially in young sexually active women. Yet, despite frequent HPV exposure at that phase of life in which the cervical transformation zone is at its most vulnerable, established expressed disease is relatively uncommon. Most studies in which the natural history of CIN is not altered by cervical biopsy reveal a progression rate from LG to HG CIN of less than one third. Where viral type is taken into account, however, the progression rate from normal but HR HPV-infected cervical epithelium to CIN 2 or 3 is higher. Despite this, most cervical abnormalities will not transform into invasive cancer, even if left untreated. The variance between the high rate of HPV infection, the intermediate rate of CIN, and the relatively low rate of cervical cancer establishes a stepwise gradient of disease of increasing severity with decreasing prevalence. In an immunocompetent host, HPV infection alone does not appear to be sufficient to induce the step from HG CIN to invasion. Epidemiological studies indicating that HPV infection with oncogenic viral types is far more common than cervical neoplasia suggest the necessity of cofactors in cervical carcinogenesis. The long time-lag between initial infection and eventual malignant conversion suggests that random events may be necessary for such conversion, and the spontaneous regression of many primary lesions suggests that most patients are not exposed to these random events. In those women who develop cervical cancer, malignant progression is rarely rapid, more commonly taking many years or decades. Malignant progression has been documented in patients who presented initially with only LG HPV-induced atypia. On the other hand, progression may be a misnomer, as “apparent” progression may really represent adjacent “de novo” development of higher grade CIN [88].

Although these fundamental steps are established, several new epidemiologic studies have shed light on the factors that influence each of these transitions. There are four major

steps in cervical cancer development: infection of metaplastic epithelium at the cervical transformation zone, viral persistence, progression of persistently infected epithelium to cervical precancer, and invasion through the basement membrane of the epithelium. The importance of the the metaplastic epithelium of the transformation zone in cervical cancer has been extended to other HPV-induced cancers such as anal or tonsillar cancers. Natural history studies show that HPV with normal cervical cytology and CIN grade 1 behave similarly, with the majority of both showing regression. Although these studies have demonstrated the importance of HPV persistence in the development of precancer CIN 3, the timing from infection to evidence of CIN 3 varies from 1 to 10 years. Whether equivalent lesions diagnosed later differ in their natural history remains unknown. Several factors have been implicated in enhancing persistence and/or progression. However, none are consistently associated with both except age: young women are less likely to show persistence and older women with persistence are more likely to be at risk of invasive cancer. Recent studies have also underscored the importance of the host immune response in clearance of established infections. Finally, data on non-cervical HPV infections, such as penile infections, are limited to date compared to cervical infections [89].

Several problems associated with HPV infection of the cervix and lower female genital tract include the definition of HPV infection and its distinction from HPV-associated neoplasia, the distinction of HPV infection from reactive epithelial changes induced by other infections, and the transmission of HPV infection via the male partner. The available evidence indicates that there are two distinct intraepithelial processes in the cervix associated with HPV: one is the classical condyloma and its counterpart in immature epithelium (atypical immature metaplasia), the other is intraepithelial neoplasia, which, like classical infection, may be mature (CIN with koilocytosis) or immature (HG CIN or CIS). Molecular hybridization studies indicate that HPV 6 and 11 are most commonly detected in the former, whereas HPV 16 and 18 DNA are most common in the latter and in invasive cancer. From the clinical standpoint, the most important distinction is between HPV-related disease (condyloma or CIN) and reactive changes associated with other pathogens, such as Chlamydia. The former should be removed from the cervix, whereas the latter should be treated medically or followed. It is stressed that therapy should not hinge upon the histological distinction of HPV infection from neoplasia and that all lesions should be removed, by conservative means if possible. This is underscored by the fact that a high proportion of CIN lesions contain areas identical to condyloma and that lesions with deep endocervical canal involvement, including those with features suggesting condyloma, should be treated by cone biopsy to exclude the presence of invasive cancer. The management of the male partner is still unsettled. However, a large proportion of male partners of these patients have penile lesions and should be included in diagnostic and therapeutic protocols of women with genital HPV infections or neoplasms [90].

The new HPV-oriented model of cervical carcinogenesis should gradually replace older morphological models based only on cytology and histology. If applied wisely, HPV-related technology can minimize the incidence of cervical cancer, and the morbidity and mortality it causes, even in low-resource settings [91]. Natural history models of HPV infection and disease have been used in a number of policy evaluations of technologies to prevent and screen for HPV disease (e.g. cervical cancer, ano-GW), sometimes with wide variation in values for epidemiologic and clinical inputs. Published data were most plentiful for natural history parameters relating to the progression and regression of CIN without HPV typing, and

data concerning the natural history of HPV disease due to specific HPV types were often lacking. Epidemiologic evidence to support age-dependency in the risk of progression and regression of HPV disease was found to be weak. No data were found on the duration of immunity following HPV infection. In the area of clinical management, data were observed to be lacking on the proportion of clinically manifest ano-GW that are treated and the proportion of cervical cancer cases that become symptomatic by stage. Knowledge of the natural history of HPV disease has been considerably enhanced over the past two decades, through the publication of an increasing number of relevant studies. However, considerable opportunity remains for advancing our understanding of HPV natural history and the quality of associated models, particularly with respect to examining HPV age- and type-specific outcomes, and acquired immunity following infection [92].

Cervical cancer and its precursor lesions (CIN) are unique in that we can study the natural history of one disease at two different levels:

1) by assessing the clinical lesions; and
2) by analysing the viral events of HPV infections, their prime etiological agent.

The outcome (natural history) of CIN has been well established by a large number of prospective cohort studies covering over 25,000 patients, and the figures for regression, persistence, and progression are well established. The outcome of HPV infections is far more complex with at least six distinct patterns being demonstrated in long-term cohort studies. There is little doubt that the mechanistic explanation for HPV clearance is by specific immunological reactions, where competent humoral and CMI mediators are needed. To understand this process in detail still necessitates a substantial amount of clinical and laboratory research, however. In general, HPV outcomes follow the pattern where a dynamic balance exists between incident infections and virus clearance. Following a rapid accumulation of incident infections after onset of sexual activity (women < 20 years of age), there is a transition of this balance in favor of virus clearance soon after age 25. This explains the constantly declining age-specific prevalence of HPV infections until menopause. Failure to eradicate the virus at postmenopause is not uncommon, however, explaining the deep second peak in HPV prevalence now reported in many different populations. The importance of HPV clearance/non clearance (= persistence) has been recognized recently, and the number of studies addressing these issues has increased substantially during the past few years. The data are now rather unanimous concerning the times and rates (usually expressed per 1,000 women/months at risk [WMR]) of HPV clearance. On the other hand, data are still incomplete and in part inconsistent as to the cofactors that regulate these events. A wide variety of variables have been explored as potential co-determinants and/or predictors of HPV clearance. Until now, all efforts attempting to identify suitable biomarkers as such predictors, have been disappointing, but fortunately, this is a largely unexplored area as yet. Similarly, data on the two extremes of life, i.e. early infancy and postmenopause, are still far too fragmentary to enable creating a comprehensive view, how these viral infections behave in early life, and what makes many women incapable of clearing their virus at postmenopause. Both issues are of utmost importance and have widespread clinical implications. We need to know how and why some infants and children contract HR HPV infections well before the onset of their sexual activity, to be able to select the proper targets for prophylactic HPV vaccination. Similarly, we need to know why some women over 55 years of age are likely to

remain HR HPV carriers, while the vast majority successfully clears their infection well before the menopausal age. Early detection of cervical cancer precursors among these elderly HR HPV positive women past the usual age of organized screening remains a major challenge also in the future [93, 94].

VIROLOGY

HPV are present in virtually all cervical cancers and cancer precursors. Understanding the epithelial virology of this group of viruses has greatly influenced current concepts of cervical carcinogenesis, has provided a framework for understanding the biologic basis of many diagnostic criteria, and has led to revised classification schemes, diagnostic testing, and modifications in clinical management [95]. HPV are small 55-nm 7.9 kb double-stranded DNA (dsDNA) tumor viruses that contain a compact and non-redundant genome. The HPV capsid is non-enveloped, having a T=7 icosahedral symmetry formed via the interaction among 72 pentamers of the major capsid protein, L1. The minor capsid protein L2 associates with L1 pentamers, although it is not known if each L1 pentamer contains a single L2 protein. The HPV life cycle strictly adheres to the host cell differentiation program, and as such, native HPV virions are only produced in vivo or in organotypic "raft" culture. Research producing synthetic HPV particles, such as VLP, HPV-based gene transfer vectors, known as pseudovirions (PsV), and HPV genome-containing quasivirions (QV), has bypassed the need for stratifying and differentiating host tissue in viral assembly and has allowed for the rapid analysis of HPV infectivity pathways, transmission, immunogenicity, and viral structure [96].

Papillomaviruses form one genus of the Papovaviridae family. They share common antigenic determinants and their DNA cross-hybridize under conditions of low stringency. The classification of Papillomaviruses is at present based on the host range and the relatedness of the nucleic acids. Isolates are considered independent types if there is less than 50% cross-hybridization in the liquid phase according to a standard protocol. The host range does not reflect the natural relationship between the viruses. Subgenera, which differ in biological properties, can be distinguished in outline. Data on overall sequence homology are insufficient for a meaningful classification because two types of virus may be closely related within one genome region and rather heterogeneous in other areas. Some new isolates appear as intermediates between previously well-separated types and complicate the system. A reasonable classification of such types of Papillomavirus should be based on homologies between genes that are relevant for differences in the biology of the viruses. A functional mapping of the rather uniformly organized, colinear genomes of Papillomaviruses has been made. Genetic studies with BPV type 1 have assigned functions in replication, transformation, gene expression, and capsid synthesis to individual open reading frames [97]. The HPV types that are causally linked to genital cancer originated in ancient times in prehuman primates. The molecular diversity of viral isolates reflects the African origins and the subsequent worldwide spread of human races. The lack of transmission of Papillomaviruses between species may support a gradual mode of molecular evolution [98].

HPV are formally described by isolation of their circular dsDNA genomes and establishment and comparison of the nucleotide sequence of these genomes. Alternatives such as serological diagnosis and maintenance of HPV in culture are neither clinically useful nor

consistently feasible. Novel HPV isolates have traditionally been described as "types". The analysis of specific HPV types is of medical importance, because HPV types typically induce type-specific lesions, i.e. they may be specific for cutaneous or mucosal epithelia, or give rise to benign warts or malignant carcinomas. Recently, it was formally decided that Papillomaviruses are a virus family separate from the Polyomaviruses. Within the Papillomavirus family, closely or remotely related types form species or genera. These formal agreements were important as they brought the taxonomy of Papillomaviruses in line with that of other viruses, bacteria, and higher organisms, although their impact on medical practice and terminology used in clinical studies is limited. Notably, however, HPV types that are closely related (i.e. form "species") are associated with similar lesions. Confusion of the terms "type" and "subtype" should be avoided, as the latter term refers to some specific but rare taxonomic assemblages. In contrast to many RNA viruses, HPV types evolve very slowly, and diverged since the origin of humans only by about 2%. These divergent isolates are called "variants". HPV evolved together with humankind and Homo sapiens was never without HPV, and consequently never without warts and cervical cancer. Variants of the same HPV type may have different pathogenicity and may account for part of the worldwide disparities in the occurrence of genital cancers [99]. It is believed that HPV intratype variants may differ in biological behavior. Recognition of the crucial role that some specific HPV types play in cervical cancer development is highly important for their prevention and implementation of public health strategies to control cervical cancer [100]. Several viruses often infect the same species and each virus is associated with a defined tissue. Recent evidence has shown that certain benign lesions can undergo malignant transformation in both animals and humans in response to genetic or environmental factors [101]. HPV itself is both ubiquitous and markedly heterogeneous but can nevertheless be classified as either a HR type or a LR type based upon its frequency of detection in cervical cancer. Given that the association between HPV and cervical cancer is causal, the classification of this virus has been strengthened by large-scale epidemiologic studies and is widely accepted across many disciplines. It is evident, however, that cervical cancer is frequently associated with multiple HPV types. Therefore, it is crucial to distinguish causal types of HPV (drivers) from noncausal types (passengers) in cervical lesions [102].

Hallmarks of HPV infection include a restricted tropism for human epithelial cells and a viral life cycle tightly linked to the differentiation program of the host keratinocyte. This particular viral cycle has hampered the study of the HPV vegetative life cycle for decades, due to the lack of suitable in-vitro culture conditions. The tissue and differentiation dependence seems to be dictated by viral transcription rather than viral DNA replication. Indeed, viral transcription is restricted to epithelial cells of human origin, more specifically to keratinocytes. In contrast, HPV genomes can replicate in various undifferentiated cell lines regardless of their natural permissiveness to infection, as long as the viral replication proteins E1 and E2 are expressed [103]. HPV, with the help of only few genes, can achieve a complete vegetative cycle specifically in the epidermal and mucosal keratinocytes. Modification of the host cell transcriptional regulation is one of the major ways to regulate the viral production and maturation. The vegetative cycle of HPV is linked to terminal differentiation of the epithelium and is dependent on the host cell regulatory networks for transcriptional control. The mucosal HR HPV 16 and HPV 18 types have been the main models to explore this transcriptional regulation mainly because they are prevalent in cervical cancer as the best studied virally induced cancers in human. In addition, the availability of cell lines, grown

from cervical cancers containing integrated HPV 16 or 18, represent versatile in vitro models for transcription studies. Another specificity of small DNA viruses is the multifunctional characteristics of their regulatory proteins due to extreme genomic constraint [104]. Recombinant DNA technology has enabled investigators to understand HPV biology. HPV DNA has been found in a variety of benign and malignant tumors. Once discovered, this HPV DNA can be extracted, produced in large quantities, and submitted to nucleotide sequence analysis. Ultimately, this research reveals the mechanisms involved in the malignant transformation of HPV-induced papillomas [105].

GENETICS

Genetic experiments have assigned oncogenic activity to the viral genes E5, E6, and E7. The encoded proteins interact with and disturb the physiologic functions of cellular proteins that are involved in cell cycle control. The proteins of HPV 16, 18, or related types are most efficient in this regard. Some of these activities lead to genetic instability of the persistently infected human cell. This enhances the probability of mutations in cellular proto-oncogenes and tumor suppressor genes (TSG) and thus contributes to tumor progression. Mutations in cellular genes devoted to the intracellular surveillance of HPV infections, integration of viral DNA, and deletions or mutations of viral transcription control sequences lead to a significantly increased expression of the E6/E7 genes, which is a consistent characteristic of HG intraepithelial neoplasia and cancers. The genetic instability caused by viral oncoproteins and the autocatalytic increase in oncoprotein expression caused by mutations in the viral and cellular genome identify the virus as a major driving force of progression [106]. The integration sites in the cellular genome of HPV are located in chromosomal regions always associated with oncogenes or other known tumor phenotypes. Two regions, 8q24 and 12q13, are common to several cases of cervical carcinoma and can have integrated more than one type of HPV DNA. These two chromosomal regions contain several genes implicated in oncogenesis. These observations strongly imply that viral integration sites of DNA tumor viruses can be used as the access point to chromosomal regions where genes implicated in the tumor phenotype are located, a situation similar to that of non-transforming retroviruses [107].

The development of HPV-immortalized cervical and foreskin cell lines represents a useful model for studying the role of HPV in cervical cancer. Studies with these cells show that HPV genes regulate epithelial cell growth and differentiation. Transfection of HPV types associated with invasive cervical cancer results in immortalization of human epithelial cells, whereas HPV not associated with cancer are ineffective. The combination of E6 and E7 genes, which are normally retained and expressed in cervical carcinomas, is sufficient for immortalization, however, the E7 gene alone induces immortality less efficiently. Although the immortalized cells actively express HPV oncoproteins observed in cervical cancer, after injection of immortal cells into nude mice, tumors are rare, having been reported only for HPV 18. Immortalized cells are resistant to terminal differentiation. In fact, HPV may contribute to the carcinogenic process by uncoupling the processes of cell growth and differentiation. Host regulation of viral genes also is important in the malignant process. Endogenous cytokines modify HPV gene expression and influence the pathogenesis of HPV

infection in the cervix. HPV gene expression is regulated by cellular transcriptional activators and repressors. This normal regulation is altered by viral integration. HPV become integrated preferentially at chromosomal regions near fragile sites and protooncogenes. In fact, immortality is associated with induction of structural rearrangements frequently affecting HPV integration sites. Structural and numerical alterations nonrandomly involve chromosomes 1, 11, 19, and 20, with chromosome 1 alteration being the most predominant. Wild-type functions of Rb and p53 are necessary to control normal cell growth, and mutation or loss of these suppressor genes often contributes to cancer development. In HPV-containing carcinomas, pRb and p53 were wild type. However, in carcinomas lacking HPV, both suppressor genes were mutated. Functional inactivation of these TSG by HPV oncoproteins E6 and E7 may explain this difference. Treatment of HPV-immortalized cells with ras or a subfragment of HSV of HPV-immortalized cells resulted in locally invasive carcinomas when the cells were implanted subcutaneously in nude mice [108].

Human epithelial cells transfected by HR-type HPV DNA can overcome apoptosis and become immortalized, but they are not capable of anchorage-independent growth or tumorigenic in nude mice, suggesting that they are premalignant and not malignant cells. These cells, due to their genetic instability, are liable to progress to anchorage-independent growth and tumorigenicity following subsequent genetic and epigenetic events. On the other hand, because immortalized epithelial cells represent an initial step in the multistep model of human carcinogenesis in vitro, they are useful in studies on the regulation by environment factors. Immortalized epithelial cells differ from normal epithelial and malignant carcinoma cells, in their response to various cytokines and growth factors, but their response is more like that of normal cells than of malignant cells. These experiments indicate that HPV integration and expression are insufficient for malignancy but that HPV do participate in the multistep development of cancer [109]. It is apparent that an intricate interplay of cellular and viral factors determines whether the outcome is active HPV infection, viral latency, or ultimately, genital cancer [110]. Both host and viral genetic factors have been postulated to be important determinants of risk of HPV progression to neoplasia among infected individuals. Epidemiological studies have evaluated the role in cervical cancer pathogenesis of genetic variation in HLA genes and in the HPV genome itself. A protective effect of HLA class II DRB1*13/DBQ1*0603 alleles is the most consistent HLA finding in the published literature. A consistent association between HPV 16 non-European variants and risk of disease is also evident from published work [111].

Research on the intratypic variability of HPV has defined variants that are associated with persistent infections and are potentially more oncogenic, translating to a higher risk of malignant disease. The genetic variability of the host also plays a role in the risk of cervical cancer, especially genes controlling the immune response, such as HLA class I and II. These highly polymorphic genes are important risk determinants of HPV persistence and disease progression. The interaction between host and viral factors is complex and needs to be further investigated, paving the way to better define the patients at the highest risk of developing malignant diseases linked to HPV infection [112]. Progression of the HPV infected cell to a malignant phenotype involves further modification of host gene expression and/or mutations. The appearance of chromosomal aberrations can lead to mutational inactivation or loss of TSG, activation and amplification of oncogenes, with importance for the process of carcinogenesis. Oncogene amplification, with exception of few reports, seems not to be a major mechanism in cervical carcinogenesis. In contrast, cytogenetic and loss of

heterozygosity (LOH) results from CIN and invasive cancer demonstrate alterations at specific chromosomal regions, pointing at localisation of TSG. Genetic alterations at chromosomes 3p, 6p, 1lq were frequently found early in tumor development. Primary invasive carcinoma showed additional allelic losses at chromosome arms 6q, 17p, and 18q. Useful biological diagnostic and prognostic markers for HR HPV infection and malignant progression may be p16INK4a, p27Kip, and NET-I/C4.8. Putative senescence genes relevant for HPV-induced carcinogenesis are localized on chromosomes 2, 4, and 10. Genes for telomerase suppression are presumably located on chromosomes 3, 4, and 6 [113].

MOLECULAR BIOLOGY

In the past years, new data have been published on the molecular biology of HPV infections and their relationship to cervical neoplasia. HPV-related genital neoplasms are one area where molecular biology has had an impact at many levels. Studies of cell transformation, gene expression, and genome organization have linked HPV to neoplasia; they have also provided data suggesting potential pathways by which the HPV genome exerts its effect on cells. Molecular epidemiological studies using clinical material have identified specific HPV types with neoplasia, profiled the populations at risk for these infections, and supported the emerging concept of latent infection. Studies using in situ hybridization (ISH) have confirmed the close relationship of neoplastic change with certain infections (such as HPV 16), and have detailed the transcription patterns of the HPV genome in warts, precancers, and carcinomas. The technology of ISH has facilitated the evaluation of archive material: using this material, the close relationship between HPV type 18 and adenocarcinomas and small-cell carcinomas has been described. Methods for expressing HPV proteins in bacteria have produced a spectrum of antisera to specific gene products, which in turn facilitate mapping their distribution in tissues, determining their biological significance, and clarifying the host immune response to genital HPV infections. These multidisciplinary approaches helped to promote an understanding of genital HPV infections and their related neoplasms as well as clarifying the role of HPV in the evolution of genital neoplasia [114]. The application of gene cloning and non-stringent hybridization has provided us with an apparently ever-increasing catalog of HPV. The recognition of multiple types of HPV has resulted in remarkable progress in the detection of persisting viral nucleic acid sequences in carcinomas. In vitro transformation studies indicate that HPV, in particular types 16 and 18, can transform primary mammalian cells in cooperation with Ha-ras. This means that these viruses have at least a c-myc like activity which is consistent with their ability on their own to immortalize primary keratinocytes. The fact that HPV 6 can also transform mammalian cells, at a low frequency, in cooperation with Ha-ras indicated the need to use appropriate cells and systems to elucidate differences in virus-cell interactions between benign HPV types 6 and 11 and the viruses associated with severe disease 16 and 18 [115].

It was recently found that infectious entry of these viruses is dependent upon a specific proteolytic event that occurs prior to viral endocytosis. Specifically, a proprotein convertase, furin or proprotein convertase 5/6, must cleave the minor capsid protein for infection to proceed [116]. Like many other viruses, HPV use the host cytoskeletal components for several steps during their life cycle. Prior to internalization, HPV particles are transported

along filopodia to the cell body. Following internalization, retrograde transport along microtubules via the dynein motor protein complex is observed. In addition, viral minichromosomes depend on the host cell machinery for partitioning of viral genomes during mitosis, which may be affected by oncoproteins E6 and E7 of HR HPV types [117].

HPV are strictly host-specific and also show a distinct tropism to squamous epithelial cells. Upon HPV infection, only a portion of the virus reaching the nucleus seems to undergo replication, suggesting that HPV replication remains confined to a small number of cells. HPV critically depend on the cellular machinery for the replication of their genome. Viral replication is restricted to differentiated keratinocytes that are normally growth arrested. Hence, HPV have developed strategies to subvert cellular growth regulatory pathways and are able to uncouple cellular proliferation and differentiation. Endogenous growth factors and cellular oncogenes modify HPV E and L gene expression and influence on the pathogenesis of HPV infections. HPV oncoproteins (E5, E6, E7) are important proteins not only in cell transformation but also in the regulation of the mitotic cycle of the cell, thus allowing the continuous proliferation of the host cells. Cyclins are important regulators of cell cycle transitions through their ability to bind CDK. CDK have no kinase activity unless they are associated with a cyclin. Several classes of cyclins exist which are thought to coordinate the timing of different events necessary for cell cycle progression. Each CDK catalytic subunit can associate with different cyclins, and the associated cyclin determines which proteins are phosphorylated by the CDK-cyclin complex. The effects of HPV on the cell cycle are mediated through the inhibition of antioncogens (mostly p53 and Rb) and through interference with the cyclins and CDK, resulting in target cell proliferation, their delayed differentiation, and as a side-effect, in malignant transformation [118].

Five of the more than 50 known genital HPV types, HPV 6, 11, 16, 18, and 31, have become the models to study gene expression. The comparison of the studies of these five viruses and analyses of the genomic sequences of those genital HPV types that have not been transcriptionally studied make it likely that genital HPV share most strategies for regulating their transcription. These strategies are quite different from those of unrelated human and animal Papillomaviruses. Among these common properties are:

1) a specific promoter structure allowing for fine-tuned negative feedback;
2) a transcriptional enhancer that is specific for epithelial cells;
3) regulation by progesterone and glucocorticoid hormones;
4) silencers, whose principal function appears to be transcriptional repression in the basal layer of infected epithelia;
5) specifically positioned nucleosomes that mediate the functions of some enhancer and the silencer factors;
6) nuclear matrix attachment regions that can, under different conditions, repress or stimulate transcription; and
7) as yet poorly understood, late promoters positioned very remote from the late genes.

Most of these properties are controlled by cellular proteins that, due to their simultaneous importance for cellular processes, may not be useful as HPV-specific drug targets. It should be possible, however, to target complex cis-responsive elements unique to these HPV genomes by nucleotide sequence-specific molecules, such as antisense RNA, polyamides, and artificial transcription factors. The application of small molecule-based drugs may be

restricted to target proteins encoded by the HPV DNA, such as the replication factor E1 and the transcription/replication factor E2 [119]. Genome amplification, which is necessary for the production of infectious virions, is prevented until the levels of viral replication proteins rise, and depends on the co-expression of several viral proteins. Virus capsid proteins are expressed in cells that also express E4 as the infected cell enters the upper epithelial layers. The timing of these events varies depending on the infecting HPV, and in the case of the HR HPV, on the severity of neoplasia. Viruses that are evolutionarily related, such as HPV 1 and Canine Oral Papillomavirus (COPV), generally organize their productive cycle in a similar way, despite infecting different hosts and epithelial sites. In some instances, such as following HPV 16 infection of the cervix or CRPV infection of domestic rabbits, Papillomaviruses can undergo abortive infections in which the productive cycle of the virus is not completed. As with other DNA tumor viruses, such abortive infections can predispose to cancer [120].

Although in benign infections the viral genome is present as an episome, in cancers it is integrated. Integration disrupts the E2 and E5 genes and viral gene regulation. Two viral early genes, E6 and E7, and an upstream regulatory region (URR) are preserved in cervical carcinoma cell lines as well as in clinical samples of cervical cancer, indicating that these regions are important in cancer development. The integration event invariably results in the expression of the two viral proteins, E6 and E7. These two proteins are capable of transforming cells individually and cooperate to immortalize primary human epithelial cells. E6 and E7 are transcribed from a promoter, P97. P97 is regulated by complex interactions between multiple, positive and negative, cellular factors and the viral E2 product. E2 disruption caused by the integration into the cellular genome may induce overexpression of E6 and E7. Molecular analysis has revealed that the E6 protein encoded by the HPV HR types prevalent in cancers forms a tripartite complex with the p53 tumor suppressor protein and a cellular protein termed E6-associated protein (E6-AP), resulting in the degradation of p53. E6-AP is an E3 ubiquitin protein ligase and is not normally involved in the regulation of p53 stability in the absence of E6. E6-AP is the prototype for the homologous to the E6-AP carboxyl terminus (HECT) domain family of E3 ubiquitin protein ligases. In the presence of E6, E6-AP catalyses the ubiquitylation and proteolysis of p53. The E7 protein encoded by HR HPV types shows high-affinity association with the pRb, and the other pRb related “pocket proteins” p107 and p130. The E7 protein associates also with other cellular factors known to play a role in cell cycle regulation [121-123]. Cellular transformation is achieved by complex interaction of these oncogenes with several cellular factors of cell cycle regulation including p53, Rb, cyclin-CDK complexes, p21, and p27. Both persistent infection with HR HPV genotypes and immune dysregulation are associated with increased risk of HPV-induced SCC [124].

The HPV 16 E7 protein has been shown to be a multi-functional protein possessing both transcriptional modulatory and cellular transforming properties similar to those described for Adenovirus E1A and Polyomavirus Simian Virus (SV) 40 large T proteins. E7 is able to transactivate the Adenovirus E2 promoter and can cooperate with an activated ras oncogene to transform primary baby rat kidney cells. The N-terminal 37 amino acids of all of the E7 proteins of the genital associated HPV contain regions which are highly conserved and which are quite similar to portions of conserved domains 1 and 2 of Adenovirus E1A. These domains in E1A are critical for cellular transformation properties and contain the amino acid sequences involved in binding the product of the pRb. Results from a collaborative study with Ed Harlow and Nick Dyson (Cold Spring Harbor Laboratory) have shown that the E7

oncoprotein of HPV 16 can associate with the Rb gene product in vitro. The ability of the E7 proteins encoded by various HPV to bind pRb has been examined using an in vitro complexing assay. E7 is not sufficient for transformation of human keratinocytes. The co-operation of the HPV 16 E6 and E7 genes has been shown to be important for transformation of these cells [125]. Like SV40 large T and Adenovirus 5 E1B, the E6 oncoprotein encoded by the HR HPV can form a complex with p53. In vitro, E6 promotes the degradation of p53 and this degradation involves the E3-ubiquitin-dependent protease system. The selective degradation of cellular proteins such as p53 with negative regulatory functions provides a mechanism of action for dominant acting oncoproteins. The relevance of the inactivation of the normal functions of pRb and p53 in human cervical carcinogenesis has been demonstrated by the analysis of these two genes and their products in a series of HPV-positive and HPV-negative cell lines. Each of HPV-positive cervical cancer cell lines expressed normal pRb and low levels of wild type p53 proteins, which are presumed to be altered in function as a consequence of association with the HPV oncoproteins. In contrast, mutations were identified in the p53 and Rb genes expressed in the HPV-negative cervical carcinoma cell lines, C33-A and HT-3. These results confirm that the inactivation of the normal functions of the tumor suppressor proteins pRb and p53 are important steps in human cervical carcinogenesis, either by mutation or through complex formation with HPV E6 and E7 oncoproteins [126,127]. This process results in impaired TSG function, involving DNA repair, decreased apoptosis, and eventual cell immortalization. Mutations causing chromosomal alterations, loss of heterozygosity, and proto-oncogene and telomerase activation in immunopermissive individuals have important roles in virus-induced cervical carcinogenesis. The so-called non-European variants of HPV 16 and 18 may increase the degradation potential of p53. HPV 16 is polymorphic and, although the evidence is controversial, the Arg/Arg genotype of p53 could have greater susceptibility to HPV E6 degradation than the other genotypes. The coincident interplay between the non-European genomic variants of HPV 16/18 and p53 Arg/Arg may explain, at least in part, the persistence of HPV infection and tumor progression in women with cervical neoplasia [128].

The E1 proteins are the essential origin recognition proteins for HPV replication. E1 proteins bind to specific DNA elements in the viral origin of replication and assemble into hexameric helicases with the aid of a second viral protein, E2. The resultant helicase complex initiates origin DNA unwinding to provide the template for subsequent syntheses of progeny DNA. In addition to adenosine-5’-triphosphate (ATP)-dependent helicase activity, E1 proteins interact with and recruit several host cell replication proteins to viral origin, including DNA polymerase alpha and replication protein A (RPA) [129].

The viral E2 protein represses transcription of the E6/E7 oncogenes and activates viral DNA replication together with the viral E1 helicase. The E2 protein is specifically inactivated in HPV 18-associated carcinoma, suggesting that it may prevent carcinogenic progression. Indeed, E2 was shown to exhibit a strong anti-proliferative action when ectopically expressed in cervical carcinoma cells, as it induces both G1 cell cycle arrest and cell death by apoptosis. While the cell cycle arrest is due to E2-mediated transcriptional repression of the viral oncogenes, the induction of apoptosis appears to be an autonomous function of E2. The amino-terminal transactivation domain (TAD) of the E2 protein is required for its pro-apoptotic activity, but transcriptional transactivation is not involved. E2 induces apoptosis through the extrinsic pathway, involving the initiator caspase 8. In addition, E2 is cleaved by caspases during apoptosis, providing an example of an apoptotic inducer, which is itself a

target for caspase cleavage. The cleaved E2 protein exhibits an enhanced apoptotic activity, suggesting that it may participate in an amplification loop [130]. The DNA binding domain of the E2 master regulator from HPV is the primary effector for most the essential activities controlled by this protein. In addition, the particular fold of the DNA binding domain only shared with the Epstein-Barr nuclear antigen (EBNA) 1, suggests a link between this unique architecture and the function of viral origin binding proteins of this kind. Finally, the E2 DNA binding domain proved to be an excellent model for addressing fundamental problems of DNA recognition mechanisms and folding of intertwined dimmers [131]. E2 proteins are sumoylated and that overexpression of sumoylation results in a dramatic increase in intracellular levels of the E2 protein. There is increased sumoylation during keratinocyte differentiation, suggesting that the levels of E2 protein may be tied to changes in the cellular sumoylation state during differentiation. In addition to itself being regulated by sumoylation, E2 appears to influence the sumoylation state of one of its binding partners, the cellular transcription factor (CTF), cytidine-cytidine-adenosine-adenosine-thymidine (CCAAT) /enhancer-binding protein (C/EBP). Overall, these observations indicate a complex interplay between this viral protein and the host sumoylation system [132]. The HPV transcription/replication factor E2 specifically up-regulates expression of the serine/arginine-rich (SR) proteins splicing factor 2/alternative splicing factor (SF2/ASF), SRp20 and SC35 in infected epithelial cells. These SR proteins are essential for viral RNA processing. SF2/ASF is a proto-oncogene that is also up-regulated in a number of cancers. For example, SF2/ASF, together with SRp20 and SC35, is selectively up-regulated in cervical tumors caused by persistent oncogenic HPV infection. However, the mode of SR protein up-regulation in tumors is different to the E2-directed transcriptional regulation in normal transient HPV infection. SR proteins could provide excellent targets for HPV antiviral therapy as well as anticancer therapy [133]. Changes in alternative splicing and the mechanisms controlling this for viral messenger RNA (mRNA) have been the subject of intense exploration. However, to date experiments have only been carried out in model systems because the genetic systems suitable for studying alternative splicing of viral RNA in the context of the virus life cycle are relatively recent and technically challenging. Now, using these life cycle-supporting systems, SR proteins have been identified as important players in differentiation-dependent regulation of HPV gene expression. Better understanding of the role of cellular factors in regulating the virus life cycle is needed as it may help development of novel diagnostic approaches and antiviral therapies in the future [134].

While the oncogenic activities of E6 and E7 are well characterized, the role of E5 is still rather nebulous. The widespread causal association of HPV with cancer makes their study worthwhile not only in humans but also in animal model systems. The BPV system has been the most useful animal model in understanding the oncogenic potential of Papillomaviruses due to the pivotal role of its E5 oncoprotein in cell transformation [135]. E5 is a highly hydrophobic membrane-bound protein of 83 amino acids associated with the Golgi apparatus, endoplasmic reticulum, and nuclear membrane in infected cells. E5 can activate epidermal growth factor receptor (EGFR) through binding to the 16 kD subunit of protein pump ATPase leading to a reduced downregulation of EGFR receptors. The activation of EGFR can initiate biochemical cascades that lead to overexpression of a variety of protooncogenes and stimulate rapid cell growth. Moreover, E5 can inhibit the expression of TSG p21((WafI/SdiI/CipI)) and impair the control of cell cycle checkpoint. E5 protein has been identified as a potential tumor vaccine target antigen [136].

The E6 protein is a major transforming protein of many types of HPV. Mechanistically, the best characterized E6 proteins are those of the HR genital HPV (e.g. HPV 16 and 18 E6), which function, at least in part, by inactivating the p53 tumor suppressor protein. Biochemical studies have shown that this occurs by targeted proteasome-mediated degradation of p53, dependent on the E6-AP ubiquitin-protein ligase [137]. E6 abrogates multiple cell cycle checkpoints and modulates apoptosis. Loss of cell cycle checkpoints contributes to genomic instability, a hallmark of cancer cells. Cancer cells also show reduced propensity for apoptotic cell death. Inactivation of the tumor suppressor p53 by E6 is an important mechanism by which E6 promotes cell growth. The molecular basis for apoptosis modulation by E6 is poorly understood. Although it is expected that inactivation of p53 by E6 should lead to a reduction in cellular apoptosis, numerous studies showed that E6 could in fact sensitize cells to apoptosis [138]. Since p53 is frequently wild type in cervical cancers, unlike other cancers in which it is often mutated, the notion has arisen that E6's activity with respect to p53 is equivalent to an inactivating mutation of p53. In addition, several studies have shown that the pathways both upstream and downstream of p53 are intact in cervical cancers. This suggests the potential importance of the E6-p53 interaction for therapeutic intervention. However, like all viral oncoproteins, E6 is a multifunctional protein and a plethora of other cellular targets has been identified. Indeed, E6's interactions with some of these additional targets appear to be equally important in the pathogenesis of HPV, and may also represent valid targets for therapeutic intervention [139,140]. However, there is still a need to further solve the structures of additional interacting complexes to identify the structural relationship that exists between proteins that simultaneously bind E6, such as E6-AP and p53 or E6-AP and post synaptic density (PSD) protein 95, Drosophila disc large (Dlg) tumor suppressor 1, and zonula occludens-1 (zo-1) protein (PDZ) domain-containing proteins, and to provide a clear picture of the interface between E6 and its ubiquitin ligase [141,142]. A unique characteristic of the cancer-causing HPV types is the presence of a PDZ recognition motif on the carboxy terminus of the E6 oncoprotein. Through this motif, E6 directs the proteasome-mediated degradation of cellular proteins involved in the regulation of cell polarity and in cell proliferation control. These include components of the Scrib and Par polarity complexes, as well as a number of other PDZ domain-containing substrates. Thus, HPV are now providing novel insights into the functioning of many of these cellular proteins, and into which of these functions, in particular, are relevant for maintaining normal cellular homeostasis [143].

The HPV E7 protein can overcome the activity of some CDK inhibitors, associate with cyclin/CDK complexes and bind to and destabilize the Rb tumor suppressor protein. These biological activities contribute to the carcinogenic potential of the HR HPV E7 proteins which are consistently expressed in HPV-positive cervical carcinomas [144]. E7 exerts its oncogenic function at least in part by modulating cellular growth regulatory pathways. Experimental evidence suggests that cell transformation by E7 is mediated by the physical association of E7 with cellular regulatory proteins, whose functions are specifically altered by E7, as exemplified by the well-known interaction of E7 with the pRb. Similar to the case of the E6 protein of HPV 16, whose interaction with p53 was long considered its major activity, it now appears that the interaction of E7 with the pRb represents just one of many distinct interactions that are relevant for cell transformation [145,146].

The regulation of host-mediated apoptosis by the E6 and E7 oncoproteins has garnered attention because it is believed to be an important strategy employed by HR HPV to evade immune surveillance. Additionally, the revelation that E5 can protect cells from tumor

necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis suggests that it may also play a role in undermining host defense mechanisms. Cellular transformation is an unintended consequence of persistent infection by HR HPV, and it is therefore likely that the primary function of E5, E6, and E7 is to regulate cell survival throughout the normal viral life cycle in order to ensure viral replication and promote the spread of progeny [147].

The minor capsid protein L2 of HPV plays important roles in virus entry into cells, localization of viral components to the nucleus, in DNA binding, capsid formation, and stability. It also elicits antibodies that are more cross-reactive between HPV types than does the major capsid protein L1, making it an attractive potential target for new-generation, more broadly protective subunit vaccines against HPV infections. However, its low abundance in natural capsids, 12-72 molecules per 360 copies of L1, limits its immunogenicity [148].

Evidence points to the existence of a host-mediated intracellular control which down-regulates these HPV genes in replicating normal cells. This control appears to be interrupted in HPV-positive carcinoma cells, probably due to structural modifications of the respective host cell genes acquired during the period of viral DNA persistence. Factors affecting genes seem to be responsible for geographic differences in anogenital cancer incidence, since HPV infections appear to occur worldwide at similar frequency [149-151]. Transformation studies using primary human cells and nontumorigenic HeLa/fibroblast hybrid cells have also suggested that chromosome 11 may be important in suppressing the HPV transformed phenotype. The transformed phenotype may therefore also involve an impaired intracellular control of persisting HPV oncogenic sequences. Although there exists no solid evidence that a cytotoxic T-lymphocyte (CTL) reaction is mounted against HPV transformed cells, there is evidence that both NK cells and activated macrophages can preferentially kill HPV transformed cells in vitro [152].

In HR HPV-associated cervical neoplasia, the two HPV-encoded oncoproteins E6 and E7 have been implicated in mitotic infidelity by their ability to induce centrosome-related mitotic disturbances. However, the mechanisms by which HPV E6 and E7 subvert centrosome homeostasis are strikingly different. Whereas the E7 oncoprotein rapidly drives centrosome duplication errors in cells that appear phenotypically normal, expression of the HPV E6 oncoprotein results in an accumulation of supernumerary centrosomes in multinucleated cells. The primary centrosome duplication errors (CDE) in HPV E7 expressing cells may be linked to the ability of E7 to disrupt regulatory nodes that govern both the host cell division cycle machinery and the initiation of centrosome duplication. Most importantly, the E7 oncoprotein has been shown to dysregulate CDK2 activity, a major determinant for the initiation of centrosome duplication. HPV-induced centrosome abnormalities, multipolar mitoses, and aneuploidy often occur at early stages during cervical carcinogenesis and increase with malignant conversion. These findings suggest that HPV oncoprotein-induced chromosomal instability increases the risk for genetic changes that may ultimately facilitate carcinogenic progression [153]. Given the role of CDK2 as a regulatory node not only for cell cycle progression but also for centrosome duplication, inhibition of CDK2 may not only retard cellular proliferation but also decrease CDE and centrosome-related mitotic defects. Compared to some conventional cytotoxic agents, which exclusively target DNA replication, modulation of CDK2 activity may hold the promise of diminishing the development of genomically unstable, aneuploid tumor cells that are frequently the source of chemotherapy resistance in malignant tumors [154]. The detailed molecular analysis of these interactions

allowed to define new biomarkers for dysplastic cervical cells. E7 for example induces increasing expression of the CDK inhibitor p16INK4a in dysplastic cells. This can be used to identify dysplastic cells in histological slides, cytological smears, or samples taken for biochemical analyses with a yet unmet fidelity. Detection of specific viral mRNA derived from integrated HPV genomes in advanced precancers can be used to identify lesions with a particularly HR for progression into invasive carcinomas (amplification of Papillomavirus oncogene transcripts [APOT] assay). These new markers will result in a modified classification of cervical precancers and improved screening assays [155].

Review of the available data indicates that telomerase is activated in the majority of cervical SCC as it is in most malignant neoplasms. Telomerase activity can also be detected in some preneoplastic cervical lesions, but the significance of this is unclear, because nonneoplastic, proliferating epithelial cells also can have telomerase activity. The bias introduced by cytologic sampling methods can complicate the interpretation of results. Quantitative telomerase assays may be useful in distinguishing nonmalignant, physiologic activation of telomerase from malignant activation. Studies evaluating telomerase component (telomerase RNA [hTR] or telomerase reverse transcriptase [hTERT]) expression by evaluation of RNA, mRNA, or antigen have yielded conflicting results, but the observation that many nonmalignant, nontelomerase active cells have detectable hTR and hTERT suggests that many cells express telomerase RNA and catalytic components, but do not have active telomerase. The implication is that a regulatory overlay must exist that controls telomerase activation. Activation of the enzyme in carcinogenesis could conceivably be a physiologic activation that normally accompanies cellular proliferation, a direct appropriation of telomerase activity by the neoplastic process, or both. The presence of inactive telomerase in many cells also raises the possibility of a noncatalytic function for the telomerase complex. An understanding of telomerase interaction with HPV infection in the pathogenesis of cervical neoplasia must await a further elaboration of telomerase regulation. Likewise, application of telomerase detection in cervical cancer screening programs must await a better integration of telomerase regulation in normal and specifically in HPV-infected squamous epithelial cells [156].

In conclusion, as molecular techniques have become more sophisticated and as the molecular knowledge of HPV infections has been pursued in greater depth, it is increasingly apparent that this human tumor DNA virus is similar to a number of other oncogenic DNA viruses that have been described and well-studied. These viruses appear to act through a common pathway of producing oncogenic proteins that interfere with key signaling elements that normally control the process of cell division. With a better mechanistic knowledge, it should be possible to design new therapeutic approaches to treating HPV-associated disease that are directed toward specific cellular events such as turning off the production of E6 and E7 proteins or restoring the activity of pRb or p53. Increased attention has also been turned to immunologic aspects of HPV infections, and a number of groups are eagerly pursuing the possibility of using simple office-based procedures to detect specific proteins encoded for by the HPV open reading frames in an attempt to determine who has been infected, is actively infected, and has proteins being produced that are indicative of neoplasia. From the clinical point of view, the use of outpatient excisional techniques such as the loop electrosurgical excision procedure (LEEP) is rapidly supplanting ablative techniques because of their superior ability to identify early invasive carcinomas and AIS that have not been detected by colposcopy [157-164].

CYTOHISTOPATHOLOGY

HPV are associated with hyperplastic (warts, condylomas), dysplastic (CIN and VIN), and malignant lesions (carcinomas) of squamous epithelium. Each HPV type is preferentially associated with specific clinical lesions and has an anatomic site preference for either cutaneous or mucosal squamous epithelium. Infection appears to begin in the basal cells. E gene expression is associated with acanthosis, and L gene expression is associated with appearance of structural antigens and virions in nuclei of cells of the granular layer, usually koilocytotic cells [165,166].

The identification of a specific marker cell, the koilocyte, has led to initial studies of frequency and biologic significance of neoplastic lesions of the uterine cervix associated with HPV [167].

Pap smears of the cervix and biopsies of the cervix, vagina, vulva, and penis are a substantial portion of the work load of any anatomic pathology laboratory (Figures 1,2).

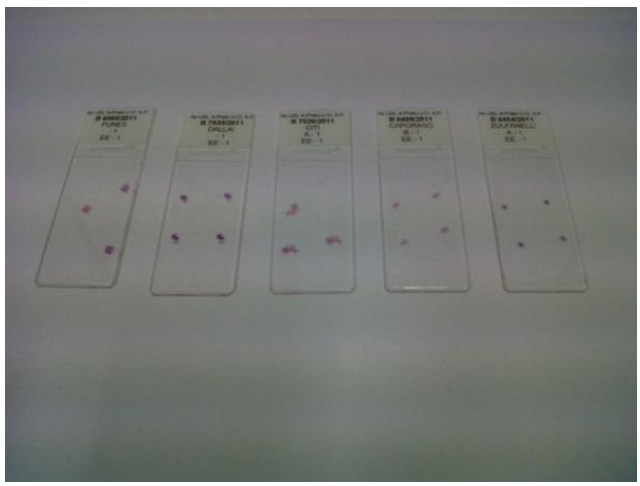


Figure 1. Bioptic slides.

(a)

(b)

Figure 2 (a,b).Anatomic pathology laboratory.

Figure 3. Automated device for cytologic assessment (ThinPrep Integrated Imager System [Hologic Inc., Bedford, MA, USA]).

The importance of samples from these sites is highlighted by the fact that their most common disease is dysplasia, which is sexually transmitted and a recognized risk factor for carcinoma. HPV is an essential co-factor for the development of lower genital tract dysplasia. Molecular testing for HPV has revealed a great deal about the pathophysiology of dysplasia and its evolution to carcinoma. Importantly, it now allows diagnostic pathologists the opportunity to be more accurate in their assessment of common conditions such as Pap smears of squamous atypia and biopsies equivocal for dysplasia [168]. Squamous dysplasia of the cervix is a morphologic continuum that is divided into a number of categories. When the severity of a morphologic abnormality is assessed, whether in a biopsy sample or in an exfoliative smear, there can be significant observer variation. The use of a binary (Bethesda) system of classification of squamous dysplasia and its validation by comparing consensus diagnoses with HPV type both in biopsy samples and in smears is advised (Table 1) [169,170].

Table 1. The Bethesda System

SPECIMEN TYPE: • Conventional smear (Pap smear) • Liquid-based preparation • Other
SPECIMEN ADEQUACY: • Satisfactory for evaluation (describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g. partially obscuring blood, inflammation, etc.) • Unsatisfactory for evaluation… (specify reason) • Specimen rejected/not processed (specify reason) • Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)
GENERAL CATEGORIZATION (optional): • Negative for intraepithelial lesion or malignancy • Other: see interpretation/result (e.g. endometrial cells in a woman ≥ 40 years of age) • Epithelial cell abnormality: see interpretation/result (specify “squamous” or “glandular” as appropriate)

INTERPRETATION/RESULT:

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY,
(when there is no cellular evidence of neoplasia, state this in the general categorization above and/or in the interpretation/result section of the report, whether or not there are organisms or other non-neoplastic findings).
ORGANISMS:
* Trichomonas vaginalis
* Fungal organisms morphologically consistent with Candida spp.
* Shift in flora suggestive of bacterial vaginosis
* Bacteria morphologically consistent with Actinomyces spp.
* Cellular changes consistent with HSV
OTHER NON NEOPLASTIC FINDINGS (optional to report; list not inclusive):
* Reactive cellular changes associated with:
- inflammation (includes typical repair)
- radiation
- intrauterine contraceptive device (IUD)
* Glandular cells status post hysterectomy
* Atrophy

OTHER:
- Endometrial cells (in a woman ≥ 40 years of age)
(Specify if "negative for squamous intraepithelial lesion")

EPITHELIAL CELL ABNORMALITIES
- SQUAMOUS CELL
* Atypical squamous cells
- of undetermined significance (ASC-US)
- cannot exclude H-SIL (ASC-H)
* Low grade squamous intraepithelial lesion (L-SIL)
(encompassing: HPV/mild dysplasia/CIN 1)
* High grade squamous intraepithelial lesion (H-SIL)
(encompassing: moderate and severe dysplasia, CIS, CIN 2 and CIN 3)
- with features suspicious for invasion (if invasion is suspected)
* Squamous cell carcinoma

- GLANDULAR CELL
* Atypical
- endocervical cells (not otherwise specified [NOS] or specify in comments),
- endometrial cells (NOS or specify in comments),
- glandular cells (NOS or specify in comments)
* Atypical
- endocervical cells, favor neoplastic
- glandular cells, favor neoplastic
* Endocervical adenocarcinoma in situ
* Adenocarcinoma:
- endocervical
- endometrial
- extrauterine
- not otherwise specified (NOS)

Table 1. (Continued)

OTHER MALIGNANT NEOPLASMS: (specify)
ANCILLARY TESTING: Provide a brief description of the test methods and report the result so that it is easily understood by the clinician.
AUTOMATED REVIEW: If case examined by automated device (Figure 3), specify device and result.
EDUCATIONAL NOTES AND SUGGESTIONS (optional): Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included).

Adapted from: International Agency for Research on Cancer (IARC), 2013.

PATHOPHYSIOLOGY

HPV replicate in the upper parts of the epithelium, where cells would normally be dying to produce a cornified layer. Therefore, they need to inhibit or delay differentiation and stimulate cell cycle progression to create an environment conducive for replication of the viral genome [171]. Genital, oral, and some rare types of cutaneous cancers have all been found to contain varying degrees of HPV DNA. In several instances, secondary tumors resulting from metastases to lymph nodes and lungs have also been demonstrated to contain HPV DNA. A major difficulty in elucidating the role of HPV in oncogenesis has been the lack of an appropriate in vitro culture system that would permit the growth of the virus and allow an analysis of its transforming properties. Nevertheless, recent advances in molecular biology have permitted the molecular cloning and amplification of HPV viral DNA, thereby facilitating its use as a probe for the detection of miniscule amounts of HPV DNA and HPV RNA in tumor biopsies. Moreover, DNA transfections of cells in culture have been extremely useful in the study of viral DNA replication and transformation properties, providing information on the maintenance and oncogenicity of HPV DNA [172].

Current data implicating the role of HPV infections in squamous cell carcinogenesis may be summarized as follows:

1) animal models have shown that Papillomaviruses can induce malignant transformation;
2) HPV involvement in both benign and malignant human squamous cell tumors has been demonstrated by morphological, immunohistochemical, and DNA hybridization techniques;
3) HPV infections in the genital tract are venereally transmitted and are associated with the same risk factors as cervical carcinoma;
4) the natural history of cervical HPV lesions is similar to that of CIN, namely, they have the potential to develop into CIS;
5) malignant transformation of HPV-induced lesions seems to depend on virus type and the physical state of its DNA, e.g. whether or not it is integrated in the host cell DNA;

6) malignant transformation most probably requires synergistic actions between the HPV and chemical or physical carcinogens, or other infectious agents;
7) genetic disposition significantly contributes to malignant transformation; and
8) immunological defense mechanisms of the host are probably capable of modifying the course of HPV infections [173].

The role of these virus infections has been established:

1) by the regular presence of HPV DNA in the respective tumor biopsy specimens;
2) by the demonstration of viral oncogene expression (E6 and E7) in tumor material;
3) by the identification of transforming properties of these genes;
4) by the requirement for E6 and E7 expression for maintaining the malignant phenotype of cervical carcinoma cell lines;
5) by the interaction of viral oncoproteins with growth-regulating host-cell proteins; and
6) by epidemiologic studies pointing to these HPV infections as the major risk factor for cervical cancer development.

In addition to cancer of the cervix, a major proportion of anal, perianal, vulvar, and penile cancers appears to be linked to the same HPV infections. In addition, close to 20% of OPCA contain DNA from the same types of HPV. Evidence also points to a possible role of other HPV infections in SCC of the skin. Modifications in host-cell genes, most likely engaged in the control of HPV gene expression in proliferating cells, emerge as important events in HPV-mediated carcinogenesis [174].

The viral capsid has evolved to fulfill various roles that are critical to establish viral infection. The particle interacts with the cell surface via interaction of the major capsid protein, L1, with heparan sulfate proteoglycans of the basement membrane. Moreover, accumulating evidence suggests the involvement of a secondary receptor and a possible role for the minor capsid protein, L2, in cell surface interactions. The entry of HPV in vitro is initiated by binding to a cell surface receptor in contrast to the in vivo situation where the basement membrane has recently been identified as the primary site of virus binding. Binding of HPV triggers conformational changes, which affect both capsid proteins L1 and L2, and such changes are a prerequisite for interaction with the elusive uptake receptor. Most HPV types that have been examined, appear to enter the cell via a clathrin-dependent endocytic mechanism, although many data are inconclusive and inconsistent. Furthermore, the productive entry of HPV is a process that occurs slowly and asynchronously and it is characterized by an unusually extended residence on the cell surface, resulting in an uptake half-time of up to 14 h. Despite the significant advances and the emergence of a general picture of the infectious HPV entry pathway, many details remain to be clarified [175]. Productive infection by HR HPV types is manifest as cervical flat warts or condyloma that shed infectious virions from their surface. Viral genomes are maintained as episomes in the basal layer, with viral gene expression being tightly controlled as the infected cells move towards the epithelial surface. The pattern of viral gene expression in LG cervical lesions resembles that seen in productive warts caused by other HPV types. HG 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. The HR E6 and E7 proteins drive cell proliferation through their association with PDZ domain proteins and Rb, and contribute to neoplastic progression, whereas E6-mediated p53 degradation prevents the normal repair of chance mutations in the cellular genome. E7 is expressed in the lower epithelial layers, and is followed eventually by the expression of E4 and L1 closer to the epithelial surface. This ordered pattern changes in characteristic ways during neoplastic progression and latency, and can be irreversibly fixed following integration of the viral genome into the host cell chromosome. Our understanding of expression patterns and their significance, is beginning to explain the nature of disease progression, and offers a rational basis for the selection of biomarkers that may be used to predict disease status and prognostic outcome. Cancers usually arise in individuals who fail to resolve their infection and who retain oncogene expression for years or decades. In most individuals, immune regression eventually leads to clearance of the virus, or to its maintenance in a latent or asymptomatic state in the basal cells [176,177].

HPV induces pathognomonic abnormality of squamous cells, known as koilocytosis, that may precede or accompany various manifestations of cancerogenesis including invasive cancer. Still, studies suggest that HPV infection is quite common in normal people and may prove to be ubiquitous. Hence, the activation of the virus and its ability to interact with cervical epithelium is likely to be due to “patient factors” rather than the presence of the virus per se [178].

Anogenital cancers develop from precursor lesions, which, for cervical cancer, are termed CIN, and are graded from 1 to 3 depending on the degree of disruption of epithelial differentiation. Viral production occurs in LG lesions that are only slightly alterated in their pattern of differentiation from normal cells. The production of viral particles, genome amplification, capsid protein synthesis, and virion assembly is dependent upon differentiation and is restricted to suprabasal cells. In carcinomas, viral DNA is usually found integrated into host chromosome, and no viral production is seen. The processes of viral transcription and replication are, therefore, intimately associated with the differentiation program of epithelial cells. In the past, studies on the life cycle of HPV have been limited due to an inability to faithfully duplicate the epithelial differentiation program in vitro. Recent advances in culture systems have overcome these problems, allowing for the propagation of HPV in vitro. In addition, insight has been gained at the molecular level regarding the mechanisms by which these viruses contribute to malignancy, centering on the action of the E6 and E7 viral oncoproteins. Evidence suggests that these oncoproteins function by inactivating the cell cycle regulators p53 and pRb, thus providing the initial event in a multistep progression to malignancy [179]. Progression of an HPV-infected cell clone to invasive growth involves consecutive modifications of a set of host cell genes. Some of these modifications suppress viral oncogene functions post-transcriptionally, and others suppress transcription via a signaling pathway stimulated by activated macrophages and possibly by additional cells [180]. The impressive technological progress in HPV virion analysis achieved over the past decades, in addition to the improvements in general methodologies for studying viral infections, provide reasons to be optimistic about further advancement of this field [175].

ENDOCRINOLOGY

If HPV is necessary for the development of neoplastic lesions of the uterine cervix, it is not sufficient. Among the cofactors involved in the malignant transformation of cells infected by HPV, sex hormones may facilitate the cervical carcinogenesis by different mechanisms, including the induction of squamous metaplasia in the transformation zone of the cervix, interactions between steroid hormones and HPV gene expression, and alterations of the local immune microenvironment [181]. Epidemiological evidence suggests that steroid hormones can increase the risk of cervical neoplasia in HPV-infected women. Steroids can interact with hormone-response elements in the viral long control region, enhancing HPV transcription and resulting in transformation of cervical cells. Subsequent malignant progression may involve virus-induced chromosomal instability, facilitating viral DNA integration and deregulation of gene expression [182]. Since OC use for long durations is associated with an increased risk of cervical cancer, it is important to know whether HPV infection is more common in OC users. In a systematic review of epidemiological studies of the risk of genital HPV infection and OC use, there was no evidence for a strong positive or negative association between HPV positivity and ever use or long duration use of OC. The limited data available, the presence of heterogeneity between studies and the possibility of bias and confounding mean, however, that these results must be interpreted cautiously. Further studies are needed to confirm these findings and to investigate possible relations between OC use and the persistence and detectability of cervical HPV infection [183].

IMMUNOLOGY

The nature of the interaction between HPV and their infected host has led to the identification of ways in which the viral oncoproteins can transform the infected host cells into cancer cells. As viral persistence is required for malignancy, and persistence requires avoidance of immune attack by the host, defining the relationship between HPV and the immune system is also paramount in understanding tumorigenesis. It has emerged that HPV have evolved several ways in which to prevent clearance by the host immune system. The limitation of the HPV replication cycle to the epithelium, together with low level expression of the virus proteins and an absence of inflammation, minimizes the exposure of virus to immune cells. In addition, more recently it has been shown that, like many other viruses, HPV can directly subvert the immune response, including interference with the interferon (IFN) pathway, modulation of antigen presentation, inhibition of interleukin (IL)-18 activity and down-regulation of MHC class I on infected cells. Collectively these mechanisms explain how HPV lesions are able to persist for long periods of time in immunocompetent hosts [184]. Although abundant circumstantial evidence exists for the involvement of the immune system in the control of HPV infection, restriction of infection to epithelium and the lack of a viral productive phase pose problems for immune response induction, and for immunological effector mechanisms. HPV antigens may be presented to the immune system on the surface of keratinocytes as well as, or in addition to, presentation by “classical” antigen presenting cells, and non-specific and specific (B- and T-cell) effector functions have putative roles [185]. Both cellular and humoral immune responses are essential for the clearance of HPV-

associated cervical lesions. There is increasing evidence that the immune system plays a pivotal role in determining the outcome of HPV infection. Viruses and associated neoplastic cells are proposed to have evolved mechanisms to avoid immune attack. T-CMI against oncogenic HPV are believed to play a central role in cervical carcinogenesis. The presence of HPV-specific CTL in a majority of human cervical cancer patients provides an approach for further study of their functional role in modulating this malignancy. Tumor-infiltrating lymphocytes (TIL) develop as manifestations of the recognition and defense against malignant cells by the host immune system. Cancer cells may overcome immune surveillance, either by downregulating the proliferation of HPV-specific CTL, or altering the effector compositions of immune cells against HPV infections. TIL in the tumor microenvironment can be functionally inhibited and lose the ability of clonal proliferation as a result of depressed expression of IL-2Ralpha. The upregulation of inhibitory signaling relates to the modulation of the virus- and/or tumor-specific immune responses. Alteration of host genetic susceptibility may also lead to abnormal immune response as a general genomic instability resulting from virus persistence [186].

HPV infection of the genital tract is common in young sexually active individuals, the majority of whom clear the infection without overt clinical disease. Most of those who do develop benign lesions eventually mount an effective CMI, and the lesions regress. Regression of ano-GW is accompanied histologically by a CD4+ T cell-dominated helper (Th)1 response: animal models support this and provide evidence that the response is modulated by antigen-specific CD4+ T cell-dependent mechanisms. Failure to develop an effective CMI response to clear or control infection results in persistent infection and, in the case of the oncogenic HPV, an increased probability of progression to HG intraepithelial neoplasia and invasive carcinoma. Effective evasion of innate immune recognition seems to be the hallmark of HPV infections. The viral infectious cycle is exclusively intraepithelial: there is no viremia and no virus-induced cytolysis or cell death, and viral replication and release are not associated with inflammation. HPV globally downregulates the innate immune signaling pathways in the infected keratinocyte. Proinflammatory cytokines, particularly the type I IFN, are not released, and the signals for LC activation and migration, together with recruitment of stromal dendritic cells (DC) and macrophages, are either not present or inadequate. This immune ignorance results in chronic infections that persist over weeks and months. Progression to HG intraepithelial neoplasia with concomitant upregulation of the E6 and E7 oncoproteins is associated with further deregulation of immunologically relevant molecules, particularly chemotactic chemokines and their receptors, on keratinocytes and endothelial cells of the underlying microvasculature, limiting or preventing the ingress of cytotoxic effectors into the lesions. Recent evidence suggests that HPV infection of basal keratinocytes requires epithelial wounding followed by the reepithelization of wound healing. The wound exudate that results provides a mechanistic explanation for the protection offered by serum neutralizing antibody generated by HPV L1 VLP vaccines [187]. The design of the HPV infection cycle is tightly fitted to the differentiation program of its natural host, the keratinocyte. This has important consequences for the role of antigen-presenting cells in the priming of antiviral immunity. The confinement of HPV infection to epithelia puts the epithelial DC, the LC, in charge of the induction of T cell-dependent immunity. Because HPV-infected keratinocytes cannot reach the regional lymphoid organs, and HPV-infection of LC does not result in viral gene expression, priming of antiviral T cells exclusively depends on cross-presentation of viral antigens by the LC. Sensitization of the immune system in the

regional lymphoid organs elicits systemic anti-HPV immunity as well as intraepithelial immune surveillance by memory-type intraepithelial T cells and locally produced antibodies. The high rate of spontaneous rejections of HR HPV-infections and HPV-positive premalignant lesions indicates that in general the LC-driven antigen presentation machinery is capable of raising an effective immune defense against HPV. Epidemiological studies also reveal that a decrease in the vigilance of the immune system is readily exploited by HPV to escape immune destruction, resulting in persistent infections and development of HPV-positive cancers [188].

The first line of defense against HPV is the innate immune system, which provides nonspecific protection against a variety of pathogens and also enhances the adaptive immune response. However, HPV-infected cells often evade innate immune recognition and elimination. HPV gene expression and release of virus occur in superficial squamous cells where virus antigens are not readily detected, and keratinocytes are not lysed during HPV infection so there is no inflammatory response. In addition, HPV E proteins inhibit specific components of the innate immune system. E6 and E7 inhibit signaling by type I IFN and decrease expression of multiple IFN-inducible genes. E5 and E7 inhibit expression of MHC class I proteins on the cell surface. HPV-infected cells are resistant to lysis by NK cells, but are sensitive to cytokine-activated NK cells. Activated macrophages also kill HPV-infected cells and control malignant development. Thus, innate immunity is important for prevention of HPV infections, but HPV often persists due to evasion or inactivation of innate defenses [189]. The immune system uses innate and adaptive immunity to recognize and combat foreign agents that invade the body, but these methods are sometimes ineffective against HPV. HPV has several mechanisms for avoiding the immune system. HPV infects, and multiplies in keratinocytes, which are distant from immune centers and have a naturally short lifespan. The naturally short life cycle of the keratinocyte circumvents the need for the virus to destroy the cell, which would trigger inflammation and immune response. In addition, HPV downregulates the expression of IFN genes. Despite viral immune evasion, the immune system effectively repels most HPV infections, and is associated with strong localized CMI [190]. Effective evasion of innate immune recognition seems to be the hallmark of HPV infections, since the infectious cycle is one in which viral replication and release is not associated with inflammation. Furthermore, HPV infections disrupt cytokine expression and signaling with the E6 and E7 oncoproteins particularly targeting the type I IFN pathway. High doses of IFN can overcome the HPV-mediated abrogation of signaling, and this may be the basis for the therapeutic effects on HPV infections of IRM such as the imidazoquinolones that induce high levels of type I IFN by activation of toll-like receptor (TLR) 7. Using the unique W12 model of cervical carcinogenesis, some of these IFN-related interactions and their relevance in the selection of cells with integrated viral DNA in cancer progression have been investigated. Data show that episome loss associated with induction of antiviral response genes is a key event in the spontaneous selection of cervical keratinocytes containing integrated HPV 16. Exogenous IFN-beta treatment of W12 keratinocytes in which the majority of the population contain episomes results only in the rapid emergence of IFN-resistant cells, loss of episome-containing cells, and a selection of cells containing integrated HPV 16 in which the expression of the transcriptional repressor E2 is down-regulated, but in which E6 and E7 are up-regulated [191].

Benign HPV-induced lesions are chronic persistent growths: high levels of viral antigen are expressed in the apparent absence of a host immune response suggesting that these viruses

have evolved efficient mechanisms of immune evasion. Cell-mediated responses are central in the pathogenesis of HPV and regression of both cutaneous and GW histologically resembles a delayed-type hypersensitivity response (DTH). In an experimental murine model DTH responses to the E6 and E7 proteins of HPV 16 can be elicited when viral antigen is presented via the epithelial route. Priming with low levels of viral antigen in this model induces non-responsiveness and the loss of DTH. In HPV-associated cancers the E6/E7 genes are expressed and an antibody response to the proteins is found in at least 50% of cases indicating that these oncoproteins are potential targets for immunotherapy [192]. During the last 25 years, monoclonal antibodies from animals immunized with denatured BPV type 1 major capsid (L1) protein have been derived, their corresponding immunodominant epitopes to within a single amino acid have been mapped, and the reactivity of authentic L1 proteins has been compared to the predicted response by collinear analysis of the amino acid sequences of the same and other Papillomaviruses. The data obtained from this approach has provided us with new insights into the sensitivity and specificity of the antibody response to viral proteins [193]. The antibody response to the HPV particle is dominated by a neutralizing antibody response to a type-specific, conformationally dependent immunodominant epitope. In humans, the serum antibody response to VLP is stable over time, also after the HPV infection has been cleared, resulting in HPV serology being used as a marker of cumulative HPV exposure in spite of the fact that a significant proportion of HPV-exposed subjects fail to seroconvert. Several HLA class II haplotypes are associated with cervical cancer: DQw3 increases and DR13 decreases the risk for cervical cancer in general (OR and 95% confidence intervals [CI]: 1.25 (1.15-1.37) and 0.69 (0.56-0.85), respectively), DR15 increases the risk for HPV 16-carrying cancer (OR: 1.47; CI: 1.20-1.81), and DR7 may be either protective or increase the risk. Most cervical cancers have downregulated the expression of at least one HLA class I antigen, whereas class II expression is increased in infected epithelium. A Th2 cytokine profile is associated with progression to cervical cancer. Specific CTL responses were originally reported in only a minority of infected subjects, typically cancer patients, but with advancing technology, specific CTL can be stimulated from about half of the women with HPV-carrying disease. In animal model systems, CTL responses can mediate clearance. The antibody response to HPV is a mediator of type-specific protective immunity. The cellular immunity to HPV is implicated as an important factor in cervical carcinogenesis [194], and is likely to be of major importance for the clearance of inapparent or overt infections caused by HPV. The highly polymorphic class I or class II HLA molecules present HPV-derived peptides to cytotoxic (CD8+) or helper (CD4+) T lymphocytes bearing specific receptors and condition the immune responsiveness to HPV infections. Recent data point to a role of an altered expression of HLA molecules in the persistence of HPV-induced cervical premalignant lesions and their progression towards invasive carcinoma. Furthermore, positive of negative associations of certain HLA alleles or haplotypes with cutaneous of cervical neoplasias have been found [195].

The mechanisms of host immunity to prevent and control HPV infection still remain unclear. The importance of ubiquitination (or ubiquitylation) as an intracellular proteasomal-mediated protein degradation pathway, and as an important modulator for the regulation of many fundamental cellular processes has been valued over the last decade. Although the molecular and cellular mechanisms are not completely established, the critical role of ubiquitination in host immune response to HPV infection has become increasingly apparent.

Targeting the components of the ubiquitin system might offer potential therapeutic strategies for HPV-related diseases in the future [196,197].

HUMAN PAPILLOMAVIRUS IN IMMUNODEFICIENCY

Immunocompromised individuals have an increase prevalence of HPV-associated lesions and neoplasia. Immunosuppressed patients (e.g. those who have undergone transplantation or those who have been treated with chemotherapy and/or radiation therapy) have a higher rate of HPV infections [198]. People with deficient CMI have an increased susceptibility to viral infections and certain cancers, particularly large cell lymphomas and cancers of the skin and anogenital region. All are linked to viral origins. Neoplasms in the immunodeficient patient often occur at a relatively young age, involve multifocal locations, tend to persist, recur, and progress rapidly. Anogenital neoplasms show a strong association with HPV infection and also persist, extend, and progress, in spite of standard therapy. Since standard therapy of anogenital HPV infection and neoplasia is often not effective in immunodeficient patients (and others with an anogenital neoplastic syndrome), special treatment is required. 5-Fluorouracil (5-FU) chemosurgery, followed by maintenance 5-FU therapy, is effective and provides field suppression against recurrent HPV infection and neoplasia, with minimal damage to affected organs. After removal of all detectable HPV infection or neoplastic lesions, immunodeficient patients require close surveillance of the entire anogenital tract. Immunodeficient patients are an in-vivo human laboratory in which to study the natural history of HPV and its oncogenic effects on the anogenital tract. The theory of HPV oncogenesis has been supported by the evidence gathered from these patients [199].

Long-term immunosuppressive drug regimes, used to prevent organ transplant rejection, are associated with an increased risk of epithelial malignancies, particularly anogenital and cutaneous cancers. Premalignant and malignant lesions of the oral mucosa have also been reported in renal transplant recipients (RTR), particularly of sun-exposed lip lesions. Many of these anogenital lesions are associated with the detection of HR mucosal HPV. Novel degenerate and nested PCR techniques have found high levels of EV HPV (HR cutaneous oncogenic HPV) in cutaneous warts, dysplastic keratoses, and SCC. Unusual appendageal and spindle cell carcinomas are being observed in RTR [200]. Frequently, there are multiple HPV types present in single skin biopsies. Typically, the prevalence of viral warts rises steadily after transplantation and a strong association exists between the number of HPV-induced warts and the development of skin cancer. The interval between the transplantation to the development of warts is clearly shorter than the interval from transplantation to the diagnosis of the first skin cancer. A comparison of transplant recipients with and without skin cancer, however, showed an equally high prevalence of EV-HPV DNA in keratotic skin lesions in both groups of patients and the detection rate and spectrum of HPV infection in hyperkeratotic papillomas, actinic keratosis (AK), and SCC was also similar. HPV DNA can frequently be detected in patients with hyperproliferative disorders like psoriasis and antibodies against HPV in patients with regenerating skin (e.g. after extensive second degree burns). Latent infection with EV-HPV seems to be widespread. The hair follicle region might be the reservoir of EV-HPV. The E6 protein from a range of cutaneous HPV types effectively inhibits apoptosis in response to UV-light induced damage. It is therefore conceivable that

individuals who are infected by EV-HPV are at an increased risk of developing AK and SCC, possibly by chronically preventing UV-light induced apoptosis [201]. The association between SCC of the oral cavity, female genital tract, and skin with HPV subtypes is well established in the general population and in solid organ transplant recipients, but no consistent link has been reported between HPV infection and SCC after allogeneic stem cell transplantation (allo-SCT). Studies are needed to determine if SCC, the most common secondary malignancy after allo-SCT, which is linked to chronic graft versus host disease (GVHD) and immunosuppressive therapy, is HPV related. Consideration should be given to assessing pretransplant HPV antibodies to identify patients at risk for HPV reactivation. If a strong relationship between HPV and second malignancies after allo-SCT exists, studies to evaluate the immunogenicity and efficacy of quadrivalent HPV vaccine (subtypes 6, 11, 16, and 18) should be considered in both male and female long-term survivors after allo-SCT [202].

As we have said, CMI likely plays an important role in prevention of HPV-associated disease, and HPV-associated SCC has been shown to occur with increased frequency among iatrogenically immunosuppressed individuals. Similarly, individuals with HIV-associated immunodeficiency have been shown to have a high prevalence of anogenital HPV infection as well as a high prevalence of HPV-associated lesions that are thought to be cancer precursors. Thus, HIV-positive women have a high prevalence of CIN, and HIV-positive men have a high prevalence of AIN. Several studies also highlight high rates of HPV infection and HPV-associated disease at sites other than the cervix and anus, including the penis and the mouth. The risk of disease in these populations appears to increase as the degree of immunosuppression increases, and these individuals are likely at risk for development of invasive SCC. This situation may reflect loss of systemic immune response to HPV antigens or local HPV-HIV interactions at the tissue or cellular level. The prevalence of oral, anal, and cervical HPV infection in HIV-positive individuals compared with HIV-negative individuals increases with progressively lower CD4+ levels, as does incident HG intraepithelial neoplasia. In contrast to intraepithelial neoplasia, development of cancer is not related to lower CD4+ level. With increasing grades of intraepithelial neoplasia and cancer, the proportion of tissues with copy-number abnormalities (CNA) increases, with one of the most common genetic changes being amplification of chromosome 3q. The presence of CNA is associated with the integration of HPV DNA into the host genome, with loss of HPV E2 and/or E2 rearrangement. This suggests a link between CNA and increased HPV-induced chromosomal instability mediated through de-repressed E6 and E7 expression consequent to loss of functional E2 protein. In addition, epigenetic changes occur with increasing frequency in HG intraepithelial neoplasia and cancer, such as hypermethylation leading to down-regulation of potential TSG. Analysis of these data together suggests that immune suppression plays a more prominent role in the earlier stages of HPV-associated disease, up to and including incident HG intraepithelial neoplasia. Persistent HG intraepithelial neoplasia and development of cancer may be more strongly related to the cumulative effect of HPV-associated genetic instability and the resulting host genetic changes. There are few data to suggest a direct role for HIV in the pathogenesis of HPV-associated neoplasia, but HIV-associated attenuation of HPV-specific immune responses may allow for persistence of HG intraepithelial neoplasia and sufficient time for accumulation of genetic changes that are important in progression to cancer. Early data suggest that at least 75% of those with H-SIL lesions do not regress while receiving highly active antiretroviral therapy (HAART). Given

that progression of H-SIL to invasive cancer may require several years, lengthened survival associated with HAART may paradoxically lead to an increased risk of anal cancer. Because these diseases are preventable, women should be screened on a regular basis with cervical Pap smears, followed by colposcopy if the Pap smear is abnormal. The potential to prevent anal cancer through detection and treatment of anal H-SIL suggests a need to screen HR individuals with anal cytology, similar to cervical cytology screening to prevent cervical cancer. Although preliminary studies indicate that anal Pap smears may also be useful for screening, further trials need to be performed, and at this time, HIV-positive men should be assessed on a regular basis with anoscopy. Cost-effectiveness analyses suggest that anal screening programs may be cost-effective in HIV-positive men. However, barriers to implementation of screening include inadequate numbers of clinicians skilled in diagnosis and treatment of H-SIL and lack of medical alternatives to surgical excision. Lesions that are detected should be biopsied for histopathologic assessment. Thorough assessment of the entire anogenital region should be performed because of the multicentric nature of HPV-associated diseases. Following treatment, rigorous follow-up should be maintained because of the high recurrence rate of HPV-associated disease in these populations. Recent progress in understanding the pathogenesis of anal squamous intraepithelial lesions (A-SIL) in HIV-positive men points to a role for decreased CMI to HPV antigens as well as the effects of the HIV-1 tat protein in modulating the biology of HPV-infected keratinocytes [203-209].

Dermatologic HPV infection in HIV patients manifests as both anogenital and nongenital skin disease. Anogenital HPV-related disease includes benign CA, the most common cutaneous manifestation of genital HPV infection; intermediate malignancy or premalignant conditions including giant CA (also called Buschke-Loewenstein tumor), AIN, penile intraepithelial neoplasia (PIN), and vaginal intraepithelial neoplasia (VAIN) or VIN, and frankly malignant disease including Bowen's disease and invasive anal, penile, or vulvar carcinoma. Cutaneous HPV-related disease in nongenital skin is also increased in HIV-positive patients, in the form of benign common warts, EV-like skin lesions, and NMSC [210].

Oral HPV infection is the principal cause of a distinct form of OPCA that has been rising in incidence in the USA since 1973, particularly among young men. The possible contribution of the HIV-epidemic to this increase is unclear. Incidence rates for HPV-related OPCA increase with age and are strongly influenced by year of birth in the USA (cohort effect). Persons with HIV/acquired immunodeficiency syndrome (AIDS) are at increased risk (approximately two to six-fold) for OPCA relative to the general population. However, this excess risk may be attributable to tobacco smoking rather than the three-fold higher prevalence of oral HPV infection in this population. Consistent with a viral attribution, however, is the apparent increase in risk of OPCA with severity of AIDS-related immunosuppression. Analogous to other HPV-related cancers (e.g. cervical and anal cancer), trends over time do not appear to be influenced by HAART. Healthcare providers may encounter HPV-related OPCA more frequently among individuals with HIV/AIDS as this population ages and due to the strong birth cohort effects observed in the general population. However, there is no evidence in support of different incidence trends over time among persons with and without HIV/AIDS [211].

An increasing body of information permits certain conclusions to be drawn about the nature and magnitude of the interactions between HPV and HIV infections and their influence on the genesis of intraepithelial neoplasia and, to a lesser extent, cancer. Importantly, findings

tend to be consistent across a number of independent studies. While HPV infection probably does not significantly alter the course of HIV infection, HIV-induced immunosuppression does increase the severity and duration of ano-GW, increase their infectiousness and reduce treatment efficacy. However, in developed countries the countervailing effects of enhanced HPV infectiousness and declining rates of unsafe sexual behavior have resulted in stable or declining incidence rates of ano-GW. Advanced immunosuppression due to HIV infections results in highly significant increases in rates of HPV-associated CIN and AIN [212]. This association is not completely due to immunosuppression. It is likely that HPV pathogenesis is altered in HIV-infected women. Preinvasive cervical neoplasia likely occurs more frequently in HIV-infected women because of several factors, including immunosuppression, viral interactions, and alterations in viral pathogenesis [213]. The presence of HPV DNA, extent of disease, and potential for malignant transformation also appear to correlate with the degree of immunosuppression. Individuals with a CD4+ cell count under 0.20 x 109/l (< 200/microliters) are at greatest risk [198]. The CDC designated moderate and severe cervical dysplasia as a category B defining condition, and invasive cervical cancer as a category C defining condition of AIDS in 1993 [214].

One difficulty complicating elucidation of the association between HIV and HPV infections is that the risk factors for acquisition and transmission of the two viruses are similar. The strength of this association represents a burgeoning health problem, yet there are no treatment guidelines aimed specifically at HIV-infected individuals with HPV-associated genital neoplasias. Treatment of HPV-associated cervical disease in HIV-infected women may be further complicated by a greater risk of treatment failure and recurrence than exists among HIV-seronegative women. A thorough understanding of the associations among HIV, HPV, and HPV-associated disease is essential to the development of effective strategies for intervention and prevention [215]. Treatment options for immunocompromised individuals remain similar to those for normal hosts. However, immunocompromised individuals have a much higher rate of recurrence. Improved immunostimulant and/or antiviral therapy is needed for HIV-seropositive individuals with widespread genital involvement. Control of both the HPV and the HIV epidemics involves at least 3 levels of intervention: better antiviral drugs, frequent monitoring for disease progression in infected persons, and better education to reduce spread of the disease [198]. Data on outcomes following therapy for anal dysplasia (infrared coagulator, high-resolution anoscopy-guided ablation) and anal cancer (chemoradiation and possibly intensity-modulated radiation therapy) have been encouraging. Treatment options for anal dysplasia and anal cancer in HIV-infected individuals are expanding and may lead to decreased morbidity and mortality [216].

Recent developments may impact on the future incidence of these cancers: the effect of HAART and the approval of vaccines to prevent initial HPV infection. HAART has limited benefit to reduce the incidence of CIN 3 and no benefit to reduce the incidence of AIN 3. Consistent with these findings, there has been no reduction in the incidence of cervical and anal cancer since the introduction of HAART [217]. HAART has not been shown to affect HPV detection, and data on its effect on the natural history of CIN are mixed. Some studies show no effect of HAART on the natural history of CIN, and others show a statistically significant but modest beneficial effect. HAART appears to have limited ability to clear HPV infection and induce regression of CIN in HIV-positive women. Combined with the high prevalence of cervical HPV infection and CIN, current data suggest that CIN should be aggressively sought and treated in HIV-positive women, including those who have responded

well to HAART with good HIV viral load suppression and increasing CD4+ levels [218]. HIV-infected men who have sex with men (MSM) remain at HR of developing anal cancer despite the widespread use of HAART. In HIV-infected women, however, there is some evidence that HAART may be associated with regression of HPV-related cervical disease. So far, epidemiologic data provided by cancer registries have shown no reduction in the incidence of cervical and anal cancer in patients with HIV infection since the initiation of HAART in 1996 [219]. HAART seems to have little, if any, beneficial effect on the natural history of intraepithelial lesions in HIV-positive women. Despite this fact, HAART does increase the life expectancy of HIV-positive women. Therefore, it remains important to closely monitor HPV-related disease in women with HIV who are receiving HAART, particularly in regions of the world where cervical screening is not available routinely [220]. Immune reconstitution-associated diseases (IRAD) are common and important consequences of HAART. The increase in HPV infection in HIV-infected individuals may be, at least partly, due to increased exposure to diverse HPV types, particularly HR types that might be able to persist for longer in anogenital regions. Alternatively, persistent/emergent HPV disease in HIV infection might represent persistent or modulated immunodysregulation after HAART and be viewed as a form of IRAD. The immunopathogenesis of HPV IRAD is fascinating and possibly determined by host genotype [221].

More encouraging is the development of highly effective preventive HPV vaccines that are projected to reduce the incidence of cervical cancer by up to 70% among vaccinated women. HIV-positive men and women remain at risk for HPV-associated cancers, even in the HAART era. Conversely, the incidence of anogenital cancers may decline in the future among HIV-positive individuals if they received the HPV vaccine before they acquired HIV infection, and studies should be done to assess the safety and efficacy of the vaccines in individuals already infected with HIV [217]. The effectiveness of a HPV vaccine to reduce the incidence of these tumors in immunocompromised individuals may depend on several factors, including the effects of immunocompromise on the response to vaccination, the extent of prior infection with the HPV types included in the vaccine, whether immunocompromised women and men have tumors that contain types of HPV not in the vaccines more often than the general population, and whether or not immunization occurs before immunocompromise is severe. Clinical studies are needed to determine HPV vaccine safety and effectiveness in different populations of immunocompromised women and men [222].

HIV-infected women in different geographic regions appear to be infected with less prevalent types of HR HPV as compared to the general population who, across all continents, are more commonly infected with types 16 and 18. Secondly, integration of HPV DNA into the host genome is no longer thought to be a necessary cause of malignant transformation of cervical cells. However, rate of integration appears to differ by the type of HPV. In fact, the types of HPV which appear to be more common in cervical dysplasia of HIV-infected women are the same types which are more likely to require integration for malignant transformation. Finally, HPV types found in HIV-infected women are relatively common and likely to persist. The most common among these types belong to the alpha-9 and -7 species which are the most carcinogenic species. Given that current vaccines target HR HPV 16/18, the findings from the above mentioned studies may have important implications for the design of HPV vaccines that target the types of HPV associated with disease risk in HIV-infected women. HPV typing and assessment of the physical state (whether it is integrated or episomal) appear to be two

valuable parameters for the prognostic evaluation of dysplastic lesions of the uterine cervix. This, however, has not yet been assessed in HIV-infected women. Recent data about the immune response in HPV/HIV co-infection may lead to understanding potential mechanisms for less virulent HPV causing malignant transformation in HIV-infected women [223].

In summary, individuals with immunosuppression caused by HIV infection or organ transplantation are at increased risk of HPV-associated anogenital cancers compared with age-matched healthy individuals. The exact role of immunosuppression in conferring increased risk is not known. Although it is unknown which stages of progression from dysplasia to cancer are most affected by immunosuppression, current data suggest that immunosuppression is most strongly associated with the early stages of dysplasia, and that progression to cancer per se is not associated with immunosuppression. Studies to determine this relationship in detail are hampered by the paucity of precise biomarkers of CMI to HPV. Questions remain about the biology of HPV infection among immunocompromised HIV-negative individuals (e.g. transplant patients) compared with those who are HIV-positive, the impact of HAART on the natural history of anogenital dysplasia and cancer among those who are HIV-positive, and whether the biology of specific HPV types is the same in HIV-positive as in HIV-negative individuals. Understanding HPV infection in those who are immunocompromised offers the potential to better understand its pathobiology in the putatively immunocompetent host [224]. HAART has had an unequivocally positive impact on morbidity and mortality in HIV-infected individuals. These benefits have clearly extended to some HIV-related malignancies, including Kaposi's sarcoma and non-Hodgkin's lymphoma. The impact of HAART on cervical cancer, however, remains uncertain. While the partial immune reconstitution afforded by HAART might be expected to decrease susceptibility to HPV infection and cervical disease, the local effects of improved immunosurveillance on the cervix are uncertain and the increased longevity of patients on HAART may increase risk of exposure to HPV and provide the time required for progression of cervical disease. Registry-based evidence has been consistent in identifying the lack of decrease in cervical cancer incidence in the HAART era. Clinical research on the subject, however, has produced conflicting evidence with regards to both the effect of HAART on HPV infection and its impact on cervical disease progression/regression. The incidence of cervical cancer has not decreased in the HAART-era. Furthermore, clinical research has not shown a clear benefit of HAART in decreasing HPV-related cervical disease in HIV-infected women. A better understanding of this subject will have an impact on cervical disease surveillance practices [225-228].

Studies that performed cytological analysis showed a higher frequency of SIL in systemic lupus erithematosus (SLE) patients compared with normal women. Moreover, some studies found a higher frequency of H-SIL. Additionally, it was observed that women with SLE had a higher frequency of HPV infection, confirmed by molecular biology techniques. Curiously, despite the above findings, no increased frequency of cervical cancer was observed in the majority of the studies which addressed this issue. Some studies observed a relationship between cervical abnormalities and previous use of immunosuppressive drugs. This suggests that SLE patients seem not to be at increased risk for developing cervical cancer. However, they should be considered at higher risk for HPV infection and cervical dysplasia than the general population. Thus, gynecological visits at shorter intervals seem to be a reasonable approach for those patients [229].

HUMAN PAPILLOMAVIRUS IN PREGNANCY

Pregnancy may foster the development of infections, in particular HPV infections. The prevalence of HPV infection in pregnancy varies between 5.4% and 68.8%. The population with the highest risk, also among pregnant women, are those under the age of 26. A number of different opinions emerge from the literature regarding the possibility of maternal-fetal virus transmission. None of the patients examined carried out treatment during pregnancy. The data reported in the literature on the relationship between HPV and pregnancy are highly discordant. This discrepancy depends on the diagnostic techniques used, the clinical history of the pregnant woman and the period of pregnancy when the sample is collected. Pregnancy enhances the development of the pathology which then often recedes in the postpartum. The possible maternal-fetal transmission of the virus is an important aspect: the latter is the main factor responsible for juvenile laryngeal papillomatosis. A number of Authors report an initial presence of HPV in newborns which often disappears within 6 months after birth [230]. HPV DNA detection in amniotic fluid, fetal membranes, cord blood, and placental trophoblastic cells all suggest HPV infection in utero, i.e. prenatal transmission.

Current evidence is strong enough to conclude that HPV can be transmitted both sexually and non-sexually. The debate on HPV infections in children still continues but it is more focused on HPV prevalence than on transmission modes. Based on recent meta-analysis, vertical transmission occurs in approximately 20% of cases. Most of the mucosal HPV infections in infants are incident, persistent infections in oral and genital mucosa being found in less than 10% and 2%, respectively. The mother seems to be the main transmitter of HPV to her newborn, but subsequent HPV infections are acquired horizontally via saliva or other contacts. Bimodal peak prevalence is seen for skin warts, oral papillomas, and RRP in younger and older age groups, suggesting similar epidemiology. Of the clinical HPV diseases, juvenile-onset-RRP and genital condylomata are problematic, the former because of its life-threatening potential and the latter because of possible sexual abuse. HPV 6 and 11 are the most common genotypes in both the lesions. Early in life, infections by the HR HPV genotypes may also remain persistent for a considerable period, and should be of considerable importance for HPV vaccination strategies [231]. Recent studies on perinatal infection with HPV have been inconclusive. It is still unclear how frequently perinatal infection progresses to clinical lesions, whether genital, laryngeal, or oral. Conflicting reports have been published on the prevalence of HPV infections in children. The current consensus is, however, that newborn babies can be exposed to cervical HPV infection of the mother. The detection rate of HPV DNA in oral swabs of newborn babies varies from 4% to 87%. The concordance of HPV types detected in newborn babies and their mothers is in the range of 57% to 69%, indicating that the infants might acquire the HPV infection post-natally from a variety of sources. HPV antibodies have been detected in 10% to 57% of the children, and there is usually no correlation between seropositivity and the detection of HPV DNA in either the oral or the genital mucosa. There is also evidence that transmission in utero or post-natal acquisition is possible. The mode of in utero transmission remains unknown, but theoretically the virus could be acquired hematogenously, by semen at fertilization, or as an ascending infection in the mother. The serologic response to HPV detected in different populations of young women or women at risk of cervical cancer might be due to genital infections, but the

possibility that HPV infection has been acquired earlier in life through the oral mucosa or respiratory tract cannot be ruled out [232].

Vaccines against HPV types 6/11/16/18 (Gardasil) and 16/18 (Cervarix) are non-viable vaccines composed of recombinant HPV proteins. As a precaution, they should not be given during pregnancy. However, some women are vaccinated shortly before conceiving or early during an undiagnosed pregnancy. After in utero exposure to the HPV 6/11/16/18 vaccine during the first trimester, animal studies, only conducted in rats, showed no increase in the risk of malformations. Five clinical trials and the latest annual update of the Pregnancy Registry for Gardasil, released in 2010 and including more than 1000 vaccinated pregnant women, showed no particular pattern of malformations with the quadrivalent vaccine. A few reports of very rare abnormalities are troubling, but they do not clearly implicate the vaccine. Most data on the HPV 16/18 vaccine come from two clinical trials comparing this vaccine with hepatitis A vaccine or placebo vaccination. Fewer than 400 pregnancies exposed to the HPV 16/18 vaccine have been studied. The rate of congenital malformations was similar to that in the control population. In practice, there are few data on exposure to HPV vaccines during the first trimester of pregnancy. There are more, relatively reassuring, data on the HPV 6/11/16/18 vaccine. Women who are vaccinated just before conceiving or early in pregnancy should receive appropriate information. Active pharmacovigilance must continue [233]. Cervical cone excision increases the risk of preterm deliveries (before the 37^{th} week of pregnancy). Vaccination against HPV 16/18 will probably prevent development of HG CIN and thereby reduce the need for cervical cone excisions. An HPV 16/18 vaccination programme may therefore also prevent some preterm deliveries. The number of preterm deliveries prevented by HPV 16/18 vaccination programme would depend on the number of preterm deliveries related to cervical cone excision (extent of the health problem), and the proportion of this health problem that could be prevented by a vaccination programme. If 2% of childbearing women are treated with cervical cone excision, between 60 and 220 preterm deliveries/100 000 births may be related to such treatment. Close to 60% (between 35 and 128 preterm deliveries) could be prevented by an HPV 16/18 vaccination programme, if the programme coverage was 90%. If 4% of women are treated with cone excision, between 70 and 257 preterm deliveries/100 000 births could be prevented. HPV 16/18 vaccination programmes may reduce the number of preterm deliveries through reducing the need for cone excision [234].

HUMAN PAPILLOMAVIRUS IN CHILDHOOD AND ADOLESCENCE

A substantial body of evidence has demonstrated that the primary means of transmission of GW in sexually active adults is through sexual contact. However, the epidemiology and social significance of anal-GW in prepubertal children is controversial. The incidence of ano-GW in prepubertal children is increasing. Modes of transmission of HPV to the anogenital area include perinatal, autoinoculation and heteroinoculation, sexual abuse, and possibly indirect transmission via fomites. Debate continues regarding the frequency with which these lesions have resulted from sexual abuse or transmission by other means. An accurate understanding of the dominant means of transmission of anal-GW in children is of particular importance because that understanding influences the extent to which child protective

services may become involved following a diagnosis. Methods for the diagnosis of child sexual abuse that have developed in the past decade form one of the bases for the evaluation of studies of the transmission of anal-genital HPV-related diseases to children [235]. The possibility that the lesions were acquired through sexual contact mandates a careful and thorough evaluation. Even then, the source of the infection may be elusive because of a long latency between inoculation and the development of lesions, the secretive milieu of childhood sexual abuse, and lack of data about modes of transmission. New molecular techniques of HPV DNA detection and typing have not proven to be helpful in determining the source of the infection but may assist in identifying children who are at risk for the development of carcinoma. Various treatment modalities have been attempted with a significant percentage of recurrences. Clinical and basic science research specifically designed to address the concerns of the pediatric age group is urgently needed [236].

It was previously thought that childhood sexual abuse was the most common mode of transmission and HPV types 6 and 11 were most often detected. Vertical transmission from mother to infant during birth is also well recognized. Laryngeal papillomas are the most common tumors of the larynx in children worldwide, and recurrent lesions are common occurrences. Ano-GW in children are problematic in that child sexual abuse is a potential means of acquisition, but many cases are acquired perinatally. Postnatal acquisition by nonsexual means also can occur. The likelihood of sexual abuse as the mode of acquisition increases with increasing age in childhood [237]. More recent studies, however, would suggest that perinatal infection and autoinoculation or heteroinoculation may be much more prevalent than originally thought. It has been increasingly reported that HPV type 2 is present in a significant proportion of cases [238].

Although spontaneous immunity can develop, as many as one third of children will have persistent HPV infection beyond 2 years. Therapeutic modalities are manifold, primarily because no therapy is universally effective [239]. Treatment modalities, although similar to adult disease, are particularly dependent on individual factors. In view of the as yet unknown risk of subsequent anogenital neoplasia, it is recommended that individuals should have regular follow-up on a long-term basis [238]. Warts continue to be a therapeutic challenge, especially widespread warts on children. A single, most effective treatment has not been defined. Conventional methods attempt to nonspecifically destroy infected tissue. Most of these procedures are painful, poorly tolerated by children, and often require multiple treatments. The efficacy of destructive techniques is impossible to verify in controlled clinical trials. Uncontrolled success rates are suboptimal and often no better than that seen with placebos. Alternative pharmacologic approaches have been designed to stimulate immunologic responses or provide anti-viral activity. Further study is needed to establish efficacy of these treatments [240].

Adolescence is a period of physical, cognitive, psychosocial, and moral development that often results in risk-taking behavior. As a consequence, adolescents are at high risk for STD. Two of the most common STD in the USA, Chlamydia and HPV, affect millions of adolescent women. Interventions that are effective in decreasing the transmission of Chlamydia, such as increasing condom use, are less effective in prevention of HPV. Efforts to increase adolescent awareness of HPV, to increase age of first coitus, and to decrease numbers of sexual partners are more effective for HPV prevention. Early screening for HPV, smoking cessation, and health promotion may be effective in decreasing the incidence of cervical cancer in young women. Nurse practitioners and other primary health care providers

need a more holistic approach to the prevention of HPV in adolescent women [241]. In fact, perhaps the most important implication of the finding that some anogenital cancers are in part infectious STD is that they may be preventable. The data overwhelmingly suggest that avoidance of exposure to HPV via abstinence or monogamy in both partners markedly reduces the risk of cervical cancer. A more realistic goal, however is prevention of HPV transmission by the use of barrier method contraceptives, which may be protective against development of cervical carcinoma. The American Academy of Pediatrics (AAP) Committee on Adolescents has outlined the obligation of pediatricians to be actively involved in adolescent education on STD. Certainly, a fundamental knowledge of HPV epidemiology, the risks of HPV-related sequelae, and prevention of HPV infection are important considerations for adolescent sexuality. Although helpful, such awareness alone falls far short of making an impact on sexual behaviors. A significant reduction in HPV infection rates could be achieved only by inundating adolescents at an early age with a highly visible society-wide campaign directed at these issues [242].

Evidence suggests that condoms provide some protection against infection and disease progression, but any genital contact is sufficient for HPV transmission. HPV is so common and transmissible that having just one sexual partner often results in infection. Indeed, cumulative prevalence rates are as high as 82% among adolescent women in select populations. Adolescents who are sexually active have the highest rates of prevalent and incident HPV infection rates with over 50-80% having infections within 2-3 years of initiating intercourse. These high rates reflect sexual behavior and biologic vulnerability. Most infections are transient in nature and cause no cytologic abnormality. However, a small number of adolescents will not clear the infection. Persistence of HPV is strongly linked to the development of H-SIL and invasive cancer. The H-SIL detected, however, does not appear to progress rapidly to invasive cancer. Understanding the natural history of HPV in adolescents has shed light into optional treatment strategies which include watchful observation of ASC-US and L-SIL. The association between age of first intercourse and invasive cancer cannot be ignored. Consequently, initiating screening at appropriate times in this vulnerable group is essential. In addition, with the advent of the HPV vaccine, vaccination prior to the onset of sexual activity is critical since most infections occur within a short time frame post initiation [243]. As such, nearly all sexually active adolescents are at HR for acquiring HPV. Most infections are asymptomatic and are efficiently cleared by the immune system. Similarly, both LG and HG lesions caused by HPV can regress in adolescent and young adult women. Treatment guidelines allow for observation of adolescent women who develop LG lesions rather than immediate colposcopy. Nonetheless, a small percentage of adolescents will develop precancerous lesions that may progress to invasive cervical cancer. Adolescents should be given appropriate education about HPV and the dangers associated with infection; they should also be encouraged to obtain appropriate gynecological care after initiating sexual activity [244]. Annual cervical cytology screening should begin approximately 3 years after initiation of sexual intercourse but no later than age 21 years. Because of the high rate of regression of L-SIL in adolescents, the cytologic study should be repeated within 6 to 12 months [245].

In summary, major differences in estrogen and progesterone levels and in vaginal/cervical cell types exist among children, adolescents, and adults, which may help explain the observed differences in the prevalence of HPV among these groups. Although sexual transmission occurs in both children and adolescents, the persistence of genital HPV in

the neonate from maternal transmission is highly questionable. The discordances in HPV types between neonatal transmission studies and the studies in older children have yet to be explained. Neonatal infections seem to be predominantly types 16 and 18 and persist for short periods in the genital area. The majority of condylomatous lesions in children are HPV types 6 and 11. Acquisition of HPV during childhood and adolescence is not an immediate cause of severe morbidity. However, significant evidence suggests that early exposure not only hastens the development of anogenital cancers but may also increase the actual risk of cancer. Future studies should include large-scale longitudinal designs to test and follow neonates with careful attention to sexual abuse issues during the prospective period so that we may confidently diagnose and appropriately treat children with HPV infections. The role of age and immunity remains confusing, supporting the need for studies to examine cervical immunity more closely [246, 247].

HUMAN PAPILLOMAVIRUS IN MEN

Although a consistent and coherent picture of the epidemiology and pathogenesis of genital HPV infections in women has developed over the past two decades, less is known about these infections in men. Available data suggest that, as with women, most genital HPV infections in men are symptomless and unapparent, and that HPV 16 is probably the most frequently detected type. In populations of similar age, the prevalence of specific HPV types is usually lower in men than in women. Whether this observation relates to lower incidence or shorter duration of infection in men than in women has not yet been determined. Seroprevalence of specific anti-HPV antibodies also seems to be lower in men than in women of similar age, a difference that might be due to lower viral load, lower incidence or duration of infection or lower antibody responses, or both, in men compared with women. Differences in sexual behavior may also be important predictors of genital HPV infection. With the availability of prophylactic HPV vaccines, it becomes increasingly important to understand the incidence and duration of HPV infections in men to develop cost-effective approaches to prevention through a combination of immunization and promotion of risk-reduction strategies [248]. In part these differences reflect difficulties in penile sampling and visual assessment of penile lesions. Anal HPV infection and disease also remain poorly understood. Although HPV is transmitted sexually and infects the genitals of both sexes, the cervix remains biologically more vulnerable to malignant transformation than does the penis or anus in men. An understanding of male HPV infection is therefore important in terms of reducing transmission of HPV to women and improving women's health. However, it is also important due to the burden of disease in men, who may develop both penile and anal cancer, particularly among HIV-positive MSM. Improved sampling techniques of the male genitalia and cohort studies in progress should provide important information on the natural history of anogenital HPV infection and disease in men, including risk factors for HPV acquisition and transmission. The impact of HPV vaccination in women on male anogenital HPV infection will also need to be assessed [249]. However, male HPV infection is also an important concern, both for the disease burden in men and for the risk of transmission to women.

HPV is associated with a variety of cancers in men, including anal cancer and a subset of penile and oral cancers. The incidence of anal and oral cancers related to HPV is increasing in

the general population and is growing even faster among individuals who are immunocompromised because of HIV infection. Penile HPV infection is very common among heterosexual men and remains high throughout a wide range of ages. Likewise, anal HPV infection and AIN are very common throughout a wide range of ages in both HIV-negative and HIV-positive MSM. Other HPV-related diseases of clinical importance in men include CA (GW) and RRP [250]. In men, 80-85% of anal cancers and close to 50% of penile cancers are associated with HPV infection. Among HPV DNA positive ano-genital cancer cases, HPV 16 is the most frequently found, followed distantly by HPV 18. In benign HPV-related diseases such as GW or RRP HPV 6 and 11, the two most frequent non-oncogenic types, are the predominant types detected. Oncogenic types are rarely detected [251]. Screening of the asymptomatic male partners of women with genital condyloma or intraepithelial neoplasia has shown that about 50% of the individuals examined present genital HPV-associated lesions. Half to two thirds of these lesions are clinically invisible and are detected only after the acetic acid test. Histological studies have shown that 20% of male partners of women with HG intraepithelial neoplasia present lesions histologically defined as HG intraepithelial neoplasia. Couples in which both partners present lesions of intraepithelial neoplasia are infected by the same potentially oncogenic HPV type in at least 50% of cases. Also, 50% of subclinical lesions showing only minimal histological changes (acanthosis and papillomatosis, without clear koilocytosis) contain HPV DNA, mostly type 42. ISH of such lesions indicate HPV-positivity, suggesting that these lesions may be infectious. Cytology does not seem to be specific enough to detect HPV infection in males. Moreover, virological studies do not confirm the hypothesis of an urethral reservoir of HPV. Morphology allows the detection of HPV-associated genital lesions in males. Although unequivocal histopathological signs of HPV influence often are absent, conventional light microscopy is usually adequate for differential diagnostic evaluation in clinical routine. ISH for the detection of HPV DNA may improve histopathologic accuracy. Current treatment protocols allow a 95% cure rate by easily applicable outpatient treatment modalities [252, 253].

The quadrivalent HPV vaccine has been shown to be highly efficacious in the prevention of GW in women and precancerous lesions of the cervix, vulva, and vagina. In addition, recent interim data have shown that the quadrivalent HPV vaccine is highly effective in reducing external genital lesions in young men. Although the protective efficacy of HPV vaccination in men has not yet been fully established, pending the outcome of public policy discussions and cost-efficacy studies, there may be a strong rationale for vaccinating boys, similar to girls, at an early age when they have had limited or no prior sexual activity [250].

HPV infection has been thoroughly demonstrated as a major factor in the pathogenesis of cervical cancer, but HPV's role in penile cancer has not been demonstrated as convincingly. Men with certain risk factors (e.g. intact foreskin, history of sexual encounters outside marriage, and history of first intercourse at a younger age) place their current female sex partners at greater risk for cervical carcinoma caused by transmission of HPV infection [254]. Penile HPV infection and disease are very common in sexually active men, and may be manifest in many forms. Flat penile lesions have similar predilection sites as HPV, often contain HR HPV as identified by DNA ISH in biopsy specimens, show a high association with HR HPV as identified by PCR in penile scrapes of lesional sites and are associated with high viral copy numbers. Absence of flat lesions is generally associated with very low HPV copy numbers or absence of HPV. Therefore, it can be argued that these lesions form the reservoir of HR HPV in men and contribute to the viral spread. Their bare visibility with the

naked eye and their high degree of spontaneous healing explain why flat penile lesions have slipped the attention of the clinician. Combining an HPV DNA test with a visual inspection after acetic acid application offers a more reliable interpretation of a positive HPV test in men, as it helps to distinguish positivity that is very likely to reflect a productive HPV infection from potentially HPV infections with very low copy numbers or HPV contamination by the sex partner. Future trials of HPV vaccines in men should take into account not only the presence of penile HPV, but also the presence of flat penile lesions as an outcome measure for the efficacy of a vaccine [255]. Emerging evidence suggests that penile cancer follows 2 etiologic pathways, one related to HPV infection and the other related to other factors including phimosis, chronic inflammation, and lichen sclerosus. HPV DNA is found in 47% to 48% of all penile tumors, and most of these cases correspond to HR genotypes, preferentially HPV 16. HPV status is associated with histologic subtype, with higher detection ratios in warty-basaloid carcinomas and lower detection ratios in keratinizing variants (i.e. verrucous, papillary, and usual SCC). It is the cell type, rather than a distinctive architecture, that is more strongly associated with HPV presence. The detection ratio is higher in tumors composed entirely or partially of cells with basaloid features. In addition, a few studies have evaluated the impact of HPV infection on the prognosis of patients with penile cancer. However, results are controversial, and more data are needed to clarify this matter. A proper understanding of the role of HPV in penile carcinogenesis might help in planning intervention strategies such as vaccination against HPV infection [256].

The unkeratinized epithelium of the inner human foreskin is thought to be more susceptible to HPV entry than the rest of the penis. However, studies exploring a potential association between male circumcision and HPV infection have produced conflicting results. Circumcision reduces the risk of HPV infection in a stage- and type-specific manner. There is no consistent association of HPV acquisition with circumcision status, indicating that circumcised men may be no more protected from initial HPV infection than their uncircumcised peers. Circumcision is not protective against nononcogenic types of HPV, but is associated with a reduced prevalence and persistence of oncogenic HPV infections. Circumcised men are also less susceptible to multiple infections. These findings indicate that circumcision modulates HPV persistence rather than acquisition. Through promoting HPV infection clearance, male circumcision could be an important adjunct to education, condom use, and vaccination in reducing the global burden of HPV morbidity and mortality [257]. Male circumcision reduces HIV acquisition in men. Randomized controlled trial data also demonstrate that male circumcision reduces penile HR HPV infection in both HIV-negative and -positive men. Male circumcision of HIV-negative men also reduces the prevalence and incidence of HR HPV infections in their female partners. However, male circumcision of HIV-positive men has no effect on HR HPV infection in their female partners. These data demonstrate that male circumcision is most effective prior to sexual debut, and the presence of the male foreskin facilitates HIV and HR HPV infection in men and their female partners. Additional studies that utilize the foreskin mucosa obtained at the time of male circumcision are needed to assess the mucosal microenvironment in HIV and HR HPV coinfections to develop additional preventive and therapeutic approaches [258].

Even though the incidence of anal cancer among MSM is higher than the incidence of cervical cancer among women, few MSM are identified as HR patients in primary care or have received vaccination for HPV. Early diagnosis and treatment of patients with HPV infection is important because this infection causes patients substantial distress even in its

benign manifestations. The incidence of HPV infection drops in women older than 30 years but remains high for MSM in all age ranges. For all of these reasons, physicians should routinely assess the sexual practices of all male patients, especially MSM, and educate them on the HPV infection risks, diagnosis, and treatment options. Physicians can have a significant impact in the primary prevention of HPV by routinely offering HPV vaccination to male patients younger than 26 years [259]. Treatment of clinically detectable lesions is advisable not only to relieve symptoms but also to prevent the spread of HPV infection to new sexual partners. Treatment of subclinical disease is more controversial but also may be advisable in some cases given the evidence that these lesions may also harbor infectious virus. In addition, subclinical disease may demonstrate intraepithelial neoplasia, which if left untreated may progress to invasive cancer in a small number of cases. Anal HPV infection and A-SIL are very common in HR homosexual and bisexual men, particularly among those who are HIV positive. Parallels with cervical HPV infection and disease suggest that anal H-SIL may be precancerous, and indeed anal cancer may be as common or more common in this HR group as cervical cancer is in women. Like cervical cancer, anal cancer may be a preventable disease, and implementation of a well-targeted screening program similar to that in place for cervical disease should be considered in the future when appropriate supporting data become available [260, 261].

Some studies have reported on the prevalence of anogenital types of the HPV in prostate cancer, benign prostatic hypertrophy, and normal prostatic tissue. When the data were combined, 32% of the cancers were positive, compared to 49% of the benign lesions and 9% of the normal tissue. The highest positivity rates were found in the earlier studies, and the more recent results have been more negative. The finding of higher rates in benign compared to malignant tissue and the failure of confirmatory studies to support earlier reports make it unlikely that the common anogenital HPV have an important role in prostate carcinogenesis. More work is needed to decide if the prostate is a significant reservoir for the HPV in the male [262].

A high incidence of HPV infection has been demonstrated in sperm from sexually active men with and without risk factors for HPV and from infertile patients. Semen infection is associated to an impairment of sperm parameters suggesting a possible role in male infertility. Interestingly, it has been demonstrated that when HPV is present in semen only a percentage of total cells are infected and the virus can be localized in sperm or in exfoliated cells with different impact on sperm motility. Moreover, infected sperm are able to penetrate the oocyte, to deliver HPV genome in the oocyte and HPV genes can be actively transcribed by the fertilized oocyte. Recently, an increased risk of pregnancy loss has been demonstrated in couples undergoing in-vitro fertilization (IVF) and particularly when HPV DNA was present in semen samples of male partners. To date, no effective treatment, control strategy, and prevention is provided for men despite the reported high incidence of HPV semen infection. Because this infection in men is also a problem for partners, and because growing evidence suggests that semen infection may cause infertility and early miscarriage, more attention should be paid to male HPV infection [263-265].

CLINICS

Over the past decade, the ever-increasing volume of evidence implicating HPV types in genital neoplasia has stimulated much research interest into all aspects of the biology of this interesting group of viruses. This has led to the identification of an ever-increasing heterogeneity of HPV types. It is not surprising, therefore, that the clinical profile of disease associated with genital HPV types is much broader than previously recognized. Knowledge of this clinical spectrum is mandatory to the understanding of the possible role of specific HPV types in human carcinogenesis [266-268]. HPV are associated with a spectrum of diseases, ranging from common warts to invasive carcinoma of the genital tract. The clinical manifestations of HPV infection depend on the viral subtype, the immune status of the patient, and environmental co-carcinogens. Infection with HPV is often asymptomatic, which makes viral detection challenging. Current therapies do not reliably eradicate HPV infection, and benign GW and genital tract intraepithelial neoplasia often recur after treatment [269].

Established associations between HPV and lower genital tract cancers provide a framework from which to evaluate a possible pathogenic role for the virus in cancers at nongenital sites [270]. Although genital HPV types produce a broad spectrum of disease, the nongenital types are a bit more predictive. Particularly in the immunocompromised patient, it appears as though when they become symptomatic they cause warts. These warts can be a particular problem with immunocompromised patients where the malignant potential can also be expressed [271-273].

Human Papillomavirus in Skin Diseases

Although HPV that populate the skin were the first identified HPV types, knowledge of the pathogenicity of HPV in the cornified epithelia stayed behind. What the majority of cutaneous HPV types do, for instance those belonging to the beta genus (beta-HPV), is largely unknown. As the number of reports that describe epidemiological associations between markers of beta-HPV infection and skin cancer gradually increases, the need for basic knowledge about these viruses grows as well [274]. There is a strong association between HPV and cutaneous SCC. If this association was merely random, one would expect an equal distribution of HPV types among affected individuals. However, only specific types of HPV are consistently found in cutaneous and genital SCC. Immunosuppressed individuals clearly have a much higher incidence of cutaneous carcinomas. Immunosuppression, either local or systemic, not only decreases immune surveillance but may also dictate the amount and type of virus each individual may carry. EV and other rare hereditary disorders that combine specific immune defects and an increased incidence of malignancy are very useful models that clearly fulfill a multistep theory of oncogenesis. Intracellular interactions with the recently described tumor suppressor proteins may prove to be the primary site of action of these oncogenic viruses. Environmental cocarcinogens and activation of oncogenes are clearly important if not essential factors in HPV-associated tumors. As our knowledge and understanding of malignant transformation grows, it becomes apparent that this is a complex multistep process [275].

A minority of HPV cause cutaneous warts and mucosal condylomata. The HPV that cause mucosal condylomata put the patient at various degrees of risk for developing cancers, particularly cervical cancer. The majority of HPV infect the skin of normal and immunocompromised individuals. In normal people, most of these HPV appear to establish a latent infection of the skin, most likely as normal flora residing in hair follicles. However, in patients with various systemic and localized depressions of CMI, some HPV infections appear to be involved in the development of NMSC and its precursor lesions in skin, usually in sunlight-exposed areas [276]. External genital and perianal warts represent a possible phenotypic expression of HPV infection and results from hyperkeratosis and hyperplasia of keratinocytes. The cell cycle disruption caused by LR anogenital HPV subtypes (e.g. HPV 6, HPV 11) is similar to HR HPV subtypes, except LR HPV E6 and E7 proteins likely bind regulatory proteins with less affinity. Although UV light clearly has a primary causal role in the development of AK, new data suggest that HPV infection, particularly with 3-HPV subtypes, may serve as a cocarcinogen. By impairing normal DNA repair and apoptotic mechanisms, HPV may set the stage for later UV-induced transformation. It also has been suggested that HPV may increase the severity of AK lesions and contribute to their recurrence following therapy [277]. Seborrheic keratoses situated in the anogenital region often resemble CA, clinically and histopathologically, yet they are benign neoplasms of unknown cause, in contrast to CA, which are hyperplasias induced by HPV. In the past, some Authors claimed to have demonstrated HPV in some anogenital seborrheic keratoses, but they failed to set forth precise histopathologic criteria for diagnosis of seborrheic keratoses. Standards by which to diagnose CA histopathologically also are wanting in many textbooks of dermatology, general pathology, and dermatopathology. In fact, in some of those books, findings proffered for histopathologic diagnosis of CA are very similar to those for diagnosis of seborrheic keratoses. All of this, as well as observations about CA and seborrheic keratoses on genital skin, prompt us to a different conclusion about the matter, namely, lesions said to be seborrheic keratoses replete with HPV are really CA [278].

HPV-induced skin warts are classically benign lesions. However, an association between specific HPV types and skin cancer becomes obvious in EV. EV is a rare autosomal recessive genodermatosis that has raised an enormous interest since it is a model of cutaneous genetic cancer induced by specific HPV. The interacting immunogenetic and environmental factors, especially UV irradiation, result in the inability of the patients' immune system to respond to EV-specific HPV. The local immunosuppression is an effect, at least in part, of the overproduction of TNF-alpha and transforming growth factor (TGF)-beta1 and of the excessive formation of cis-urocanic acid. EV is a model not only of cutaneous viral oncogenesis but also of local defense mechanisms in the progression of HPV-associated cancers [279]. The analysis of this disease suggests that lesions infected with HPV types 5 and 8 carry a HR of developing SCC.

The oncogenes of EV-viruses appear to be E2 and E6, rather than E7. The HR EV-viruses, HPV 5, 8, and 47, differ from related HPV types in the transforming activity of the E6 gene and in the density of positive transcription control elements in the non-coding region (NCR) of the genome. The extrachromosomal viral DNA in cancers may show deletions affecting regulatory sequences. EV-specific lesions occasionally occur in immunosuppressed patients and HPV 5 or 8 persist in some of the skin cancers to which these patients are prone. DNA of HPV 2, 16, 34, or 41 were identified in few premalignant and malignant skin tumors

of the general population [280]. New insights in EV pathogenesis have been gained from the following recent observations:

1) EV-specific HPV (beta-HPV) are defective for an important growth-promoting function encoded by an E5/E8 gene present in other HPV, and inactivation of EVER proteins may compensate for the missing viral function; and
2) the transmembrane viral E5/E8 and cellular EVER proteins interact both with the ZnT1, and are likely to modulate Zn homeostasis.

EV may thus represent a primary deficiency in intrinsic, constitutive immunity to beta-HPV, or constitute a primary deficiency in innate immunity (or both). Keratinocytes, the home cells of HPV, are likely to play a central role in both cases. An important issue is to establish which cellular genes involved in intrinsic and innate antiviral responses play a part in the outcome of infections with other HPV types, such as genital oncogenic HPV [281]. Despite its rarity, EV was addressed in depth in recent literature. Patients are afflicted by persistent HPV infections and develop cutaneous malignancies more frequently and younger than in the general population.

The disease is therefore considered a model for a viral role in cutaneous oncogenesis, although implication is controversial. Susceptibility loci for EV were mapped and encoded protein functions are becoming better understood, but a unified genetic theory for EV is lacking. EV-HPV, originally thought present only in EV, is now considered ubiquitous, its role still being elucidated. Numerous therapies for EV lesions were proposed, although there is no consensual first-line treatment strategy. Discoveries of novel mutations and further study of EV-HPV in lesional and nonlesional skin of EV patients and the general population may generate a cohesive theory regarding a viral role in cutaneous oncogenesis. Future understanding of the disease may yield an optimal approach to treating EV patients [282, 283].

NMSC is the most frequent cancer among Caucasians worldwide. The lesions occur preferentially on sun-exposed sites of the body.

The role of HPV in the etiology of carcinoma of the genital tract is well established. EV has been regarded as a model for NMSC developing on sun-exposed sites. Infection with a specific group of HPV types has been associated with the benign and malignant lesions occurring in these patients. Recent studies using improved detection methods, as well as re-examining material used in previously published studies, reported the presence of HPV DNA in NMSC from immunocompetent patients, as well as more than 90% of NMSC occurring in organ transplant recipients. Five HPV types were identified as the most prevalent in these tumors, i.e. HPV 20, HPV 23, HPV 38 and two newly identified HPV types, DL40 and DL267. These and other HPV types were also demonstrated in normal skin biopsies (35%) and a small number of melanomas.

The frequent presence of more than one HPV type within a lesion was noticeable, with at least one type belonging to the EV-associated HPV types. Present data indicate that the primary infection with the majority of, if not all, HPV types, apparently occurs early in life, after which it remains latent. Prolonged UV radiation is needed either to activate viral gene functions, and/or to inactivate cellular genes responsible for controlled cell growth [284]. It is difficult, however, to interpret these findings against a background of low-level infections with multiple HPV types from supergroup B (HPV 4-related and EV HPV), probably

acquired by everyone early in and throughout life. Thus far, no HR HPV types have been identified. Because of the low copy numbers of HPV DNA in skin cancers, probably not every tumor cell contains a viral genome, which is compatible with cutaneous HPV being possibly important for tumor initiation and progression, but not for maintenance of the malignant phenotype.

The question with regard to HR types should, therefore, be readdressed in case-control studies on the basis of serology, which can reveal viral activities over years. The viruses lingering in all people are apparently activated by sunlight (UV) exposure, by immunosuppression, and by hyperproliferation of the epithelium (psoriasis) and/or in the specific genetic background of the host (EV). It is intriguing that most of these factors are established risk factors in skin carcinogenesis.

The weak transforming activity of cutaneous HPV in vitro compared with the transforming activity of genital HPV may explain the need for activators and synergistic factors. The antiapoptotic activities of E6 proteins of cutaneous HPV could be relevant to oncogenesis in the interplay with UV exposure. Prospective studies should determine the kinetics of HPV activation relative to tumor development [285-288].

Human Papillomavirus in Ocular Diseases

While no definitive answer exists at present, several lines of evidence indicating a viral role in human ocular tumors include the well-documented role of viruses in causing animal ocular tumors, evidence for an association of certain viruses with human ocular tumors, and virally induced animal models of human ocular tumors, Rb, and ocular malignant melanoma [289]. The role of HPV infection in eye disease is controversial.

However, a recent case illustrates the possible role of HPV in conjunctival squamous carcinoma and the potentially devastating effects of this disease. The development of two vaccines to prevent infection with HPV types most commonly associated with anogenital cancers has led to debate about the pros and cons of a national immunization programme to prevent cervical cancer. The introduction of such a vaccination programme may have an additional beneficial effect on the occurrence of some head and neck, including ocular, cancers [290].

Human Papillomavirus in Head and Neck Diseases

HPV has been associated with tumors of the head and neck, being associated with more than 50% of oral and nasal carcinomas, as well as with carcinomas of the larynx and esophagus [291]. Reports demonstrating the presence of HPV in benign and malignant tumors of the head and neck, lung tumors, and tumors of the aerodigestive tract are increasing. The phylogenic groups of HPV also showed a definite correlation with the morphology of head and neck tumors. The groups A6, A7, and A9 include viruses that are frequently demonstrated in basaloid and verrucosus SCC known to associate with HPV infection [292]. Because of different methods used, all varying in sensitivity and specificity, a critical evaluation of results is required. Although the majority of papillomatous lesions of the oral

cavity and the larynx contain HPV DNA sequences, other benign and malignant lesions still remain negative.

The identification of a new HPV from an agressively growing inverted papilloma of the paranasal sinuses could lead to the isolation of HPV which could play a role in the etiology of these benign and malignant lesions [293]. Therefore, any patient with persistent lesions, ulcers, swallowing difficulty, change in voice, or neck mass needs prompt referral to an otolaryngologist-head and neck surgeon [294].

HPV of different types are associated with a variety of rare and uncommon oral lesions, and there has been increasing suspicion that they may be implicated also in some premalignant and malignant oral lesions [295]. However, since it is now clear that a variant of HPV 16 is harbored by normal oral mucosa, as well as by premalignant and malignant lesions, such associations may not necessarily always be causal [296].

These HPV-associated lesions can be classified into two broad types on the basis of their biologic behavior, benign lesions and premalignant malignant or malignant lesions. Benign oral lesions include squamous cell papilloma (SCP), verruca vulgaris (VV), CA, and focal epithelial hyperplasia (FEH). Of these entities, VV, CA, and FEH demonstrate characteristic HPV-induced cytopathic effects, whereas SCP infrequently shows such changes. All of these lesions show a clear association with HPV. Premalignant and malignant oral lesions include leukoplakia and SCC. Koilocytosis is the most common cytopathic effect seen in both groups of lesions. Even though it is sometimes difficult to distinguish between hyperplastic lesions such as SCP, VV, and CA, clinical and certain histologic features can facilitate the diagnosis. Although exceptions do exist, each of the two classes of lesions is most commonly associated with particular HPV types.

The benign oral lesions are associated with HPV 2, 4, 6, 11, 13, and 32, the malignant oral lesions are associated with HPV 16 and 18. No preferential association has been demonstrated between specific HPV types and a particular oral lesion [297]. The most common conditions induced by oral HPV infection are usually benign-like oral papillomas, oral condylomas, and FEH. Oral HPV infection has been found to be associated with some cases of OPCA, but it is not the main risk factor for this kind of cancer. HPV is been proved to be the causative agent in causation of cervical cancers without doubt, but its role as a etiologic agent in causing oral cancers needs to be evaluated and studied more to come into any conclusion [298].

Oral SCC is one of the most common cancers in some areas of the world (i.e. India). Although tobacco and alcohol are the main etiologic factors for nearly three-fourth of these cancers, no definite etiologic factor can be identified in one-fourth of the cases.

There is growing evidence that HPV may act as a cocarcinogen, along with tobacco, in the causation of oral cancers. The role of HPV in the etiology of anogenital cancers has been firmly established, and infection with this virus has also been shown to have prognostic significance. However, there is no clear evidence to support its involvement in oral carcinogenesis. Association of HPV is strongest for OPCA, especially cancers of the tonsils, followed by those of the base of tongue. HR HPV 16 is the predominant type: it commonly affects the younger age-groups, with males appearing to have a predisposition for infection with this strain. Its prevalence increases from normal to dysplasia and finally to cancer [299]. HPV 16 is the most frequently detected HPV type in oral SCC and is present in up to 22% of cases, either alone or in combination with other HPV types. HPV 18 is present in up to 14% of cases. HPV 16 and HPV 18 are present together in approximately 6% of cases. However,

HPV 16 and 18 are also detected in normal oral mucosae (10% and 11% of subjects, respectively). These data suggest that HR HPV infection may be a co-factor in oral carcinogenesis and that latent HPV infection of the oral mucosa is common. A role for HPV infection in oral carcinogenesis is supported by the ability of HR HPV to immortalize oral keratinocytes in vitro. Immortalization may involve:

1) deactivation of pre-formed tumor-suppressor proteins by viral oncoproteins;
2) blocking of TSG transcription as a result of HPV oncogene insertion; or
3) stimulation of cellular oncogene transcription by the upstream insertion of HPV-derived transcription activating sequences.

Hence, infection of oral keratinocytes with HR HPV may be involved in the pathogenesis of some oral SCC, although the evidence implicating HPV in oral carcinogenesis is, at present, mainly circumstantial [300]. HPV prevalence has been reported to be twice as high in premalignant lesions as in normal mucosa and is nearly five times higher in oral SCC. The overall prevalence of HPV in oral SCC ranges between 20-50%. Oral SCC associated with HPV have been found to have better outcomes, being more responsive to radiotherapy and showing higher survival rates. In view of the association of HPV with oral SCC, it should be worthwhile to conduct further experimental studies to elucidate its role in oral carcinogenesis [299,301-306].

Laboratory evidence indicates that the virus is integrated and that HPV oncogenes are transcriptionally active in these tumors. Many aspects of the association remain to be investigated, including the epidemiology and natural history of HPV infection in the mouth, the role of cofactors, and the potential use of HPV testing and vaccines in the prevention of these tumors. An analogous role for the virus at other anatomic sites in the upper aerodigestive tract (UADT) such as the larynx is less clear. The relationship between HPV infection and laryngeal cancer is of particular interest, given that RRP is clearly caused by benign proliferative growths induced by HPV 6 or 11 infection of the laryngeal epithelium. Although HPV genomic DNA has been detected in a proportion of laryngeal cancers and despite the many efforts made during the last 25 years, there is not yet compelling evidence that HPV plays a substantial role in laryngeal cancer [307]. Epidemiological and experimental evidence lend some support to this possibility. Increased risk of cancer of the oral cavity, pharynx, and larynx subsequent to the occurrence of cancer of the cervix has been found and suggests common etiological factors besides smoking. HPV has been found in a substantial proportion of benign UADT lesions, most notably laryngeal papillomas and oral verrucal-papillary lesions. Largest and most accurate case series (based on best HPV detection techniques) showed HPV DNA in 46% of cancers of the oral cavity and pharynx, 15% of cancers of the esophagus, and 24% of cancers of the larynx, with however, great discrepancies from one study to another. An additional case series with a comparison group of noncancer patients revealed approximately a 4-fold higher HPV prevalence in UADT cancer tissues than in normal ones. Strictly designed case-control studies dealt with cancer of the oral cavity and provided inconclusive results, possibly because of interference of primary treatment with HPV detection in buccal exfoliated cells. An increasing bulk of experimental and in vitro evidence suggests that at least a proportion of UADT cancers harbor a relatively high copy number of HPV DNA. E6/E7 region transcripts and a clonal association with HPV have been demonstrated in these tumors. Allowance for the strong effect of smoking, alcohol

drinking, and betel quid chewing on UADT cancer and exclusion of noncausal associations are the most difficult challenges of such studies [308].

There is a strong and consistent association between HR HPV types, specifically HPV 16, a known human carcinogen, and these distinctive OPCA with molecular characteristics indicative of viral oncogene function. Risk for HPV-head and neck SCC is increased by certain sexual behaviors after consideration of alcohol and tobacco exposure, consistent with an extensive literature that has established HPV infection as a STD. Furthermore, exposure to HPV 16 has been associated with increased risk for subsequent development of OPCA. Prophylactic and therapeutic vaccines targeted against the viral capsid components and oncoproteins will provide the ultimate evidence for a role for HPV in head and neck SCC, if demonstrated to be effective in the prevention or therapy of this disease. It is time for clinician scientists to translate knowledge of this newly recognized disease entity into potential applications for the prevention, detection, and treatment of HPV-head and neck SCC [309]. The strength and consistency of HPV DNA presence in OPCA bolster the argument that this association is likely causal. HPV-positive tonsillar cancer in particular is emerging as a specific disease entity with distinct molecular, pathologic, and clinical characteristics. Recent data suggest that the incidence of tonsillar carcinoma in the USA is increasing, despite a decline in tobacco use, supporting the existence of other important risk factors such as HPV infection. Individuals with a history of an HPV-associated anogenital cancer and HIV-infected men are at increased risk for tonsillar carcinoma [310]. The mere presence of the virus in tumor specimens, by itself, does not imply a causal relationship. However, recent studies support an etiologic role for HPV in a subset of head and neck SCC, particularly poorly differentiated tumors arising from Waldeyer's tonsillar ring. Epidemiologic studies have shown that exposure to HPV increases the risk of head and neck SCC, and HPV infection may interact with alcohol and tobacco exposure in tumor promotion. Molecular studies indicate that transcriptionally active virus is confined to tumor cells. It will be important to clarify further the role that HPV has in head and neck SCC development, because HPV-based therapeutic vaccines which are currently being developed for cervical cancer may also be of benefit in the management of head and neck SCC [311].

The great majority of HPV-related carcinoma of the oropharynx is nonkeratinizing SCC. More recently, an increasing number of SCC variants that are HPV positive are being reported in the oropharynx, as well as in other head and neck sites. As a result, several clinical and pathologic questions have emerged. Importantly, questions raised include whether the virus is biologically active and involved in the pathogenesis of these tumors, and whether there are clinical implications with regard to patient outcome and treatment modality changes that may be needed in HPV-related variants. Examples of HPV-related SCC variants include: basaloid SCC, undifferentiated carcinoma, adenosquamous carcinoma, papillary squamous carcinoma, and small cell carcinoma. Some investigations have suggested a favorable prognosis in some variants, analogous to that of the conventional nonkeratinizing (basaloid) carcinoma, while others showed poorer outcome. So far, the number of studies on this subject is limited and the number of cases evaluated in each investigation is few. Because of this, it is prudent at this stage not to alter management protocols as a result of identification of HPV in these variants and to await additional studies [312].

Researches are focused on the development of HPV detection assays specially designed for head and neck SCC. The HPV diagnosis in these tumors is relevant to prognosis even in an already-developed tumor, whereas in the cervix, where the HPV is the cause of almost all

tumors, this information has less clinical relevance. The better outcome of HPV-associated head and neck SCC raises the question about the best methodologies to distinguish between HPV and non-HPV-associated SCC. However, no consensus has been reached on the optimal way to identify HPV-associated SCC and ancillary studies have utilized many different methodologies, including HPV PCR testing, HPV ISH analysis, immunohistochemical staining for p16, and newer techniques that are currently under investigation. Although currently insufficiently specific due to the possibility of HPV infection originating at other sites, methodologies utilizing serum and plasma to measure HPV infection have potential future development and use. Finally, DNA/RNA microarray platforms have the capacity to identify the profile of molecular changes in any particular HPV+/HPV- cancer. In this way, it is expected to be possible to correlate the appropriate transcriptome-based diagnosis to the patients' specific cancer risk [313]. HPV testing is now recommended as part of the work up for patients with oropharyngeal SCC and those patients with cervical lymph node metastasis of unknown origin. The laboratory testing strategy should accurately assess the presence or absence of oncogenic HPV infection in routinely collected tumor samples that are subject to standard fixation protocols, alcohol-fixed cytological preparations, and formalin-fixed tissue samples. The HPV status should correlate with biologically relevant outcome measures such as overall, disease-specific, and disease-free survival. Whilst increased expression of p16 by immunohistochemistry (IHC) is considered to be a surrogate marker of oncogenic HPV infection and is a validated independent prognostic biomarker, only HPV specific tests provide definitive evidence of the etiological agent. HPV specific testing is the starting point for developing increasingly informative biomarker panels in the context of "stratified medicine". Only a testing strategy that includes HPV specific tests can deliver more effective care for patients with oropharyngeal SCC. The international head and neck oncology community should work together to clearly define the minimum requirements for assigning a diagnosis of HPV-related oropharyngeal SCC in order to ensure consistent reporting of this emerging and increasingly prevalent disease [314].

Imaging, especially contrast-enhanced computed tomography (CECT) for anatomy and positron emission tomography (PET) with labeled (18)F fluorodeoxyglucose for physiologic detail, is critical for staging carcinomas of the oropharynx. As the incidence of HPV infection and related carcinomas of the tonsil and base of tongue (BOT) increases, experience with CECT and PET for staging HPV+ tumors is growing. No imaging modality, however, can determine whether the tumor is HPV+. There are some unique challenges posed by HPV+ oropharyngeal SCC. In most locations of the head and neck, a malignancy enhances more than surrounding normal structures, which facilitates tumor mapping. Unfortunately, normal lymphoid tissue of the oropharynx, in the BOT and palatine tonsillar fossa, enhances on CECT and gadolinium enhanced magnetic resonance imaging (MRI) in a manner similar to SCC. The primary tumor may be small or even occult at presentation, and easily over-looked on CECT. PET coupled with CECT has made a true "unknown primary" very rare, as the metabolically active tumor is almost always detectable on PET. The nodal metastases, so common with HPV+ SCC, can be truly cystic, and as such, can be misdiagnosed as a second branchial cleft cyst, a congenital benign lesion. These pitfalls, coupled with the complex anatomy of the UADT, make staging these tumors difficult [315].

Management of this disease is controversial. Traditional open surgical techniques gave way to concurrent chemoradiotherapy following several American and European organ-preservation trials suggesting that both modalities were equally efficacious. More recently,

minimally invasive surgical techniques have gained popularity. These techniques provide an opportunity to achieve a complete surgical resection without the treatment-related morbidity associated with open surgery. Proponents of this technique contend that transoral surgical techniques provide a means to analyze the tumor tissue, prognosticate, and personally direct therapy. Skeptics suggest that HPV-associated OPCA responds well to chemoradiotherapy and that surgery may not provide a treatment advantage. Both approaches provide a unique perspective and both are currently being studied under trial [316].

In conclusion, the causative relationship between HR HPV and oral SCC is well-established. HPV-associated oral SCC represents a distinct disease entity compared to tobacco-associated ones. These virus-associated cancers continuously express the HPV E6 and E7 viral oncogenes even in advanced stages, and repression of viral oncogene expression can prevent the growth or survival of cancer cells. This finding raises the possibility that even late-stage HPV-associated oral SCC can be cured by HPV-targeted approaches, such as medicines that interfere with the expression or function of viral oncoproteins, and therapeutic vaccines that elicit a cytolytic immune response to cells expressing these oncoproteins. The demonstration that HR HPV are causally associated with a subset of oral SCC has allowed the development of preventive and therapeutic strategies aimed at reducing the incidence and mortality of this disease. The better outcome of HPV-associated oral SCC raises the question as to whether similar results can be achieved with less treatment. An important aim of novel approaches for favorable-prognosis, HPV-associated cancers will be minimization of devastating side effects of intensified treatment developed for poor prognostic subsets. Clinical trials are studying the potential for de-escalation of radiation therapy in HPV+ oral SCC in the setting of different chemoradiotherapy regimens. The role of cetuximab in HPV-associated oral SCC needs to be explored in prospective clinical trials [317-335].

Human Papillomavirus in Lung Diseases

Lung cancer is the leading cause of cancer related death in Western countries. Several factors have been implicated in its etiology: cigarette smoking, environmental pollution, asbestos, and genetic factors. The possible involvement of HPV in bronchial squamous cell lesions was first suggested in 1979 by Syrjänen. Since then, several studies have confirmed the presence of HPV DNA in about 20% of lung cancer cases examined, with HPV 16 and 18 as the two most frequently found oncogenic viral types. More recently, these data have been supported by the detection of E6 and E7 transcripts in HPV-positive lung cancer cases, reinforcing the hypothesis that oncogenic HPV could act as cofactors in bronchial carcinogenesis [336]. Nonsmall cell lung cancer is a heterogeneous disease. The most common histologic subtypes include SCC, adenocarcinoma, and large cell carcinoma. Despite different histologies, nonsmall cell lung cancers are often classified together because of similarities in approach and management of the disease [337]. Adenocarcinoma is the most common histological subtype among non-smoking women. Previous studies showed that HPV infection may relate to the tumorigenesis of pulmonary adenocarcinoma. Women with anogenital malignancy have a higher risk of lung cancer, which raises the possibility of HPV transmission from the cervix to the lung. Two postulated pathways are the following: first, HPV may infect the female cervix and then move to the lung by blood circulation; the second transmission route is the HPV infection of oral cavity resulting from dangerous sexual

contacts, and subsequently transmitted to the lung. Future studies are needed to demonstrate the causal inference between HPV infection and the risk of female lung adenocarcinoma [338,339].

Human Papillomavirus in Breast Diseases

Breast cancer is the leading female cancer and the third most common cause of cancer deaths worldwide. Many studies have suggested a possible link between breast cancer pathogenesis and viral infection, particularly mouse mammary tumor virus, SV40, Epstein-Barr Virus (EBV), and HPV. A significant number of recent studies have reported that approximately 29% of human breast cancer tissues were positive for HR HPV subtypes, especially HPV subtypes 16, 18, or 33. In contrast, several other investigations did not detect any HPV subtypes in either breast cancer tissue or normal breast tissue from patients diagnosed with breast cancer. Given these conflicting data and the established complexity of the association between HPV with other cancers, a definitive relationship between human breast cancer and HPV infection has not been determined. Recent advances in laboratory methodologies aim to overcome the inherent challenges in detecting HPV in breast cancer tissue. There is an urgent need to obtain additional evidence in order to assess the possibility of breast cancer prevention using HPV vaccines [340]. Despite an increase in the number of molecular epidemiological studies conducted in recent years to evaluate the association between HPV infection and risk of breast carcinoma, the studies remain inconclusive. A meta-analysis conducted to estimate the prevalence of HPV in breast carcinoma and test the association revealed that 24.49% of the breast carcinoma cases were associated with HPV, 32.42% occurred in Asia and 12.91% in Europe. The four most commonly identified HPV types, in the order of decreased prevalence, were HPV 33, 18, 16, and 35. The detection of HPV was mostly influenced by publication calendar period and PCR primers used. In addition, the analysis of ten case-control studies showed a significant increase in breast carcinoma risk with HPV positivity (OR 3.63, 95% CI 1.42-9.27). These results suggest that it's difficult to rule out the possibility of the association of HPV and breast carcinoma at present according to available publication proofs [341].

Human Papillomavirus in Gastroenteric Diseases

Esophageal SCC is an invasive neoplastic disease generally associated with poor survival rates. The incidence of esophageal SCC is characterized by marked geographic variation, with highest rates noted in developing Southeastern African, Central and Eastern Asian countries. In the developed Western European and North American regions where there is a low disease incidence, heavy alcohol and cigarette consumption constitute major risk factors. The toxic effects of both these risk factors cause chronic irritation and inflammation of the esophageal mucosa, while at the cellular level they further confer mutagenic effects by the activation of oncogenes (e.g. k-ras mutations), inhibition of TSG, and profound DNA damage. Viral infections, particularly with HPV, may activate specific antiapoptotic, proliferative, and malignant cellular responses that may be intensified in combination with the effects of alcohol and tobacco [342]. HPV has been implicated in esophageal SCC, particularly the sub-

types 16 and 18. Transforming proteins E6 and E7 from these HR sub-types, interact with p53 protein and Rb protein respectively, leading to loss of function of these TSG products. These interactions further lead to inactivation of the growth suppressive effects of the p53 and Rb proteins, resulting in abnormal proliferative states. p53 protein expression has been found in both HPV-positive and -negative tumors, indicating that HPV and p53 protein expression are not mutually exclusive and can occur together in the same tumor. It has been observed that HPV plays a more significant role in esophageal carcinogenesis in geographic areas with a high prevalence of the disease. A variation in the association between HPV and esophageal SCC worldwide may be due to environmental and geographic factors, or to genetic susceptibility to esophageal HPV infections. Variations in the sensitivity of techniques used in the detection of the virus and in the methodology for processing the tumor tissues, may also be responsible for global differences. Esophageal carcinogenesis is a complex multistep process with a multifactorial etiology. Infection with oncogenic HPV types may be an integral part in a multistep process that leads to esophageal SCC [343]. In countries with a high esophageal SCC incidence, low socioeconomic status and an inadequate diet of poorly preserved food are combined with basic nutritional deficiencies and inadequate medical treatment. These conditions are favorable to the above-mentioned risk factors implicated in esophageal SCC development, which may be present and/or habitually used in certain populations. New perspectives in epidemiological studies of esophageal SCC development and its risk factors allow genome-wide research involving specific environments and habits. Such research should consist of adequately large and representative samples, should use newly designed informative genetic markers, and apply genomic variation analysis of the functional transcripts involved in malignant cell cycle regulation and neoplastic transformation in the multi-step process of esophageal SCC carcinogenesis [342,344].

Twenty-one studies investigating a possible correlation between HPV infection and colon cancer have been published. HPV was detected in the majority of reported series with a significant difference in HPV infection between tumors and disease-free controls or tumor-adjacent tissue: the HPV mean detection rate within carcinomas was 41.7%, comparing to a mean detection rate of 32.8% in adjacent colic mucosae, and 5.8% in disease-free controls (Chi-square test, $p = 0.001$). The correlation between HPV infection and c-myc amplification, k-ras mutation, and p53 polymorphism or mutations has been investigated, however, the possible role of HPV in colorectal carcinogenesis was not defined. HPV has been detected in the majority of reported series, but published literature lacks in definitive data regarding standard methods of investigation and stratification of groups and population. These data encourage further studies with the aim to investigate the presence of the virus in larger series, its possible role in oncogenesis, the integration in host genome, the expression of viral oncoproteins, the mutations in HPV positive cancers, and routes of colon infection (hematologic/lymphatic spreading or perineal diffusion) [345].

Human Papillomavirus in Proctologic Diseases

The infection caused by HPV in the anogenital area is considered the most common sexually transmitted infection in the world. Although anal cancer is relatively uncommon in the general population, there has been a significant increase in incidence in recent years. The existence of previous genital neoplasia associated with HPV promotes the development of

anal lesions, especially in younger patients, and a poor immune status contributes to the appearance of this pathologic finding [346]. Rectovaginal examination with gloves contaminated with vaginal secretions might increase the risk of HPV inoculation of the rectum in women with genital HPV infections. Because of the high prevalence of asymptomatic genital HPV infections, and the association between HPV and colorectal malignancy, examination gloves should be changed between vaginal and rectal examinations [347].

AIN is a consequence of chronic HPV infection in the anal canal and appears to be driven by high viral loads of HPV. In MSM with multiple sexual partners prevalent HPV infection does not decline with age, in contrast to heterosexual patients. AIN is equally prevalent in different age groups of MSM, but in other respects what is known of its natural history resembles that of CIN. LG lesions frequently resolve, but HG lesions are much more stable. HIV-positives who practise receptive anal intercourse are at highest risk of AIN. Screening is easy to perform using cytology, the limitations of anal cytology being similar to those of cervical cytology. Patients with any grade of cytological abnormality require further investigation, ideally with high-resolution anoscopy, every 6 months. Successful treatments for individual small to medium-sized HG lesions include trichloroacetic acid (TCA), infra-red coagulation, and laser. In HIV-positive patients, the development of new lesions elsewhere is very likely. Topical agents for multifocal disease include imiquimod and cidofovir. There is a need for large prospective cohort studies in MSM and HIV-positive patients to further our understanding of this disease and to evaluate treatment strategies [348]. Anal cancer is a rare disease in the general population, but the incidence of anal cancer is higher in certain at-risk groups, such as MSM and immunosuppressed individuals, including those with HIV infection. Among HIV-positive MSM, the incidence of anal cancer may be as high as 10 times greater than current rates of cervical cancer in the general population of women. Anal cancer is associated with HPV infection and may be preceded by HG AIN. HG AIN and anal HPV infection are both highly prevalent in groups at risk for anal cancer. Current issues include determining the effect of antiretroviral therapy on the natural history of HG AIN and the incidence of anal cancer, optimizing diagnostic and therapeutic approaches to HG AIN, and determining the potential for prophylactic HPV vaccines to prevent anal HPV infection and anal cancer in at-risk groups [349].

Human Papillomavirus in Urologic Diseases

Sexually transmitted HPV are linked to both benign and malignant lesions of the genitourinary tract. Evidence links oncogenic HPV types with carcinomas of the penis and urethra. An association with other common sites of urologic malignancies (prostate, bladder) is controversial. Whereas the screening of sexually active females for HPV has received substantial attention, the presence of a potential male carrier state has received little scrutiny. Systemic immunotherapies based on expression of HPV-related proteins by infected or transformed human epithelia, however, may be possible in the near future [350].

Bladder cancer remains an important cause of oncological morbidity and mortality in women. Known etiological agents include smoking and exposure to certain industrial chemical compounds, though the origin of the majority of cases remains unknown. HPV infection is also common in women and has been closely linked to the development of

carcinoma of the cervix. It has been suggested that infection with HPV may also be an important factor in the subsequent development of bladder cancer. A number of studies using various techniques of molecular biology have looked at the relationship between HPV infection and bladder cancer. Although the results are somewhat conflicting, the overall picture would suggest little involvement of HPV in the evolution of bladder cancer, except possibly in a small group of patients who are immunocompromised [351]. HPV DNA has been originally detected in urothelial carcinomas of the bladder in immunocompromised patients. Studies from the general population showed a variable incidence of HR HPV DNA which ranged from 2.5% to 81%, with HPV 16 DNA occurring more frequently. HPV DNA was detected in both papillary and invasive cancers, although the overall incidence was low. Most HPV positive cases were of HG and stage with significant reduced survival or increased recurrence rate after transurethral resection. These results indicate an additional prognostic value of viral infection in bladder cancer. In addition, molecular studies suggest that the HPV related oncoproteins E6 and E7 play a role in bladder carcinogenesis via inactivation and/or degradation of p53 and pRb suppressor gene-associated proteins [352].

Psychosocial Issues

Biobehavioral and psychosocial research is uniquely capable of addressing many of the issues raised by HPV and its link with cervical cancer [353]. Psychosexual vulnerability increases with number of recurrences of HPV infections. Depression, anxiety, and anger are the emotions most frequently reported. However, to date, there is no conclusive evidence of a specific correlation between HPV infection and a specific female sexual disorder. The relationship between HPV and vulvar vestibulitis/vulvodynia-related dyspareunia seems not to be direct.

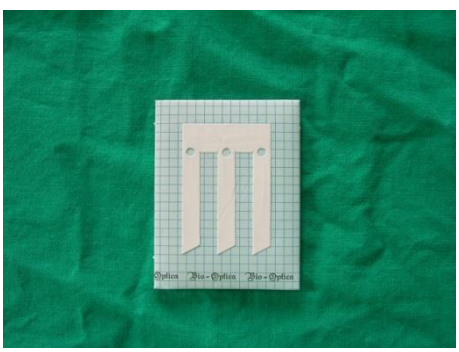
The evidence of psychosexual consequences of HPV-related GW and intraepithelial lesions is limited. Specific research on the sexual impact of GW and intraepithelial HPV-related lesion in women is urgently needed [354]. Psychosocial associations have been observed with level of cervical dysplasia or “pre-cancer” and invasive cervical cancer (related to HPV infection). Psychoneuroimmunological relationships have been observed in HIV type 1 infection, which is being described in an increasing number of women. Relationships of psychosocial factors and level of cervical dysplasia were similarly observed with reference to immunological and health status in asymptomatic and early symptomatic HIV 1 infected homosexual men, suggesting that a potentiating effect may occur in HIV 1 and HPV co-infected women. Consistency of relationships across studies appeared to be enhanced by the use of a biopsychosocial model integrating the effects of life stressors, social support, and coping style as well as psychiatric disorders. Research is indicated on the relationships between psychosocial factors, immunological status, and clinical health status in this group of women. Because of the high prevalence of psychosocial risk factors for chronic psychological distress in these women and the known immunological and health status decrements occurring with progression of these two infections, a clinical screening program based on the biopsychosocial model is recommended as a means of secondary prevention. If effective in generating treatment referrals, such a program would likely improve quality of life and could aid in the determination of relationships with immunological and health status as well [355].

DIAGNOSIS

GW are easily diagnosed by clinical criteria, however, the more prevalent subclinical infections generally require laboratory diagnosis. Satisfactory methods for culture of HPV do not exist, and cytologic and histopathologic methods are the most widely available diagnostic techniques (Figure 4) [356].

The importance of samples from these sites is highlighted by the fact that their most common disease is dysplasia, which is sexually transmitted and a recognized risk factor for carcinoma. Molecular testing for HPV has revealed a great deal about the pathophysiology of dysplasia and its evolution to carcinoma. Importantly, it now allows diagnostic pathologists the opportunity to be more accurate in their assessment of common conditions such as Pap smears of squamous atypia and biopsies equivocal for dysplasia [357]. A variety of ancillary tests useful in the diagnosis of HPV infection are currently at the clinician's disposal. Use of 5% acetic acid (VIA) or Lugol's iodine (VILI) for detection of inapparent HPV infection is a simple procedure that can be performed in any office setting. Colposcopy is useful alone or in combination with the use of acetic acid but may not be as readily available. Use of laboratory-based tests has gained popularity as an adjunctive measure, particularly in combination with Pap smears, for the detection of cervical carcinoma or intraepithelial neoplasia. Although the most readily available ancillary laboratory tests are the immunologically based tests, they suffer from lack of sensitivity.

The more sensitive and specific DNA hybridization tests, such as the dot blot (DB) and the SB, have been adapted for general clinical use. These hybridization tests allow routine screening of patients for infection with potentially oncogenic HPV types.



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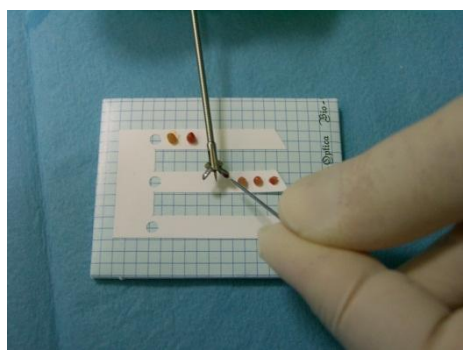

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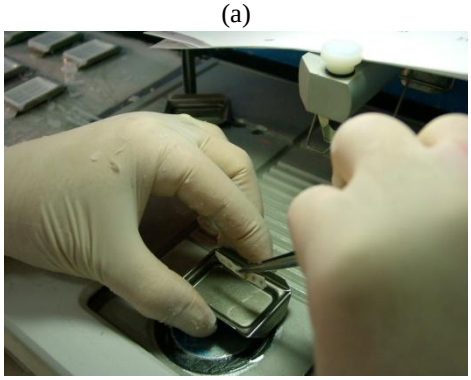

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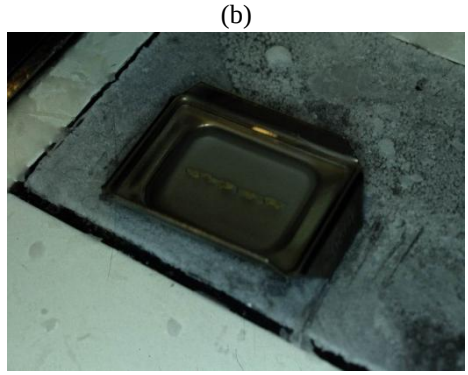
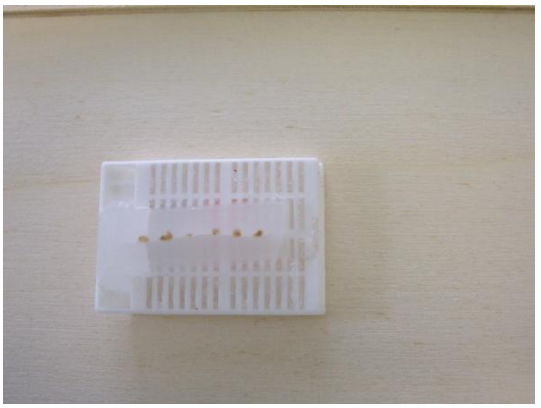


(d)

(e)



(f)

(g)

Figure 4 (a-g). Histological evaluation of bioptic samples.

The PCR is perhaps the most exciting new development in hybridization technology. This technique is the most sensitive technique available, allowing amplification of amounts of HPV DNA as small as a single copy. With further development and automation of this technique, the clinician has an almost unlimited ability to diagnose occult HPV infection [358]. HPV detection assays are almost exclusively based on the detection of viral nucleic acids, mostly viral DNA. The HPV detection methods that are nowadays in use can broadly be subdivided into target amplification methods and signal amplification methods [359].

The viruses do not replicate in culture without extraordinary measures, and virtually all studies to date have used molecular methods to elucidate their biology and natural history. Tests of choice for detecting HPV from clinical specimens are based on nucleic acid probe technology. Until recently, most epidemiologic and molecular studies employed SB, DB, and ISH. With the exception of ISH, which continues to have many uses and a strong following in the pathology community, SB and DB have been essentially replaced by PCR and the HC System. These in vitro probe tests have proven to be accurate and robust workhorses for epidemiologic and clinical use. [360]. Testing for HPV relies exclusively on techniques of molecular biology using nucleic acid probes. Tests for HPV using nucleic acid probes have been commercially available since the late 1980s, but early tests were cumbersome, involving

the use of nucleic acid probes labeled with radioactive phosphorus (32P). These early HPV tests did not achieve widespread use because they did not detect all oncogenic HPV genotypes. The current commercial HPV detection kit, Digene's HC 2 kit, detects virtually all HR oncogenic HPV types, as well as most LR nononcogenic HPV genotypes. The HC 2 test format is a proprietary nucleic acid hybridization signal amplification system owned by Digene Corporation. Virtually all test formats for DNA sequence analysis are amenable to applications intended to detect and perhaps quantify the various HPV genotypes. These methods can involve direct hybridization with complementary DNA probes, such as SB or ISH, signal amplification, such as the HC 2 method or target nucleic acid amplification, most notably the PCR. PCR has been used for HPV detection, genotyping, and viral load determination. General or consensus primer-mediated PCR assays have enabled screening for a broad spectrum of HPV types in clinical specimens using a single PCR reaction. Following amplification using consensus primers, individual HPV genotypes are identified using a variety of methods. Using consensus primers in a test format known as real-time quantitative PCR (RQ-PCR), it is possible to generate viral load (concentration) data from reaction curves generated by monitoring PCR reaction kinetics in real time [361,362].

The issue of determining which HPV is present in a clinical specimen (typing specimens for HPV) has received attention because HPV cause CA and are associated with the continuum of disease which ranges from dysplasia to invasive genital cancer. Morphological inspection of precancerous lesions is not sufficient to determine which lesions will progress and which will not. A number of research tools based primarily on DNA hybridization have been developed. These permit identification and typing of HPV in genital tract scrapings or biopsies. Some HPV types (e.g. HPV 16 and HPV 18) have been identified in HG dysplasias and carcinomas more commonly than other types (e.g. HPV 6) and have been designated HR types for cervical cancer. Thus, the question arises whether HPV typing would improve patient management by providing increased sensitivity for detection of patients at risk or by providing a prognostic indicator. Analysis of the typing data indicates that, while HPV types can be designated HR and LR, these designations are not absolute and thus the LR group should not be ignored. In addition, interpretation of the data is complicated by finding HR types in individuals with no indication of disease. Insufficient data exist to indicate whether knowledge of the presence of a given HPV type is a better prognostic indicator than cytological or histological results. Thus, more research is needed before it can be determined whether typing information will augment the method currently in use for deciding treatment regimen and whether it warrants widespread use [363]. Five main groups of commercial assays for the multiplex detection of alpha HPV are currently available. DNA-based screening assays, which test for the presence of 13-14 HPV without determination of HPV type, have been the standard for HPV detection in the last decade. Assays that combine testing for 14 HPV and HPV 16 and HPV 18 genotyping are the standard for HPV detection. Recently, one of the mRNA-based assays showed equal clinical sensitivity but higher clinical specificity for CIN 2+/CIN 3+ in comparison with the validated DNA-based assay. ISH are too laborious and have insufficient clinical sensitivity to be used in routine screening. Automation, price reduction, and improvement of clinical specificity are the main goals for the development of HPV assays [364]. Currently, molecular detection methods are the gold standard for identification of HPV. The three categories of molecular assays that are available are based on the detection of HPV DNA and include:

1) non-amplified hybridization assays, such as Southern transfer hybridization (STH), DB hybridization, and ISH;
2) signal amplified hybridization assays, such as HC assays (HC 2); and
3) target amplification assays, such as PCR and in situ PCR.

STH requires large amounts of DNA, is laborious and not reproducible, while ISH has only moderate sensitivity for HPV. The sensitivity of the HC 2 assay is similar to that of PCR-based assays, with high sensitivity being achieved by signal rather than target amplification. PCR-based detection is both highly sensitive and specific. Since PCR can be performed on very small amounts of DNA, it is ideal for use on specimens with low DNA content. In the future, with the advance of technology, viral DNA extraction and amplification systems will become more rapid, more sensitive, and more automated [365]. Accurate genotyping of a HPV isolated from clinical specimens depends on molecular identification of the unique and exclusive nucleotide base sequence in the hypervariable region of a highly conserved segment of the HPV L1 gene. Among other options, a heminested (nested) PCR technology using two consecutive PCR replications of the target DNA in tandem with three consensus general primers may be used to detect a minute quantity of HPV DNA in crude proteinase K digestate of cervicovaginal cells, and to prepare the template for genotyping by automated direct DNA sequencing. A short target sequence of 40-60 bases excised from the computer-generated electropherogram is sufficient for BLAST determination of all clinically relevant HPV genotypes, based on the database stored in the GenBank [366].

To date, investigators have relied primarily upon the detection of viral DNA in clinical specimens as evidence of HPV infections. Such assays cannot determine whether past infections with HPV have occurred which have subsequently resolved. Latent infections and current infections might also evade detection because of sampling errors or because of suboptimal sensitivity of DNA detection methods. Efforts to develop HPV serological assays have been hampered by the lack of appropriate viral antigens, since HPV cannot be propagated in tissue culture and virions are not abundant in infected human tissues. Using HPV-encoded proteins expressed in Escherichia coli, assays to measure human antibodies that react with HPV proteins have been developed. Human antibodies to L gene products (L1 and L2) of genital-type HPV were more prevalent than antibodies to E gene products. However, approximately 15% of sera contained antibodies that reacted with the HPV 16 E7-encoded protein, a gene product that has been implicated in HPV 16-mediated cellular transformation. The human antibodies appeared to be type or “serotype” specific, because the antibodies did not cross-react with homologous proteins encoded by other HPV types. Antibodies to proteins encoded by HPV types 6 or 11 were detected in approximately 70% of adults, while antibodies to proteins encoded by HPV type 16 were found in approximately 50% of adults. Antibody prevalence was not associated with measures of sexual activity. There was also no significant difference between the prevalence of antibodies to HPV types 6 or 16 proteins in children when compared to the antibody prevalence in sexually active adults. These results suggest that infections by genital HPV types are widespread and frequently cause clinically inapparent infections. The viruses have a broad tropism for mucosal epithelium and are likely to be acquired by other modes, as well as by sexual transmission [367]. A variety of serological assays to detect antibodies to genital-type HPVs have been developed. Bacterially expressed fusion proteins, synthetic peptides and HPV 11 virus propagated in a xenograft system have been the most commonly used antigen targets in either

Western blot (WB) assays or enzyme-linked immunosorbent assay (ELISA). HPV antibodies have been readily detected and most studies suggest that they are type-specific. Primarily, antibodies appear to be directed against the capsid antigens. The presence or titer of antibodies to the HPV 16 E7 protein is strongly associated with cervical cancer in approximately 25% of cases. The significance of antibodies to other HPV antigens, or of antibodies which recognize conformational epitopes, is less clear [368].

Laboratories contemplating either the addition of new molecular tests or modifying methods approved by the FDA for HPV testing should be aware of a variety of procedural, performance, and regulatory issues surrounding such activity. Diagnostic medical laboratory testing in the USA is regulated by the Centers for Medicare and Medicaid Services (CMS), an agency formerly known as the Health Care Finance Administration (HCFA). The regulatory vehicle of the CMS is manifested in the Clinical Laboratory Improvement Amendments (CLIA). The CLIA program has put into place specific regulations for laboratory quality control, which includes specific recommendations for method validation. Regulations that must be followed regarding personnel, quality control, quality assurance, method validation, and proficiency testing depend on the complexity category of the individual test. All molecular diagnostic tests, including those for HPV, are considered high complexity. The CMS retains the authority to allow private, national accreditation organizations to "deem" that a laboratory is compliant with CLIA '88 requirements. Accreditation organizations, such as the Joint Commission for Accreditation of Hospitals (JCAHO), the Commission on Office Laboratory Accreditation (COLA), and the CAP, as well as several state medical laboratory-accrediting agencies, possess the authority to deem laboratories as "CLIA-approved". The CAP, through its Laboratory Accreditation Program, has promoted standards for laboratory performance and method validation. In general, guidelines set forth in the CAP Laboratory Accreditation Program checklists specify that all clinical laboratory testing must essentially meet those requirements defined for high-complexity testing under CLIA '88, including test validation standards, reportable/reference ranges, performance criteria, and proficiency testing [369]. International reference materials such as International Standard (IS) reagents facilitate quality assurance of essential biopharmaceutical products and related in vitro diagnostic tests. Standardization of antibody and DNA measurements and harmonization of laboratory procedures are key to the success of cancer prevention strategies through screening methods as well as for development and implementation of vaccination against the HPV. The WHO supported the preparation and initial analysis of a panel of candidate serological and DNA reference reagents aimed at facilitating inter-laboratory comparisons and detection of HPV worldwide. Two international collaborative studies assessed the performance of various HPV antibody and HPV DNA detection assays and examined the feasibility of generating HPV antibody and DNA standard reagents. These studies showed that improvement in performance and comparability of assays is urgently needed and that the use of the same IS reference reagent could significantly improve performance and comparability. It is hoped that the establishment of International Units (IU) and IS for HPV antibody and DNA analysis will be pursued with high priority [370-374].

Colposcopy plays an important role in the investigation and direct management of HPV infection of the female lower genital tract. The colposcopic appearances of subclinical HPV disorders resemble intraepithelial neoplasia, and the matter is further confused in that subclinical lesions are often accompanied by intraepithelial neoplasia into which they grade with indefinite borderlines. High accuracy in prediction of histologic diagnosis in the

spectrum that ranges from subclinical HPV infection to major grades of intraepithelial neoplasia may be achieved by a system of grading of colposcopic appearances [375]. The exact histologic diagnosis of CIN lesions is essential for a differentiated therapy. Data indicate that mild and moderate CIN lesions are detected more frequently by Pap smear and colposcopically directed punch biopsy than by cervical smears alone. Histological diagnosis and HPV-typing by ISH offers the possibility to establish a differentiated therapy, e.g. by way of close follow-up, local destruction, or cone biopsy. Main attention should be directed at early detection of dysplasia, at timely institution of follow-up programs and a possibly conservative therapeutic management [376]. Target biopsy and transformation zone ablation on excision is the mainstay of management for cervical lesions that can be adequately visualized. For the physician who does not progress beyond whimsical estimates of lesion prominence (which cannot differentiate minor- from major-grade lesions), the colposcope will never be more than a simple aid to the collection of directed biopsy specimens. Unfortunately, in situations in which the clinician's only option is passive response to the histopathology report, optimal management of the patient's disease will not occur. Indeed, the best prospect for diagnosing or prognosticating confusing disease patterns can sometimes be lost by blind precipitous intervention. Because the most prominent areas of colposcopic change do not necessarily coincide with the areas of greatest histologic abnormality, less experienced colposcopists may not be able to select the most abnormal sites for target biopsy. Peripheral areas of prominent aceto-whitening tend to be overinterpreted, and the subtle acetowhitening of HG CIN near the canal tends to be easily overlooked. This problem is best solved by using colposcopic criteria that are based on critical analysis, rather than "pattern recognition". It is easy to derive the four proven colposcopic criteria, and they can be quickly compiled into an index that helps the clinician recognize lesion severity while he or she is performing the colposcopy. Of course, using the colposcopic index to infer approximate histologic findings does not eliminate the importance of biopsy. Skilled physicians will conscientiously follow the triage rules, including the need to collect carefully sited target biopsy specimens [377,378].

SCREENING

The ASCCP National Consensus Conference for the Management of Women With Cervical Cytological Abnormalities and Cervical Cancer Precursors was held on the National Institutes of Health (NIH) campus in Bethesda, Md, September 6^{th}-8^{th}, 2001. The conference was attended by representatives from national organizations interested in cervical cancer screening issues. For the first time, guidelines for the management of women with abnormal cervical cytology, developed from evidence-based literature, were presented to delegates from the majority of organizations with interest in cervical cancer screening, voted on, and revised when necessary to achieve a majority two-thirds approval. This development of consensus-approved guidelines is likely to be considered one of the most important milestones to date in the management of women with abnormal cervical cytology. The timing of this Consensus Conference resulted from the convergence of many different factors, including new cytologic terminology developed at the Bethesda 2001 workshop and publication of the enrollment data from the National Cancer Institute (NCI)'s ASC-US)/L-SIL Triage Study, otherwise known

as ALTS. Additionally, new preliminary longitudinal ALTS data provided much of the information on the natural history of abnormal Pap tests and CIN, as well as data on the performance of both new liquid-based cytology (Figures 5,6) and HPV DNA testing in the management of women following colposcopy. The result was a large database of new information that provided the foundation for the ASCCP Consensus Conference. The recommendations of the ASCCP Guidelines were based in large part on the results of the ALTS trial. Therefore, the focus is on the management of women with equivocal (ASC-US) and LG (L-SIL) cytologic abnormalities. Management of women with these cytologic abnormalities has been particularly problematic, because individually these women are at least risk for CIN 3 and cancer, yet their sheer numerical dominance ensures that they account for the majority of HG CIN detected in the USA in the follow-up of abnormal cervical cytology. Data from ALTS confirmed that women with ASC-US could be safely managed by any of the conventional approaches (repeat Pap test, immediate colposcopy, or HPV testing), but that the preferred management approach for women having an ASC-US report from liquid-based cytology was to assess the patient's risk by testing for HPV. Additionally, longitudinal ALTS data determined that repeat liquid-based cytology at 6 and 12 months and an HPV test at 12 months were nearly equivalent options in the follow-up of women referred for HPV-positive ASC or L-SIL, yet not found to have CIN 2+ at initial colposcopy.

Therefore, all follow-up recommendations for women with CIN 1 or lower postcolposcopy findings include these 2 options [379]. Risk management efforts in the cytology laboratory must address the gap between what can be achieved with medical history's most effective cancer screening test, the Pap test, and even higher entrenched public expectations. Data from the ALTS now provide level I clinical evidence from a large, randomized, controlled, multicenter clinical trial that reflex HPV DNA testing of ASC-US cases is generally the preferred method for initial assessment of the most prevalent category of abnormal Pap interpretation. The proposed combination of HPV DNA testing with cytologic Pap testing, the DNA Pap test, further shows the potential to nearly eliminate false-negative screening results, based on sensitivity and negative predictive values (NPV) reported in available studies. HPV DNA testing also appears to represent a significant enhancement for detection of endocervical adenocarcinomas, which are difficult to detect and prevent. HPV DNA testing, when used in conjunction with cervical cytology, can significantly reduce risk to both the patient and the laboratory [380].

The results of the ALTS, the only randomized longitudinal trial in cytopathology, have provided a wealth of data to benchmark practice and stimulate discussion regarding the usefulness of HPV testing. These landmarks aided the ASCCP's broad-based consensus conference in integrating the Bethesda deliberations and ALTS data into clinical management.

The outcomes of these open- and wide-ranging discussions have a direct impact on all who practice in this area [381]. The conclusions of the workshop were that HPV DNA testing may be added to cervical cytology for screening in women aged 30 years or more. Women whose results are negative by both HPV DNA testing and cytology should not be rescreened before 3 years. Women whose results are negative by cytology, but are HR HPV DNA positive, are at a relatively LR of having HG cervical neoplasia, and colposcopy should not be performed routinely in this setting. Instead, HPV DNA testing along with cervical cytology should be repeated in these women at 6 to 12 months. If test results of either are abnormal, colposcopy should then be performed.

This guidance should assist clinicians in utilizing HPV DNA testing in an effective manner, while minimizing unnecessary evaluations and treatments [382].

Figure 5. ThinPrep kit for liquid-based Pap test (Hologic Inc., Marlborough, MA, USA).

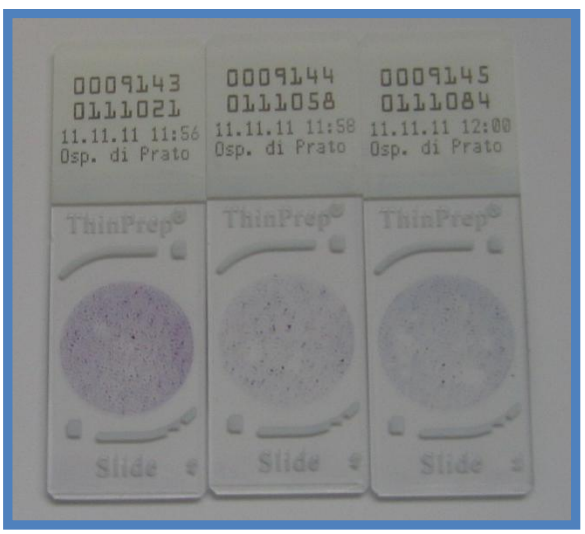


Figure 6. ThinPrep slides.

Screening for cervical cancer precursors by cytology has been very successful in countries where adequate resources exist to ensure high quality and good coverage of the population at risk. Mortality reductions in excess of 50% have been achieved in many developed countries, however, the procedure is generally inefficient and unworkable in many parts of the world where the appropriate infrastructure is not achievable. Four possible clinical applications of HPV DNA testing are:

1) triage of women with equivocal or LG cytological abnormalities;
2) follow-up of women with abnormal screening results who are negative at colposcopy/biopsy;
3) prediction of the therapeutic outcome after treatment of CIN; and, most importantly,

4) primary screening HPV DNA test, solely or in combination with Pap smear to detect cervical cancer precursors.

There are clear benefits for the use of HPV DNA testing in the triage of equivocal smears, LG smears in older women, and in the post-treatment surveillance of women after treatment for CIN. However, there are still issues regarding how best to use HPV DNA testing in primary screening. Primary screening with HC 2 generally detects more than 90% of all CIN 2, CIN 3, or cancer cases, and is 25% (95% CI: 15-36%) relatively more sensitive than cytology at a cut-off of ASC-US (or L-SIL if ASC-US unavailable), but is 6% (95% CI: 4-7%) relatively less specific. Several approaches are currently under evaluation to deal with the lower specificity of HPV DNA testing as associated with transient infection. These include HPV typing for HPV 16 and 18/45, markers of proliferative lesions such as p16 and mRNA coding for the viral E6 and/or E7 proteins, with a potential clinical use recommending more aggressive management in those who are positive. In countries where cytology is of good quality, the most attractive option for primary screening is to use HPV DNA testing as the sole screening modality with cytology reserved for triage of HPV-positive women. Established cytology-based programmes should also be gradually moving towards a greater use of HPV DNA testing to improve their efficacy and safely lengthen the screening interval. The greater sensitivity of HPV DNA testing compared to cytology argues strongly for using HPV DNA testing as the primary screening test in newly implemented programmes, except where resources are extremely limited and only programmes based on visual inspection are affordable. In such countries, use of a simple HPV DNA test followed by immediate "screen and treat" algorithms based on visual inspection in those who are HPV-positive are needed to minimize the number of visits and make best use of limited resources. The fact that HPV is a sexually transmitted infection may lead to anxiety and concerns about sexual relationships. These psychosocial aspects call the need for more information and educational programmes about HPV [383].

USA-based guidelines recommend that screening should be initiated approximately 3 years after initiation of sexual intercourse, but no later than age 21 years and be continued at least until age 65 or 70. Annual screening is recommended by the ACS and the American College of Obstetricians and Gynecologists (ACOG), although in women aged ≥ 30 years with ≥ 3 negative Pap tests, screening may be conducted every 2 to 3 years. HPV testing has been approved by the USA FDA and most USA guidelines say that it is reasonable to consider HPV testing, in combination with triennial cytology screening. Pharmacoeconomic analyses indicate that combined cytology and HPV testing every three years in women aged ≥ 30 years is comparable in sensitivity to annual liquid-based cytology for the detection of cervical cancer precursors and is more cost-effective. Both surgical and nonsurgical therapies are commonly employed in patients with HPV lesions although papilloma recurrence is not uncommon. Treatment should be individualized based on the extent of disease and the needs of the patient. Current treatment of cervical cancer reflects the stage of the disease and should take into account patient- and tumor-related factors to ensure optimal patient outcomes [384]. HPV testing is more sensitive for the detection of cervical precancer and cancer than cervical cytology. The increased sensitivity of HPV testing and cytology combined ("cotesting") compared to cytology alone permitted professional societies to recommend 3-year screening intervals among the cotest-negative results. However, there is an increasing recognition that

both clinical sensitivity and specificity of cervical cancer screening are important to patient safety and must be considered in the context of using current and future HPV DNA tests. Exquisite analytic sensitivity for HPV does not increase clinical sensitivity of an HPV test but does result in excessive test positivity and decreased clinical specificity. A recent USA FDA-approved HPV test, Cervista (Hologic, Bedford, MA, USA), demonstrated excessive test positivity-2 to 4 times more positive than the other FDA-approved HPV test-from its premarketing approval trial. The poor specificity of Cervista raises questions about the safety and applicability of using this test in routine cervical cancer screening. These data provide a didactic example of the potential dangers of mistaking excellent analytic sensitivity and even clinical sensitivity for good clinical performance [385]. HPV tests in combination with Pap tests are 96% to 100% sensitive for detection of CIN 2/3 and cancer. However, because HPV infection is common in young women and most commonly transient, HPV testing is not recommended as part of primary cervical screening for women younger than 30 years of age. HPV testing is recommended for women of any age for the clarification of ASC-US and as an option for follow-up of women with HPV-positive ASC-US, ASC-H, or L-SIL not found to have CIN 2/3. HPV testing is also recommended as an alternative to colposcopy and/or cytology for follow-up of treated cases. Proper use of HPV testing improves the management of women with cytologic abnormalities. In addition, a negative HPV test in combination with a normal Pap test result in women age 30 and older allows the safe extension of the interval between cervical screenings. Thus, when used properly, HPV testing may reduce morbidity and mortality and do so in a cost-effective manner [386].

Primary prevention is now possible via immunization with highly efficacious HPV vaccines, and secondary prevention has gained impetus with the advent of sensitive HPV-DNA testing to improve traditional Pap cytology screening programs. Although universal vaccination of teenagers and young women is a desirable policy, cost remains a key obstacle. To achieve cost-effective reductions in cervical cancer burden, prevention initiatives must consider screening and immunization as integrated and organized approaches that take advantage of HPV testing as a primary screening test followed by triage with Pap cytology. This strategy has the added benefit of providing immunosurveillance of vaccinated populations [387]. Screening for precancerous lesions cannot be discontinued because vaccination will not protect against HPV types not included in the first generation of vaccines. Moreover, protection for the target types, 16 and 18, which are responsible for most cases of cervical precancerous lesions and cancer, and 6 and 11, which are responsible for a substantial proportion of LG lesions, cannot be expected to be absolute, and the likely implementation of HPV vaccination in young women will not impact older groups initially. Cervical cancer control programs will need to be re-evaluated because the addition of HPV vaccination will make the existing approach of high-frequency screening by cytology too costly and inefficient for most public health budgets. Simply making cytology screening less frequent may not be a viable strategy in light of potential problems that may plague cytology performance in conditions of low lesion prevalence. HPV testing has the performance characteristics that would make it an ideal primary screening test in such conditions. Cytology should be reserved for triage of HPV-positive cases because it is more likely to perform with sufficient accuracy in high-prevalence conditions. Another advantage of using HPV testing as a primary screening tool is the opportunity to create infection registries that can link test results from the same women over time, thus allowing an efficient and low-cost strategy to monitor long-term protection among vaccinated women [388].

The clinical application of molecular tests for HPV detection for screening purposes has been of considerable interest. DNA amplification methods allow the use of self-collected (SC) samples (including urine) from material collected away from the original disease site. For screening of cervical pathology, detection of HPV DNA in urine would be useful only if it represents cervical HPV infection and/or HPV-related cervical pathology [389]. The detection of HPV DNA in urine, a specimen easily obtained by a non-invasive self-sampling method, has been the subject of a considerable number of studies. Urine sampling, storage conditions, sample preparation, DNA extraction, and DNA amplification may all have an important impact on HPV DNA detection and the form of viral DNA that is detected. Possible trends in HPV DNA prevalence in urine could be inferred from the presence of risk factors or the diagnosis of cervical lesions. HPV DNA detection in urine is feasible and may become a useful tool but necessitates further improvement and standardization [390].

Cervical screening by Pap smear involves a high rate of false negatives, necessitating frequent testing. Because women do not like the sampling procedure, many avoid being screened. Testing for the causative HR HPV types, by PCR or other technologies, on SC (tampon) samples permits women to be monitored non-invasively. The high NPV of HPV testing means a greater interval between tests, and thus reduces costs. HPV testing lends itself to primary screening. A kit for self-collection and return to a testing laboratory, followed by practitioner notification and follow-up if required, should result in wider participation. The higher accuracy of HPV testing should lead to improved cervical cancer prevention [391]. The concordance between samples collected by patients and those obtained by clinicians was reasonably high in the majority of cases. Women in many countries across wide age ranges were successful in collecting samples for HPV DNA testing. In some studies, the quality of the cytology from patient samples was as good as clinician samples, with more than 95% of samples yielding HPV DNA results. The studies that examined acceptability found that women were generally very positive about collecting their own samples, although some concerns were noted. No study evaluated the effect of HPV DNA self-sampling on screening participation rates, early detection, survival, or quality of life. Self-sampling for HPV DNA testing is a viable screening option, but there is insufficient evidence to conclude that self-sampling for HPV DNA testing is an alternative to the Pap test. Although HPV DNA testing using SC samples holds promise for use in under-resourced areas or for women who are reluctant to participate in Pap testing programs, the evidence supporting it is limited. Further definitive research is needed to provide a solid evidence base to inform the use of self-sampling for HPV DNA testing for the purpose of increasing screening rates, especially in women who are never or seldom screened [392].

The current prevention of cervical cancer and elimination of its precursors is predicated on the identification of cervical cytologic abnormalities and their histologic confirmation. This strategy, although effective, depends on both sensitivity and specificity of cytology and precise histologic distinction between precursor lesions and their mimics during biopsy interpretation. The effective application of diagnostic criteria is operator dependent and varies as a function of experience and training. However, because HPV are causative agents and alter the cell cycle in cervical neoplasms, host genes interacting directly or indirectly with HPV oncoproteins have been identified in vitro. Recent research has centered on identifying the host genes upregulated in association with HPV infection, determining their suitability as “surrogate markers” for HPV infection, and using these markers to identify HPV-associated epithelial lesions in tissue or cytologic specimens. Candidate biomarkers include proliferating

cell nuclear antigen (PCNA), Ki-67, cyclin E, p16INK4a, MN antigen, carcinoembryonic antigen (CEA), and telomerase in the recognition of preinvasive cervical neoplasia. Based on current studies, the strongest candidates for diagnosis and screening are p16 and cyclin E (squamous) and MN (glandular) biomarkers. As new genes are identified and tested, the concept of biomarkers as tools in primary screening and lesion recognition will continue to mature [393]. p16INK4a has emerged as a valuable surrogate marker for HR HPV infection and shows increased immunoexpression with worsening grades of CIN. Numerous studies have emerged in recent years supporting its role in the detection of HG dysplasia and have lead to the use of p16INK4a IHC in many laboratories. However, only a few studies have examined the possible predictive or prognostic value of p16INK4a in CIN or cervical cancer [394]. Biomarkers indicating the initiation of neoplastic transformation processes in HPV-infected epithelial cells are moving into the focus of cancer prevention research, particularly for anogenital cancer, including cancer of the uterine cervix. Based on the in-depth understanding of the molecular events leading to neoplastic transformation of HPV-infected human cells, the CDK inhibitor p16INK4a turned out to be substantially overexpressed in virtually all HPV-transformed cells. This finding opened novel avenues in diagnostic histopathology to substantially improve the diagnostic accuracy of cervical cancer and its precursor lesions. Furthermore, it provides a novel technical platform to substantially improve the accuracy of cytology-based cancer early-detection programs [395]. There is evidence to suggest that detection of viral gene expression both directly and indirectly may constitute a more specific approach for delineating clinically significant infection compared with HPV DNA-based assays. HPV oncogene expression and evidence of its deregulation can be monitored through direct detection of viral mRNA transcripts or through detection of the cellular protein p16. For both approaches, commercial assays have been introduced and numerous studies have been conducted. Currently, there is promising data indicating that HPV mRNA and p16 might play an important role in future cervical cancer screening scenarios. Still, large randomized studies are necessary to confirm the preliminary data [396].

Despite the morbidity associated with anogenital condylomas and the mortality associated with anal, penile, and cervical carcinoma as a direct consequence of HPV, the USA CDC and Prevention currently does not recommend routine screening for HPV in immunocompetent men. However, findings of emerging research focusing on the HR populations of MSM and men who test positive for HIV, in whom HPV infection is pervasive and persistent, suggest that these populations may benefit from screening. Therefore, HPV screening, including anal cytology, should be considered for these men in settings where appropriate follow-up, including high-resolution anoscopy, is available [397].

There are critical statistical and study design issues related to measurement of HPV infection status and its cervical lesion outcomes that need to be considered for the appropriate interpretation of the association of HPV infection and its determinants with cervical cancer in epidemiologic studies, and of screening effectiveness by HPV testing in clinical and intervention trials. These statistical issues may affect the validity of epidemiologic and screening studies and have led to inconsistent results in the literature. Meticulous attention to study design and laboratory detection issues helps to minimize the impact of such measurement errors and detection biases. Simple statistical analysis techniques are also available to correct or to control for these biases if they were to be identified and additional information is known concerning the expected test performance [398].

n conclusion, even in the era of highly effective HPV prophylactic vaccines, substantial reduction in worldwide cervical cancer mortality will only be realized if effective early detection and treatment of the millions of women already infected and the millions who may not receive vaccination in the next decade can be broadly implemented through sustainable cervical cancer screening programs. Effective programs must meet three targets:

1) at least 70% of the targeted population should be screened at least once in a lifetime;
2) screening assays and diagnostic tests must be reproducible and sufficiently sensitive and specific for the detection of HG precursor lesions (i.e. CIN 2/3); and
3) effective treatment must be provided.

HPV DNA screening from swabs collected by the women in their home or village is sufficiently sound for consideration as a primary screening strategy in the developing world, with sensitivity and specificity for detection of CIN 2/3 as good or better than Pap smear cytology and VIA. A key feature of a SC HPV testing strategy is the move of the primary screening activities from the clinic to the community. Efforts to increase the affordability and availability of HPV DNA tests, community education and awareness, development of strong partnerships between community advocacy groups, health care centers and regional or local laboratories, and resource appropriate strategies to identify and treat screen-positive women should now be prioritized to ensure successful public health translation of the technologic advancements in cervical cancer prevention [399].

Guidelines for cervical cancer screening continue to evolve, as knowledge of the pathogenesis of the disease as well as the role of HPV expands. Oncogenic HPV typing has proven effective in cancer screening and follow-up in certain situations, and its uses will undoubtedly increase. Patients and providers may be reassured with negative HPV testing, but long term management of positive HPV testing (especially in conjunction with negative cytology) is unclear. At present, however, numerous worldwide, prospective studies involving HPV testing and cervical cancer screening will, we hope, provide some of the answers regarding optimal management of women who remain persistently HPV positive [400-406].

THERAPY

HPV infections are very common, yet no uniformly effective therapy exists. Current treatments do not selectively inhibit viral processes but destroy the infected epithelial cells. Treatment of ano-GW is difficult and no completely satisfactory treatment modality is currently available, and no one form of therapy is effective. Treatment modalities used include cytotoxic agents (podophyllin, podophyllotoxin, 5-FU), and destructive procedures (scissor excision, cryotherapy with liquid nitrogen, electrocautery, carbon dioxide [CO_2] laser). Therapeutic options include chemotherapeutic agents, cryotherapy, and surgery, but all these treatments are anti-tumor, not anti-viral [407]. Nanotechnology tools employed in the development of vaccines and a noninvasive treatment may provide a significant advancement in the global combat against this disease [408]. Unfortunately, the technology for effective treatment of these conditions has not advanced as rapidly. There are a host of therapeutic modalities available for the treatment of HPV-associated conditions, ranging from simple

topical medication like podophyllin to sophisticated combination therapies involving CO_2 laser destruction in conjunction with IFN. Regrettably, there is no "magic bullet" that is totally effective for any of the HPV-associated diseases. This makes it more important to weigh the benefits and morbidity of treatment regimens before they are used. Until the success rate of available therapies improves, it is important to have definitive goals for therapy, because it is currently impossible to completely eradicate HPV from tissues. Examples of therapeutic goals might range from removal of visible lesions or treatment of symptomatic areas to treatment of premalignant or malignant lesions. At present, perhaps the most important service we can provide for patients is close follow-up of HPV-associated diseases in an attempt to prevent the development of invasive malignancy. Regardless of the choice of treatment, the primary objective of the clinician should be to help the patient but to do no harm [409].

The goal in treating noncervical HPV infection is the elimination of lesions, eradication of the virus is not yet possible. Current forms of treatment include cryotherapy, podophyllum resin, podophilox, TCA, laser ablation, LEEP, 5-FU, and alpha-IFN. Success in treating condyloma may be increased if the area is first soaked with 5% acetic acid to more clearly show the extent of the local infection. Recurrence is a problem no matter what form of therapy is used [410]. Unfortunately, effective therapy is currently not available. Therapeutic options are limited, expensive, and often ineffective. They comprise cytodestructive and cytotoxic substances, surgical methods, laser, and cryotherapy. Immunotherapy seems to be one of the more promising options. IFN combined with ablative methods such as CO_2 laser can prevent recurrence of disease. Cytokine (CK) enhancement is an old concept that has shown effectiveness with imidazoquinolinamine derivatives (imiquimod). Imiquimod was released recently for therapy of GW. Currently, HPV vaccines have been developed to protect against infection with HPV and to treat existing warts and other HPV-associated lesions. Some processes in the HPV infection cycle have been determined as possible targets for the development of specific antiviral agents. Cidofovir is a primordial antiviral substance active against HPV. Unfortunately, there is a potential risk for side effects [411].

Since IFN have antiviral effects in vivo and in vitro, it was hypothesized that they might be useful for treating HPV-induced conditions. IFN have now been demonstrated to be effective in several forms of HPV infection. In vitro, chronic treatment of BPV-transformed cells led to the loss of the Papillomavirus genomes and return of the cells to a normal morphology. In humans, IFN have been used for treating laryngeal papillomatosis, cutaneous and ano-GW, and EV. Partial and total remissions have been achieved with both intralesional and systemic administration. Ongoing studies aim to identify which conditions are most responsive, the optimal dosage and regimen, and the most effective class of IFN [412]. IFN objectively regress HPV-induced warty disease and affect the regrowth of the transformed epithelium. IFN effectively control the most serious and potentially life-threatening HPV-associated diseases, respiratory papillomatosis, and GW, but not without the anticipated side effects associated with this potent biological weapon. It is entirely possible and even likely that individual species of IFN act preferentially on certain cells, tissues or organs in response to particular viruses [413].

Three major classes of IFN have been identified (alpha, beta, and gamma). All three have been tested in clinical trials in CA, or GW, with positive results. Administration by topical, intralesional, intramuscular, and subcutaneous routes results in regression of HPV genital disease. Significant reduction in measurable lesions occurs in some patients within days of

initiation of therapy. Responses appear to be both time and dose dependent. Although disease resolution is highly variable from patient to patient, approximately 75% to 80% of all persons show clear clinical benefit at low doses. Biologic side effects of IFN are tolerated well at these doses and occur following systemic or local administration of IFN. In general, the IFN are active and safe therapeutic agents for genital HPV infections. Results of controlled trials in refractory GW have proved that IFN alpha provides significant clinical benefit for the majority of subjects with severe disease. Current studies show that it can be combined safely and effectively with other conventional treatment modalities, such as laser or podophyllin [414]. IFN treatment of existing genital HPV lesions has had mixed results. While HPV proteins down-regulate the expression of IFN-inducible genes, IFN treatment ultimately induces their high-level transcription after a delay. Cells containing complete HPV genomes that are able to undergo productive replication upon differentiation are sensitive to IFN-induced growth arrest, while cells from HG cancers that only express E6 and E7 are resistant. Recent studies indicate this sensitivity is dependent upon the binding of the IFN-inducible factor, p56, to the E1 replication protein. The response to IFN by HPV proteins is complex and results from the action of multiple viral proteins [415].

Cidofovir, a nucleotide analog with antiviral activity against a broad range of DNA viruses including HPV, is available off label to clinicians. Cidofovir, as a result of its antiviral and antiproliferative activity and its ability to induce apoptosis, can offer a solution for the treatment of severe recurrent HPV-induced lesions. It can also be used to attempt to treat dysplastic lesions and as an adjuvant treatment. The long-lasting antiviral activity allows infrequent dosing. As a rule, cidofovir applied on the skin is well tolerated, even in long-term treatment. The dose-limiting nephrotoxicity of the drug is not a concern in patients with a glomerular filtration rate within the normal range. Cidofovir has clearly influenced the landscape of refractory and dysplastic anogenital CA and its use has increased over the last decade. However, further controlled clinical trials are needed to assess the role of cidofovir and its derivatives [416,417].

Some studies highlight the importance of dietary vitamin A (retinol) and other retinoids in maintaining normal cervical cell function and in inhibiting the growth of cervical tumors. Based on these results it can be concluded that:

1) HPV 16-immortalization enhances cervical cell sensitivity to retinoids;
2) cytokeratin expression may be useful as a marker for evaluating the success of retinoid therapy in vivo;
3) retinoids do not necessarily act to inhibit proliferation of HPV-immortalized cervical cells via effects on HPV E6 and E7 RNA levels; and
4) retinoids may act to inhibit cervical proliferation by “suppressing” the activity of the EGF and insuline-like growth factor (IGF) signaling pathways.

Based on these and other results, it is worth considering the possibility that vitamin A or related retinoids could be administered therapeutically, early in the neoplastic process (either systemically or locally), to inhibit the progress of the disease. These results also suggest that combined IFN/retinoid therapy may provide an enhanced beneficial effect to reduce cervical tumor size due to the fact that each agent is inhibiting cervical cell proliferation via distinct, but reinforcing, pathways (i.e. IFN gamma reduces E6/E7 expression, vitamin A inhibits the function of the EGF and IGF1 signaling pathways) [418].

Estrogen interacts with HPV at a cellular level causing cell growth and inhibition of apoptosis. Indole derivatives, formed during digestion of cruciferous vegetables, have been shown to have chemopreventative properties inhibiting HPV transcription and influencing estrogen metabolism. Further clinical research is required to evaluate the chemopreventative properties of these agents [419].

Evidence for the assumption that cervical carcinoma, among other malignancies such as melanomas, renal malignancies, and Kaposi sarcoma, are immunogenic is provided by the fact that these malignancies grow more rapidly in the presence of systemic immunosuppression. Spontaneous regression for these tumor types is also described and immunohistochemical studies show extensive infiltrates in the tumor, consisting of immunocompetent cells. It is thus postulated that CMI, and mainly the T-cell system, plays an important role in the antitumor defense in cervical carcinoma [420]. Failure of the immune system to launch a strong and effective immune response to HR HPV is related to viral persistence and the development of anogenital premalignant lesions such as VIN. Different forms of immunotherapy, aimed at overcoming the inertia of the immune system, have been developed and met with clinical success. Unfortunately, these, in principal successful, therapeutic approaches also fail to induce clinical responses in a substantial number of cases. The potential mechanisms involved in the escape of HPV-induced lesions from the immune system indicate gaps in our knowledge. There are a number of pre-existing conditions that determine the patients' responsiveness to immunotherapy. An immunotherapeutic strategy in which different aspects of immune failure are attacked by complementary approaches, will improve the clinical response rate [421]. There are two licensed prophylactic vaccines, both target HPV 16 and 18, the two most pathogenic, oncogenic types and one, additionally, targets HPV 6 and 11 the cause of genital warts. The approach of deliberate immunization with oncogenic HPV E6 and/or E7 proteins and the generation of antigen-specific cytotoxic T-cells as an immunotherapy for HPV-associated cancer and their HG pre-cancers has been tested with a wide array of potential vaccine delivery systems in phase I/II trials with varying success. Understanding local viral and tumor immune evasion strategies is a prerequisite for the rational design of therapeutic vaccines for HPV-associated infection and disease [422].

Gene therapy of human cancer is likely to be most effective when it is directed at targets that are expressed in cancer cells but are lacking from other cells. HPV can provide such targets, since these viruses are present in many cervical and oral cancers, and are likely to be etiological agents of the tumor. Continued expression of HPV genes is probably necessary for the growth of these cancers, and effective gene therapy could consist of antisense or ribozyme molecules directed against these genes. Some HPV gene products are antigenic, and immunotherapy based upon these antigens might prove clinically beneficial. HPV have specific promoters, are linked to toxin genes, the toxin may be selectively expressed by tumor cells where the virus genes are active. Thus, there are several approaches for the development of specific gene therapy for human cancers that contain HPV [423]. Two novel approaches for inactivating gene expression involve ribozymes and oligonucleotides. Methods for identification of target genes involved in neoplastic transformation and tumor growth have been established, and these will lead to therapeutic approaches without any damage to normal cellular RNA molecules, which is often associated with conventional therapeutics. Ribozymes and oligonucleotides represent rational antiviral approaches for inhibiting the growth of cervical lesions and carcinomas by interfering with E6/E7 RNA production. The E6 and E7 genes of HR HPV cooperate to immortalize primary epithelial cells and because they are

found in cervical cancer are considered the hallmark of cervical cancer. The use and modification of ribozymes and antisense oligodeoxynucleotides can inhibit the growth of HPV 16 and HPV 18 immortalized cells, and tumor cells by eliminating E6/E7 transcript. Hammerhead and hairpin ribozymes have been widely studied because of their potential use for gene therapy and their place as therapeutic tools for cervical cancer is being evaluated. Although antiviral ribozymes and antisense molecules have been effective as in vitro or in vivo inhibitors of HR HPV-positive cells, none is currently in clinical trial. There are, however, a number of other antisense therapies in phase I-III clinical trial for several oncogenes [424]. Evidence from in vitro studies shows that when expression of these genes is inhibited by gene therapy approaches such as antisense RNA, ribozymes, or small interfering RNA (siRNA), the transformed phenotype of the cells is lost. Although it seems possible that clinical applications of this approach could help in the management of cervical and oral cancers, there have been no clinical trials of gene therapy for HPV-associated cancers. Since the basic information is now available, a shift to translational research would be greatly welcomed [425].

A growing understanding of the molecular biology of HPV infection has identified several viral protein functions that may serve as drug targets. Among these are the HPV E1 and E2 proteins, which are necessary for viral genome replication and partitioning, and the E6 and E7 proteins, which are necessary for cell proliferation and apoptotic inhibition. With the exception of E1, these proteins lack enzymatic activity and achieve their effects by interacting with cellular proteins. Protein-protein interactions are in general quite difficult to inhibit using conventional small molecule drugs, but are amenable to inhibition using intracellular antibodies or intrabodies, which bind the viral proteins and sterically inhibit their association with cellular partners. The lack of homology between viral and cellular proteins, and the fact that HPV infections can be treated topically, makes them particularly well suited to the intrabody approach [426]. The E2 proteins from some HPV types induce growth arrest and apoptosis, and these proteins could be used as therapeutics for HPV-induced disease. A major obstacle to this approach concerns the delivery of the protein to HPV-transformed cells and/or HPV-infected cells in vivo. One possible solution is to use recombinant viruses to deliver E2. Another possible solution is to use purified E2 proteins or E2 fusion proteins. The HSV VP22 protein is one of a small number of proteins that have been shown to cross the cell membrane with high efficiency. VP22-E2 fusion proteins produced in bacterial cells are able to enter mammalian cells and induce apoptosis. This suggests that VP22-E2 fusion proteins could be topically applied as a treatment for HPV-induced diseases, most probably post-surgery [427].

FOLLOW-UP

According to the current guidelines in most Western countries, there is a general agreement concerning the role HPV DNA testing in the follow-up period after conservative treatment for CIN. There is a marked heterogeneity in the design, population, intervention, and follow-up policy across different studies.

The sensitivity of HPV DNA testing in detecting treatment failures was quite good in most studies, reaching 100% in some of them, whereas the specificity of the test differed across the studies, ranging from 44% to 95%. Among women in whom the treatment was

considered to be successful, 84.2% had a negative postoperative HPV DNA test and 15.8% a positive one.

The corresponding rates for cases with treatment failures were 17.2% and 82.8%, respectively. It seems that a positive HPV test, even in the presence of normal cytology, may pick up early and accurately a treatment failure. Cytology and colposcopy may still be needed in order to rule out false positive and false negative results [428].

In a meta-analysis, the NPV for recurrent/residual disease of HR HPV testing was 98% (95% CI 97-99%), that of resection margins 91% (95% CI 87-94%), and that of cervical cytology 93% (95% CI 90-95%). When HR HPV testing was performed in conjunction with cytology, the sensitivity was 96% (95% CI 89-99%), specificity was 81% (95% CI 77-84%), the associated positive predictive value (PPV) was 46% (95% CI 38-54%), and the NPV was 99% (95% CI 98-100%). Combined HR HPV and cytology testing yielded the best test characteristics. It has been proposed to include HR HPV testing in conjunction with cytology for monitoring women treated for CIN 3. Some follow-up visits for women testing negative for both HR HPV and cytology can be skipped. In Western countries, this could mean that for women double negative at 6 months, retesting at 12 months should be skipped while keeping the 24-month follow-up visit [429,430].

PROGNOSIS

Conflicting evidence on the prognostic influence of some of the clinical and histopathological variables in cervical cancer of the HPV status and type and chemotherapeutic response prompted a number of reviews from nearly 40 year-experience in a tertiary referral centre. The collation and analyses of these data with those from recent literature allow some proposals to be made. The disease is more prevalent in the young women in whom, in many centers, the mortality is also higher: the latter may be related to the reported increase in both small cell types and adeno- and adenosquamous carcinoma, a finding more marked in the young. Lymph node metastases, related to increasing grade, size, stage, and lymph space invasion (Table 2), are unequivocally associated with a worse prognosis [431]. The HPV DNA status of lymph nodes in women with cervical cancer is being explored as a potential marker of “occult” metastases. Although the presence of HPV DNA in lymph nodes usually correlates with its metastatic involvement, there is always a subgroup of HPV-positive but histologically negative lymph nodes.

The significance of HPV in negative lymph nodes remains uncertain, although several studies have concluded that HPV is a risk factor of recurrence. A small group size and a short follow-up are the main limitations for drawing any conclusion concerning prognostic significance of the presence of HPV DNA in lymph nodes [432]. Resolution of the exact nature of the intimate association of this disease with the HPV remains to be resolved as does the influence on prognosis of the tumor HPV status and that of the different oncogenic types. Reports on the efficiency of neoadjuvant platinum based combination chemotherapy are generally promising but vary considerably depending on the regimen used [431]. Studies have suggested that the presence in tumors of nucleic acids from HPV constitutes a prognostic marker of disease severity in cervical cancer. There are two conflicting lines of evidence in this regard.

Table 2. FIGO Cervical cancer staging

Stage 0 (Tis, N0, M0): The cancer cells are only in the cells on the surface of the cervix (the layer of cells lining the cervix), without growing into (invading) deeper tissues of the cervix. This stage is also called carcinoma in situ (CIS) which is part of cervical intraepithelial neoplasia grade 3 (CIN 3). Stage 0 is not included in the FIGO system.
Stage I (T1, N0, M0): In this stage the cancer has grown into (invaded) the cervix, but it is not growing outside the uterus. The cancer has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IA (T1a, N0, M0): This is the earliest form of stage I. There is a very small amount of cancer, and it can be seen only under a microscope. The cancer has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IA1 (T1a1, N0, M0): The cancer is less than 3 mm (about 1/8-inch) deep and less than 7 mm (about 1/4-inch) wide. The cancer has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IA2 (T1a2, N0, M0): The cancer is between 3 mm and 5 mm (about 1/5-inch) deep and less than 7 mm (about 1/4-inch) wide. The cancer has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IB (T1b, N0, M0): This stage includes stage I cancers that can be seen without a microscope as well as cancers that can only be seen with a microscope if they have spread deeper than 5 mm (about 1/5 inch) into connective tissue of the cervix or are wider than 7 mm. These cancers have not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IB1 (T1b1, N0, M0): The cancer can be seen but it is not larger than 4 cm (about 1 3/5 inches). It has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IB2 (T1b2, N0, M0): The cancer can be seen and is larger than 4 cm. It has not spread to nearby lymph nodes (N0) or distant sites (M0).
Stage II (T2, N0, M0): In this stage, the cancer has grown beyond the cervix and uterus, but hasn't spread to the walls of the pelvis or the lower part of the vagina. Stage IIA (T2a, N0, M0): The cancer has not spread into the tissues next to the cervix (called the parametria). The cancer may have grown into the upper part of the vagina. It has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IIA1 (T2a1, N0, M0): The cancer can be seen but it is not larger than 4 cm (about 1 3/5 inches). It has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IIA2 (T2a2, N0, M0): The cancer can be seen and is larger than 4 cm. It has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IIB (T2b, N0, M0): The cancer has spread into the tissues next to the cervix (the parametria). It has not spread to nearby lymph nodes (N0) or distant sites (M0).
Stage III (T3, N0, M0): The cancer has spread to the lower part of the vagina or the walls of the pelvis. The cancer may be blocking the ureters (tubes that carry urine from the kidneys to the bladder). It has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IIIA (T3a, N0, M0): The cancer has spread to the lower third of the vagina but not to the walls of the pelvis. It has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IIIB (T3b, N0, M0; OR T1-3, N1, M0): either: The cancer has grown into the walls of the pelvis and/or has blocked one or both ureters (a condition called hydronephrosis), but has not spread to lymph nodes or distant sites. Or: The cancer has spread to lymph nodes in the pelvis (N1) but not to distant sites (M0). The tumor can be any size and may have spread to the lower part of the vagina or walls of the pelvis (T1-T3).
Stage IV: This is the most advanced stage of cervical cancer. The cancer has spread to nearby organs or other parts of the body. Stage IVA (T4, N0, M0): The cancer has spread to the bladder or rectum, which are organs close to the cervix (T4). It has not spread to nearby lymph nodes (N0) or distant sites (M0). Stage IVB (any T, any N, M1): The cancer has spread to distant organs beyond the pelvic area, such as the lungs or liver.

Adapted from: American Cancer Society (ACS), 2013.

First, the presence of HPV 18 is equated to rapid progression through early disease stages, possibly resulting in a more aggressive clinical course. Although fragmentary, in terms of the clinical and epidemiological basis, this line of evidence has some experimental support. Second, the absence of HPV from the tumor would confer a worse prognosis than if any viral types were present. Unlike the former, the latter line of evidence is not bolstered by experimental data but emerged from persuasive clinical studies, which had adequate sample sizes, used survival end points, and controlled for confounders. The absence of HPV in some tumors could indicate that they originated through different oncogenic mechanisms, perhaps resulting in different cell proliferation rates and, consequently, distinct clinical behavior. On the other hand, HPV detectability could simply be a correlate of other genuine prognostic characteristics, which would explain its association with survival [433].

PREVENTION

Until recently, cervical cancer strategies focussed on screening. However, as adolescents become sexually active at a much younger age, the focus is on the use of vaccination as an effective measure to prevent progression of HPV infection to cancer. Primary prevention through vaccination is now possible in Europe using either the quadrivalent HPV vaccine, Gardasil, or the bivalent HPV vaccine, Cervarix, which are both highly immunogenic, with their effects persisting for at least 5 years. HPV vaccines are well tolerated, with serious vaccine-related events occurring in less than 0.1% of patients for both vaccines. The European Academy of Pediatrics (EAP) Scientific Working Group on Vaccination concluded that the use of HPV vaccines will have a significant impact in primary prevention of cancers and other HPV-related disease [434].

In June 2006, a quadrivalent HPV 6/11/16/18 vaccine (Gardasil) was licensed in the USA: subsequent approval has been granted in the European Union (EU) (September 2006). It has since been approved in 121 countries with over 74 million doses distributed globally as of March 2011 [435]. HPV quadrivalent recombinant vaccine is a mixture of VLP derived from the L1 capsid proteins of HPV types 6, 11, 16, and 18. It is administered intramuscularly in a three-dose regimen, with the initial injection followed by subsequent doses at months 2 and 6. The vaccine is indicated for use in the prevention of cervical cancer, vulvar and vaginal precancer and cancers, precancerous lesions, and GW associated with HPV types 6, 11, 16, or 18 infection in adolescents and young women. The quadrivalent vaccine has demonstrated good immunogenicity in young women (16-26 years) and male and female adolescents (aged 9-15 years), inducing high and persistent anti-HPV antibody titers. In a randomized phase III trial designed to bridge efficacy in young women to adolescents (using immunogenicity as a surrogate), the quadrivalent HPV vaccine in adolescents was at least as immunogenic as that in young women. In randomized, double-blind, placebo-controlled trials in > 20 000 young women (aged 16-26 years), the vaccine was highly effective in preventing cervical dysplasia of any grade and external genital lesions related to HPV types 6, 11, 16, and 18 infection. These women were followed-up for an average of 2 years. The vaccine was well tolerated, with injection-site reactions and fever being the most common vaccine-related adverse events [436]. In placebo-controlled clinical trials, quadrivalent HPV vaccine administered as three doses over 6 months provided high-level protection against infection or disease caused by the

vaccine HPV types over 2-4 years of follow-up in females aged 15-45 years who were naive to the vaccine HPV types. A degree of cross-protection against certain other non-vaccine HR HPV types was also observed. The vaccine is not effective against current infection with a vaccine HPV type. Girls or women with current infection with one or more of the vaccine HPV types gained protection from infection or disease caused by the remaining vaccine HPV types and they were also protected against reinfection with the same HPV type after clearance of an infection caused by a vaccine HPV type. High seroconversion rates and high levels of anti-HPV antibodies were observed in all vaccinated individuals of all age ranges from 9 to 45 years. No correlation was found between antibody levels and protective efficacy of the vaccine. Rechallenge with quadrivalent HPV vaccine produced a potent anamnestic humoral immune response. The vaccine is projected to be cost-effective in most pharmacoeconomic models. Therefore, quadrivalent HPV vaccine offers an effective means, in combination with screening programmes, to substantially reduce the burden of HPV-related precancerous lesions and cancer, particularly cervical cancer, as well as ano-GW [437,438]. Recommendations by the ACIP on the use of a quadrivalent HPV vaccine licensed by the USA FDA on June 8th, 2006 advise its use for vaccination of females is 11-12 years. Vaccine can be administered as young as age 9 years. Catch-up vaccination is recommended for females aged 13-26 years who have not been previously vaccinated. Vaccination is not a substitute for routine cervical cancer screening, and vaccinated females should have cervical cancer screening as recommended [439].

The AS04-adjuvanted HPV 16/18 vaccine (Cervarix) is a noninfectious recombinant vaccine produced using purified VLP that induce a strong immunogenic response eliciting high levels of anti-L1 VLP antibodies that persist at levels markedly greater than those observed with natural infection. The vaccine adjuvant (AS04) is composed of monophosphoryl-lipid A, which enhances cellular and humoral immune response, adsorbed to aluminium hydroxide. The vaccine is indicated for the prevention of premalignant cervical lesions and cervical cancer causally related to certain oncogenic HPV types in females aged ≥ 10 years. The AS04-adjuvanted HPV 16/18 vaccine administered in a three-dose schedule over 6 months (0-1-6) elicits a high immunogenic response and is highly protective against CIN and infection causally related to HR oncogenic HPV types. In well designed clinical trials in young women aged 15-25 years who were HPV 16/18 seronegative and DNA negative to 14 HPV HR types, high levels of immunogenicity and protection were sustained for follow-up periods of up to 8.4 years. High and persistent immunogenicity against infection with HPV 16/18 has also been demonstrated in older and younger females (aged 10-55 years) who were seronegative for vaccine HPV types. The AS04-adjuvanted HPV 16/18 vaccine elicited a greater immunogenic response than the quadrivalent HPV vaccine in women aged 18-45 years who were seronegative and DNA negative for HPV 16/18. The AS04-adjuvanted HPV 16/18 vaccine confers cross protection against certain non-vaccine, HR HPV types. A rapid and strong anamnestic humoral immune response was elicited following a fourth dose of the vaccine. The AS04-adjuvanted HPV 16/18 vaccine is generally well tolerated, and pharmacoeconomic analyses have demonstrated the potential for public health benefits and cost-effectiveness when vaccination programmes are run in conjunction with screening programmes. Thus, the AS04-adjuvanted HPV 16/18 vaccine prevents cervical disease associated with certain oncogenic HPV types, thereby reducing the burden of premalignant cervical lesions and, very likely, cervical cancer [440,441].

Recent data indicate that persistent HR HPV infections represent a significantly increased risk of developing incident HG CIN and cervical cancer. Accordingly, 6-month (6M+) or 12-month (12M+) type-specific persistence of HR HPV have been proposed as powerful surrogates of progressive disease. Because of substantial practical impact in future HPV vaccine trials using non-HPV 16/18 vaccines, studies on HR HPV persistence as a surrogate endpoint of progressive CIN have been subject to a comprehensive meta-analyses recently. Based on a large number of relevant studies, there remains little doubt that persistence of HR HPV for 6+ or 12+ months is associated with a significantly increased risk of developing incident HG CIN. However, some data also disclosed several important issues that need to be carefully considered and/or adequately resolved before adopting 6M+ or 12M+ HR HPV persistence as a surrogate of progressive disease. These include:

1) definitions of HPV persistence;
2) HPV detection techniques;
3) testing intervals;
4) length of follow-up;
5) diagnosis of the surrogate endpoints;
6) other study characteristics; including
7) the type of reference category used in calculating the risk estimates.

Of major impact seems to be the reference category used to calculate these risk estimates, as evident from the New Independent States of the Former Soviet Union (NIS)-Latin American Screening (LAMS) cohort. Taken together, it is suggested that in all future studies using the 6M+ or 12M+ HR HPV persistence as a surrogate endpoint of progressive disease, a "gold standard" should be used in calculating the risk estimates. In addition to deciding:

1) whether to use 6M+ or 12M+ persistence criteria;
2) cytological, histological, or combined surrogate endpoints (SIL, CIN 1, CIN 2, CIN/SIL); one should
3) use exclusively the HPV negative reference group in calculating the risk estimates for viral persistence endpoints.

This is supported by the data from the recent meta-analysis as well as from the combined NIS-LAMS cohort, both implicating that the most consistent association to progressive disease is obtained when women with persistent HR HPV are compared with HPV-negative women. The two other reference categories (HPV transient and HPV mixed outcome) are far too heterogeneous and subject to potential misclassifications to give consistent and reproducible risk estimates for HR HPV persistence as a surrogate endpoint of progressive CIN [442].

The USA FDA has approved both a bivalent and quadrivalent HPV vaccine. The ACIP has recommended that HPV vaccination routinely be given to girls when they are 11 years or 12 years old. The vaccine can be given to individuals as young as 9 years; catch-up vaccination is recommended in females aged 13 years through 26 years. The ACOG endorses these recommendations. Although obstetrician-gynecologists are not likely to care for many girls in the initial vaccination target group, they are critical to the catch-up vaccination period. Both HPV vaccines are most effective if given before any exposure to HPV infection (i.e.

before sexual activity). However, sexually active girls and women can receive some benefit from the vaccination because exposure to all HPV types prevented by the vaccines is unlikely in females aged 13 years through 26 years. Vaccination with either HPV vaccine is not recommended for pregnant women. It can be provided to women who are breastfeeding. The need for booster vaccination has not been established but appears unnecessary. Health care providers are encouraged to discuss with their patients the benefits and limitations of the HPV vaccine and the need for routine cervical cytology screening for those aged 21 years and older [443]. HIV-infected populations are at an increased risk for HPV-related cancers. None of the efficacy trials for these vaccines included HIV-infected populations. However, studies in HIV-infected children and adult men show that the vaccine is safe and highly immunogenic. Studies evaluating the vaccine in HIV-infected women are in progress. Based on these studies, the ACIP recommends HPV vaccination for all HIV-infected children and young adults up to age 26 years. HPV vaccine policies in resource-limited countries, many of which have a high prevalence of HIV infection, are still being developed. Future studies should examine the role of HPV vaccination for older HIV-infected adults who likely have ongoing HPV infection [444]. Both sexes play a role in transmission of the disease, but the cost-effectiveness of HPV vaccination differs between them. It is necessary to determine the best allocation of limited resources between these two populations to produce the most effective strategy for reducing the burden from HPV-related disease. Current outreach in the USA is based on vaccination against HPV as a means for combating cervical cancer in women. If we are to include males, however, new marketing strategies must focus on educating patients about the full range of the vaccine's benefits. MSM are also unprotected against HPV in the current system. Social considerations alone may not be enough, however, as economic prediction models suggest that the associated costs outweigh the benefits in most circumstances. The most prudent programs will include physician involvement in patient education and the implementation of structured vaccination and screening programs. Unfortunately, many countries do not have the necessary resources to undertake national vaccination programs. HPV testing and cytology screening for women and MSM may be the most financially reasonable option for many countries [445].

In April 2009, experts on STD were convened to review updates on STD prevention and treatment in preparation for the revision of the CDC and Prevention STD Treatment Guidelines. A new patient-applied treatment option for GW, sinecatechins 15% ointment, is available and recommended for treatment of external GW. This product is a mixture of active ingredients (catechins) from green tea [446]. On October 25th, 2011, the ACIP recommended routine use of quadrivalent HPV vaccine (HPV 4, Gardasil) in males aged 11 or 12 years. ACIP also recommended vaccination with HPV 4 for males aged 13 through 21 years who have not been vaccinated previously or who have not completed the 3-dose series, males aged 22 through 26 years may be vaccinated [447]. Recent evidence shows that the quadrivalent HPV vaccine prevents several HPV-related diseases in men. However, despite the licensure of the vaccine in the USA for men 9 through 26 years of age, rates of male vaccination are very low. Research on acceptability, in general, indicates strong interest in vaccination among men, parents, and healthcare providers, though female vaccination is typically seen as a higher priority. Cost-effectiveness studies indicate that in the context of modest female vaccination rates and with the specification of a broad range of disease outcomes (e.g. GW, anogenital cancers, and OPCA), male vaccination can be quite cost-effective. Men are at high risk for HPV infection and can benefit from vaccination, but vaccination rates among men

remain extremely low. More research needs to be done on the predictors of uptake of HPV vaccine among men and on the development of interventions to increase male vaccination [448]. Despite recommendations from the ACIP for routine vaccination of adolescents with the HPV vaccine, USA coverage among women remains less than 50%, with that of adolescent men below 2%. Ongoing studies document the efficacy and safety of the HPV vaccine. Nevertheless, misconceptions and negative attitudes persist and serve as barriers to vaccine uptake. Additionally, other factors such as age, insurance status, poverty status, and racial or ethnic background have been associated with diminished vaccine uptake and poor completion rates. Internationally, HPV vaccination programs and school-based programs have achieved increased rates of uptake and series completion. HPV vaccination coverage may also be facilitated by improving communication between physicians, parents, and adolescents as well as by addressing common misconceptions about the vaccine [449].

The currently licensed HPV vaccines are safe and highly effective at preventing HPV infection for a select number of HPV types, thus decreasing the incidence of precursors to cervical cancer. It is expected that vaccination will also ultimately reduce the incidence of this cancer. The licensed HPV vaccines are, however, type restricted and expensive, and also require refrigeration, multiple doses, and intramuscular injection. Second-generation vaccines are currently being developed to address these shortcomings. New expression systems, viral and bacterial vectors for HPV L1 capsid protein delivery, and use of the HPV L2 capsid protein will hopefully aid in decreasing cost and increasing ease of use and breadth of protection. These second-generation vaccines could also allow affordable immunization of women in developing countries, where the incidence of cervical cancer is high [450]. Prevention and treatment of HPV infection may be revolutionized using nanotechnology tools such as vaccines based on VLP and nanoscale drug-delivery systems. Advances in both VLP design and noninvasive delivery of antiviral protein drugs, such as IFNalpha, may provide new opportunities to take on the challenge of global elimination of HPV infections. Biphasic vesicle cream formulation, representing a new class of dermal delivery system for protein drugs, is an alternative to injectable dosage form to deliver IFNalpha for the treatment of HPV infections, showing efficacy in L-SIL of the cervix [451].

HPV vaccination is expected to reduce the burden of cervical cancer in most settings, however, it is also expected to interfere with the effectiveness of screening. In the future, maintaining Pap cytology as the primary cervical screening test may become too costly. As the prevalence of cervical dysplasias decreases, the positive predictive value of the Pap test will also decrease, and, as a result, more women will be referred for unnecessary diagnostic procedures and follow-up. HPV DNA testing has recently emerged as the most likely candidate to replace cytology for primary screening. It is less prone to human error and much more sensitive than the Pap smear in detecting HG cervical lesions. Incorporating this test would improve the overall quality of screening programs and allow spacing out screening tests, while maintaining safety and lowering costs. Although HPV testing is less specific than Pap cytology, this issue could be resolved by reserving the latter for the more labor-efficient task of triaging HPV-positive cases. Because most HPV-positive smears would contain relevant abnormalities, Pap cytology would be expected to perform with sufficient accuracy under these circumstances. HPV Pap triage would also provide a low-cost strategy to monitor long-term vaccine efficacy. Although demonstration projects could start implementing HPV testing as a population screening tool, more research is needed to determine the optimal age to

initiate screening, the role of HPV typing and other markers of disease progression, and appropriate follow-up algorithms for HPV-positive and Pap-negative women [452].

Immunological intervention against HPV can be envisaged at two levels, prophylactic and therapeutic. Different therapeutic strategies have been developed including peptide immunization-based therapies, DNA vector-based therapies, viral/bacterial vector-based therapies, IRM, photodynamic therapy (PDT), and T-cell receptor based therapy. The existing immunotherapies are not completely effective, nor are they durable. In addition, natural history studies associated with spontaneous regression have provided little guidance to the design of successful interventions. This state of knowledge has encouraged efforts towards the development of novel immunotherapeutic strategies [453]. The therapeutic vaccines constructed to counteract tumors which are already developed utilize two nonstructural early proteins coded by HPV, the products of their E6 and E7 oncogenes. These E6/E7 oncoproteins are the only HPV-coded proteins expressed in cervical cancer: they are involved in malignant transformation of HPV-infected cells, their presence is necessary for the maintenance of the malignant phenotype of the cells, and their expression correlates with the transforming potential of HPV. Therefore, the E6/E7 oncoproteins are used for the construction of therapeutic vaccines against HPV-associated neoplasms [454].Successful preclinical studies of therapeutic vaccine candidates have led to clinical studies for a variety of HPV-associated indications, such as ano-GW and CIN and AIN. Specifically, chimeric molecules comprised of mycobacterial heat-shock proteins (HSP) and HPV 16 E7 appear promising [453]. At present, the design of therapeutic vaccines to control the growth of HPV-induced tumors has focused on utilization of E6 and E7 proteins or peptides as vaccine antigens. Human trials are the most important test for the efficacy of HPV 16/18 E6 and E7 proteins as immunotherapy for cervical cancer [455]. A therapeutic HPV DNA vaccination strategy uses the HPV 16 E7 antigen fused to the invariant chain to enhance the E7-specific CD8+ and CD4+ T cell immune responses, resulting in a potent anti-tumor effect against E7-expressing tumors. Continued exploration of HPV therapeutic DNA vaccines may lead to eventual clinical application [456]. A majority of clinical trials examining therapeutic vaccination have shown limited efficacy due to examining patients with more advanced-stage cancer who tend to have decreased immune function. Current trends in clinical trials with therapeutic agents examine patients with pre-invasive lesions in order to prevent invasive cervical cancer. However, longer follow-up is necessary to correlate immune responses to lesion regression. Meanwhile, preclinical studies in this field include further exploration of peptide or protein vaccination, and the delivery of HPV antigens in DNA-based vaccines or in viral vectors. As long as pre-clinical studies continue to advance, the prospect of therapeutic vaccination to treat existing lesions seem good in the near future. Positive consequences of therapeutic vaccination would include less disfiguring treatment options and fewer instances of recurrent or progressive lesions leading to a reduction in cervical cancer incidence [457].

In summary, effective vaccination against HPV represents an opportunity to restrain cervical cancer and other important cancers. The FDA recently approved the HPV vaccine Gardasil for the preventive control of HPV, using HPV VLP to generate neutralizing antibodies against major capsid protein, L1. However, prophylactic HPV vaccines do not have therapeutic effects against pre-existing HPV infections and HPV-associated lesions. Furthermore, due to the considerable burden of HPV infections worldwide, it would take decades for preventive vaccines to affect the prevalence of cervical cancer. Thus, in order to speed up the control of cervical cancer and treat current infections, the continued

development of therapeutic vaccines against HPV is critical. Therapeutic HPV vaccines can potentially eliminate pre-existing lesions and malignant tumors by generating cellular immunity against HPV-infected cells that express early viral proteins such as E6 and E7. Though their development has been challenging, many therapeutic HPV vaccines have been shown to induce HPV-specific antitumor immune responses in preclinical animal models and several promising strategies have been applied in clinical trials. With continued progress in the field of vaccine development, HPV therapeutic vaccines may provide a potentially promising approach for the control of lethal HPV-associated malignancies [458]. Among the various forms of therapeutic HPV vaccines, DNA vaccines have emerged as a potentially promising approach for vaccine development due to their safety profile, ease of preparation, and stability. However, since DNA does not have the intrinsic ability to amplify or spread in transfected cells like viral vectors, DNA vaccines can have limited immunogenicity. Therefore, it is important to develop innovative strategies to improve DNA vaccine potency. Since DC are key players in the generation of antigen-specific immune responses, it is important to develop innovative strategies to modify the properties of the DNA-transfected DC. These strategies include increasing the number of antigen-expressing/antigen-loaded DC, improving antigen processing and presentation in DC, and enhancing the interaction between DC and T cells. Many of the studies on DNA vaccines have been performed on preclinical models. Encouraging results from impressive preclinical studies have led to several clinical trials [459]. DNA vaccination has emerged as a particularly promising form of therapeutic HPV vaccines due to its safety, stability, and ability to induce antigen-specific immunity. Improving the potency of therapeutic HPV vaccines can be reached through modification of DC by:

1) increasing the number of antigen-expressing/antigen-loaded DC;
2) improving HPV antigen expression, processing, and presentation in DC; and
3) enhancing DC and T cell interaction.

Continued improvement in therapeutic HPV DNA vaccines may ultimately lead to an effective DNA vaccine for the treatment of HPV-associated malignancies [460-483].

PHARMACOECONOMICS

The monetary and personal costs to society of HPV infection are enormous. The total cost of HPV-related diseases accounts for euro 200-250 million of which euro 210 million is absorbed by the prevention and treatment of precancerous lesions and cervical cancer. Although both available, HPV vaccines are below the threshold value for economic convenience (euro 9,569 and euro 26,361 per quality-adjusted life year (QALY)-gained for the quadrivalent and bivalent vaccines, respectively), at this point in time long-term economic models developed for Italy seem to indicate the quadrivalent vaccine as the most cost-effective option. Recent publications by official bodies, including the WHO and the Supervisory Authority for Public Contracts in Italy, recommend that the decision-making process be based on both the quality of goods and services as well as the best achievable price [484].

Patient-applied therapies offer patients the possibility of convenient and, on the whole, pain-free treatment. Podofilox (podophyllotoxin) and salicylic acid for genital and extra-GW, respectively, have the additional advantage of being the most cost-effective treatments and, on this basis, they are to be recommended as appropriate first-line agents. The second-line treatment of choice for common warts is cryotherapy. For recalcitrant common warts possible options include inosine pranobex with cryotherapy or electrosurgery, imiquimod with paring and occlusion, intralesional bleomycin, or diphencyprone. Alternative first-line and second-line treatments for genital warts would be either some form of surgical removal or imiquimod. The first option may be the cheapest but this has to be balanced against a degree of post-operative morbidity. Limited data from comparative studies do not show any clear difference in efficacy between cryotherapy, TCA, scissor excision, electrosurgery, and laser surgery in the treatment of GW, and the cost effectiveness of these therapies is probably similar to that of imiquimod. Cryotherapy and TCA are relatively expensive and inconvenient for patients and should be reserved as third-line treatments with certain exceptions, such as cryotherapy for meatal warts. The duration of treatment is significantly related to the number of warts present, the area covered by the warts, and the length of time the warts have been present. For recalcitrant anogenital warts third-line treatment options that show promise include surgery in combination with imiquimod or cidofovir cream. For SIL that cannot easily be excised or physically ablated current treatment options include imiquimod and 5-FU cream. The latter is an inexpensive option but causes the greatest morbidity. It is hoped that cidofovir may be added to this list if it becomes commercially available, and that protective and therapeutic HPV vaccines will transform the management of HPV in the future [485].

The cost-effectiveness of HPV screening depends on the interval of the established Pap screening strategy. Results for settings with annual Pap screening are based on models assuming 100% screening coverage. The introduction of HPV-based screening programs is cost-effective if the screening interval of the established Pap program exceeds 2 years. In settings with biennial Pap screening, introduction of HPV-based screening is unlikely to be cost-effective. Results also suggest cost-effectiveness of HPV-based screening in settings with annual Pap screening. However, this finding should be confirmed under realistic screening adherence assumptions [486]. For women with ASC-US $\geq$ 30 years, HPV triage is the least costly alternative, whereas immediate colposcopy with biopsy provides the most effective option at an incremental cost-effectiveness ratio (ICER) of SEK 2,056 per additional case of CIN 2+ detected. For L-SIL (all age groups) and ASC-US (23-60 years and < 30 years), HPV triage is dominated by immediate colposcopy and biopsy. Model results were sensitive to HPV test cost changes. With improved HPV testing techniques at lower costs, HPV triage can become a cost-effective alternative for follow-up of minor cytological abnormalities. Today, immediate colposcopy with biopsy is a cost-effective alternative compared to HPV triage and repeat cytology [487].

The recent approval of HPV vaccine means that decision makers need information beyond that available from RCT to recommend funding for this vaccination programme. Modelling and economic studies have addressed some of those information needs. Although the studies used different model structures, baseline parameters and assumptions, all studies showed that vaccination would decrease rates of HPV infection, precancerous lesions, and cervical cancer. Studies had a consistent message with respect to cost-effectiveness: a female-only vaccination programme is cost-effective compared with the current cytology-based Pap smear screening programme, while the cost-effectiveness of a male and female vaccination

programme is generally not cost-effective compared with female-only vaccination [488]. Given the absence of data on the long-term effectiveness of HPV vaccination, a number of mathematical models have been developed to provide insight to policy makers by projecting the long-term epidemiologic and economic consequences of vaccination and evaluate alternative vaccination policies. Three types of HPV mathematical models have been reported in the literature: cohort, population dynamic, and hybrid. All have demonstrated that vaccination can significantly reduce the incidence of cervical cancer in the long term. However, only the cohort and hybrid models have evaluated the cost-effectiveness of vaccination strategies for preventing cervical cancer. These models have generally shown that vaccinating females can be cost-effective. None has accounted for the potential benefits of vaccinating the population to reduce the burden of RRP and cancers of the vagina, vulva, anus, penis, and head/neck. Given that only the population dynamic model can account for both the direct and indirect (i.e. herd immunity effects) benefits of vaccination in the population, future research should focus on further development of dynamic models by expanding the range of epidemiologic outcomes tracked and including the ability to assess the cost-effectiveness of alternative vaccination policies [489]. The costs of developing and producing new-generation vaccines have increased compared to many of the older, "traditional" vaccines because of new technologies and regulatory requirements. While the public sector often supports basic research costs, private manufacturers are usually responsible for the investments in product development and production scale-up. When considering investments, firms evaluate the probability of a market. Unfortunately, the developing country vaccine market is small (in revenue terms) and often unpredictable, particularly given inaccurate forecasting in the past. Low-income developing countries expect low prices. Demand (actual decisions to pay for and introduce the vaccine) is almost always lower than need (estimates of requirements to achieve optimal public health outcomes), a distinction that may be even more significant for HPV vaccines given the number of new vaccines against priority diseases that will become available over the coming 5 years. One new mechanism under consideration to address some of these challenges is Advanced Market Commitments (AMC). By providing an assured price subsidy for developing country purchase of a future vaccine meeting predefined standards, an AMC would provide industry with greater assurances of earning a reasonable return on their investment to serve the poorest developing countries. The AMC mechanism could provide critical motivation for increased industry (private) investment that would otherwise not occur. HPV vaccines are one of six vaccines being considered for a possible AMC pilot [490].

Considerable variation was observed across HPV vaccine cost-effectiveness models in a number of influential assumptions. Holding constant factors for which current data are lacking, the combined impact of assumptions made for the remaining parameters examined would appear to tend toward underestimation of the cost-effectiveness of HPV vaccination within existing studies. However, uncertainty concerning parameters, such as the duration of vaccine protection and acquired immunity following HPV infection, and the relationship between age and HPV virulence, complicates precise estimation of the cost-effectiveness of HPV vaccination and rigorous evaluation of the validity of existing modeling results [491]. There are large within-study variations in estimates of the cost per QALY gained. The most influential source of uncertainty is the duration of the vaccine protection. Between-study variations are mainly due to three causes: methodological differences, assumptions, and local conditions in the application area. We find large variations between studies for a given

country. Economic evaluation models are increasingly sophisticated, but scientific treatment of epidemiological and market uncertainty does not compensate for the lack of basic information. The large disparities in cost per QALY estimates of massive vaccination programs around the world may be attributed to several critical sources (unavoidable and avoidable) of uncertainty. An asset of economic evaluation is the ability to highlight the areas of research that could be undertaken to reduce uncertainty [492]. The studies were very heterogeneous because of different assumptions. Nevertheless, a substantial reduction in cervical cancer is reported consistently and a (smaller) reduction in precancerous lesions and HPV prevalence. Cost-effectiveness ratios are also very diverse and dependent on the assumptions made. An HPV vaccine can be profitable if duration of vaccine-related immunity is high, efficacy is high, price is low, screening is reduced, administration is before sexual activity, discount rate is not too high, or if there is herd immunity. HPV vaccines have the potential to reduce cervical cancer by at least approximately half of its current incidence, and this might be cost-effective if there is high efficacy with a long-lasting immunity [493].

Vaccines have demonstrated cost-effectiveness in managed care through the prevention of disease. As new vaccines for previously untargeted conditions are developed, pharmacoeconomic modeling is becoming even more critical for the quantification of value in the health care industry. Two recently developed vaccines aimed at prevention of infection from HPV types 16 and 18 have proven to be highly efficacious. Therefore, prevention of cervical cancer via HPV vaccination may have a significant financial impact. A direct quantitative comparison of model results is challenging due to the utilization of different model types as well as differences in variables selected within the same model type. Each model produced a range of cost-effectiveness ratios, dependent on variables included in sensitivity analyses and model assumptions. All models of female adolescent vaccination were able to produce vaccination strategies that would be cost-effective according to this definition in addition to many strategies that would be cost-prohibitive. Variables influential in determining cost-effectiveness of HPV vaccination included the frequency of accompanying cervical screening, the age at which screening is initiated, vaccination efficacy, duration of vaccine protection, and the age range of females to be vaccinated. The actual effectiveness of HPV vaccination in the female population will also depend on levels of vaccine uptake or coverage and compliance in completing all vaccine doses. Clinical studies have shown HPV vaccination to be highly efficacious and potentially lifesaving if administered to females naive or unexposed to vaccine HPV types. Modeling studies have also shown that HPV vaccination can be cost-effective with an ICER of $100,000 or less per QALY gained if administered to females aged 12 years in the context of cervical screening intervals typically greater than 1 year. Catch-up vaccination through 21 years of age increases the cost per QALY to more than $100,000. Until real-world coverage rates increase, cost-effectiveness modeling of HPV vaccination underestimates the actual cost per QALY [494]. Many countries have adopted a vaccination programme for adolescent females based on results of cost-effectiveness analyses. However, given the new indications for use of the vaccine in males, decision makers require information on the cost-effectiveness of vaccinating males in order to make policy decisions on whether or not to fund such programmes. While different model structures, input parameters and baseline assumptions were used, the consistent message in studies that focused on female-only vaccination programmes was that routine vaccination of females is cost effective compared with cervical cancer screening alone. Based on the currently available literature, it appears that the addition

of boys to a vaccination programme generally exceeds traditional cost-effectiveness thresholds. The MSM population represents a potential additional target for routine HPV vaccination, however, more cost-effectiveness studies are required before making such a policy change [495,496].

ETHICAL ISSUES

In contrast to most vaccine-preventable diseases, which are transmitted by air or casual contact, HPV is primarily transmitted by sexual contact. An analysis that applies ethical theories, such as utilitarianism, rule of double effect, and principlism, is needed for policy considerations. These analyses reveal that HPV vaccination can be recommended universally, including at ages 11-12 years. However, given concerns for autonomy, justice, as not all persons are at risk, and non-maleficence, HPV vaccine should not be mandated for school entry. Economic justice indicates a need to provide vaccination for the disadvantaged [497]. When the first preventive HPV vaccine became available in 2006, it drew both enthusiasm and multiple ethical problems. In the case of HPV vaccination, there is a clear conflict between the scientific data that claim a definitive advantage for preventing HPV infection in the exposed population and the ethical and moral issues resulting from a compulsory program. Despite the evident success of routine and compulsory vaccination in young women, there is increasing concern about safety, efficacy, and equity of the vaccine and to close the knowledge "gaps" about HPV infection and consequent health outcomes. Some of these fears are expressed particularly in conservative groups that link these arguments to those of religious and moral issues contending that HPV vaccination is an indirect license for liberal sexual activity in youths, resulting in promiscuity and/or less participation in cervical cancer screening. It has been well demonstrated that HPV infection can lead to harm through the induction of premalignant and cancerous lesions. Therefore, any proven method for preventing infection, such as HPV vaccines, should be used in persons at risk. These policies, however, should be strictly linked to cervical cancer screening programs [498]. The announcement of an experimental vaccine against HPV has raised great hopes and expectations. Promising trial results, however, should not obscure ethical issues related to a vaccine's ultimate dissemination. Although lay media might view an HPV vaccine as a panacea, a more complicated ethical reality exists, touching upon public knowledge, health care disparities, and parental consent for childhood vaccination [499].

In 2007, many legislatures considered, and two enacted, bills mandating HPV vaccination for young girls as a condition of school attendance. Such mandates raise significant legal, ethical, and social concerns. Some Authors argue that mandating HPV vaccination for minor females is premature, since long-term safety and effectiveness of the vaccine has not been established, HPV does not pose imminent and significant risk of harm to others, a sex specific mandate raises constitutional concerns, and a mandate will burden financially existing government health programs and private physicians. Absent careful consideration and public conversation, HPV mandates may undermine coverage rates for other vaccines [500]. Compulsory HPV vaccination of young girls has been proposed as a public health intervention to reduce the threat of the disease. Such a program would entail a symbiotic relationship between scientific interests in reducing mortality and morbidity and philosophical

interests in promoting morality. This proposal raises the issue of whether government should use its police powers to restrict liberty and parental autonomy for the purpose of preventing harm to young people. Applying a principle-based approach to moral reasoning, it can be concluded that compulsory HPV vaccinations can be justified on moral, scientific, and public health grounds [501]. The CDC and Prevention ACIP recommends HPV vaccination of 11- to 12-year-old girls, with catch-up vaccination for girls and women aged 13 to 26 years. Although compulsory HPV vaccination is not currently mandated for any USA population, immigrant women aged 11-26 years are now required to receive the first injection of the vaccine (the full series consists of three doses) as a result of the 1996 Illegal Immigration Reform and Immigrant Responsibility Act (IIRIRA). According to this law, immigrants applying for visas to enter the USA or to adjust their immigration status must receive the inoculations that the ACIP recommends for USA residents. In the case of HPV, this law represents not only an undue burden on immigrant women, but also raises scientific and ethical questions regarding the benefit of vaccination in this population. Given these issues, immigrant women should not be required to provide documentation of HPV vaccination at the time of visa application or adjustment of immigration status [502].

COUNSELING

Educational counseling has an important role in managing patients who have viral sexual transmitted infections, such as genital HPV infections. Given the lack of a curative therapy, patients may require long-term management and may need to be attentive to recurring symptoms. In addition, diagnosis may raise issues of persisting infectiousness along with a need for patient counseling about the potential risk to partners and risk reduction strategies. Lastly, there is a growing psychosocial literature on genital HPV. Clinicians can make a significant difference in patient adjustment to the diagnosis of an sexual transmitted infection, and addressing these challenging issues will benefit patients greatly [503]. Public awareness of HPV is generally very low, particularly with respect to its relation to abnormal smears and cervical cancer although knowledge levels vary to some extent according to sociodemographic characteristics. There is also much confusion around which types cause warts and the types that can cause cancer. The sexually transmissible nature of the infection is of major concern and confusion to women. Due to the lack of current awareness of HPV, significant education initiatives will be necessary should HPV vaccination and/or HPV testing be introduced. Organized edification of health-care workers and the media, who constitute the two most preferred sources of information, will be crucial [504]. Recent studies document stark knowledge gaps about HPV at all levels, among policy makers, healthcare providers, parents, and teens, in both the industrialized and developing worlds. Pharmaceutical companies, public health advocates, medical trainers, and health educators need to understand their diverse audiences and respond appropriately to the needs of each. They also must use research-based communication strategies and materials to most effectively, and accurately, convey the need for an HPV vaccine and to manage expectations about how the vaccine can, and cannot, protect women and men [505]. The potential for HPV DNA testing in cervical cancer prevention programs has been a topic at the forefront of cervical cancer policy discussions in recent years. To prevent some of the anxiety and psychological distress often

experienced on HPV diagnosis and during the period of management, mass patient education must accompany the incorporation of HPV DNA testing into screening protocols [506].

New vaccines designed to prevent HPV infection have the potential to reduce the incidence of serious illness and death worldwide among women, substantially reduce the emotional suffering associated with abnormal Pap test results and the diagnosis of cervical cancer, and save significant health care dollars. However, these benefits may not be fully realized until the vaccine is accepted by patients, parents, and health care practitioners. Furthermore, there may be unique issues related to the acceptance of a vaccine designed to prevent a sexually transmitted infection that is poorly understood by many women. Among the acceptance issues are: individual comfort with a sexually transmitted infection vaccine, parental comfort with vaccination of their preadolescent/early adolescent daughters, physician comfort with recommending a HPV vaccine to women and parents of preadolescents, and physician communication skills related to talking with women and parents about the vaccine. Potentially difficult as it might be to implement a vaccination program, vaccination and prevention of HPV-associated disease are still infinitely preferable to observation and treatment [507]. The success of future HPV vaccination programs will depend on individuals' willingness to accept vaccination, parents' willingness to have their preadolescent and early adolescent children vaccinated, and health care providers' willingness to recommend HPV vaccination. The research published to date suggests that there is a good deal of misunderstanding about HPV infection, cervical cancer screening, and the sequelae of HPV infection. However, the majority of research studies to date indicate that young women, parents, and health care providers are interested in vaccines that prevent HPV and other sexually transmitted infections. Of particular note are the consistent findings that providers are less comfortable vaccinating younger versus older adolescents and that endorsement of vaccination by a professional organization is of great importance. Furthermore, research suggests that most parents are interested in having their preadolescent and adolescent children vaccinated against HPV. Parents value the information and recommendations provided by their children's health care providers. To the extent that providers are concerned about potential negative reactions of parents to a recommendation of HPV vaccination, these findings should provide reassurance. At the same time, health care providers will need to be prepared to provide patients and parents with information about HPV and HPV immunization and to respond productively to the rare parent who expresses opposition to HPV vaccine or any other vaccine [508]. The news coverage of HPV vaccine provides information on the experimental status and efficacy of the vaccine, explains link between HPV and cervical cancer, and reports the manufacturers by name, as well as relies on them for a news source. Detailed information about HPV, however, was frequently missing which could lead to an incomplete picture or lack of understanding of the complexity of HPV and cervical cancer. As a major source of medical information, the media can be particularly important in educating policy makers and the general public about new scientific advances. Public health officials may wish to collaborate with journalists, health educators, healthcare providers, and women's health advocates to ensure that future educational initiatives explain the complexity of the association of HPV and cervical cancer and to stress the importance of continued cervical cancer screening [509].

Educational strategies are essential, given several new and highly effective technologies to prevent HPV and related diseases such as cervical cancer. Although little has been published regarding adolescent knowledge about HPV and HPV vaccines, studies conducted

primarily in adult women demonstrate that knowledge generally is poor. Studies of adolescent attitudes about HPV vaccines have identified several modifiable factors associated with intention and confidence in one's ability to receive the vaccine, including higher perceived severity of cervical cancer and fewer barriers to vaccination. Studies of clinician attitudes about HPV vaccines have demonstrated that although clinicians generally support vaccination, some report concerns; for example, adolescents may practice riskier sexual behaviors after vaccination. Studies also show that clinicians believe that educational materials developed specifically for adolescents are essential. The recent literature on adolescent knowledge about HPV and attitudes about HPV vaccines supports the importance of designing developmentally appropriate educational materials for adolescents about HPV and HPV vaccines, and provides guidance for the development of key educational messages [510].

CONCLUSION

HPV are newsworthy in this new millennium. Numerous articles have appeared in the lay press ranging in style and quality from informative essays to sensationalized exposes. Women, sensitized by confusing information, are asking obstetricians hard questions about HPV transmission and prevention, partner notification, the need for HPV testing, and methods of treatment. These questions are difficult because none of the answers are clear cut [511]. More than 20 years ago, a relationship between HPV infection and cervical cancer was recognized. Since then, important strides in understanding the virus have been made, particularly in the following areas: modes of transmission and risk factors associated with transmission, the oncogenic potential of specific viral types and the mechanism by which they cause cancer, and the spectrum of infection, ranging from asymptomatic carrier states to overt warts, preneoplastic lesions, and invasive cancer. Sophisticated new tests for the detection of HPV for improved screening for cervical cancer precursors and invasive cancer and for the triage of abnormal cervical cytology also have been developed. Understanding the immunology of HPV has allowed the development of new and more effective treatment modalities for HPV infection and the development of primary prevention modalities, including HPV vaccines [512].

During the past decades, much has been learned about the natural history of HPV. Most infections occur early in one's sexual life. The overwhelming majority of infections are cleared by the host immune system and never present as warts or neoplasia. Certain patient behavior such as smoking, frequent sex with many different partners, other STD, especially HIV, and immune-suppressive drugs promote HPV expression and cause persistent infection. Persistent HPV infection is very strongly related to neoplasia. In addition to HR HPV types, variant subtypes have been identified that interact with the host immune system to subvert host immunity and encourage viral persistence. New treatment programs rely on drugs that modulate the immune system and disrupt viral persistence [513]. Effective primary and secondary cancer prevention programmes are key to improve public health. Cervical cancer is preventable if high quality screening programmes, diagnosis and treatment are offered to female populations at high coverage. Nevertheless, it continues to be a public health problem, and screening programmes need improvements. To date we count two clinically validated and

approved HPV technologies, available to prevent cervical cancer, and other diseases caused by these carcinogenic viruses: Prophylactic vaccines for primary prevention, and HPV DNA tests for secondary prevention, to detect life threatening infections by carcinogenic HPV types, allowing timely diagnosis and clinical management of precancerous lesions. The new technologies will help improve the health of the public if made widely accessible. Similar to vaccination programmes, systematic and well organized cervical screening programmes, with high quality validated HPV tests, can save more lives than ever and improve women's health, in an effective manner [514]. New methods for preparing probes with higher sensitivities for hybridization tests allow use of in-situ methods on formalin-fixed tissues and are the method of choice. Antigen detection systems are not available except for antisera directed against the common structural antigens. The most useful immunologic test are directed toward detection of nonstructural antigens in fixed tissues: such a system could also be useful for virus typing. Therapies based on use of these antigens to stimulate the immune system are applicable as an alternative to current therapies. Most intriguing has been the realization of a vaccine based on either structural or nonstructural viral antigens. It has been estimated that as many as 20% of female cancer deaths worldwide are associated with HPV. Thus, use of an effective vaccine will relieve considerable human suffering [515].

In conclusion, much has been learned about the biology of HPV during the last decades, and great progress has been made in the development of rational and effective patient management strategies. With stronger evidence supporting a causal role for HPV in the development of genital tract and anal cancers, and with the availability of effective therapies, it is time to recommend widespread screening for detection of HPV DNA or RNA with the molecular hybridization tests commercially available. Pap smear screening for detection of early precancerous changes of the cervix and referral for colposcopy and biopsy of areas of epithelium that are suspicious for intraepithelial neoplasia of the cervix, vagina, vulva, penis, or anus remain the cornerstones of genital tract and anal cancer prevention. The patient care implications of subclinical persistent HPV infection of the genital tract are not well understood. For this reason, and because none of the available therapies are curative, treatment of large areas of normal-appearing genital tract epithelium also cannot be recommended at this time. It is hoped that, with the growing research focus on therapies that have the potential for virologic cure, someday effective treatment for subclinical infection will be available. Until that time, patients with recalcitrant or recurring GW may benefit most by the sequential application of different treatment modalities [516,517]. Academic research has made a significant advancement in understanding the viral causes of cervical cancer and generating the technology for prevention, both at the primary and secondary levels. HPV have been recognized as the first necessary cause of cervical cancer, the second most common cancer in women worldwide. DNA probes of HR HPV types in different formats have been fully validated as primary screening tests, as secondary triage tests and as a prognostic marker following treatment of H-SIL. They consistently showed significant superiority over the conventional Pap smears. Biomarkers of the activation of oncogenes (HPV mRNA, p16, and other) are being tested as screening options to improve in sensitivity and specificity, with promising results. HPV vaccines against the two most common HPV types in cancer have completed their phase III trials with excellent results in efficacy and safety. Combined strategies of HPV vaccination and HPV-based screening tests could theoretically control cervical cancer in any population in which a large coverage with both preventive options is ensured. Accessibility of developing countries to vaccination and low-cost HPV screening

options are the barriers to overcome at present. The available technology for prevention and its developments allows real opportunities for cervical cancer elimination in defined populations to be foreseen [518].

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Chapter 2

HUMAN PAPILLOMAVIRUS AND NON-CODING RNAS: FROM BASICS TO DIAGNOSTIC

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ABSTRACT

Human Papillomavirus (HPV) is the etiological agent of cervical cancer. Like other oncogenic viruses, HPV encode oncoproteins whose role in cellular immortalization and transformation has been extensively investigated. HPV E6 and E7 proteins disable tumor suppressors p53 and Rb and up-regulate telomerase, fundamental changes for cell immortalization. Another important step in the induction of cancer by oncogenic viruses seems to be the specific interaction of some viral proteins with mitochondria, an organelle that has been implicated for decades in carcinogenesis. The identification of non-coding RNAs (ncRNAs) has opened new research areas and there has been an explosive increase of reports showing that the expression of these RNAs is deregulated in many different human diseases, including cancer. The ncRNAs can be classified into two groups based on their length: small transcripts (20–200 nucleotides), such as microRNAs (miRs), piwi-interacting RNAs and long transcripts (higher than 200 nucleotides). While the function of small ncRNAs has been well documented, the role of long ncRNAs is still not completely understood. MicroRNAs are small 21–22 nt non-protein-coding RNAs that regulate mRNA translation and decay. It has become evident that miRs plays a pivotal role in the development of human cancer. Some miRs have been characterized as tumor suppressors and others as oncogenic (onco-miRs). MiR patterns are tissue specific, and the expression profile could allow to distinguish carcinomas from normal cells. Moreover, miR expression profiles of cervix, head and neck cancers have been carried out in different studies associated to HPV infection. In this scenario, the HPV E5, E6 and

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E7 proteins modulate the expression of several cellular miRs. Deregulation of miRs expression might be used to identify cancer progression and also as potential target for therapy against HPV infection and cervical cancer development. The differential expression of a family of long non-coding mitochondrial RNAs (ncmtRNAs) in response to HPV infection has been recently reported. The expression profile of these transcripts allows distinguishing between normal, pre-tumoral and cancer cells. One of these transcripts, SncmtRNA-1, has been characterized as a regulator of cell cycle progression while two others, ASncmtRNA-1 and 2, has been suggested to act as tumor suppressors. HPV E2, E6 and E7 modulate the expression of this family of mitochondrial long ncRNAs. Further evidence suggests that other viruses such a Hepatitis B virus, Human T-cell lymphotropic Virus type 1 and Epstein Barr virus can also modulate the expression of these long ncRNAs in human cells. In this chapter we will discuss how the differential expression of both, microRNAs and long ncRNAs, in response to HPV infection, might serve as early biomarkers for progression of cervical dysplasia in PAP smears and biopsies allowing the detection of precursor lesion of cervical cancer.

NON-CODING RNAS AND CANCER

The central dogma of molecular biology indicates that the functional importance of genetic information lies in proteins encoded within it [1]. According to this concept, cellular functions would be defined by the presence or absence of certain proteins and RNAs would be mere intermediates in the process of gene expression [2]. Nevertheless, after the publication of the human genome in 2001 and a profound analysis of open reading frames (ORFs) of different organisms, it has been concluded that more than 90% of the genome are non-protein-coding sequences [3, 4]. Within the non-coding sequences, elements non-translated into proteins, we can find several RNAs with structural or regulatory functions [5-7]. Among the structural RNAs we can mentione for example transfer RNAs (tRNAs), ribosomal RNAs (rRNAs) and small nuclear RNAs (snuRNAs) [5-7]. On the other hand, regulatory RNAs, or non-coding RNAs (ncRNAs) which modulate gene expression and can be classified according to their sizes, as small non-coding RNAs (sncRNAs) [8, 9] and long non-coding RNAs (lncRNAs) [6, 10, 11]. Small ncRNAs can be subdivided in two groups: microRNAs (miRs) with an average size of 22-23 nt and piwi-interacting RNAs (piRNA) with an estimated size of 26-31 nt [12-14]. Conversely, long ncRNAs correspond to molecules with a size over 200 nt long [15, 16]. There is a group of lncRNAs that correspond to antisense RNAs, which are complementary to coding transcripts, and thereby induce their degradation or negatively regulate their translation [17-19]. Regarding the synthesis of ncRNAs, the participation of the enzymes RNA pol II and RNA pol III have been described [6, 20-22]. Moreover, there are reports indicating that these classes of transcripts are processed including splicing, polyadenilation and addition of 5'cap [7, 23]. Giving the increasing importance that ncRNAs and their relationship to cancer during the past years (Figure 1), this chapter will be focused on the state of the art on this topic, with emphasis on the role of miRs and lncRNAs in cancer development associated to the HPV.

MICRORNAS AND THEIR ROLE IN CANCER

MiRs are small non-coding RNAs, which regulate gene expression at a post-transcriptional level [12]. The first findings that indicate the abundance of miRs genes came from sequencing small RNAs from mammals, flies, and worms [13, 14]. Hundreds of mammalian miRs have now been identified by Sanger sequencing of cloned small RNA-derived cDNAs [12-15, 17, 24, 25]. Some miR, however, are expressed only in a limited number of cells or at a limited time during development, arising a problem for their detection. MiRs are involved in a series of cellular processes such as differentiation, proliferation and apoptosis [13, 14]. Interestingly, the expression profile of these molecules is deregulated in a large number of pathologies, such as psoriasis [26], cardiovascular disease [27, 28] and also in different types of cancer [29-32].

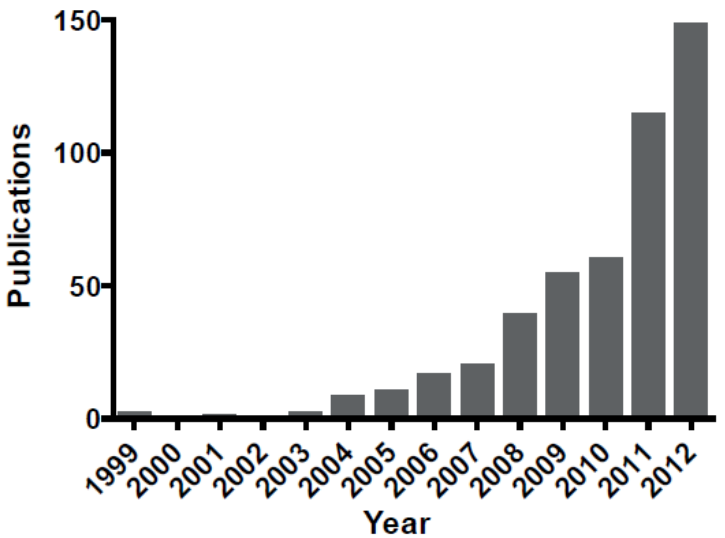

Figure 1. Number of publications related to non-coding RNAs associated to cancer. A search on the Pubmed database from the National Center for Biotechnology of Information (NCBI) was performed using the terms "ncRNA" or "non-coding RNA" or "noncoding RNA" or "non-protein-coding RNA" AND cancer, with limit date December 31, 2012. Data was corroborated performing the same search on the Gopubmed database. The data was plotted as number of publications per year, starting from 1999.

The genesis of these molecules involves RNA pol II that transcribes a primary miR (pri-miR), which contains one or more segments that fold into an imperfect hairpin (Figure 2). For canonical metazoan miRs, the RNase III enzyme Drosha together with its partner, the RNA-binding protein DGCR8, recognize the hairpin and subsequently Drosha cleaves both strands 11 bp from the base of the stem, leaving a 5' phosphate and 2-nt 3' overhang [33, 34]. The released precursor miR (pre-miR) hairpin is then exported to the cytoplasm by Exportin-5 [35, 36], where the RNase III enzyme Dicer cleaves off the loop of the pre-miR, ~22 nt from the Drosha cut again leaving a 5' monophosphate and 2-nt 3' overhang [37, 38]. The resulting miR:miR* duplex, comprising ~22-bp is associated with the Argonaute (Ago) proteins in such a manner that the miR strand is usually the one that is stably incorporated, while the miR* strand dissociates and is subsequently degraded. In addition to canonical miRs, some

miRs mature through pathways that bypass Drosha/DGCR8 recognition and cleavage [39]. Finally, partial complementarity of the miR and its mRNA target, usually at the 3' UTR, induce translational repression and in some cases, degradation of the transcript [29, 30].

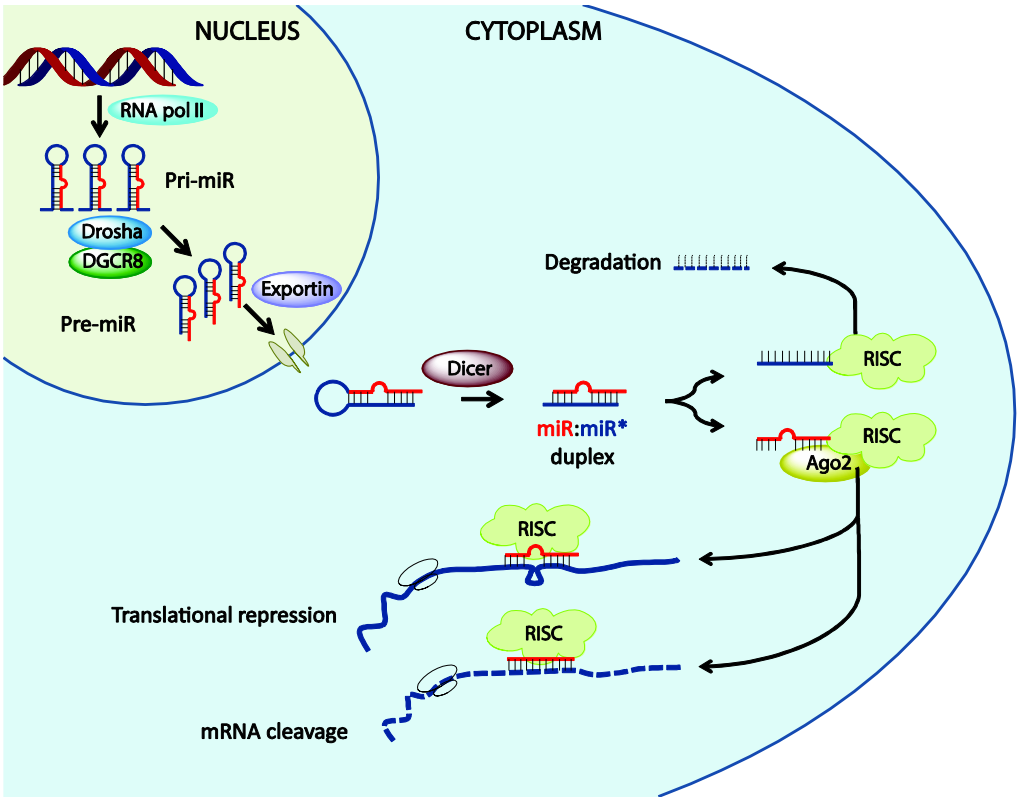

Figure 2. Biogenesis and functions of cellular microRNAs. In the nucleus, RNA pol II transcribes a pri-miR, which folds as an imperfect hairpin. Drosha, together with its partner DGCR8, recognizes the hairpin and subsequently cleaves both strands from the base of the stem leaving a 5' phosphate and 2-nt 3' overhang in a structure named pre-miR, which is exported to the cytoplasm by Exportin-5. There, Dicer cleaves off the loop of the pre-miR, 22 nt from the Drosha cut, again leaving a 5' monophosphate and 2-nt 3' overhang. The resulting miR:miR* duplex, comprised about 22-nt taken from each arm of the original hairpin, associates with the Ago protein in such a manner that the miR strand is usually the one that is stably incorporated, while the miR* strand dissociates and is subsequently degraded. The mature miR will then associate with other proteins of the RNA Induced Silencing Complex (RISC), which directs it to a target mRNA. Finally the perfect base complementarity of the miR and its target, usually the 3' UTR of an mRNA, induce the degradation of the transcript, while partial complementarity induces translational repression.

Since the first reports describing the existence of miRs in mammals [39, 40], an increasing number of publications showing a deregulation of a great number of miRs associated to human cancer is found in the literature [29, 30]. Some of the different types of cancer associated to deregulation of miRs are non-melanoma skin cancer [41], thyroid [42], breast [43], ovarian [44], glioblastome [45], lung [47], gastric [48, 49], melanoma [50], kidney [51] and cervical cancer [52, 53]. Some of the best-characterized miRs involved in cancer are summarized in Table 1. In all these types of cancers there appears to be a common feature: deregulation of miRs that participate in tumorigenesis [54, 55], apoptosis [56, 57] and

in metastasis capacity [58, 59]. Interestingly, up or down-regulation of miR expression associated to malignant processes now is being used to improve diagnostic and prognosis of different types of cancer. For examples: miR-200, has being used in ovarian cancer diagnostics [44], miR-34c, miR-375, miR-17-5p in lung cancer [47, 60, 61], miR-148b in breast cancer [62], miR-146, miR-371a-3p and miR-372 in thyroid cancer [42, 63]. Additionaly, miR-182 and miR-96 has being studied for their role in ovarian cancer development [64, 65], miR-148a, miR-210, miR-21, miR-10b, miR-8, and let-7 in breast cancer [66, 67], miR-10b, miR-34a, miR-376a, miR-21 and miR-211 in glyoblastoma [68-72]. A number of miRs related to cervical cancer have been analyzed and the great majority of them participates in cancer development or has metastatic potential. Furthermore, miR-21, miR-143, miR-200a, miR-345, miR-9, miR-372, miR-218, miR-133b, miR-19a, miR-19b and miR-203 have been implicated in different aspects of cervical cancer development and most of them are deregulated by one of the HPV oncoproteins and are potential tools for diagnostic and prognosis [52, 53, 73-75].

Table 1. Representative microRNAs associated with human cancer

Name	Use and/or Function	Associated cancer	References
miR182	Cancer progression	Ovarian	64, 65
miR96	Cancer progression	Ovarian	64, 65
miR371a-3p	Diagnostic/Cancer progression	Thyroid	42, 63
miR19a	Diagnostic/Cancer progression	Adenomas/Cervical	52, 53
miR200a	Cancer progression	Cervical	52, 53
miR146b	Diagnostic/Cancer progression	Thyroid	42, 63
miR210	Diagnostic/Cancer progression	Lung	47
miR183	Diagnostic/Cancer progression	Lung	47
miR200	Diagnostic/Tumor suppressor	Lung/Ovarian	44, 47
miR486-5p	Diagnostic/Cancer progression	Lung	61
miR148b	Diagnostic/Cancer progression	Breast	62
miR17-5p	Diagnostic/Cancer progression	Lung	47
miR34	Diagnostic/Tumor suppressor	Gastric/Lung	48, 60
miR133b	Cancer progression	Cervical	52, 53
let7	Tumor suppressor/ Cell differentiation	Breast	66, 67
miR125b	Cancer progression	Cervical	79, 86
miR21	Diagnostic/Cancer progression	Glioblastoma/Cervical	45, 208
miR143	Diagnostic/Cancer progression	Cervical	52, 53
miR23b	Cancer progression	Cervical	204
miR29	Tumor suppressor	Cervical	80
miR27a	Cancer progression	Gastric	49

MicroRNAs involved in cell proliferation, such as miR-15a, miR-16, miR-17 and miR-148a are up-regulated in cervical cancer cell lines [53, 76-78]. Interestingly, miR-29, miR-218, miR-125b, miR-145, miR-328, miR-199, miR-574 and miR-455, which suppress cell proliferation, are down-regulated [76, 79-81]. Concerning the ability of papillomaviruses to deregulate the expression of miRs, the oncoproteins E5, E6, E7 and the capsid protein L2 are involved in down or up-regulate of a subset of miRs [82-87]. The functional importance of these processes during viral replication and malignization of cervical cells is discussed in

detail in the following sections of this chapter. Currently, worldwide researchers are investigating the ability of oncogenic and not oncogenic viruses to modulate the expression of cellular miRs and the ability to generate their own repertoire of miRs in order to regulate the transcriptional machinery of the infected cell. The consequence is the ability to escape immune vigilance, to maintain long latency periods or to regulate their own transcriptional machinery [88-90].

LONG NON-CODING RNAS AND THEIR ROLE IN CANCER

LncRNAs are considered molecules with a size over 200 nt [16, 91]. Currently more than 3,000 lncRNAs have been described in humans [17-19, 24, 25]. The majority of lncRNAs are transcribed by RNA pol II and then processed as an mRNA precursor, including splicing, polyadenylation and 5′-capping [6, 7, 21, 22, 26]. In addition, some types of lncRNAs are transcribed by RNA pol III indicating an over activation of lncRNAs in cancer cells [20, 92, 93]. BC200 is a cytoplasmic lncRNA found in neurons of primate nervous systems and human cancers, but not in non-neuronal organs [92, 93]. Unlike the majority of lncRNAs, BC200 is transcribed by RNA pol III and shares unique homology with human Alu elements [92, 93]. Similarly, the lncRNA HULC also shares homology with mobile DNA, containing a long terminal repeat (LTR) retroelement [94, 95]. The BC200 lncRNA has been characterized as a negative regulator of eIF4A-dependent translation initiation [96]. Since that many whole transcriptome sequencing methods were developed to enrich poly(A) purified transcripts, RNA pol III transcripts may have been excluded from previous analysis. This suggests that other, as yet unidentified RNA pol III depending lncRNAs, deregulated in cancer and that participate in tumorigenesis [97].

Similar to miRs, many of the lncRNAs are expressed in different stages of development or are tissue-specific [98-102]. The majority of them is involved in cellular differentiation and also has been described in human pathologies including cancer, participating in pathogenesis and maintenance of the tumorigenic potential of transformed cells [103, 104]. In the past few years a large number of lncRNAs have been identified, and the specific function of only some of them are known [6]. The expression profiles of well characterized lncRNAs such as HOTAIR [22, 105], ANRIL [97, 106-109], MALAT-1 [97, 109-114], AK023948 [115], PANDA [116], SRA-1[117], PCAT [118], MEG3 [97, 109], GAS5 [97, 109, 119, 120], H19 [121, 122], UCA-1 [123], P15AS [124], HULC [94] and CDRU [125] have been associated to different types of cancer and are summarized in Table 2.

An increasing number of lncRNAs correspond to antisense ncRNAs with perfect complementarity with their corresponding mRNA. The formation of a double-stranded RNA can negatively regulate gene expression by inducing degradation of the mRNA [100, 101]. This antisense lncRNAs can act in cis, when transcribed from the opposite strand of the same genomic loci, or in trans, when transcribed from a distant one [100-102]. Many tumor suppressor genes express antisense ncRNAs, such as p15INK4B/ ANRIL and AIR/HOTAIR [98, 99]. Another interesting example is the tumor suppressor PTEN (Phosphatase and Tensin) and PTENP1 (PTEN pseudogene 1). PTENP1 is a lncRNA transcribed from a pseudogene with a high complementarity to PTEN. This tumor suppressor is involved in cell cycle regulation, preventing cell division and is one of the targets of the oncomiR miR-21

[102]. PTENP1 acts like a "sponge" trapping miR-21 diminishing the effect of oncomiRs on PTEN [102]. PTENP1 is down-regulated in prostatic and lung cancer [102, 105]. A new family of sense and antisense non-coding transcripts derived from mitochondria has been described. Within this family, Sense non-coding mitochondrial RNA (SncmtRNA) is associated to cellular proliferation and is found over-expressed in leukemia, breast, prostatic, colon, hepatocarcinoma, ovarian, brain, kidney cancer, lymphoma and cervical cancer [126]. Antisense non-coding mitochondrial RNA (ASncmtRNA) have a potential role as tumor suppressor and they are found repressed in leukemia, breast, prostatic, colon, hepatocarcinoma, ovarian, brain, kidney cancer, lymphoma and cervical cancer [126], representing a universally suppressed molecule across a high number of different types of cancer. The role of these molecules in cervical cancer is discussed in detail on this chapter.

Efforts to evaluate the diagnostic potential of the expression profiles of lncRNAs have been reported. Thus, expression patterns of MALAT-1, HOTAIR y HULC have been used to evaluate the prognosis of patients with lung cancer [102, 105], and hepatocarcinoma [94, 102, 105]. The expression patterns of the SncmtRNA and ASncmtRNAs are also being evaluated, mainly in progression of cervical cancer [126], as well as in other pathologies of the urinary tract [127]. Probably, in a near future the use of expression profiles of ncRNAs will be as important or complementary to current detection systems based on the presence or absence of proteins or in morphological changes analyzed by H&E [128].

NON-CODING RNAS ENCODED BY ONCOVIRUSES

The First report on agents capable of transmitting cancer was described in 1909 by Rous [129]. Up to date, it is recognized that about 20-25% of human cancers are associated with viral infections [130]. Human T-cell Leukemia Virus type 1 (HTLV-1) was the first described retrovirus associated to adult T-cell leukemia [131, 132]. Later, another retrovirus, HTLV-2, was also associated with human diseases [133]. Another group of viral agents involved in human pathologies are the DNA viruses, responsible of a great number of mammalians cancers. Thus the Herpes Simplex Virus type 2 (HSV2) was associated to cancer development, including cervical cancer [134, 135]. Years later, HPV, another DNA virus, was found to be the etiological agent of cervical cancer [136]. This major breakthrough in cervical cancer carried out by zur Hausen was recognized by the Nobel Academy in 2008.

Depending on the type of genetic materials, human oncogenic viruses can be divided into DNA or RNA oncogenic viruses. Examples of the first group are the Epstein-Barr virus (EBV), the etiological agent of Burkitt lymphoma, nasopharyngeal carcinoma (NPC) and a type of Hodgkin disease [137-144], the Kaposi Sarcoma associated Herpesvirus (KSHV), involved in the development of Kaposi´s sarcoma and a type of B-cell lymphoma [145-147], the Hepatitis B virus (HBV), the etiological agents of liver cancer [148-150] and HPV, the etiological agent of cervical cancer [136, 151, 152]. The RNA oncogenic viruses include HTLV-1, associated to Adult T-cell leukemia/lymphoma and Tropical Spastic Paraparesis [131, 153] and Hepatitis C virus (HCV), whose persistent infection causes cirrhosis and liver cancer [154-156].

Table 2. Some long non-coding RNAs associated with human cancer

Name	Function	Associated cancer	References
PTENP1	Tumor suppressor	Prostatic/Lung	102, 105
HULC	Metastasis	Liver/Colorectal	94
HOTAIR	Metastasis	Breast/Colorectal/Pancreatic/Liver	22, 105
ANRIL	Cancer progression	Prostatic/Glioma/Leukemia/Breast	97, 106-109
MALAT1	Metastasis/Cancer progression	Lung/Colon/Prostatic/Cervix/Liver	97, 109-114
AKO23948	Tumor suppressor	Thyroid	115
PANDA	Resistence to chemotherapy	Breast	116
SRA1	Metastasis	Breast	117
PCAT1	Cell proliferation	Prostatic	118
GAS5	Tumor suppressor	Prostatic/Melanoma/Lymphoma/Breast	97, 109, 119, 120
H19	Cell proliferation	Breast/Colon	121, 122
UCA1	Cell proliferation/Cancer progression	Bladder	123
P15AS	Cell proliferation	Leukemia	124
CUDR	Resistence to chemotherapy/Cancer progression	Squamous carcinome	125
MEG3	Tumor suppressor	Brain/Bladder/Liver	97, 109
SncmtRNA	Cell proliferation	Leukemia/Cervical/Breast/Prostatic/liver/Ovarian	126, 213
ASncmtRNA	Tumor suppressor	Leukemia/Cervical/Breast/Prostatic/liver/Ovarian	126, 213

Table 3. Principal microRNAs encoded by human oncogenic viruses

miRNA	Virus	Function	References
BART-5	EBV	Antiapoptotic	163, 164, 165
BART-6	EBV	Latency	163, 164, 165
BART-2	EBV	Immune evasion	163, 164, 165
BHRF-1	EBV	Latency	163, 164, 165
miR-k12	KSHV	Viral replication	159, 160, 161
miR-k2	KSHV	Viral replication	159, 160, 161
miR-k4	KSHV	Latency	159, 160, 161
miR-k8	KSHV	Viral replication	159, 160, 161
miR-k7	KSHV	Latency	159, 160, 161
miR-k5	KSHV	Antiapoptotic	159, 160, 161
miR-k9	KSHV	Antiapoptotic	159, 160, 161
miR-k3	KSHV	Antiapoptotic	159, 160, 161
miR-k1	KSHV	Antiapoptotic	159, 160, 161
miR-k10	KSHV	Antiapoptotic	159, 160, 161
miR-k10	HSV	Viral replication	167, 168
miR-H6	HSV	Viral replication	167, 168
miR-k12	HSV	Viral replication	167, 168
miR-H3	HSV	Neurovirulence	167, 168
miR-H4	HSV	Neurovirulence	167, 168
miR-93	HTLV-1	Antiapoptotic	173, 174
miR-130b	HTLV-1	Antiapoptotic	173, 174
miR-21	HTLV-1	Antiapoptotic	173, 174
miR-24	HTLV-1	Antiapoptotic	173, 174
miR-146A	HTLV-1	Antiapoptotic	173, 174
miR-155	HTLV-1	Antiapoptotic	173, 174
miR-29	HTLV-1	Immune evasion	173, 174
miR-142-5p	HTLV-1	Latency	173, 174

MICRORNAS AND ONCOVIRUS

EBV was the first reported oncogenic virus that encodes miRs [157, 158]. Currently, there are more than twenty five described miRs encoded by EBV [158], sixteen miRs encoded by HSV-1, thirteen miRs encoded by HSV-2 and twelve miRs encoded by KSHV [158]. In contrast to other DNA viruses, HPV is a paradigm due to the fact that it is the most characterized etiological agent associated with cervical cancer development and yet, there are no reports revealing miRs encoded by this virus [158]. A summary of the main miRs encoded by human DNA and RNA oncoviruses is shown in Table 3 and we will briefly describe some of their main functions.

During the latency period, KSHV differentially express twelve miRs besides the latency proteins [159]. Thus, miR-K4 and miR-K7 regulate the expression of viral mRNAs and the

later is involved in immune evasion [160, 161]. On the other hand, miR-K5, miR-K9 and miR-K10 inhibit the apoptotic ability of the infected cells [161]. MiR-K10 and miR-K12 are involved in the latency-lytic replication transition [159]. Furthermore, small antisense RNAs that target miR-K2, miR-K4 and miR-K8 are expressed from the viral genome and are associated with the restriction of lytic replication [159]. MiR-K1, miR-K3 and miR-K10 are involved in apoptosis inhibition [159] and attenuation of cell cycle arrest [159], besides inhibiting the p21 protein located down-stream in the activation pathway of P53 [162].

EBV encodes three miR-BHRF1s and twenty-two miR-BARTs, which are differentially expressed during the viral cycle [163-165]. MiR-BHRF1 is expressed during late latency and miR-BART, in previous phases [163-165]. MiR-BART2 has a role in the immune evasion of infected cells [163-165]. EBV also induces over-expression of miR-155, a cellular transcript involved in viral replication [166].

HSV express six miRs, involved in different stages of the viral cycle. MiR-H6 maintains a latent infection and miR-H10 and miR-H12 are associated to the activation of the lytic phase of viral replication [167]. HSV-1 and 2 encode miR-H3 and miR-H4, which are associated to the neurovirulence [168] and HSV-2 also expresses miR-H6, which participates in viral replication [168].

Although there are no reports on HCV-encoded miRs, the virus induces over-expression of miR-122, a cellular transcript necessary for viral replication [169, 170]. The cellular miRs, miR-196b, miR-199a-3p and miR-29 are also involved in antiviral defense, inhibiting replication and viral pathogenicity [171].

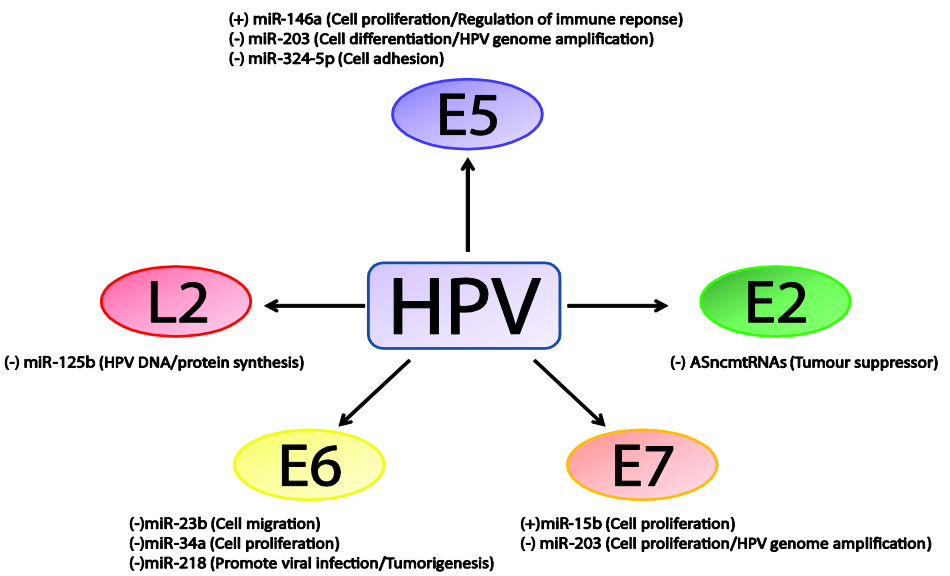

Figure 3. Principal microRNAs modulated by HPV proteins. Only HPV proteins that directly influence the expression of cellular miRs are indicated. In brackets, the putative function of these miRs are shown.

To date, there are no reports on the existence of HBV-encoded miRs. Nevertheless, this virus down-regulates the expression of cellular miR-199a-3p, miR-210, miR-575, miR-4294 and miR-125a-5p, which inhibit HBV replication [172]. HBV also induces over-expression of the cellular transcripts miR-122, miR-22 and miR-99 [172].

HTLV-1 encodes miR-93 and miR-130b that target the tumor suppressor TP53 [173] and induces over-expression of the cellular miR-93, miR-21, miR-24, miR-146a, miR-155 and miR-130b involved in the restriction of tumor suppressor activity [174]. Interestingly, cellular miR-15a and miR-16-1 are down-regulated in leukemia cells [173].

LONG NON-CODING RNA AND ONCOVIRUS

In addition to microRNAs, EBV encode two lncRNAs, named EBER1 and EBER2, which are highly expressed in latently infected cells [175]. They form complexes with many cell proteins, such as RNA-activated protein kinase R (PKR) [176, 177], ribosomal protein L22 [178-180], La [181] and the retinoic acid-inducible gene I (RIG-I) [182]. In this manner, EBERs act upon direct binding with their targets [175]. It has been demonstrated that EBERs confer resistance to interferon (IFN-gamma)-induced apoptosis by binding PKR and inhibiting its phosphorylation [177]. Although EBERs are not essential for cell transformation [183], it has been reported that a recombinant EBV, lacking EBERs, have a diminished capacity of transformation compared to wild-type EBV [184]. Although there is a debate concerning the real tumorigenic potential of EBERs, it has been demonstrated that EBER2 plays a critical role in efficient B-cell growth transformation, whereas EBER1 is dispensable [184].

To date, there are no reports on lncRNAs encoded by HBV. However, this virus is capable to modulate the expression of the lncRNA known as HULC (highly up-regulated in liver cancer), which is strongly associated to the development of liver cancer and is over-expressed in HBV-infected cells [185]. A new lncRNA related to liver cancer and HBV pathologies and referred to as lncRNA-HEIH (long non-coding RNA highly expressed in hepatocarcinoma) is polyadenilated and it might be transcribed by the polymerase II [186]. LncRNA-HEIH is found in the nucleus and the cytoplasm, where promotes cell proliferation and control of cell cycle arrest [186].

KSHV encodes a lncRNA known as PAN RNA (poly-adenylated nuclear RNA) [187], which is polyadenilated and transcribed by RNA polymerase II. In the nucleus, PAN forms a speckled pattern typical of U snuRNA [187], and the KSHV protein ORF57 induces the nuclear accumulation of PAN [187]. ORF57, also known as MTA, is the earliest KSHV regulatory genes to be induced [188, 189] and required for lytic virus replication, suggesting that PAN RNA is involved in this process [190, 191]. Whether the study of ncRNAs encoded by viruses will be in the future used as tools for virus related cancer diagnostic is still a matter of discussion.

DEREGULATION OF MICRORNAS BY HUMAN PAPILLOMAVIRUS

HPV is the etiological agent of cervical cancer. Neoplastic progression occurs through the concerted action of the viral oncoproteins E6 and E7, which bind and stimulate the degradation of the tumor suppressor p53 and retinoblastoma (Rb), respectively [192-195]. These two events lead the cell to genomic instability due to an impaired DNA response to damage and a dysfunctional G_1/S checkpoint. However, increasing amounts of evidence suggest that HPV infection by itself is not enough to provoke malignant changes and thereby additional host genetic variations induced by the viral oncoproteins would be important in the development of cervical cancer. In this section we will discuss the basic biology related to deregulation of miRs expression in response to HPV infection. The potential use of these differential profiles as a tool for diagnosis, prognosis and management of cervical dysplasia and cancer is discussed in the final section of this chapter. Some of the best-characterized microRNAs modulated by HPV proteins are summarized in Figure 3.

The E6 Protein and MicroRNAs

Recently, studies on the role of cellular miRs in the regulation of the HPV replicative cycle and carcinogenesis have been reported. The relationship between HPV-16 infection and deregulation of miR-218 was reported by Martinez et al. [83]. Down-regulation of miR-218 was induced only by the expression of the E6 protein from HPV-16. In striking contrast, the E6 protein from low-risk (lr) HPV-6 did not alter the expression levels of miR-218. Interestingly, the target of miR-218 is the mRNA of laminin 5 β3 (LAMB3), a protein that when over-expressed can potentiate viral infection of surrounding tissues and tumorigenesis [83]. A recent clinical study evaluates the expression levels of miR-218 in 78 samples from patients with different degrees of cervical intraepithelial neoplasia (CIN). The results show that miR-218 was down-regulated in samples from patients infected with high-risk (hr) HPV compared to those infected with low or intermediate-risk HPV, or non-infected samples [196]. A later study also shows down-regulation of miR-218 in samples from patients with HPV-associated cervical cancer tissues. In addition, miR-433 was also down-regulated, while miR-16, miR-21, miR-106b, miR-135b, miR-141, miR-223, miR-301b and miR-449a were over-expressed in cancer and cervical dysplasia compared to normal cervical tissue [197]. Zheng et al. described up-regulation of miR-15b, miR-16, miR-146a and miR-155 whereas miR-126, miR-143, miR-145, miR-424 and miR-218 were down-regulated in samples obtained from cervical cancer biopsies. Interestingly, no differences in expression patterns could be observed when comparing cells with integrated or episomal HPV genome [87]. From the list, miR-143 and miR-145 were analyzed in detail and they were found to be suppressors of cell growth, while miR-146a is involved in cell proliferation, suggesting that the differential expression of these molecules is a fine-tuning in carcinogenesis. Furthermore, miR-143 and miR-145 were down-regulated in HPV-induced pre-neoplastic lesions, suggesting that down-regulation of these molecules is an early step in cancer development and thereby can be potentially used as early markers [87].

A recent study established the miRs expression profiles of biopsies obtained from patients with oral (OSCC) and pharyngeal cell carcinoma (PSCC), compared to normal

epithelium. Surprisingly, many of the miRs that presented deregulation associated to HPV infection were similar to those found in cervical cancer samples. Thus, miR-145, miR-125a, miR-126, miR-127-3p and miR-379 were down-regulated in cancer tissues [198]. Up-regulation of miR-363 was also found in PSCC and confirmed by the group of Khan et al. [199]. This group determined that miR-363, miR-33 and miR-497 were up-regulated, whereas miR-155, miR-181a, miR-181b, miR-29a, miR-218, miR-222, miR-221 and miR-142-5p were down-regulated in HPV-positive cells compared to both HPV-negative SCC of head and neck (SCCHN) and normal oral keratinocytes. HPV E6 silencing in SCC2 cells or expression of E6 in HFK cells allowed the authors to suggest that E6 protein is involved in deregulation of the above-mentioned miRs [199]. More recently, down-regulation of miR-145 in HPV-positive cancer cells was proposed as a novel target for cervical cancer therapy due to its role in the suppression of the p53 protein [200].

In 2009, Zheng et al. described that E6 protein from HPV-16/18 inhibits the expression of the tumor suppressor miR-34a. MiR-34a is a transcriptional target of the transcription factor p53, and thereby the destabilization of p53 by E6 results in promotion of cell proliferation [201]. Reinforcing this data, the expression level of pri-miR-34a was found reduced in cervical tissues of patients with cervical cancer and pre-neoplastic lesions compared with normal cervical epithelium. Down-regulation of miR-34a also correlates with the malignancy of the lesion, from CIN 1 to CIN 3 [202]. Thus, expression levels of miR-34a can be used as precancerous lesion detection, even before observation of morphological changes.

Finally, a recent report indicates that HPV-16 E6 decreases the expression levels of miR-23b, which has been previously associated to cervical cancer [203]. The target of miR-23b is the mRNA of urokinase-type plasminogen activator (uPA), and consequently down-regulation of miR-23b induces up-regulation of uPA, increasing migration of human cervical carcinoma cells. Interestingly, p53 acts as transcriptional factor for expression of miR-23b. Thus, degradation of p53 mediated by HPV E6 indirectly affects miR-23b expression. In this manner, the p53/miR-23b/uPA triad is involved in cervical cancer development [204].

The E7 Protein and MicroRNAs

In 2010, a report indicated that the E7 protein from HPV-16 and 31 was responsible for down-regulation of miR-203, with E6 having a minimal effect. One target of miR-203 is the transcription factor p63, which regulates the balance between epithelial proliferation and differentiation. Thus, in HPV-infected epithelia, the expression of E7 blocks expression of miR-203, which leads to increased levels of p63, causing cells to remain active in cell cycle and HPV genome amplification [84]. Another interesting finding is that miR-34b, miR-124a and miR-203 gene are hypermethylated in samples from cancer precursor lesions and cervical tumors versus normal control samples, demonstrating that epigenetic alterations of miRs are also involved in cervical carcinogenesis associated to hr-HPV genotypes [205]. Moreover, miR-15b was up-regulated in biopsies from patients with anal carcinomas associated to hr-HPV infections, which leads to a down-regulation of cyclin E1, which plays a crucial role in G_1/S transition. Knockdown of E7 induce down-regulation of miR-15b with a simultaneous down-regulation of cyclin E1, as observed in CasKi cells, suggesting a role for this molecule as an inhibitor of cell cycle progression [85].

Other HPV Proteins Involved in MicroRNAs Deregulation

E6 and E7 proteins are key players during cellular transformation upon HPV infection, and thereby a direct modulation of miRs expression by these proteins would be expected. Thus, it was described that the expression of miR-125b was diminished in CIN 1 lesions, specifically in koilocytes, where the productive infection of HPV takes place. Remarkably, down-regulation of miR-125b was associated to the structural protein L2. The expression of HPV-16 L2 protein in NIH3T3 cells was enough to induce down-regulation of miR-125b. This process occurs in cells involved in productive infection of HPV virions and thereby a role of miR-125b in regulation of the viral DNA and proteins synthesis was suggested [86].

In 2011 the group of Auvinen et al. described for the first time the involvement of the E5 protein in deregulation of miRs. A direct correlation between down-regulation of miR-203 and miR-324-5p and up-regulation of miR-146a in HaCaT cells, depending on E5 expression was found [82]. It is interesting to note that down-regulation of miR-203 is also a property of the E7 protein [84], suggesting that the oncogenic properties of both proteins are at least partially, mediated by miR-203. Interestingly, up-regulation of miR-146a was also observed in breast, pancreas and prostate cancers [206], suggesting that cellular factors none related to HPV E5 are able to induce similar molecular changes on cell transformation.

Another interesting finding is the discovery that in beta HPV-8 infected cells, miR-17-5p, miR-21 and miR-106a were up-regulated while, miR-155 and miR-206 were down-regulated in HPV8-CER (E6/E7/E1/E2/E4=CER) mice after UV irradiation being the first report on how a cutaneous HPV modulate the expression of miRs [207]. Other authors reported up-regulation of miR-21 in cervical cancer lesions associated to HPV, a molecule that regulates proliferation, apoptosis and migration of HPV-16 cervical squamous cells and probably by targeting Chemokine (C-C motif) ligand 20 (CCL20) [197, 208]. Thus, miR-21 could also represent a universal miR target for potential clinical diagnosis of different types of HPV.

Two independent investigations made by the group of Xie et al. demonstrated deregulation of miR-100 and miR-29 [80, 209]. Mir-100 was reduced in cervical cancer in a gradual tendency from low-grade CIN to high-grade CIN and carcinoma *in situ* associated to HPV infection. Interestingly, changes in miR-100 expression were not attributable to E6 or to E7 oncoproteins [209]. On the other hand, miR-29 was down-regulated in samples from patients with different degrees of lesion suggesting that this molecule could be associated to cell cycle progression and promotion of malignant transformation induced by HPV [80].

Conclusively, miR expression profiles will be in the near future a complementary diagnostic or prognostic tool in cancer associated to HPV. In addition, rigorous and strict detection techniques and controls must be used in order to corroborate up-or down-regulation of a specific miR, since expression levels of these molecules are quite variable between tissues. The use of miR arrays has also been proposed, due to the inherent problem of the internal controls when using qRT-PCR [210]. Much work has to be done in this area, but we anticipate that increasing amount of clinical investigations will improve the diagnosis and prognosis of HPV-associated cancer based on miR expression. Principal cellular microRNAs deregulated by HPV are summarized in Table 4.

NON-CODING MITOCHONDRIAL RNAS

More than ten years ago, a research group described for the first time a particular mitochondrial non-coding RNA in mouse cells [211]. This transcript contains a region corresponding to the 16S mitochondrial ribosomal RNA (16S mtrRNA) and an inverted repeat (IR) linked to the 5' end of the 16S mtrRNA and generated by transcription of the L strand of the mtDNA [211]. A related transcript associated to cell proliferation was described in human [212]. Later, it was reported that human cells express a family of ncmtRNAs, which are differentially expressed in normal, and tumor cells [126]. This family of long ncmtRNAs comprises sense and antisense transcripts. The Sense ncmtRNA-1 (SncmtRNA-1) contains an IR of 815 nt linked to the 5' end of the 16S mtrRNA [213]. The IR is fully complementary to an internal region of the 16S mtrRNA, forming a long stem-loop structure resistant to RNase A digestion. The SncmtRNA-1 is expressed in normal proliferating cells and tumor cells but not in resting cells. Furthermore, normal proliferating cells express two antisense transcripts named ASncmtRNA-1 and -2 that contain IRs of 316 and 545 nt, respectively [126, 212]. In striking contrast, in tumor cell lines as well as in tumor cells present in human biopsies of different types of cancer the ASncmtRNAs are down-regulated [126]. These observations suggest that down-regulation of the ASncmtRNAs is an essential step during neoplastic transformation and progression and led the authors to suggest the possibility that these transcripts are tumor suppressors derived from the mitochondrial genome [126, 213]. Down-regulation of the ASncmtRNAs is very similar to the fate of protein tumor suppressors [214] or miRs [215] during neoplastic transformation. This is due to the fact that tumor suppressors lose their function as a result of mutations or deletions of the coding locus. Furthermore, the synthesis of ncmtRNAs require mitochondrial transcription since HeLa cells treated with ethidium bromide, a chemical agent capable of blocking mitochondrial transcription [216-218], leads to down-regulation of the SncmtRNAs, without changes in the expression of nuclear transcripts, such as 18S rRNA [212]. Additionally, these mitochondrial transcripts exit the organelle and are found in the cytoplasm and in the nucleus associated to chromatin and nucleoli [219].

HPV E6 and E7 Induce Expression of a New Sense ncmtRNA

The ability of the hr-HPV oncogenes E6 and E7 to block the function of the tumor suppressor p53 and pRb together with up-regulation of telomerase are critical steps for human keratinocytes immortalization [136, 152, 220-222]. Keratinocytes immortalized with HPV-16/18 express high levels of the SncmtRNA-1 and down-regulate the expression of ASncmtRNAs, like their tumor counterparts SiHa and HeLa cells (derived from patients with cervical carcinoma infected with HPV-16 and HPV-18, respectively)[213]. The exclusive expression of both, hr-HPV E6 and E7, is not enough to down-regulate the ASncmtRNAs in human keratinocytes [213]. Therefore, down-regulation of ASncmtRNAs seems to be one key early event during immortalization and neoplastic transformation. In addition, these results reinforce the hypothesis that the antisense transcripts would be acting like tumor suppressors, because the virus suppresses its expression in a similar manner to the functional deletion of the classic tumor suppressors p53 and pRb. Interestingly, keratinocytes expressing hr-HPV

E6/E7 or hr-HPV-immortalized keratinocytes also express a second sense transcript, named Sense ncmtRNA type 2 (SncmtRNA-2) [213]. This transcript is over-expressed in HPV-16/18 immortalized cells, but not in normal or tumor cells, such as HFK, SiHa and Hela cells. Since the SncmtRNA-2 exhibits 99,7% identity with the sequence of the human 16S mtrRNA gene and 100% identity with SncmtRNA-1, one would expect that this transcript will be localized in mitochondria, as is the case of SncmtRNA-1 and the ASncmtRNAs. Interestingly however, SncmtRNA-2 is found only in the cytosol fraction. The IR of SncmtRNA-2 is 63 nt shorter than the IR of the SncmtRNA-1 (752 and 815 nt, respectively), suggesting that the SncmtRNA-2 originates from SncmtRNA-1 [213]. Therefore, we postulated it seems reasonable to hypothesize that SncmtRNA-2 could originate from SncmtRNA-1 by an unknown reaction similar to the cleavage-and-ligation reactions (editosome) necessary for the edition of kinetoplastid transcripts in Trypanosoma and Leishmania [223-225]. Furtheremore, the results suggest that this type of processing occurs outside mitochondria [213].

Previous reports have described miRs encoded within lncRNAs or functional regulation of cellular and viral miRs by the lnc transcripts [226]. *In silico* analysis of this 63 nt sequence revealed that this fragment is highly complementary to miR-620 which is involved in the silencing of more than 100 target mRNAs, including the mRNA of promyelocytic leukemia (PML) protein. These proteins are the scaffold of PML nuclear bodies found in tumor cells [227, 228]. PML nuclear bodies are important structures involved in HPV replication and several reports indicate that the E6 and E7 oncoproteins are localized in these nuclear structures [229-231]. E6 and E7 are needed for the expression of SncmtRNA-2 and therefore it is tempting to suggest that the 63-nt fragment released from SncmtRNA-1 is required to work as "sponge" [232-234] to trap miR-620 and consequently relieve the negative effect of this miR on the expression of PML protein (Figure 4). Interestingly, a ncRNA encoded by Herpesvirus saimiri binds and induces degradation of miR-27 to facilitate infection and viral transformation [235].

Villota et al. showed that over-expression of RAS induces cell transformation of HPV-immortalized cells together with down-regulation of SncmtRNA-2 (Villota, unpublished results). The expression of HPV oncoproteins in addition to the activation of oncogenes, such as telomerase and RAS, are crucial for cervical transformation [236]. Therefore, it is possible that telomerase and RAS might block the expression of SncmtRNA-2 in HPV-infected tumor cells [213]. Why both oncoproteins are needed for the expression of SncmtRNA-2 is unclear but it is of great interest that efficient keratinocytes immortalization as well as full cell transformation also requires both E6 and E7 expression. The fact that SncmtRNA-2 is expressed in immortalized but not in tumorigenic cells might contribute to the screening of early cervical intraepithelial premalignant lesions, as discussed later [213].

HPV E2 IS INVOLVED IN DOWN-REGULATION OF THE ASNCMTRNAS

Villota et al. described for the first time that HPV oncoproteins are involved in modulation of the ncmtRNAs expression during viral infections [213]. These results indicate that E2 is involved in down-regulation of the ASncmtRNAs in keratinocytes immortalized with HPV-16 and HPV-18. E2 is essential for viral genome replication and regulation of E6

and E7 expression in early stages after HPV infection [237]. In addition, regulation of transcription factors, cell proliferation, apoptosis, cell differentiation and chromosome instability seem to be the most significant functions of E2 [237-239]. The structure of the amino (N) and carboxyl (C) domains of E2 are relatively conserved among human and animal papillomaviruses, whereas the Hinge (H) domain is highly variable in sequence and length. Little is known about the function of the H domain, and it is generally considered a flexible linker between the two functional domains [240-242]. Interestingly, E2 seems to have an oncogenic potential by itself. Expression of the HPV-8 E2 gene in transgenic mice results in increased skin cancer development, which is enhanced by UV irradiation [243]. On the other hand, the low risk HPV virus HPV-11 E2, does not induce cell transformation [244]. Whether the induction of skin cancer is related to the ability of HPV-8 E2 to induce knockdown of the ASncmtRNAs warrants future research. Interestingly however, the ASncmtRNAs are also down-regulated in 293T cells (transformed with large T antigene of SV40) and the lymphoma cell line Devernelle (transformed with EBV) (Villota et al.., unpublished results). These results suggest that oncogenic proteins of these viruses could be involved in down-regulation of the ASncmtRNAs. Previous works have shown nucleo-cytoplasmic localization of HPV-16/18 E2 [245]. E2 does not interact with mitochondria and therefore a pertinent question is how E2 alone or in combination with other cellular factors induces down-regulation of ASncmtRNAs. In addition, cellular localization of hr-HPV E2 protein is different to that of lr-HPV E2. The E2 protein of HPV-6 and HPV-11 viruses is exclusively nuclear, whereas E2 proteins from high-risk viruses are located in both, the nucleus and in the cytoplasm. HPV-18 E2 protein actively shuttles between the nucleus and the cytoplasm of infected cells and seems that partial cytoplasmic localization of hr-HPV E2 proteins correlates with the induction of apoptosis involving caspase 8 activation. In contrast, lr-HPV E2 proteins do not induce apoptosis due to their exclusive nuclear localization [245], determined by the H domain of HPV-11 E2 [246]. Electron microscopy ISH showed that the SncmtRNAs and the ASncmtRNAs in normal human kidney exit the organelle and are found localized in the cytoplasm and nucleus associated to chromatin and nucleoli [219]. In renal cell carcinoma, the SncmtRNA shows similar localization, while few copies of the ASncmtRNAs are mainly found in the cytoplasm [219]. Therefore, an intriguing question is how these mitochondrial transcripts containing long double-stranded regions can escape from the processing activities of Dicer and Drosha [224]. Hypothetically, the double-stranded region of these mitochondrial transcripts binds to the double stranded binding domain (DRBD) of Dicer and/or Drosha [223, 224] resulting in inhibition of their activities. Adenovirus ncRNAs containing double-stranded structures bind and inhibit Dicer or Drosha [223, 224]. Another interesting example is rncs-1 ncRNA of *Caenorhabditis elegans*, which contains a long double-stranded structure and binds and inhibits Dicer [247]. Perhaps then, the ASncmtRNAs in keratinocytes are able to form complexes with Dicer inhibiting its dicing activity. Hypothetically then, the expression of E2 in HPV-immortalized keratinocytes would relieve the inhibition on Dicer resulting on the degradation or processing of the double-stranded structure of the ASncmtRNAs. Nevertheless, down-regulation of ASncmtRNAs seems to be a potent diagnostic tool for early cervical cancer detection.

NON-CODING RNAS IN HPV-ASSOCIATED CANCER DIAGNOSTICS, PROGNOSIS AND MANAGEMENT

Cancer is the result of alteration of many cellular processes, which have been classified as cancer hallmarks by Hanahan and Weinberg [248]. Genes that induce cell proliferation or oncogenes are usually up-regulated whereas tumor suppressors genes are down-regulated [248]. As described before, ncRNAs represent a large family of molecules that behave as tumor suppressors or oncogenes roles. The expression profiles of ncRNAs revealed correlation with cancer progression and the potential use of these molecules as biomarkers has been extensively described over the past five years [248]. In fact, many clinical studies quantified miRs levels in different types of cancer as biomarkers of progression of the disease or predictors of conventional treatments. In the last section of this chapter, we will focus on recent published studies on the expression profiles of ncRNAs and their potential use as biomarkers of cancer prognosis, diagnostic or even management.

MicroRNAs and Their Clinical Potential

Analysis of the expression profiles of one hundred sixty six miRs in six tumor cell lines (SiHa, C33A, SW756, CasKi, C41 and ME-180) and five normal cervical samples, revealed that let-7b, let-7c, miR-23b, miR-196b and miR-143 were down-regulated in tumor cells [203]. In contrast, miR-21 was up-regulate in cervical tumor cell lines compared to normal tissues [203]. These results were confirmed later [52, 197, 198]. Remarkably, and in addition to HPV-associated cancer, miR-21 is also found up-regulated in glioblastoma, breast cancer and other cancer cell lines, while miR-143 is down-regulated in colorectal tumors, sarcomas, breast and prostatic cancer [67, 249]. The expression profiles of miR-21 and miR-143 were recently evaluated by Deftereos et al. *(*2011) in HeLa, SiHa and Caski cell lines, normal samples infected and not infected by HPV and one hundred thirty three clinical samples including CIN 1 to 3 and cervical cancer. Interestingly, miR-21 was up-regulated in severe lesions such as CIN 2 and higher compared to normal tissues and moderate dysplasia. Conclusively, despite the fact that miR-21 and miR-143 do not seem to be a specific biomarker for cervical cancer, up-regulation of miR-21 and down-regulation of miR-143 fulfill the requisites to be considered as markers to assess the development of the disease.

Analysis of miRs expression in ten invasive squamous cervical carcinoma (ISCC) samples and ten normal cervical tissues showed that sixty eight miRs were up-regulated in ISCC compared to normal tissues, including miR-9, miR-127, miR-133a, miR-145, miR-199 and miR-214. Only miR-149 and miR-203 were down-regulated in ISCC samples. The authors concluded that miR-127 could be a useful marker for lymph node metastasis of ISCC tissues. Up-regulation of miR-9 and down-regulation of miR-203 was later confirmed [52]. However, other groups reported down-regulation of miR-145 in cervical cancer [83, 87, 198]. The obvious concern on the analysis of this kind of transcripts is the technique used to identify their expression the use of appropriate experimental controls. Quantitative real-time RT-PCR (qRT-PCR) is the primary technical choice used to determine the expression profile of microRNA, but there are inherent problems with internal controls of this technique [210, 252]. In our opinion, intense research in qRT-PCR applied to clinical specimens has to be

carried out to establish miRs expression profiles and probably more than one technique will be required.

Alteration of miRs expression in cervical cancer cell lines (CasKi, SiHa, HeLa, C33A and W12) with episomal or integrated HPV genome, as well as three CIN 3 samples and five cervical carcinomas, revealed that miR-126, miR-143, miR-145 and miR-195 were down-regulated in integrated HPV-16 cervical cell lines, whereas miR-182, miR-183 and miR-210 were up-regulated. Surprisingly, miR-218 was down-regulated in CINs and cervical cancer samples compared to the normal cervix [83]. From this list, miR-218 appears to be an excellent candidate for cancer diagnostics since down-regulation of this transcript in cervical cancer associated to HPV has been well documented [52, 83, 87, 197, 199].

In a small cohort study of five HPV-negative cervical squamous carcinoma and five normal samples it was reported that miR-494 and miR-61 were down-regulated in tumor tissues, while miR-189, miR-202, let-7c and let-7a were up-regulated in tumor cells [254]. A year later, Hu et al. (2010) described a study with one hundred and two cervical samples, using 60 samples as training for their predicting model and finally 42 for testing [52]. By using qRT-PCR they identified miR-9, miR-21, miR-200a, miR-218 and miR-203 associated to cancer survival [251]. The authors concluded that the expression of miR-200a and miR-9 are predictive of patient survival since down-regulation of these miRs is associated to metastasic potential [52].

In oral and pharyngeal carcinoma, changes in miRs expression profile compared to controls patients were repoted [198]. One hundred and fourteen miRs were differentially expressed between OSCC and normal epithelium, and the most significant result was down-regulation of miR-375 and up-regulation of miR-31 in OSCC compared to normal tissues. As discussed before, HPV infection induces up-regulation of twenty one miRs and down-regulation of miR-127-3p, miR-125, miR-126, miR-145 and miR-363. Interestingly, miR-187, miR-181b, miR-21 and miR-345 were up-regulated in all OSCC samples. This is an important finding since suggests that miRs expression profiles are in somehow similar between cervical and non cervical cancer associated to HPV infection [198]. Pereira et al. analyzed the miRs expression profiles in four cervical squamous cell carcinomas, five high-grade intraepithelial lesions, nine low-grade intraepithelial lesions and nineteen normal cervical tissues [53]. The results indicate that miR-26, miR-99a and miR-513 were down-regulated in pre-neoplastic and cancer compared to normal cervical tissues. Down-regulation of miR-513 suggest that this molecule has tumor suppressor activity because targets the oncogene K-ras, was also down-regulated. In addition, miR-106a, miR-205, miR-197, miR-16, miR-27a and miR-142-5p were down-regulated in normal and dysplasia samples, but up-regulated in cervical cancer.

A recent analysis of one hundred and fifty oropharyngeal SCC samples, including 101 cases for training and forty nine cases to validate a new miR-based prognostic system, revealed that six miRs retained their prognostic significance [255]. The expression of miR-142-3p, miR-31, miR-146a, miR-26b, miR-24 and miR-193b were up-regulated in surviving patients, whereas miR-31, miR-24 and miR-193b were over-expressed in patients with low survival rate. Interestingly, the expression of miR-9, miR-31, miR-223, miR-155 and miR-18a correlates with HPV transcriptional activity, suggesting that miR-31 has a prognostic value in OSCC while miR-9 represent a good prognostic target in cervical cancer induced by HPV [255]. Taken together, the results described in this section suggest that the expression

Table 4. Cellular microRNAs deregulated by HPV infection

Methodology	Up-regulated	Down-regulated	References
miRNA array	miR-193b, miR-200c, miR-205	miR-143, miR-145,miR-218,miR-368,miR-497	83
qRT-PCR			
Northern Blot			
miRNA array	miR-15b, miR-16, miR-146a, miR-155	miR-126, miR-143, miR145, miR-218, miR-424	87
Northern Blot			
Northern Blot		miR-34a	201
miRNA ligation assay			
qRT-PCR		miR-203	84
Northern blot			
In situ RT-PCR		miR-125b	86
qRT-PCR	miR-363, miR-33, and miR-497	miR-155, miR-181a, miR-181b, miR-29a,	199
miRNA array		miR-218, miR-222, miR-221, miR-142-5p	
miRNA array		miR-23b	204
qRT-PCR			
qRT-PCR		miR-100, miR-29	80, 209
miRNA array	miR-16, miR-21, miR-106b, miR-135b,	miR-218, miR-433	197
qRT-PCR	miR-141, miR-223, miR301b, miR-449a		
miRNA array	miR-146a	miR-203, miR-324-5p	82
qRT-PCR			
qRT-PCR	miR-17-5p, miR-21, miR-106a	miR-155, miR-206	207
In situ hibridization			
qRT-PCR	miR-15b		85
miRNA array			
miRNA array	miR-363	miR-145, miR-125a, miR-126, miR-127-3p, miR-379	198
qRT-PCR			
miRNA array	miR-181a, miR-125a-5p, miR-502-3p,	miR-558, miR-576-3p, miR-606, miR-886-3p,	210
	miR-923, miR-92a-1, miR-500	miR-888, miR-1255a, miR-1274b	
qRT-PCR	miR-21		208
Northern Blot			

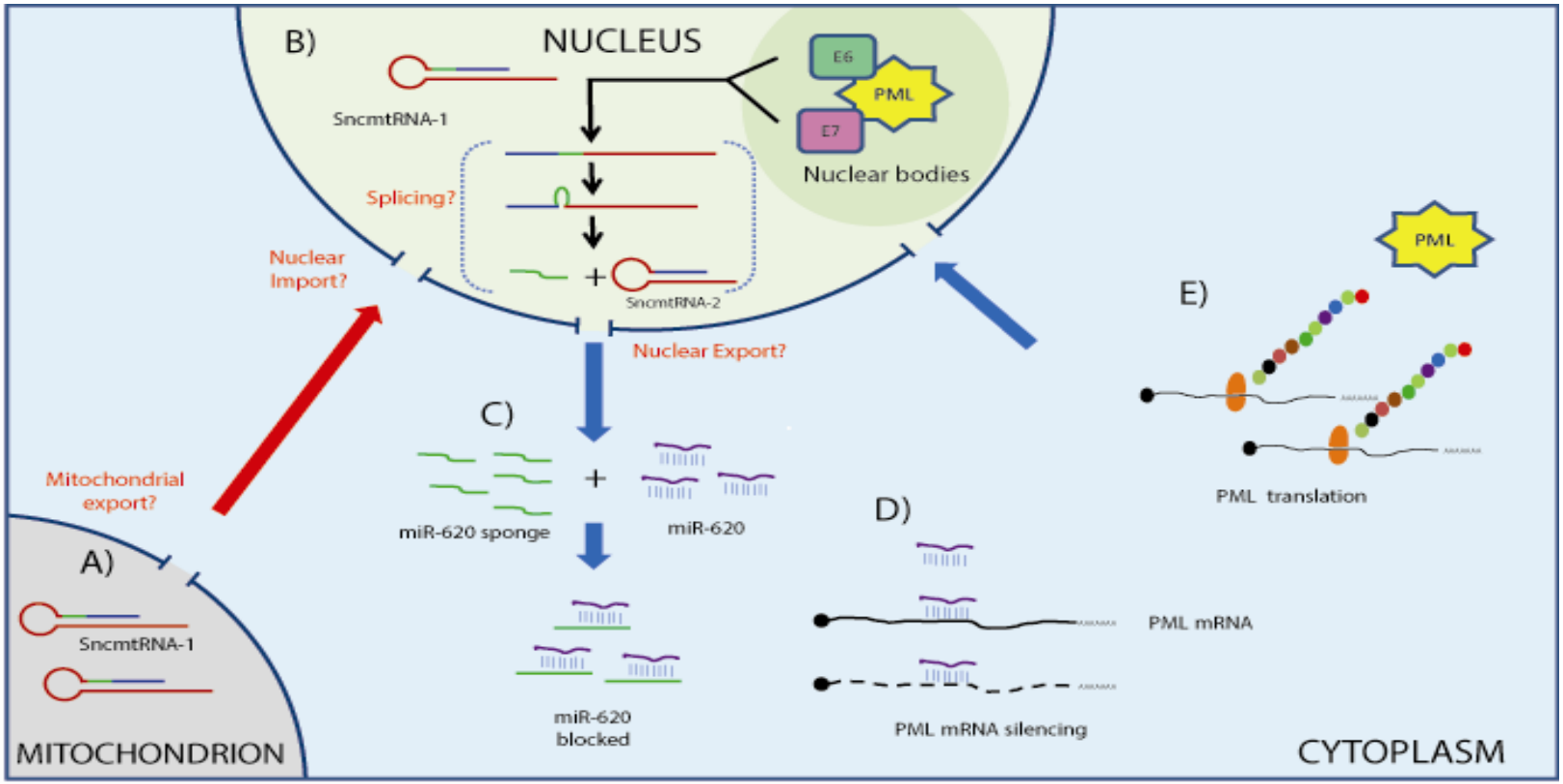

Figure 4. Hypothetical mechanism on the modulation of SncmtRNAs by HPV. A) The SncmtRNA-1 is synthesized in the mitochondria and then the transcript exits the organelle to the cytoplasm and to the nucleus. B) HPV E6 and E7 proteins induce processing of SncmtRNA-1 to synthesize SncmtRNA-2 together with a 63 nt RNA fragment. C) The 63 nt RNA fragment would act like a miR "sponge" specific to trap miR-620. D) In a normal condition, miR-620 induces inhibition of translation and degradation of the mRNA of PML, hindering production of PML proteins. E) The 63 nt RNA fragment traps miR-620 allowing the synthesis of PML proteins which are required for HPV replication.

profile of miRs may be useful to differentiate cervical cancer and dysplasia from normal cervical tissue.

Long Non-coding RNAs and Their Clinical Potential

Recently, the relationship between cervical cancer progression and expression of lncRNAs was reported. Gibb et al. analyzed lncRNA expression profiles in four normal samples, three CIN1 and 2 and six CIN3 samples by Long Serial Analysis of Gene Expression (L-SAGE) [108]. Analysis six hundred and sixty eight lncRNAs expression profiles revealed that one hundred twenty three lncRNAs were differentially expressed between CIN1-3 compared to normal tissue, while thirteen aberrantly expressed lncRNAs were common to all CIN grades. While Xist (X-inactive specific transcript) is expressed "constituvely" in all samples, MALAT1 is down-regulated in all CIN2 and higher lesions and GAS5 was down-regulated from CIN1 to cervical cancer. The deregulation of MALAT-1 and GAS5 in CIN may be contributing to the dynamics of precursor cervical cancer lesions. Taken together, these results suggest that the lncRNA expression profiles can be used to differentiate normal tissue from dysplasia and high-grade lesions [108].

MITOCHONDRIAL LONG NCRNAS AND THEIR CLINICAL POTENTIAL

Recently, Villota et al. (2012) described for the first time that HPV oncoproteins modulate the expression of mitochondrial ncRNAs in keratinocytes immortalized with the virus. Immortalization with the whole genome of HPV-16 or 18 induces down-regulation of the ASncmtRNAs and the E2 oncogene is involved in this process (Villota et al., 2012). Taken together, these results indicate that the differential expression of the SncmtRNA and the ASncmtRNAS offers a potent opportunity to detect tumor cells or cells at early stage of neoplasic transformation. Indeed, we have used the differential expression of these transcrits to detect tumor cells in voided urine of patients with bladder cancer (BC). A pilot study using FISH shows that in the urine of 24 patients with BC there are cells that express the SncmtRNA but not the ASncmtRNAs and therefore, they correspond to tumor phenotype [127]. In contrast, hybridization of the few exfoliated cells recovered from healthy donors revealed no expression of these mitochondrial transcripts [127]. Preliminary studies show that the differential expression of the mitochondrial transcripts can also be used to detect tumor cells present in cervical cells (liquid cytology). The ncmtRNAs expression profile has also been analyzed in normal, CIN1, 2, 3 and invasor squamous carcinoma (Villota et al., unpublished results). The results showed that SncmtRNA-1 expression is maintained in all tissues (normal to ISCC) whereas the ASncmtRNAs are down-regulated from CIN1 to ISCC.

On the other hand, the oncoproteins E6 and E7 are both involved in induction of SncmtRNA-2 in HPV-immortalized cells. Interestingly, SncmtRNA-2 is expressed in CIN 1 and 2. Normal tissues, as well as CIN 3 and ISCC do not express this transcript. These results support the idea that expression of SncmtRNA-2 might contribute to the screening of early cervical intraepithelial lesions. Taken together, the data indicates that microRNAs and

lncRNAs expression profiles could potentially help to differentiate between normal, dysplasia and high-grade lesions of cervical cancer.

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Chapter 3

HUMAN PAPILLOMAVIRUS INFECTION IN ADOLESCENTS

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ABSTRACT

Human papillomavirus (HPV) is one of the most common causes of sexually transmitted infection (STI) in both men and women around the world, with prevalence rates varying according to the studied population and geographical localization. It is estimated that over 80% of sexually active women are exposed to the virus and acquire the infection 3-4 years after sexual debut. Most HPV infections are transient and asymptomatic without causing any clinical manifestation. Young women are more vulnerable and often become infected by multiple types of the virus. The high prevalence of HPV in young women underscores the vulnerability of adolescents to HPV, while the low prevalence of cervical cancer in this group underscores the benign nature of initial HPV infections. Evidence indicates that there are risk behaviors and / or biological risks that make the adolescents vulnerable not only to HPV infection but also to the persistence of the HPV that is strongly linked to the development of high-grade squamous intra-epithelial lesions and invasive cancer. Among the biological factors that predispose an individual to HPV we can highlight the immaturity of both the epithelium and the immune system. It has been observed that the association between early age of first sexual intercourse and HPV infection was partially mediated by a set of risk behaviors and related conditions, including the number of sexual partners, history of STI, risk

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behaviors such as the consumption of alcohol and illicit drugs, and behavior of sexual partners. Anovulatory menstrual cycles and large glandular aversion of the cervix with active squamous metaplasia, common in adolescent girls after menarche, are associated with the lack of cyclic progesterone production, which may lead to the decreased production of cervical mucus, which is thought to serve as a protective barrier against infectious agents. In addition, the adolescent cervix is characterized by a large area of cervical ectopy, defined as the area of immature columnar and metaplastic cells on the ectocervix. HPV infects epithelial basal cells and its replication is dependent on the active cell division and differentiation, which occurs normally in pubertal adolescent girls. These cells may be particularly vulnerable to infection with HPV. There is rapid change in the cervical epithelium during puberty as squamous metaplasia occurs, the process by which the columnar epithelium is transformed into mature squamous epithelium. In this chapter we discuss the natural history of HPV infection in adolescents and the factors that may increase the vulnerability of this group of women to HPV.

1. INTRODUCTION

Human papillomavirus (HPV) is one of the most common causes of sexually transmitted disease in both men and women around the world, especially in developing countries, where the prevalence of asymptomatic infection varies from 2 to 44%, depending on the population and studied geographic region [1]. The prevalence rates of HPV infection also vary broadly, according to age, which seems to largely reflect differences in sexual behavior in the different geographical regions [2]. Most HPV infections are transient and clear spontaneously but some persist [3, 4]. Studies show that the majority of sexually active individuals are exposed to and acquire infection from this virus at some phase in their lives [4-6]. HPV infection is more prevalent in young women, at the beginning of their sexual activity, with a subsequent decline in the prevalence rate with increasing age, likely due the reduction of sexual activity or as a result of development of an immune response against the virus [2, 7, 8, 9].

HPV belongs to the family Papillomaviridae, which consists of small nonenveloped DNA viruses infecting birds and mammals. Members of this family are distributed across multiple genera but the individual viruses are highly host-specific and tissue-restricted [10]. Currently, over 150 different HPV types have been cataloged, all of which are epitheliotropic viruses and can be subdivided into two groups, mucosa and cutaneous, based on their ability to infect keratinocytes of the mucosa or of the keratinized surface of the skin [11]. HPVs are not only species-specific but also display a marked tropism for squamous epithelia.

Despite having similar genomic sequences, different HPVs infect epithelia of distinct anatomic sites. About 40 are sexually transmitted and infect the anogenital tract, oral mucosa, and other mucosal sites in the human body [12, 13]. Mucosal HPV types can be further clinically subdivided into high-risk (HR-HPV) and low-risk (LR-HPV) according to their relative propensity to cause HPV-associated lesions and their tendency to undergo malignant progression. This classification has been most compellingly established for mucosal HPV. LR-HPV types, such as HPV 6 and 11, are associated with a spectrum of genital warts, generally benign or hyperproliferative lesions with very limited tendency to malignant progression, while infection with HR-HPV types, highlighting HPV 16 and 18, are tightly associated with cervical cancer and their precursor lesions [5, 11, 13].

HPVs are classified into five genera designated by Greek letters: *alpha, beta, gamma, Mu* and *Nu*, according to the identity of the sequence of their genomes, phylogenetic characteristics, and pathology [14, 15]. The *Alphapapillomavirus* genus is associated with infections of the anogenital tract and oral mucosa and covers a group of HR-HPV that causes virtually all cases of cervical cancer, and a smaller proportion of cases of other cancers of the genital tract such as vulvar and penile, and some types of extragenital cancers, such as head and neck carcinomas.

In addition, this genus contributes to about 40% of oral cancers [16, 17]. The *alpha* genus covers three main branches (clades) where all HPV types meet with carcinogenic potential, forming a single high-risk clade composed of five species groups ("species"): α-5, α-6 α-7 α-9, and α-11. Although these five species belong to the same high-risk clade, each of which has a different risk profile, with α-9 being the most important species, consisting almost entirely of carcinogenic types, having HPV16 as the main type of specie but also covering several other types of high-risk HPV 16-related species. [18].

Persistent infection with HR-HPV is unequivocally established as a necessary cause of invasive cervival cancer, being responsible for virtually all of more than 500,000 cases of disease per year worldwide [4, 19]. The critical molecules for initiation and progression of cancer are the viral oncoproteins, E5, E6, E7, which act largely by avoiding the negative regulation of the growth of the host-cell by cellular proteins p53 and pRb, generating a genomic instability, which is the hallmark of the HPV-associated cancers [20, 21].

The sexual contact appears to be the primary route of transmission of genital HPV infection, although the transmission by nonpenetrative sexual contacts is well documented. The infection in women with only one sexual partner is also a reasonably common occurrence [22, 23]. Genital HPV infection is a very common event among sexually-active women, especially the younger, with higher prevalence rates in those with up to 30 years of age, followed by a decline until around age 45-50, and a second peak of incidence can be observed in the peri- or post-menopausal period [2, 9]. Most of the infections seem to be transitory and of little clinical significance [24]. However, some women persistently infected with high-risk HPV, particularly the non-European HPV16 variants, are at a greater risk of progression to high-grade cervical lesions, mainly those with a high viral load [25, 26]. Tumor progression is a complex phenomenon that depends on multiple factors, such as the type and variant of virus, in addition to the combination of environmental factors, and the host itself, including genetic background, immune status, behavior and sexual activity, as well as reproductive activities [26, 27]. In this chapter we discuss the genital HPV infection in adolescent girls, emphasizing aspects of the natural history, including biological and behavioral factors that may increase the vulnerability of this segment of the female population, not only for HPV infection but also for dysplasia, as well as some aspects related to the prevention and treatment.

2. Structure and Productive Life Cycle of Virus

In its episomal state, HPV is a relatively small virus with a double-stranded, circular DNA, associated with histone-like proteins. In its infectious state, the viral genome is surrounded by a capsid of icosahedral symmetry, constituted by 72 capsomers.

Each capsomer is composed of five monomeric units of a protein of 55kDa that join to form a pentamer corresponding to the major protein capsid, L1. The L1 pentamers are distributed forming a network of intra- and interpentameric disulfide interactions which serve to stabilize the capsid [28, 29]. L2 protein is the secondary component of the viral capsid with about 75kDa located within the virion. To assemble the viral capsid, the pentamers join to approximately 12 copies of L2 that occludes the center of each pentavalent capsomere [11, 30, 31]. Each capsid consists of 360 copies of L1 that come together to form 72 pentameric units or capsomers. Thus, each virion contains 72 pentameric units of L1 and a variable number of copies of L2, assembled within the virion, forming a particle with icosahedra symmetry of approximately 55 nm in diameter [29, 32, 33].

The genome of HPV contains approximately 8000 base pairs and harbors an average of eight open reading frames (ORFs), divided into three regions: The first is a noncoding upstream regulatory region which has been referred to as the long control region (LCR), varying in size and nucleotidic composition for the different HPV types, whose main function is the regulation of the transcription of the viral genes. The second is an early region (E), which encodes no structural proteins involved in viral replication and oncogenesis (E1, E2, E4, E5, E6, and E7). The third is a late region (L), which encodes the structural proteins L1 and L2, which together form the viral capsid [34, 35, 36].

Only one of the two strands of the circular DNA is actively transcribed and serves as the template for viral gene expression, coding for a variable number of polycistronic mRNA transcripts [37]. The regulation of the viral gene expression is complex and controlled by viral and cellular transcription factors. Most of these regulations occur within the LCR region, which contains a variety of cis-active elements regulating viral replication and gene expression. These sequences are bound by a number of cellular factors along with the viral E2 gene product [38]. Also found in the LCR region are the viral replication origin and many transcriptional regulatory elements. An early promoter, a differentiation-dependent late promoter, and two polyadenylation signals define three general groups of viral genes that are coordinately regulated during the differentiation process of the host cell. The E6 and E7 genes maintain replication competence and E1, E2, E4, E5, and E8 genes are involved in viral DNA replication and transcriptional control, as well as other late functions. The late gene products, L1 and L2, are responsible for the assembly of viral particles [39].

The regulation of expression of the late genes in genital HPVs is not well understood. However, it has been shown that the promoter of these genes is activated in a differentiation-dependent form. Once activated, the later promoter directs transcription from a heterogeneous set of start sites and will serve to produce a set of transcripts that facilitate the translation of L1 and L2 proteins [11]. Activation of the later promoter is accompanied by acceleration of viral DNA replication and by high levels of viral protein expression. As a result, the virus copy-number amplifies from 50 copies to several thousands of copies per cell. Therefore, when a late promoter is activated, the expression of the structural genes occurs, leading to production of the L1 and L2 proteins, which join to assemble the capsids and to form new virions [37].

Most viruses infect the target cell and produce progeny viruses in this same cell. In the case of HPV, the synthesis of new virions occurs only after the infected cell undergoes mitosis and one of the infected daughter cells goes through the process of differentiation [40]. The HPV life cycle begins with the infection of stem cells in the basal layer of the epithelium probably through micro-wounds. HPV replication begins with host cell factors which interact

with the LCR region of the HPV genome and begin transcription of the viral E6 and E7 genes [41]. The viral replication is tightly linked to the differentiation program of the epithelial cell. Initially, HPV infects only dividing basal epithelial cells [20]. HPV DNA replicates at the same time as the DNA of the basal cells in the splitting process and viral replication is considered nonproductive, with the virus settling in a low-copy-number of episoms by using the host machinery cell to replicate its DNA on average once per cell cycle [42, 43, 44].

The expression of the E1 and E2 viral genes is required to maintain a low number of copies of the genome per cell. These proteins bind to the viral origin of replication and recruit cellular DNA polymerases and other proteins necessary for viral DNA replication [45]. The viral genome is maintained in basal cells as stable episomes and these infected cells will serve as reservoirs of the virus for later, performing productive replication [46]. The products of the viral genes, E1 and E2, support viral DNA replication and its segregation such that the infected cells can be maintained in the lesion for a long period. The viral DNA replication depends almost totally on the host transcription cell factors, except for the viral helicase E1. E2 and other early genes, E6 and E7 are required to coordinate the host cell environment so that it is suitable for viral DNA replication [47].

Occuring in the more differentiated cells of the suprabasal layers of the epithelium are the activation of the differentiation-dependent promoter and the maintenance of the gene expression of E1, E4, and E5, and then the expression of genes L1 and L2 on the upper layer of the epithelium [44]. The E6 and E7 oncoproteins act to enhance the cellular proliferation and therefore the number of cells infected by HPV in the epithelium, resulting in an increase in the number of cells that eventually produce infectious virions [45]. The E4 gene product induces amplification of the replication of the viral DNA, resulting in an increase in the number of the genome copies per cell. With this, the virus switches to a mode of "rolling-circle" replication of viral DNA amplifying the viral genome, and resulting in a high copy number per cell at the same time that the expression of genes L1 and L2 occurs [44, 48]. In the granular layer, the products of late genes, the proteins L1 and L2 of the viral capsid, gather to assemble the viral capsids, resulting in the formation of new virions. Lastly, the newly formed viral particles reach the cornified layer of the epithelium, where they are released [44].

3. Natural History of HPV Infection in Adolescents

3.1. Biologic Vulnerability

During the early stage of the fetal formation, urogenital squamous epithelium migrates to replace the majority of the müllerian columnar epithelium that originally coats the cervix and vagina. This replacement is usually incomplete, which results in an acute transition called the *original squamocolumnar junction* [49].

During the rest of fetal life, the process of squamous metaplasia begins and continues so that, at birth, the newborn presents a new squamocolunmnar junction. The process whereby undifferentiated cells of the columnar epithelium transform themselves into squamous epithelium is referred to as squamous metaplasia and this constitutes a determinant of the characteristics of cervical epithelium in adolescent women [49].

After birth, most girls exhibit an abrupt squamocolumnar junction visible on the ectocervix. This junction remains quiescent until puberty when estrogen and the increased acidity of the vagina induce basal cells of the columnar epithelium to become squamous cells through the process of squamous metaplasia. Thus, the cervix in the adolescent is predominantly made up of columnar and metaplastic cells, whereas in adults the cervix is predominantly covered by squamous epithelium [50].

With the onset of puberty, under the influence of estrogen, the squamous epithelium of the vagina and cervix thickens, and the process of squamous metaplasia is reactivated. The junction between the columnar and squamous epithelium is now separated by large areas of squamous metaplastic epithelium, also known as the transformation zone (TZ). Small changes in estrogen levels, such as those seen in early puberty, result in the induction of squamous metaplasia and commonly precede other evidence of puberty, including breast changes. The activation of squamos metaplasia may also be influenced by factors other than those inherent in puberty. It has been observed that the cervixes of sexually active girls were in general more mature than those of non-sexually active girls of the same age. It is believed that certain factors such as penile trauma, exposure to sperm, and sexually transmitted diseases may affect the rate of squamous metaplasia [49].

Studies show that female adolescents constitute a vulnerable group with regard to infection by sexually transmitted agents, including HPV. Among the factors that can increase the risk of infection is the TZ of the cervical epithelium physiologically increased with active squamous metaplasia. The thinly layered columnar epithelium appears to be especially vulnerable to HPV, because it allows the virus to have direct access to the basal cells through a wound or abrasion [51, 52]. These characteristics of the relative immaturity of the genital tract epithelium increase their propensity to undergo trauma and inflammation, which probably facilitate the access of the virus to basal layer cells [53].

Apart from great physical, emotional, and psychosocial changes faced by the adolescent women, there are also high-risk behaviors for their health. The highest rates of prevalence and incidence of HPV infection found in adolescent sexually active are probably due to factors related to sexual behavior and biological vulnerability, with greater risk during the first three years of active sex life, with many acquiring the infection early, within the first 18 months after menarche [54]. Risk behaviors and / or biological risks make the adolescents more vulnerable not only toinfection, but also to the persistence of the HPV, that is strongly linked to the development of high-grade squamous intra-epithelial lesions and invasive cancer [39, 55]. In addition, some conditions typical of adolescence can also increase the risk for HPV infection: (1) immature physiologically epithelium with large gland aversion of the cervix and active squamous metaplasia, marked by a lack of cyclic progesterone secretion early in puberty due to anovulatory cycles; (2) inadequate production of cervical mucus favored by anovulatory cycles, which could act as a barrier against infections; (3) immaturity of the immune system with incomplete local immune response; and (4) increased susceptibility to trauma during sexual intercourse [54, 56].

It is speculated that metaplastic epithelium may be more vulnerable to wounds induced by intercourse, douche, or tampons, leading to the appearance of microdamage in the epithelium that can facilitate HPV access to basal layer cells. However, no study to date has shown that ectopy is a risk for HPV acquisition, although Castle et al. (2006) [57] had found that infections with high-risk HPV types were more common in women with large areas of ectopy, compared with those with mature cervixes. In a recent study Hwang et al., (2012a)

[58], found no association between the extent of the cervical ectopy and HPV acquisition in healthy adolescents. This observation might be related to age and immunologic memory, which goes hand in hand with cervical immaturity. It is possible that dynamic metaplasia rather than the sheer extent of the ectopy is the cause of the increase risk for HPV infection in healthy young women. This is consistent with the HPV life cycle, during which host cell proliferation and differentiation support viral replication [59]. Thus, cells that are rapidly undergoing division and differentiation are more likely to have dysplasia [60].

Since the process of squamous metaplasia appears to be most active during adolescence, it may represent more vulnerability to the establishment of HPV. Among the triggers of the squamous metaplasia and acceleration of the maturity of the epithelium is sexual activity. Adolescents who have ever had multiple sexual partners exhibit cervixes that resemble those of adults with small areas of ectopy.

Women with multiple partners are more exposed to STIs, which induce repair and inflammation, and to semen itself that induces metaplasia; perhaps for this reason, they have higher rates of prevalence of HPV infection. Whatever the reason, rapid proliferation and differentiation of populations of squamous metaplastic cells presumably makes these cells more vulnerable to HPV infection. As HPV replication and patterns of transcription are dependent on the differentiation program of keratinocytes, it is intuitive that squamous metaplasia is particularly favorable to HPV installation. The environment is favorable to viral replication, and possibly for persistence. The high rates of dysplasia found in adolescent populations reinforce this idea [55].

The constant changes in the cervical epithelium increase the vulnerability for disruption of the epithelial barrier integrity, facilitating invasion by pathogens, including HPV. These metaplastic conversions are influenced by the acidification of the vaginal pH and by traumas caused by sexual activity [61].

It is believed that the metaplastic epithelium is associated with a deregulated production of receptors, adhesion molecules, and soluble mediators of the inflammatory response, such as cytokines, chemokines, prostaglandins, and growth factors. These molecules might not only exercise influence on epithelial differentiation, but also alter the local antiviral immune response, favoring HPV infections [39].

It has been observed that sexually active healthy adolescents are undergoing an active process of epithelial maturation of the cervix over a relatively short time. Contraceptive pills and smoking are identified as important accelerators of this maturation. The process of cell proliferation is a dynamic response of the epithelium that may increase its vulnerability to HPV infection and DNA damage of the cell [60]. Thus, the adolescents who are still in the active process of maturation of the epithelium of the cervix have a physiological condition that creates a microenvironment favorable to the installation of HPV, which increases the risk of infection by this pathogen.

3.2. Risk Factors

The risk factors for the acquisition of HPV infection and development of cervical lesions, including cervical cancer, are the same classic risk factors for other sexually transmitted diseases, with the number of sexual partners being the most well documented. However, other indicators of sexual behavior and reproductive activities, heredity, immune and nutritional

status, and smoking can contribute in some way to the development of cervical cancer [62, 63, 64].

The highest incidence of HPV infection in adolescents has been frequently assigned mainly to sexual behavior. However, cultural factors that influence social behavior including habits, customs, attitudes and practices may make a person more exposed to the virus, increasing the chance of acquiring the infection. Similarly to what occurs with other STIs, an increased risk of acquiring HPV genital infection is associated with many factors, including the social nature. The lack of parental accompaniment, the difficulty of parent-adolescent communication, insufficient sex education, the lack of health insurance and confidential source of health care even if insured, dating violence, and incarceration. Some research suggests that sexual content in the media can increase the prevalence of risky sexual behavior, although sometimes what adolescents see on television is helpful in providing information or stimulating discussion [65].

It has been suggested that women whose columnar epithelium of the cervix is more exposed due to ectopy, which is a common finding in adolescents, exhibit an increased biological risk for acquiring HPV, although this has not been fully proven. However, it is believed that the infection appears to be more likely to occur in this epithelium, when it is exposed rather than concealed in the endocervix [66]. Other possible biological factors include the lack of protective antibodies resulting from previous infections, decreased levels of IgG during the follicular phase of the menstrual cycle in adolescents, compared with adults, and smaller protective action of hydrogen peroxide-producing lactobacilli [65].

Among the other described risk factors that may increase the likelihood of acquiring a genital HPV infection, the most clearly well established is the increased number of lifetime sexual partners, likely due to an increased probability of exposure to the virus. Other risk factors may include early age of first sexual intercourse, the behavior of the partners, an older age of the male sexual partner, and smoking. Thus, young female adolescents have an increased susceptibility to HPV infection due to biological and behavioral risk factors [8, 67]. In addition, another factor that can increase the susceptibility of female adolescents to HPV infection is the physiology of the cervical epithelium, due to its immaturity, and the large TZ, which is undergoing active squamous metaplasia. The thinly layered columnar epithelium appears to be especially vulnerable to HPV and allows the virus direct access to the basal epithelial cells through a wound or abrasion [51, 52].

The interval between menarche and age of first sexual intercourse has been reported as a risk factor for HPV infection and cervical intraepithelial neoplasia, once that the interval may be a proxy measure for the biological maturity of the cervix. Furthermore, early age of first intercourse may be related to an increased risk of HPV acquisition not only because of the potential for a higher number of sexual partners lifelong but also because young adolescents are much more likely to have a large area of metaplasia at the TZ of the cervical epithelium [68]. In a recent study it was shown that a short interval between menarche and first sexual intercourse was a risk factor for cytologic abnormalities and high-grade cervical disease [69].

Other risk factors for HPV infection may include inadequate production of cervical mucus, which may act as a barrier against patohogens, incomplete local immunity against certain infections, and increased susceptibility for undergoing small trauma during sexual intercourse [56]. Thus, young female adolescents have increased susceptibility to HPV infection due to biological and behavioral risk factors [59].

In a recent study, the detection of any HPV type in the cervix was strongly associated with increasing number of lifetime and recent sexual partners in vaginal intercourse, anal and oral sex, and history of Chlamydia infection [70].

Some conditions are risk factors not only for HPV infection but also for viral persistence. Persistence is consistently associated with high-risk HPV types, particularly HPV 16, and is strongly linked with development and progression of cervical lesions, which have been associated with multiple sexual partners, early age of first intercourse, cigarette smoking [8, 64], immune suppression [71] and use of oral contraceptives [52]. Thus, although acquisition of HPV is generally related to sexual activity, clearance of HPV and the regression or progression of cervical lesions is primarily determined by the host immune response, but other factors also influence this process including relationships with multiple sexual partners, alcohol consumption, having an uncircumcised male partner, and prolonged oral contraceptive use [52, 71, 72]. A direct association has also been observed between detection of any HPV type in the cervix of female adolescents with increasing number of lifetime and recent male vaginal sex partners, anal intercourse, oral to anal sex, and history of C. trachomatis infection [70, 73].

Several factors are thought to play a role in the progression of HPV infection, including individual susceptibility, immune status and nutrition, endogenous and exogenous hormones, tobacco smoking, parity, co-infection with other sexually transmitted agents such as HIV, herpes simplex virus type 2, and *C. trachomatis*. Other factors include viral characteristics such as HPV type and variant, concomitant infections with more than one HPV type, viral load, and viral integration [74, 75, 76].

3.3. Acquisition and Evolution of HPV Infection

HPV infection, especially at the basal cell layer of the transformation zone (TZ), is located in the boundary between the squamous epithelium of the ectocervix and the columnar epithelium of the endocervix. Basal cells in the TZ retain the ability to differentiate, a necessary requirement for virion production [77]. In addition, the presence of local hormones, such as estrogen and progesterone, which orchestrate cervical changes during menstruation and in pregnancy, can help in both acquisition of the HPV infection and lesion development [78, 79].

Two types of cells are present in the basal layer of the cervix. The first type is the transit amplifying (TA) cells that are proliferating and have the capacity to undergo terminal differentiation. TA cells split and differentiate, and are the main cells present in the suprabasal layers. The second type of basal cells is the stem cells, which have unlimited proliferation potential but divide only rarely to replenish the TA pool, serving as reserve cells for the long-term maintenance of the tissue. Only one daughter cell, resulting from the stem cell division, becomes a TA cell, while the other remains a stem cell. It is unclear which of the cells in the basal layer is the target of HPV infection, and perhaps both may be infected. If this is true, infection of stem cells could lead to one long-term persistent infection, whereas infection of TA cells could lead to short-term infections, followed by a cure [80].

Presumably, the genital HPV infection requires direct contact of the viral infectious particles with the dividing basal cells of the stratified epithelia, which occurs due to the presence of trauma or inflammation [81]. The virus does not bind directly to keratinocytes.

Instead of this, it must first attach to a heparin sulfate proteoglycan receptor in the segment of the basement membrane exposed after epithelial trauma. This binding induces conformational modifications, which affect the viral capsid proteins (L1 and L2), and such changes might allow HPV to interact with an elusive cell surface receptor on basal keratinocytes [82]. After entering the basal cells, the viral genome initially replicates together with the host DNA without triggering high levels of viral genome replication and capsid production in these cells. These non-productive infections can switch to active virus replication in terminally differentiated cells of the intermediate and superficial epithelial layers [81].

The genital infection by HPV is predominantly, but not exclusively, an STI. Penetrative vaginal or anal sexual intercourse is not a necessary prerequisite for acquiring the infection by this virus. The transmission can also occur by the direct contact with skin or mucosa during intimate contact of the infected genitalia or other mucosal surfaces, beyond casual physical contact and perinatal vertical transmission [25, 83, 84]. Genital HPV infection has been diagnosed in teen girls who have claimed to have never had vaginal or anal sexual intercourse with men, which supports the idea of the existence of alternative modes of transmission of the HPV [85]. This suggests that even if the adolescent girls abstain from sexual intercourse but not from other forms of sexual behavior, they remain at risk of acquiring HPV. On the other hand, adolescents who use condoms during sex may still acquire HPV infection at epithelial sites outside of the area covered by a condom [22, 23].

HPV is highly infectious with an incubation period ranging from 3-4 weeks to months or years, and the duration of this period is probably related to the dose of virus received. Eventually, for reasons not yet very well understood, the cell becomes permissive and viral growth commences, so that viral DNA can be detected and infectious virus is produced and released. This phase of active replication also remains for a variable length of time, but eventually the vast majority of infected individuals develop an effective immune response becoming viral DNA negative with subsequent sustained clinical remission from disease [86]. Consistent immunity depends on a cell-mediated effective response to the early proteins, mainly E2 and E6. When it is present, regression of the lesion occurs accompanied or followed by seroconversion with neutralizing antibody production for the major capsid protein L1 [87].

The adolescent population is disproportionately affected by HPV and their injuries therefore continue to be a major public health problem of global importance. [88]. Acquisition of HPV infection occurs soon after onset of sexual activity, and more than 50% of the sexually active adolescents will acquire a genital HPV infection within five to seven years after the first sexual intercourse [52]. The prevalence rates of HPV infection are highest in the adolescent populations with cumulative prevalence rates reaching values of up to 82% in certain groups. In sexually active adolescent females, the HPV infection prevalence is extremely high, involves multiple HPV types, and frequently results in cervical dysplasia [98]. In a longitudinal study involving adolescents, it was reported that among those initially HPV negative 55% acquired HPV infection within three years [90]. A recent study involving adolescent females in New York, US, found an overall prevalence rate of HPV-DNA detection at enrollment of 53.5% in the cervix, 41.5% in the anal canal, and 19.6% in the oral cavity [70]. In another study with adolescents in England, ranging between 13-24 years of age, an overall prevalence rate of 57.2% was found, being 22.6% in adolescent girls between 13-15 years of age and a significant higher rate (34.6%) among those between 16-24 years of

age. High-risk HPV prevalence increased by year of age in females 13 to 24 years old, with a peak of 39% in those 19 years old [91].

It has been reported in another study with adolescent female students who were HPV negative and reported had never had sexual intercourse, before being included in the study, that approximately 30% acquired HPV infection within 12 months after initiating intercourse and more than 50% became HPV positive within four years [22]. Also, in a longitudinal study it was shown that almost all cases of infections by low-risk HPV type and two-thirds of high-risk HPV were eradicated over a 24-month period as demonstrated by repeatedly negative tests for HPV. It has been seen still that women with persistently positive tests for HPV are at higher risk of having squamous intraepithelial lesions (SIL) of low-grade lesions (LSIL) and of high-grade lesions (HSIL) than women who revert to negative HPV tests. A study of adolescent and young women showed that 92% of LSIL cases regressed within 36 months of observation and only 3% progressed to HSIL [55]. Considering that most young women who had positive tests for several years did not have HSIL, the longer duration indicates that factors other than persistence are probably important in HSIL development.

3.4. Persistence vs Clearance

In general, in healthy individuals, incident HPV infections last at least 12-18 months, but most often the virus is cleared by the immune system within 30 months after early infection [92, 93]. This occurs especially with low-risk HPV types, which are less likely to persist [94]. However, approximately 10% of women fail to clear the virus and develop a persistent infection, whose main consequence is the development of lesions that may progress to intraepithelial neoplasia and cervical cancer [21, 39, 95].

Persistent HPV infection is generally defined as continuous detection of the same HPV genotype in the same person for at least two consecutive follow-up visits 4-6 months apart. However, as the interval between follow-up visits varies in different studies and there are many unknown questions regarding the natural history of HPV, it is difficult to distinguish persistent from transient infections. Thus, an undetectable HPV infection can be a viral latency, in which the HPV levels are below the detectable threshold of current HPV DNA assays, instead of representing a cleared host [6]. Details about the immune response that results in clearance of HPV infection are still unknown. The HPV clearance seems to result from a long-term immune response involving humoral and/or cellular mechanisms against re-infection by the same HPV type, but it is still not known whether this protection is for entire life [96]. Although the term clearance is used when an HPV infection can no longer be detected using sensitive test methods, the HPV presence might not be completely excluded because the latent state of this virus is still poorly understood. Reappearance of HPV from "latency", even in the absence of definite immunosuppression is a common event, but in most cases it seems to be benign [96]. However, in some cases the risk of intraepithelial neoplasia and cancer can increase dramatically for the HPV infections that persist detectably for more than a few years [18]. This occurs due to the characteristics of the infection and the strategy adopted by the viruses to evade the defense mechanisms of the host which becomes indifferent to the presence of the virus.

A feature of persistent HPV infection is the integration of the viral DNA into the genome of the host cell, which causes disruption of the E2 viral gene and the consequent loss of the

regulation of the viral genome with overexpression of the genes E6 and E7. The products of these viral genes, the proteins E6 and E7, will interact with the cellular proteins p53 and pRB, whose functions are stopping the cellular cycle of the dividing cell for repair of possible errors. The abrogation of cell-cycle checkpoints through the degradation of p53 and pRB family members allows division of cells with chromosomal abnormalities [20]. The viral proteins E5, E6, and E7 can induce cellular abnormalities, including mediated-E5 fusion between cells, generating aneuploidy and chromosomal instability. In addition, abnormal centrosome reduplication can also occur, leading to an abnormal number of centrosomes. These conditions lead to a genomic instability in the HPV-infected cell, increasing the risk of occurrence and of accumulation of genetic changes that extend over a long period of time. The combination of genetic changes and the deficiency of the immune response to detect and eliminate these altered cells create a favorable condition for the development of cervical neoplasia and cancer. In adolescents, the persistent HPV infection with longer periods of virus detectable in the genital tract was associated with sexual behaviors potentially modifiable, such as less frequent condom use and other concurrent sexually transmitted agents. Although the mechanism as this occurs is not known, it is believed that the role of changes in local immunity and the presence for cervical inflammation are quite likely [97].

It has been proposed that contact of semen with the mucosa of the female genital tract is associated with a marked postcoital inflammatory response by the presence of transforming growth factor beta (TGFβ), which appears to play an important role in inducing local immune hyporesponsiveness [98]. This hypothesis is enhanced by the observation of a link among increased levels of condom use with increased clearance of HPV infection [99]. In addition, it has been observed that condom use is associated with faster regressions of cervical intraepithelial neoplasia [100]. The existence of inflammation in female cervical mucosa could explain the association of concurrent infection with *Chlamydia trachmatis* or *Trichmonas vaginalis* with the higher duration of HPV infection [97]. It is believed that inflammation associated with these co-infections may favor a cytokine milieu compatible with prolonged HPV infection [99]. Infections with multiple HPV types have been detected in many adolescent females. Some, but not all studies, have concluded that infection with multiple HPV types are related with the development or progression of cervical dysplasia [73, 101]. It has also been proposed that infection with multiple types prolongs duration of infection by HPV, especially when it involves genotypes with high oncogenic potential [76]. Further investigations about the significance of infections by multiple HPV types in female adolescents are needed for a better understanding of HPV epidemiology. Finally, the characteristics of HPV infection, including the differentiation-dependent life cycle of the virus, maintaining a low number of copies in the undifferentiated cells, is another strategy adopted by the virus to evade the host defense mechanisms. This makes the organism become indifferent to the presence of the virus, facilitating the persistence.

3.5. Clinical Manifestations

It is widely known that genital HPV is associated with anogenital warts as well as with precancerous and cancerous lesions of the cervix. Moreover, HPV is also responsible for a significant portion of vulvar, vaginal, penile, and anal cancers and has been associated with other malignancies such as skin and pharyngeal cancer [63].

Low-risk HPV types cause benign lesions, including anogenital warts and low-grade lesions, but these are not found in genital cancers. High-risk HPV types cause both low- and high-grade precancerous lesions, and invasive cancers [21, 63]. Incubation periods for developing clinical symptoms following HPV infection are highly variable. Genital warts may appear within 30 months of infection, whereas development of cervical cancer usually takes up to decades. Nonetheless, most HPV infections are asymptomatic and are detected only when HPV DNA testing has been performed [92].

Genital warts are the most common disease manifestation of HPV. The vast majority are caused by the viral types of low-risk 6 and 11. These lesions have variable appearance but typically present as colored tissue growths that may be cauliflower shaped, papular, pedunculated or flat topped [67]. Sometimes they are higher; others are flat, single or multiple, small or large and can be found on the vulva, vagina, cervix perineum, and urethra. Genital warts are often asymptomatic, but may include pain or pruritis. Up to 30% regress spontaneously within months, but most individuals will require treatment [102]. Occasionally, they may be indistinguishable from dysplasia or cancer and may require a biopsy to confirm the histological diagnosis. A healthcare provider should carry a higher index of suspicion of malignancy if the lesion appears atypical or pigmented, the patient is immunocompromised, or the warts are refractory to treatment [103].

Besides the genital warts, HPV infection can have different forms of clinical presentation ranging from asymptomatic cases to invasive cervical cancer, including precancerous lesions of various disease. Viral proteins expressed during the productive cycle of the virus induces pathologic changes including basal cell proliferation, causing nuclear enlargement, koilocytosis, and abnormal mitoses [104]. These changes are defined as lesions which are characteristically visualized only with the aid of colposcopy and with acetic acid; however, they are most frequently diagnosed by cytology or Pap smear test, with the recognition of abnormal cellular characteristics best defined by the Bethesda System for rating of cytology [105].

Based on the Bethesda System, the changes associated with HPV infection are classified into the following types, according to their degree of severity: atypical squamous cells of undetermined significance (ASCUS), equivalent to squamous atypia; low-grade squamous intraepithelial lesion (LSIL), equivalent to koilocytic atypia, and cervical intraepithelial neoplasia of grade one (CIN1), also referred to as mild dysplasia; high-grade squamous intraepithelial lesions (HSIL), equivalent to cervical intraepithelial neoplasia of grade two (CIN2), also referred to as moderate dysplasia, and cervical intraepithelial neoplasia of grade three (CIN3), also referred to as severe dysplasia or carcinoma in situ; and, finally, invasive cervical cancer [49].

Similarly to acute infection of the genital tract by HPV, the majority of LSIL and some HSIL will regress without any treatment. In adolescents and young women, about 92 to 94% of cases of LSIL regress spontaneously [106]. In addition, a high proportion of HSIL will also regress in young women, but the actual rates are unknown because adolescents are generally excluded from comprehensive natural history studies [107]. These high rates of LSIL and HSIL regression have led to proposing more reasonable guidelines for adolescents. However, studies of population comprisement showed that up to 77% of adolescents with ASCUS were positive for high-risk HPV types. This has called into question the use of HPV triage tests in adolescent populations without first performing the cytology [108].

Thus, according to these population-based studies, the ideal would be that only those adolescents with injuries classified as ASCUS or more severe lesions were incorporated into the schemes of screening tests for HPV.

Performing cytology every 6 to 12 months is now more advantageous than HPV triage. Similar conservative observations of LSIL by cytology are also recommended with repeat cytology 6 to 12 months later rather than immediate referral to colposcopy. Since the ASCUS associated to high risk HPV type (ASCUS-HR) has a history similar to that of LSIL, similar strategies can be adopted for those lesions, if HPV testing is performed on the ASCUS smear. At first repeat cytology, HSIL or higher should be referred to colposcopy. At second repeat cytology, any abnormality in repeat cytology for ASCUS, ASCUS-HR, and LSIL follow-up leads to referral to colposcopy. Appearance of HSIL in cytology at any follow-up visit should be referred to colposcopy. Because of the frequency of HPV infections in adolescents, HPV testing for primary screening is not recommended [55].

Adolescents with LSIL and HSIL cytology are submitted to a significant risk for development of high-grade cervical lesions. Overall, 18% of teenagers with LSIL and 51% with HSIL were eventually found to have a high-grade lesion. These rates are similar to those previously reported in adult populations. Thus, adolescents and adults with LSIL cytology carry a similar risk for underlying cervical intraepithelial neoplasia [109]. Though most LSIL regress completely, the persistence of these lesions and the presence of HSIL in sexually active adolescents are of clinical significance because those adolescents have a substantially increased relative risk of developing invasive carcinoma compared with the SIL-negative population [110].

3.6. Immune Response

In the infections caused by viruses, the first line of defense is one's innate immune system, a cell-mediated response with cytokine production that activates responsible mechanisms by slowing viral replication, while adaptive immunity is activated to eliminate the virus. The immune response to HPV infection is often insufficient to eliminate the virus such that some infected individuals do not heal and develop persistent infection. The main reason for this is an ingenious strategy developed by this virus in which viral DNA replication and virus assembly occur only in a cell that will be terminally differentiated and will die by natural causes [111]. In HPV infection there is no viral-induced cytolysis, necrosis, or inflammation during most of the infectious cycle of HPV. Besides, there is little or no release into the local milieu of pro-inflammatory cytokines, which are important for antigen presenting cell (APC) activation and its migration. This way, the central signals to kickstart the immune response in squamous epithelia are absent [112].

One aspect that should be emphasized is the complexity of the innate immune system which includes innate effector mechanisms such as inflammation, chemotaxis, and complex patterns of pathogen recognition involving toll-like receptors (TLRs). These molecules play a key role in innate immune responses, in view of their role in the activation of the production of type 1 interferons. It was found that the clearance of infection by HPV16 was associated with increased expression of several TLRs compared to women who do not clear, including the four TLRs known as being important for recognition viral nucleic acids-TLR1, TLR3, TLR7, and TLR8 [113].

As HPV infections are exclusively intraepithelial, theoretically, an HPV attack would be detected by the professional APC cells of squamous epithelia such as the Lanherhans cells (LCs), which are intraepithelial dendritic cells (DCs). The virus capsid entry is usually an activating signal for DCs, but there is evidence that LCs are not activated by the uptake of HPV capsids [111]. The life cycle of HPV is organized so as to limit the viral antigen synthesis in undifferentiated cells, and high-expression is restricted to highly differentiated cells. Also, there is no viremia, cell lysis, necrosis, or any other signals to trigger an inflammatory response [114].

The healing of the HPV-induced lesions is dependent on the mechanisms of the cell-mediated immune response and involves interactions between lymphocytes TCD4+ and TCD8+. LCs are the major dendritic cells found in squamous epithelia, and these are probably responsible for triggering an anti-HPV immune response, but the function of LCs is disrupted by the HPV at several levels [111].

The uppermost epithelial layers of the ectocervix and vagina constitute a unique microenvironment, where the lack of tight junctions and permeability to large-molecular-weight immunological mediators suggest that this region plays an important role in host defense against microbial pathogens [115]. The epithelium of this region appears to be immunologically hyporesponsive, and HPV can induce a local immune deficiency by lymphocyte depletion, LCs, and CD4, as well as down-regulating the cytokine production of cells [116].

Furthermore, the vaginal epithelium presents a major DCs subset that does not express langerin (Lang $^{-}$ DC), and exert a downregulating activity on mucosal citotoxic T lymphocytes (CTL), by a mechanism that may involve IL-17 and, to a lesser extent, IL-10 [116].

Natural HPV infection induces weak immunity because the action of proteins encoded by the HPV, that activate multiple mechanisms to prevent initiation of a robust immune response. Among these mechanisms are the depletion of the LCs mediated by the viral protein E6, the downregulation of MHC molecules of class I by the E5 protein, facilitating the evasion of CTL attack, and blocking the signaling pathway of type 1 interferons (IFNs) by E7 [117].

Furthermore, downregulation of the expression of IFNs by the E6 and E7 viral proteins results in the lack of co-stimulatory signals by inflammatory cytokines including IFNs during antigen recognition which may induce immune tolerance rather than the appropriate responses [118]. These mechanisms of immune evasion may eventually support the establishment of persistent HPV infection, leading to a condition which favors the progression of the lesions and thus increasing the risk of development of cancer.

It is well known that keratinocytes constitutively express low levels of several cytokines that are upregulated following virus infection [119]. When infected with HPV, these cells show significantly reduced expression of a wide range of inflammatory cytokines including IL-1, IL-6, TNF-α, and TGF-β; at the same time, expression of the anti-inflammatory cytokine IL-10 is increased [119, 120] It is believed that IL-10 and possibly TGF-β contribute to the stimulation of specific regulatory T cells (Treg) lymphocytes that can suppress the activity of antitumor effector cells (CD8 CTL lymphocytes). [121]. Thus, HPV infection induces alteration in cytokine production, reducing the ability of immune cells to infiltrate the infected tissue. Keratinocytes also constitutively express low levels of interferons α, β, and κ.

The expression of interferon κ is suppressed in HPV positive cells and this could contribute to the inhibition of expression of interferon-inducible genes [122].

Studies in mice and in humans with HPV-induced tumors support the idea that the local environment in persistent HPV infection and in HPV-related cervical cancer is characterized as anti-inflammatory. These studies provide evidence that HPV E7-driven hyperplastic epithelium can actively recruit suppressive T cells to murine skin and that NK cells paradoxically use a pro-inflammatory cytokine, IFN-α, to suppress local skin immunity [123].

Thus, both the innate and adaptive arms of the immune response can be impaired by the persistent HPV infection, favoring the progression toward cervical cancer [123].

In a recent study it was observed that IL-10 levels detected in cervical secretions of patients with HPV positive lesions were significantly greater in comparison with those obtained from patients with similar HPV negative lesions, with the levels of IL-10 in cervical secretions significantly higher than in their sera. Furthermore, higher levels of IL-10 were observed in secretions of patients who had progressive cervical lesions (HSIL and cervical cancer stages) that were HPV positive. No difference was observed between the levels of TNFα in cervical secretions of patients with HPV positive lesions and those with similar HPV negative lesions. Also, no difference was found in the levels of TNFα in secretions and serum in the two groups of patients [124].

In general, the higher levels of IL-10 than of TNFα suggest a potential down-modulation of tumor-specific immune responses to HPV-infected lesions [124]. This condition seems to create a microenvironment favorable to progression of those lesions, increasing the risk of development of tumors in these patients.

Under normal conditions, the levels of cervicovaginal cytokines are higher in women with more immature columnar and metaplastic cervical epithelium compared to a more mature squamous epithelium. As adolescent females have an immature cervical epithelium, they exhibit significantly higher levels of cytokines such as IL-1α, IL-1β, IL-6, IL-8, MIP-1α, RANTES, TNFα, IL-10, IL-12 and IFN-γ, compared to women with mature epithelium. This cytokine profile in healthy adolescents may foreshadow effective responses to eliminate pathogens [125]. This could explain, at least in part, the fact that despite the high prevalence rates found for HPV infection in adolescent populations, most cases heal spontaneously in approximately 24 months [50]. However, it was found that new HPV infections occur repeatedly among young females with 28% to 48% of them acquiring another infection within a period of 12 months. Having recent new sexual partners with a laboratory-documented STI further increased the risk of have repeated HPV infection and viral persistence [126].

Thus, it is believed that the repeated acquisition of HPV infections in young women is influenced by more exposure to the virus due to a greater possibility for the sex partner's variation and may also reflect a dysfunction in immune response, which at the same time increases the risk of acquisition of the infection and the inability to clear the virus.

4. PREVENTION

Education has played an important role in the implementation of an HPV vaccination program. In a recent study in the United States [127] was found that only 35% of adolescents who had not yet received the HPV vaccine were willing to be vaccinated.

The lack of knowledge about HPV infection was attributed, at least in part, to this hesitancy. In view of this, the health-care providers must be proactive in educating and discussing benefits of vaccination. This must be done emphasizing the high effectiveness of the vaccine, high likelihood of HPV infection, and physician guidance to remove barriers to vaccination. It is important to inform parents that the vaccine is most effective prior to sexual activity and HPV exposure. It is necessary to emphasize that the vaccine does not replace routine screening and to offer continued education with guidance about cervical cancer screening, aiming to make it permanent [127]. The HPV vaccine is currently available for international distribution. However, understanding the attitudes, beliefs, barriers, and other critical components in the parents, which can influence the decision to vaccinate their daughters, is necessary for the best acceptability of the vaccine and reduction of HPV infection rates [88].

In a study assessing the adherence of mothers to vaccinate their daughters showed that mothers were moderately willing to vaccinate their sons against HPV if the vaccine were free and much less willing if it cost money. This suggests that cost and health insurance coverage will be important factors in determining whether sons will be vaccinated. When mothers were informed that the vaccine may benefit their sons and sexual partners, an increase was observed in the number of mothers willing to vaccinate their sons, showing that improving their knowledge about HPV and the HPV vaccine could increase the willingness to vaccinate their sons [128]. The parent beliefs about the HPV vaccine are important for the vaccination of their daughters. These beliefs are potentially modifiable targets that could increase HPV vaccination rates, although they may differ in importance between racial groups and regions [129]. Although there may be different effects in different populations due to cultural and ethnic differences and religious beliefs, this information should be more widespread among the health professionals and accordingly work best in the communities to improve the acceptability of the HPV vaccine.

The continued detection of the cervical and extra-cervical HPV infections after vaccination is important for evaluating the real-world impact of vaccinating high-risk adolescent populations potentially prior exposures to HPV types included in the vaccine, as well as to related HR-HPV types. These studies are important to document the continued burden of HPV and to properly design future multi-type prophylactic HPV vaccines and continued screening strategies to prevent HPV-related disease [70]. Furthermore, estimates of the prevalence of the HPV types included in the vaccine and other genotypes in young, sexually active women are important because they may be used to define vaccination policies, as a baseline against which to measure the impact of the immunization program and participation of vaccine-type and non-vaccine-type HPV infections. The inclusion of this data in mathematical models can help predict the impact of the immunization program on HPV-related cervical disease in future years [91].

The mechanisms by which sexual behaviors and concurrent STI influence the release of HPV infection are not well understood. Changes in the local immune response and

inflammation of the cervix may have some role [97]. The presence of semen in the female genital tract is associated with a marked postcoital inflammatory response in which transforming growth factor ß appears to have an important role in inducing local immune hyporesponsiveness [98]. Evidence of this is the association between levels of condom use with increased clearance of HPV infection which are also consistent with observations that condom use is associated with faster regression of cervical intraepithelial neoplasia [100].

The efficiency of latex condoms for reducing the risk of contracting HPV infection is not well established, although they can probably offer a certain degree of protection. In vitro studies demonstrate the impermeability of latex condoms to HPV during conditions simulating sexual intercourse [99]. A meta-analysis study [130], found no evidence that the use of condom reduces the risk of a person becoming HPV DNA positive. However, condom use was effective in overall reduction of genital warts, dysplasia, and HPV-associated cervical cancer. Thus, although the condom is not effective in protecting against the acquisition of HPV infection, studies have shown that use of condoms can reduce the acquisition of genital warts and cervical dysplasia and may allow HPV-related lesions to regress faster [100, 130].

A study in the UK provided evidence that adolescents, particularly those over 16 years of age, were more likely to make HPV vaccination decisions, revealing that parents' attitudes may not be as relevant for this age group. It was also found that specific religious groups may be less likely to accept HPV vaccination [131]. However, others believe that parents still exert an important influence on girls at this stage of their development by talking to them and encouraging them to think about the importance of vaccination and, having begun a vaccine course, to complete it. Health professionals should correct the misconceptions perpetrated by girls and also by some parents, thereby reducing uninformed discussion and helping girls to come to their own decision about HPV vaccination [132].

A study involving a group of 14- to 16-year old 1800 girls, prior to HPV vaccination, observed that in that age group, eligible for vaccination, the prevalence rates of HPV infection were low [85].

Among girls with a positive test for high-risk HPV, approximately a third (32%) were seropositive and showed no signs of current infection. Because of the expected increase in age-related HPV infection in these girls, due to increased sexual activity, this monitoring becomes especially important for evaluating vaccine effectiveness. In the long run, the impact of vaccination can also be measured. In addition, this monitoring allows the detection of acquiring new HPV infections in girls previously negative, in relation to their status of vaccination. Thus, risk factors and serologic status can be studied longitudinally. In addition, such study offers the opportunity to improve the knowledge of antibody response following natural infection and vaccination [85].

The vaccines are recommended preferably for young adolescent girls who develop a high titer of antibody at or before the onset of puberty and are effective only if the recipient is not already infected with HPVs employed in the vaccine, prior to vaccination [133]. Cultural factors that can have an impact on the acceptance of the HPV vaccine, particularly in minority communities with existing disparities and deficient medical assistance, have attracted the attention of investigators [134, 135]. It has been observed that racial, ethnic, and socioeconomic differences affect the knowledge, attitudes and practices related to the HPV vaccine, with differences in experience and in parents' strategies [136, 137, 138].

Socioeconomic and cultural aspects in low resource regions increase the complexity of introducing a new vaccine from the perspectives of the target population of children, their

parents, and the community as a whole [139]. The maximum feasibility, acceptability, and lower cost for HPV vaccination could be achieved through national immunization programs and by partnering with other sectors, such as non-governamental organizations. It is also important to strengthen existing human resources and cold chain infrastructure, and schools should be considered for reaching target populations [140]

Physicians play a critical role in HPV immunization, starting with conversation about the benefits of immunization as well as offering support for parenting strategies aimed at protecting daughters by vaccination. Discussing culturally relevant aspects with mothers, doctors can facilitate an understanding of the mothers about the connection between cervical dysplasia, cervical cancer and HPV as well as about the need for immunization [137, 141]. Previous experience of the mothers in prevention of HPV-related diseases, such as the Pap test, provides the opportunity to explain about HPV and to educate the mother about need for immunization of their daughters [142]. Thus, the physicians can engage in culturally relevant vaccine promotion in the neediest communities by initiating discussion of HPV immunization, convincing the mothers to acknowledge the role of HPV in dysplasia and cervical cancer, and supporting parenting strategies that aim to protect their daughters against HPV such as participating in the immunization program [143].

The evaluation of the real-world impact of vaccinating the high-risk adolescent population prior exposures to HPV vaccine can be critical to document the continued burden of HPV and to properly design future multiple-type prophylactic HPV vaccines and continued screening strategies to prevent HPV-related disease [70]. It was reported that the HPV vaccine was effective for young, high-risk adolescents, who were recruited from a clinical setting and had variable rates of completion of the vaccination. The authors emphasized the need for early and catch-up vaccination in all adolescent and young adult women. Furthermore, they suggested to clinicians and parents that the targeted vaccination population should include all 11- to 12-year-old to maximize its impact rather than waiting until the adolescent is older [144].

Despite this lack of protection against HPV infection, contraceptive barrier methods remain of particular importance for members of this age group as they begin sexually active lives. Therefore, education and counseling on prevention by the contraceptive barrier should continue. It has been proposed that the cytological examination must be performed every three years, under the condition that two annual Pap tests in a row were normal. For the particular group of female adolescents, some studies seem to support this attitude, taking into account that no high-grade cervical lesion was detected in their setting. However, some individuals with normal cytology have high- or intermediate risk for HPV. These individuals are clearly at a greater risk for cervical intraepithelial neoplasia and, according to the new guidelines, should be counseled to have the cytological examination with shorter intervals. In order to contribute to the resolution of the particular problem of HPV infection, health education and counseling for female adolescents should stress the importance of having regular Pap tests [145].

Adolescent females who are sexually active should be encouraged to seek gynecologic care for screening for HPV and cervical cancer, and all should be properly educated regarding HPV and the risks associated with infection. Condom use remains important in the control of HPV infection and HPV-disease progression, and thus should be recommended to all sexually active adolescents. Once that the LSIL lesions are usually transient in adolescent populations, an immediate treatment is not necessary. It is advised to repeat the cytology or perform HPV

DNA testing. Immunocompromised patients are at an elevated risk for developing HPV-associated disease, and should be monitored [126].

Despite the efforts of parents and educators in the field of sex education to convince young people of the need to practice safe sex through the correct use of condoms during sexual intercourse, they have not been successful in decreasing the incidence of genital HPV infections among adolescents. Although it is known that a condom protects only the area that is covered and is not totally reliable, the doctor who meets adolescent patients should not only recommend the use of condoms, but must also play a key role in providing sex education for these young people. It is important to provide adolescents with preventive guidance and to stimulate thinking about the consequences of their own behaviors and sexual practices.

There is no doubt that the key point in preventing cervical cancer and its precursor lesions is vaccination against HPV. However, the still high cost of the current vaccines hinders their use on a large scale, especially in less developed regions of the world, so that large portions of the world population still have no access to this benefit. Thus, the application where possible of prophylactic vaccines before first sexual intercourse, education for the consistent and proper use of condoms in sexual relations, and early detection and treatment of cervical lesions produced by HPV are needed to significantly reduce the incidence of cervical cancer.

5. TREATMENT

HPV-related lesions in adolescents have been the interest of numerous studies. In general, it is thought that the adolescent is less likely to have cancerous lesions; therefore, conservative management is suggested in cases of cervical intraepithelial neoplasia grade 2 (CIN2), as the majority of them regress after 2 - 3 years [146]. Conservative management is further encouraged given the negative consequence of the use of excisional cervical techniques, since they can have obstetric implications such as the increased risk of premature labor [147].

The research on transcripts of the viral genes E6/E7 has identified them as a possible prognosis marker, and girls presenting high expression of these genes are more prone to the development of high grade lesions and cancer [148]. The inclusion of the mRNA test within the current protocol of follow-up could be efficient and could allow earlier prediction of the risk of residual/recurrent cervical abnormalities after conization. This molecular strategy would also reduce overtreatment, particularly in patients above 30 years of age [149]. However, it is unclear how one should act with the detection of the mRNA expression of the E6/E7 oncogene among adolescents. Only with the follow-up of girls with the mRNA positive test will it be possible to check whether results are consistent with regard to the natural history of mRNA expression in adolescent girls in the near future [148].

It has been suggested that CIN in adolescents is more likely to regress without treatment. Although less intensive intervention for adolescents with LSIL and HSIL certainly seems reasonable and prudent, it should be considered that these adolescents still have a risk of developing malignant lesions and, therefore, require close follow-up. Counseling for contraceptive use during the time of follow-up would certainly seem reasonable. Considering the lack of consensus regarding the treatment of cervical lesions in adolescents, there is a

clear need for prospective studies to evaluate alternative strategies for helping adolescents with LSIL and HSIL [109].

Decision-making concerning treatment of the cervical dysplasia in teen girls may have important implications. The overtreatment and the relative risk on future childbearing as well as the emotional implications of labeling an adolescent with a potential precancerous lesion can have a strong negative impact on the lives of these young girls. The probability of emotional problems should be considered, given that adolescence is a time of heightened concern for self-image and emerging sexuality. A meta-analysis study reported that women undergoing an electrosurgical excision procedure (LEEP) had lower prenatal mortality, lower preterm delivery, and lower birth weight compared to those undergoing cold knife conization. However, LEEP was not completely free of adverse outcomes [150]. A significant increase in premature births in women previously treated with excisional procedures for dysplasia was also reported in a recent study [147]. Therefore, in teenagers the use of LEEP should be limited.

The new guidelines from the ACG establish that cervical cancer screening should begin at age 21 regardless of the age of onset of sexual intercourse. This recommendation is based on the high prevalence of HPV infection, high rate of regression of low-grade intraepithelial lesion (LSIL), and very low incidence of cervical cancer in adolescents and young women [151]. The update of guidelines of the American Society for Colposcopy and Cervical Pathology (ASCCP) recommend treatment for CIN 2/3 lesions in adolescents must be done by observation-colposcopy and cytology or treatment using excision or ablation of the T-zone. However, it also stated that when CIN 2 is specified, observation is preferred. When CIN 3 is specified, or the colposcopy is unsatisfactory, treatment is recommended [152]. The high rates of reported regression support the guideline of not recommending immediate excision for teenagers. Based on the literature, the treatment of CIN 2 lesions in adolescents is not necessary. Therefore, the recommendation of carefully following the ASCCP guidelines and for the limited use of LEEP for CIN 3 or persistent CIN 2 lesions should be adopted [153].

CONCLUSION

The incidence of HPV infection and its injuries is higher in adolescent population, and constitutes a public health problem of global importance. Apart from the great physical, emotional, and psychosocial changes faced by adolescent women, there are also high risk behaviors for their health.

The uterine cervix in the adolescent is predominantly made up of columnar and metaplastic epithelium. With the onset of puberty, small changes in estrogen levels, such as those seen in early puberty, result in the induction of squamous metaplasia. The immaturity of the epithelium with active squamous metaplasia and the large gland aversion with inadequate production of cervical mucus due to anovulatory cycles, associated with the immaturity of the immune system, create a microenvironment conducive to virus. Thus, it is possible that factors related to attitudes, behaviors, the physiology of the cervical epithelium, and the biologic risks, make adolescents more vulnerable not only to HPV infection, but also to the viral persistence.

Although no relationship between ectopy and the increased risk of acquiring HPV infection has been found, it is speculated that the metaplastic epithelium may be more vulnerable to wounds induced by intercourse, douche, or tampons, leading to the appearance of microdamage in the epithelium that can facilitate access of HPV to basal layer cells. It has been observed that adolescent women have higher levels of cervicovaginal cytokines compared to adult women.

This could explain why although they have greater ease for acquiring HPV infection, most of them heal without treatment. However, a portion of them acquire infections repeatedly and cannot eliminate the virus. This could be explained by more exposure to the virus, due to the higher variation of sexual partners, but may also reflect a dysfunction in the immune response, which at the same time increases the risk of acquisition of the infection and inability to clear the virus.

Several factors, including sexual behavior, may contribute to the increased risk not only for the acquisition of HPV infection, but also for the progression of lesions in the adolescent population. However, other factors such as social aspects related to the individual's culture - among them habits, customs, attitudes, and practices - may increase the risk. In addition, biological factors can make the person more exposed to the virus, increasing the chance of acquiring the infection as well as developing disease. The lack of parental accompaniment, the difficulty of parent-adolescent communication, insufficient sex education, and sexual content in the media may all play an important role. The higher probability of variation of sexual partners among adolescents, early age of first sexual intercourse, and the small interval between menarche and first sexual intercourse are also considered risk factors. Furthermore, the sexual behavior of the partners, older and uncircumcised sexual partners, smoking, alcohol consumption, high parity, prolonged oral contraceptive use, and infection with *C. trachomatis* may also constitute risk factors. Individual susceptibility, immune status, and nutrition, as well as endogenous and exogenous hormones, may also have a role in the progression of the lesions.

The characteristics of HPV infection and the strategy adopted by the virus to evade the host defense mechanisms make the organism stay indifferent to the presence of the virus, facilitating the persistence. Among these mechanisms are the differentiation-dependent organization of the viral life cycle, strategies to maintain a low number of genome copies in the undifferentiated cells, and avoidance mechanisms of the innate and adaptive immune surveillance. Targeting the innate and adaptive immune responses and different signaling pathways, as well as interfering in cell cycle and death signals, high-risk HPV types are strong carcinogens to humans.

These viruses have evolved different ways to evade the immune system. For example, virtually no inflammatory response is induced, due to the lack of cell lyses, necrosis, or clear tissue damage, as occurs in other infections. Despite this, accumulating evidence shows that inflammation plays a role in the HPV-induced carcinogenesis. Cellular, innate, and adaptive responses, in which several cytokines are secreted, play a role in HPV-associated disease progression.

The prevention of HPV infection in adolescents and, consequently, of cervical dysplasia and cancer is a complex task that involves multiple factors and conditions which intertwine and exert influence, one over the other. Although there is no evidence that it provides effective protection against the acquisition of HPV infection, condom use was generally effective in reducing the acquisition of genital warts and cervical dysplasia and may allow

HPV-related lesions to regress faster. Thus, condom use remains important for control of the infection and HPV-associated disease progression, and thus should be recommended for all sexually active adolescents.

Socioeconomic, cultural, and religious aspects increase the complexity of the prevention of HPV infection. Therefore, the introduction of the vaccine must be made from the perspectives of the target population of children, their parents, and the community as a whole.

Despite the high efficacy of prophylactic vaccines, one limitation that must be overcome is the high cost of currently available vaccines that hinders their large-scale use, especially in the poorest regions of the world. The feasibility, maximum acceptability, and lower cost of HPV vaccination could be achieved through national immunization programs and by partnering with other sectors, such as non-governmental organizations. Physicians play a critical role in HPV immunization by offering support to parents and focusing on the culturally relevant aspects that can facilitate an understanding about the connection between cervical dysplasia and cervical cancer with HPV as well as about the benefits and need for immunization.

Although the intervention for adolescents with cervical dysplasia HSIL is less intensive, it seems reasonable and prudent to consider that they have a considerable risk of developing malignant lesions and, therefore, require close follow-up. The counseling for condom use during the time of follow-up also seems reasonable. However, decision-making related to treatment of the cervical dysplasia in teens may have important implications from the viewpoint of emotional and fertility. The probability of experiencing emotional problems should be considered, given that adolescence is a time of heightened concern for self-image and emerging sexuality. Guidelines for treating dysplasia in adolescents recommend observation-colposcopy and cytology or treatment using excision or ablation of the T-zone. However, these guidelines also state that when CIN 2 is specified, observation is preferred.

When CIN 3 is specified, or colposcopy is unsatisfactory, treatment is recommended. However, close and careful monitoring, rather than immediate excision, is recommended for teenagers.

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Chapter 4

CLINICAL UTILITY OF HPV TESTING IN CERVICAL CANCER SCREENING

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ABSTRACT

Cervical cancer remains the second most common cause of cancer death among women around the world. It has been recognized as a rare outcome of Human Papillomavirus (HPV), a common sexually transmitted infection. Persistent infection with high-risk oncogenic HPV types is a known cause of cervical cancer. The benefits of cytological screening for cervical cancer are well known. However, the necessary resources, infrastructure and technological expertise, together with the need for repeated screening at regular intervals, make cytological screening resource intense. HPV DNA testing is a viable alternative to cytological screening. With optimal testing systems, HPV DNA can be identified in nearly all specimens of invasive cervical cancer and in the vast majority (>95%) of the immediate cervical cancer precursors, namely high-grade squamous intraepithelial lesions (HSILs). HPV DNA testing has been shown to decrease cervical cancer mortality. In this chapter, the clinical utility of HPV DNA testing for cervical cancer will be reviewed with a focus on primary screening, combination with cytology (co-testing), triage for abnormal cytology, and post-treatment surveillance. The review focuses on cost and patient outcomes in a wide variety of resource settings, contrasting HPV testing to other screening strategies. Technology exists today, which could eradicate death from cervical cancer. It is time to act.

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INTRODUCTION

Oncogenic human papillomavirus (HPV) has a causal role in nearly all cervical cancers and in many vulvar, vaginal, penile, anal, and oropharyngeal cancers. The epidemiologic association between HPV infection and cervical cancer fulfills all of the established epidemiologic criteria for causality [1, 2]. It is important to note that the mere presence of HPV DNA at the cervix does not imply a woman is destined to develop cancer. The natural history of a genital HPV infection involves changes in the presence of HPV, HPV types, and number of HPV genomes over time. Most genital HPV infection is transient [3, 4]. Persistent and not transient infection with HPV high-risk types is crucial for malignant transformation. Cervical infection with HPV requires a co-factor before progression to significant disease. Possible biological co-factors include viral load, viral persistence, HPV variants, immune function, other infections, and tobacco smoking.

The objective of this chapter is to review the data related to various cervical cancer screening methods with a focus on HPV testing. This will include the clinical applications of HPV testing for primary cervical cancer screening, HPV testing in combination with cytology (co-testing), triage for abnormal cytology, and post-treatment surveillance. Unlike other reviews, we will focus on the logistical issues related to each method in a comparison of high- and low-resource settings. Not every setting can implement a high-resource system like that which exists in the United States and the European Union. Even high-resource settings need to consider whether the current system is cost effective given the new technologies available. In order to accomplish this, clinicians and policy makers first need to understand the setting of screening for cervical cancer as diagramed in Figure 1.

Screening benefits all sexually active women (with a cervix) by providing a means of early detection of cervical cancer and immediate treatment. One of the major logistical issues related to cervical cancer is simply having access to a screening system. In settings where health care is only available to those who can pay, most women will not have access to cervical cancer screening. The critical policy issues involve reducing the cost of the screening test, screening women in their local environment, and utilizing the available resources. Potential resources that may affect care include access to clean water, power, laboratory and processing equipment, and specifically cold chain (temperature-controlled supply chain). Additionally, traditional screening requires trained health care providers who have the minimum equipment required to perform the pelvic examination, including speculum, examination light, Pap smear supplies, and personal protective equipment. In some environments, there is also a cultural component of personal privacy, which may prevent women from submitting to a pelvic examination.

After screening, the diagnosis needs to be confirmed and then, if necessary, the woman will be treated. If the screening test requires hours or days to return results, the woman may incur the logistical issue of either staying in a city away from her home or returning for a follow-up evaluation. Moreover, in a system that mandates confirmation of the screening test with a diagnostic evaluation such as colposcopy with biopsy, this introduces even more follow-up with the risk of women not coming back plus the increased cost. A potential option to decrease this need for follow-up evaluation is to establish a system of screening that treats all positive screens in the same visit as highlighted by the arrow in Figure 1. The cost for a screen-and-treat policy is over-treating disease that would resolve without treatment. This

approach has been proven safe and effective [5]. And finally, for women who have been treated, a system then needs to be in place to monitor their response. In summary, screening for cervical cancer is not just the delivery of the screening test. It includes the management of women who screen positive. Without a systematic delivery of all components to identify women at risk, screen, treat, and response surveillance, there will not be a reduction in the mortality from cervical cancer.

Figure 1: Overview of the setting of screening for cervical cancer.

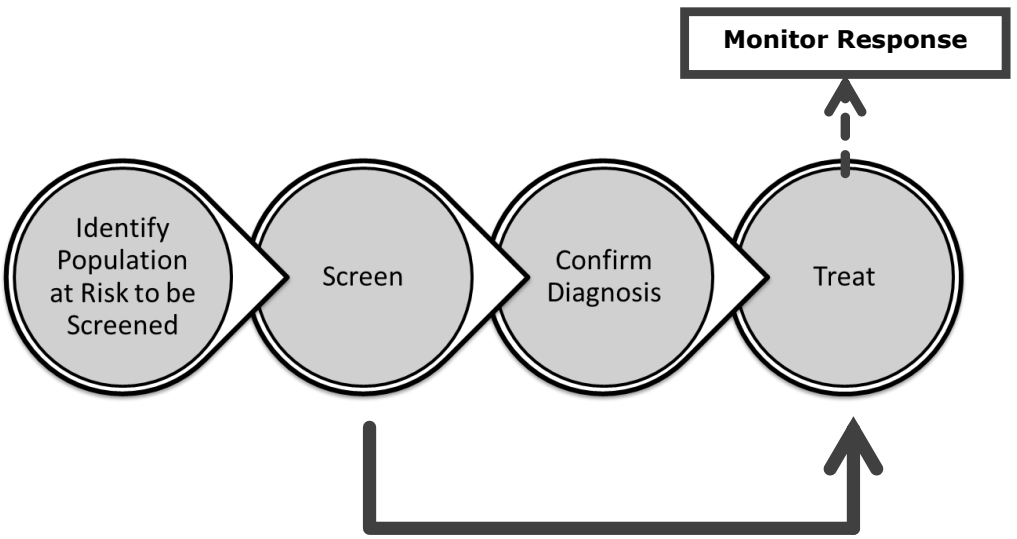

Figure 1. Overview of the setting of screening for cervical cancer.

HPV AND CERVICAL CANCER

In 2008, there were about 530,000 new cases of cervical cancer, with almost half (250,000) of which died from the disease worldwide [6]. Prevalence of cervical cancer varies widely, with 85% of incidence and mortality occurring in developing countries [6]. HPV infection is the most prevalent sexually transmitted virus and its persistent infection is required for the development of cervical dysplasia and potential invasive cervical cancer [7].

A more in depth discussion on the epidemiology of cervical cancer can be found in Chapter XX.

Hpv Genotypes

HPV "types" refer to "genotypes" that are classified based on sequence similarity. Over 120 HPV genotypes and have been categorized into two main groups, "low-risk" and "high-risk" [8]. Currently, 15 "high risk" HPV genotypes have been identified, namely HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 [9]. HPV 16 and HPV 18 are the two highest-risk types, found in over 70% of cervical carcinomas and its immediate precursor, cervical intraepithelial neoplasia (CIN) grade 3 [10]. Persistent infection with high-risk HPV subtype is the single greatest risk factor for invasive cervical cancer [11].

CURRENT METHODS FOR CERVICAL CANCER SCREENING

In order to develop cost effective screening programs internationally, countries need to balance the accuracy and efficacy of tests with the affordability and availability of their resources and treatment options. The following section highlights the various methods of screening for cervical cancer used worldwide, with and without HPV testing. An overview of the characteristics of these screening modalities is presented in Table 1.

Visual Inspection

Visual inspection of cervix with acetic acid (VIA) is currently a popular method of cervical cancer of screening test in low-resource countries. VIA, a non-cytological test is a simple and inexpensive test that can be performed by paramedical personnel with brief training. Using the naked eye or magnification (VIAM), the cervix is examined after application of acetic acid or Lugol iodine (VILI) for lesions of the squamocolumnar junction. The use of acetic acid on the cervix provides an immediate result (an acetowhite area), and the use of Lugol's iodine produces an immediate yellowish nonstaining area. The sensitivity of both for detection of high-grade lesions varies widely in studies in different settings is mostly reported in the 70-85%, which is roughly comparable to cervical cytologic examination [12]. The specificity also varies, from 66.7-89.7%, and of concern, is a low positive predictive value (PPV) of 6.6% [13]. The negative predictive value (NPV), however, is 99.2% [13]. Importantly, the application of VIA resulted in only a 32% reduction in CIN2+ [13]. The screen positive rate in newly trained screeners ranged from 25-35%, but it has been as high as 55% [14], but the accuracy of detection increases with experience [13].

Studies in India and South Africa have demonstrated the superiority of HPV testing over VIA in screen-and-treat settings. The use of VIA in South Africa using a screen-and-treat approach did show a 32% reduction in CIN2+ but was not as effective as HPV screen-and-treat [15]. In a cluster-randomized trial of 131,746 healthy women between the ages of 30-59 years in India, the population of VIA-screened women and the population of women screened with cervical cytology did not have a significant reduction in cervical cancer mortality compared to the unscreened women [16]. Other studies have demonstrated an effective screening performance for VIA in terms of sensitivity, specificity, PPV, and NPV compared to cervical cytology [13]. However, there are no other studies that have addressed the critical issue of reduction in mortality in cervical cancer from VIA screening.

Conventional Cytology (Pap Smear)

The Papanicolau test, or Pap smear, has been the standard screening test for much of the last half-century since its introduction in 1943 [17]. Cervical epithelial samplings are fixed, stained, and then checked for morphological abnormalities. This conventional cytologic (CC) testing has a specificity of 94-97% in distinguishing CIN2 and CIN3 though with a sensitivity of only 70-80% [18]. Uneven sampling of cervical epithelia and sample loss during

preparation of cytology slides limit this technique's decreased sensitivity and frequent false-negative results.

Liquid-Based Cytology

Liquid-based cytology (LBC) has emerged as an alternative to CC. Improvements in sample preparation, however, have not significantly raised overall sensitivity. In comparison in a recent meta-analysis, relative sensitivity of LBC versus CC, using the CIN2+ cut-off was 2.74 (95% confidence interval [CI] 1.66-4.53). The PPV of LBC and CC for CIN2+ was 48% and 38%, respectively [12]. Due to the long lead-time associated with cervical precancers, regular intervals of screening using LBC or conventional methods have shown to both be effective. However, routine screening places a large burden on patients and their respective health systems.

Cervical Cytology: Self or Health Care Worker Collected

In the traditional approach to screening for cervical cancer, a health care worker performs a pelvic examination and collects exfoliated cells directly from the cervix. This approach is not always feasible due to the lack of trained health care workers, inadequate or absence of facilities and equipment (private area, light, table, and speculums), and poor acceptance of the examination by women. Self-collection, on the other hand, involves women placing a cotton swab in the cervicovaginal area for several minutes. This method is advantageous in that it eliminates the need for the above-mentioned resources and is more acceptable to women [19].

Table 1. Performance And Characteristics Of Different Screening Methods

Screening Test	Sensitivity	Specificity	Characteristics
Conventional cytology	Moderate (44-78%)	High (91-96%)	Requires adequate health care infrastructure; laboratory based; stringent training and quality control
HPV DNA testing	High (66-100%)	Moderate (61-96%)	Laboratory-based; high throughput; objective, reproducible and robust; currently expensive
Visual inspection methods			Low technology; low cost
VIA	Moderate (67-79%)	Low (49-86%)	Linkage to immediate treatment possible; suitable for low-resource settings
VIAM	Moderate (62-73%)	Low (86-87%)	
VILI	Moderate to high (78–98%)	Low (73-93%)	

From reference [20]

VIA: Visual Inspection with Acetic Acid

VIAM: Magnified Visual Inspection with Acetic Acid

VILI: Visual Inspection with Lugol's Iodine

Compared to provider-collected samples, the reported sensitivities for cytology self-collection techniques range from 55-100%, specificities range from 84-100%, NPVs range from 85-91.9%, and false-negative rates range from 2.8-46.7% [19]. Self-collected samples for cervical cytology have not been promoted given these weak performance characteristics and the need for a well-trained cytologist to review the sample. Notably, the self-collected specimen can be used for cervical cytology or HPV testing as well as testing for other sexually transmitted infections.

HPV Testing Methods

Thus, the detection of HPV infection relies mainly on a molecular approach by either amplifying the viral genome or HPV mRNA, or detection of viral protein using immunoassays. All HPV assays currently in use rely on the detection of viral nucleic acids since HPV cannot yet be cultured. The most commonly used HPV test in clinical trials is the Hybrid capture (HC2). HC2 is based on liquid phase hybridization using long synthetic RNA probes complementary to the genomic 13 high-risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 5 low-risk types (6, 11, 42, 43, and 44) [21].

Target Regions

The most commonly used target for HPV genome amplification from clinical samples is the L1 region. However, this region reveals only HPV types that pre-dominate a co-infection. The E6 and E7 regions are alternative targets that are both constitutively retained and thus not affected by viral genome integration that would miss a small proportion of invasive cervical cancer samples the way L1 is subject to [22, 23]. Persistent expression of the E6 and E7 oncoproteins is an indicator of progression from intraepithelial neoplasia to invasive cancer [24, 25]. Several studies have compared E6/E7 mRNA assays to HC2 and demonstrated higher specificity and PPV for high-grade lesions [26-29]. In a review on 11 studies examining the three commercially available HPV mRNA assays, the sensitivities for CIN2+ ranged from 41-86% for the PreTect Proofer (Norchip, Klokkarstua)/NucliSENS Easy Q HPV (BioMerieux, Marcy-l'Etoile), and 90-95% for the APTIMA assay (GenProbe, San Diego)[28]. Note the Pretect Proofer and Easy Q are the same test marketed under different brand names in different countries.

Several commercially available DNA and mRNA assays have been developed based on various combinations of high-risk HPV types and are listed in Table 2.

Comparing HPV Detection Technologies

Many of the licensed HPV technologies use signal amplification assay that requires less DNA. The nucleic acid amplification assays use probe amplification such as polymerase chain reaction (PCR). These types of assays can be multiplexed and provide very high sensitivity, output genotype, and viral load. However, these assays also require high skill levels, extensive resources, and have the potential for contamination with previously amplified material that can lead to false positives. In Table 2, the common commercially available technologies are listed with the type of assays, national approval group, and genotypes detected.

In regards to utility in a low-resource setting, the DNA-based careHPV test (careHPV™ test; QIAGEN Gaithersburg Inc., Gaithersburg, MD) has more recently been shown to be a simple and cost-effective test in countries lacking the needed infrastructure, trained personnel, and other required follow-up methods [30-32]. CareHPV is the only HPV DNA test that is designed to be run by a health care worker with minimal laboratory training in settings with no running water or electrical power. In addition, the CareHPV samples can be run manually in lower number of samples with easily interpreted results within two-and-half hours. Currently, no other HPV detection technology has such unique logistical features that meet the needs of low resource settings.

Table 2. Summary of Commercially Available HPV Detection Methods.

Assay	Method	Approvals*	Low-Risk Strains	High Risk Strains
Cobas HPV Test (Roche)	Nucleic Acid Amplification	FDA CE	N/A	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 (not genotype specific)
INNO-LIPA HPV Genotyping Extra (Innogenetics)	Nucleic Acid Amplification	CE	6, 11, 40, 43, 44, 54, 70	16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 71, 73, 74, 82 (genotype specific)
PapilloCheck (Greiner Bio-One)	Nucleic Acid Amplification	CE	6, 11,40, 42, 43, 44	16, 18, 31, 33,35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82 (genotype specific)
GenoID Real-Time HPV Assay (GenoID)	Nucleic Acid Amplification	CE	6, 11, 42, 43, 44	16, 18, 31,33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 (genotype specific)
Digene Hybrid Capture 2 (Qiagen)	Signal Amplification	FDA	6, 11, 42, 43, 44	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 (not genotype specific)
CareHPV (Qiagen)	Signal Amplification	**	N/A	16,18,31,33,35,39,45,51,52, 56,58, 59, 66, 68 (not genotype specific)
Cervista HPV HR (Hologic)	Signal Amplification	FDA	N/A	16,18,31,33,35,39,45,51,52, 56,58, 59, 66, 68 (not genotype specific)
Cervista 16/18 (Hologic)	Signal Amplification	FDA	N/A	16,18 (not genotype specific)
APTIMA HPV assay (GenProbe)	Transcription-mediated amplification		N/A	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 (not genotype specific)
PreTect HPV-Proofer (Norchip) or NucliSENS EasyQ HPV (BioMerieux) ***	Transcription-mediated amplification		N/A	16,18, 31, 33, 45 (limited type differentiation)

*CE, which is the "Conformité Européenne" or "European Conformity," is a declaration by the manufacturer that the product meets all the appropriate provisions of the relevant legislation implementing certain European Directives. CE marking gives companies easier access into the European market to sell their products without adaptation or rechecking.

FDA, which is The United States Food and Drug Administration, is the federal governmental agency charged with protecting the health and welfare of the American public through the regulation of food, drugs, and medical devices, among other responsibilities. When we say that the test kit is FDA-Approved, it means that every aspect or component of the test system, including any claim found on our kit boxes, has been scientifically proven to be valid, safe, and effective.

** Moving into use in India and China 2013.

*** Same technology marketed under different brand names in different countries

HPV: Self or Health Care Worker Collected

HPV self-tests offer a promising strategy for reaching women who do not receive regular Pap smear tests since they can be used at home and returned by mail. HPV self-tests have only slightly lower sensitivity (86%) and specificity (81%) for detecting cervical disease compared to clinician-collected samples [33], and many women actually prefer the option of completing HPV self-tests rather than getting Pap smear tests [34, 35]. Multiple self-test devices exist, and women may prefer certain devices over others [36, 37]. In a recent review, women found self-collection for HPV detection to be acceptable in a variety of studies [38].

HPV TESTING AS A PRIMARY SCREENING FOR CERVICAL CANCER

A growing body of evidence demonstrates that HPV testing has a higher sensitivity and higher NPV compared to cytology in cervical cancer screening. [16, 30, 39-47]. This improved ability of HPV testing to detect pre-invasive cervical cancers compared to cytology supports its use in a variety of settings. In the next section, we review the data in low- and high-resource countries with HPV testing as a primary screening for cervical cancer and as an adjunct method to cytology to safely extend screening intervals. Most trials examined were based on HPV DNA-based screening though several emerging studies have also confirmed promising results using newer and more specific mRNA-based assays.

Primary Screening with HPV in Low Resource Countries

The use of HPV testing as a primary screen for cervical cancer is particularly important for its potential in low-resource countries. Because developing countries lack effective screening programs for cervical cancer, mortality from cervical cancer remains prevalent. The majority of cervical cancer cases (85%) occur in the developing world, where they also account for 88% of the deaths [6]. Overall, women in low-income nations are at a 35% greater lifetime risk of cervical cancer than women in high-income countries [48].

There are several barriers to screening in low-resource countries. Apart from civil strife and poverty, many people have limited access to health care, particularly in rural areas. Many countries have inadequate facilities for screening and treatment, and limited-to-no availability for a pathologist or cytotechnician. Also, competing health care needs, such as communicable diseases or maternal mortality, often take precedence in resource allocation. Another issue is that women are generally less educated about preventative care, and in many societies, their status is considered secondary to men. And importantly, for many low-resource countries, it is challenging to establish a systematic national screening program of high quality. Cervical cytology screening programs not only require an infrastructure for sample processing and interpretation but also resources for colposcopy and treatment of pre-invasive disease [49].

The use of a reproducible, objective test such as HPV testing could supplant the cost and resources of cervical cytology in low-income countries. The evidence on the use of HPV testing for cervical cancer screening in low-resource countries is reviewed below.

India

In 2008, India remained one of the countries with the highest number of cases (134,000) and deaths (73,000) from cervical cancer, representing 25% [50] of the cervical cancer burden globally [6]. Based on this high incidence and mortality rate, a task force for the government of India recommended a VIA-based screening program in primary health centers over the course of five years. [49]. To investigate the various cervical cancer screening methods in India, 131,746 women ages 30-59 were randomly assigned to four screening groups: HPV DNA testing (HC-2), cytology, VIA, or standard care (control). After eight years of follow up, the investigators found a decreased incidence of advanced cervical cancer and decreased cervical cancer mortality in the HPV testing group compared to all the other groups. The increased cost of the HC-2 ($20-30 USD) remains a consideration, but instead we suggest the use of a quicker and more affordable test such as the careHPV test developed by Qiagen [16]. As mentioned earlier in the chapter, careHPV test has been designed as a screening test specifically for low-resource settings, allowing for screening and treatment to occur at the same visit. Based on these results, an HPV DNA test could be considered an alternative to a VIA-based screening strategy for women over 30 in low-resource countries.

China

Approximately 70% of China's population lives in rural areas, where cervical cancer incidence is the highest [51]. To address this disparity, the Chinese government initiated a cervical cancer screening program in 2009. The goal was to screen 10 million women living in rural China with either VIA or a Pap smear over three years [52]. An analysis on 17 cross-sectional, population-based studies including a total of 30,371 Chinese women [53] compared HPV DNA testing (HC-2, careHPV), LBC, or VIA. HPV DNA testing overall had a higher sensitivity for detection of CIN3+ compared to cytology (97.5% vs. 87.9%) across different locations and age groups. However, HPV DNA testing did have a lower specificity (85.1%) than cytology (94.7%) or VIA (89.9%). Specificity varied with age, with the highest specificity for HPV testing in women younger than 35 years (89.4%). In another study, the Shenzhen Cervical Cancer Screening Trial 1, the sensitivity values of LBC, DNA-based HC2, and the mRNA-based APTIMA HPV assay were found to be 66.7%, 88.9%, and 100%, respectively. The specificity values were 95.5%, 84.5%, and 91.2%, respectively [29]. Both sets of data again demonstrates the higher sensitivity of HPV testing compared to other cervical cancer screening methods, supporting the use of HPV DNA or mRNA testing as a single-visit screening method for women in rural China.

Nigeria

The highest rates of cervical cancer mortality in the world are in found in Eastern Africa, Western Africa, and Southern Africa [54], and the utility of the careHPV test is particularly important in these regions. The use of the careHPV test was compared to VIA, LBC, and HPV PCR (MY09/MY11) test in a rural Nigerian village. Among 1,500 women ages 15 and older that received an initial screen, including VIA, LBC, and the PCR-based HPV testing, women with any positive screening result and a random selection of screen-negative controls were referred to colposcopy, where a careHPV test specimen was taken from all women. When comparing the careHPV test with HPV DNA testing by PCR, the assays had high intra-rater and inter-rater agreement, particularly among women 30–45 years old. Overall, the

careHPV test had a sensitivity of 80% to detect CIN2+, and 83.0% specificity in ruling out high-grade disease. The high NPV of careHPV at 98% indicates the majority of women are at minimal risk of developing cancer over a period of time (up to 10 or more years). This method of screening would be highly advantageous in a low-resource region where women undergo screening only once or twice in their lifetime [30].

Mexico

In the early 1980s in Mexico, there was an increase in cervical cancer-related mortality, which began to decrease at the beginning of the 1990s due to an increase in Pap smear coverage [55]. The Morelos HPV Study compared HPV DNA testing (self-collected vaginal and clinician-collected cervical) to CC in 7,732 Mexican women [39]. HPV DNA testing, both self-collected (71.3%) and clinician-collected (93.1%) had a higher sensitivity for detection of CIN2+ than CC (59.4%). In a cost-effectiveness analysis comparing no screening, CC, self-administered HPV, clinician-administered HPV, or clinician-administered HPV plus CC, the two strategies of clinician-administered HPV testing ($52.46 USD per case detected) or the combination of clinical-HPV testing plus CC ($54.92 USD per case detected) were the most cost-effective screening options for women between the ages of 30-80 [56]. In a more recent community-based trial in Mexico, 12,330 women ages 25-65 years were randomized to vaginal HPV testing or cervical cytology. Compared to cytology, HPV testing of vaginal self-collected samples had a relative sensitivity about 3.5 times greater for detection of CIN2+ and 4.2 times greater for detection of invasive cancer [57]. A newer trial has compared HPV testing (HC 2) to the APTIMA assay for the detection of CIN3+ in 2,049 Mexican women ages 30-50. While both assays had equal sensitivities for CIN3+ detection in endocervical (100%) and self-collected vaginal specimens (62.5%), the APTIMA assay was more specific than HC 2 for endocervical (93.5% vs 92.2%) and self-collected vaginal specimens (93.0% vs 90.5%) [58]. Therefore, self-collected vaginal HPV testing may be a promising option for primary cervical cancer screening in low-resource countries, but more studies will need to compare the cost-effectiveness of the different methods.

Ongoing Challenges for Screening in Low-Resource Countries

The challenges of creating high-quality, systematic screening programs in low-resource countries continue as more accurate and affordable screening tests become available. Although the natural course of HPV is a decline in prevalence in women over the age of 30, in some developing countries the prevalence still is around 20% [54]. With such a high prevalence level, there will be more women receiving follow-up triage testing or treatment, given the lower specificity of the HPV test. Although newer HPV mRNA-based assays have demonstrated increased specificity compared to LBC or HPV DNA testing as discussed earlier, it will be important for these countries to identify regional and age-specific prevalence patterns of HPV. Also, the benefit of HPV testing is the very high NPV, which will allow a single round of HPV testing in resource-limited countries where women undergo few lifetime examinations. To minimize cost and loss to follow up, women who test positive for HPV could be screened and treated in one visit. Furthermore, an infrastructure for treating women who test HPV positive will need to be developed, as treatment options such as cryotherapy are not widely available [59].

For cervical cancer screening programs to be successful, they must also address obstacles on an individual and population level. Project Screen Soweto was an example of a pilot project in Soweto, South Africa designed to address an increase in incidence in cervical cancer in the 1980s and the poor quality of the existing screening program. To accomplish this goal, laboratory capacity for Pap smear screening was increased from 3,000 to 90,000 Pap smears per year. After implementation, however, the number of Pap smears actually decreased and the numbers of new cervical cancer diagnoses declined. This failure was largely attributed to the lack of a public education campaign and no consumer demand for screening [49]. A contrasting example was a program in Sarawek, Malaysia, that not only trained staff in early detection of both breast and cervical cancer but also started a public awareness program. They also improved the referral systems for detected cancers and were successful in decreasing the late-stage presentation of breast and cervical cancers by almost 50% [48].

Primary Screening with HPV in High-Resource Countries

Northern and western Europe, the United States, and Canada, have demonstrated a strong correlation between the decline in cervical cancer-related mortality and the institution of organized cytological screening [60-62]. However, there are still challenges to organized screening with cytology. In these countries, screening is lower in areas of poorer socioeconomic status where women remain underscreened and undertreated [63-65]. Because of the more subjective nature of cytology, quality control and quality assurance is also more challenging to sustain. Poor screening coverage and inadequate follow up of women with abnormal cervical cytology continues to be a problem in some European countries. In Finland, a cytologic laboratory was found to perform poorly with interpretative results, resulting in a subsequent increase in the cervical cancer incidence. A Swedish audit also showed that there were significant regional differences in coding and logging of data, which affects subsequent treatment and referrals [66]. Given these challenges, the European Union guidelines on cervical cancer screening determined evidence was sufficient to recommend for the initiation of pilot implementation trials with HPV testing [67].

Italy

The New Technologies for Cervical Cancer (NTCC) study was a large clinical trial designed to compare CC to HPV DNA testing (HC-2). The experimental group was divided into two phases: Phase 1 was combined LBC and HPV DNA testing and Phase 2 was HPV DNA testing alone.

During Phase 2, 49,196 women ages 25-60 years old were randomized into either the CC arm or to HPV DNA testing alone by HC2 and then subsequently tested in two rounds. For women between ages 35-60, the sensitivity with HC2 was significantly higher than CC, both for CIN 2 or greater and CIN 3 or greater. Approximately twice as many cases of CIN 2/3 or greater were detected in the HPV testing group than in the cytology group after a single round of screening. After the second screening round (using cytology only in both groups) about 3.5 years later, the cumulative relative detection of CIN 3 or greater was still higher in the original HPV testing group. Furthermore, combining HPV DNA testing with cytology did not seem to increase the detection of persistent lesions versus HPV DNA testing alone.

For women aged 25-34 years old, a higher sensitivity with HPV DNA testing was also demonstrated compared to CC, both for CIN 2 or greater and CIN 3 or greater. Of note, the increase in sensitivity with HPV DNA testing was much larger among this younger group of women compared to the older age group. We attribute this difference partly to different management strategies employed for managing HPV positive women in this younger age group in Phase 1 and Phase 2. However, because most abnormalities in young women regress spontaneously, we recommend that HPV-positive women aged 25-34 should be referred to colposcopy only if cytology is also abnormal or if infection persists after one year. These results show additional evidence for the use of HPV testing as it detects clinically relevant pre-invasive cancers earlier than cytology alone [41].

United Kingdom

Two trials in the United Kingdom have evaluated the use of HPV DNA testing in conjunction with cervical cytology, but neither of these trials evaluated the use of HPV DNA testing alone compared to cytology.

In the HART (HPV in Addition to Routine Testing) study, 11,085 women ages 30-60 years received both cytologic screening and HPV DNA testing (HC 2) concurrently. HPV testing had higher sensitivity for the detection of CIN2+ compared to cytology (97% vs. 77%) but had slightly decreased specificity (93.3% vs. 95.8%). In women with mild cytologic abnormalities, all 20 of the women with high-grade lesions had originally tested HPV positive at baseline. Similarly, all high-grade lesions in women with borderline cytology were strongly HPV positive at baseline. Finally, there was no high-grade disease in the 178 women with borderline cytology who were HPV negative [40]. After six years of follow up, women with a negative HPV test result had a substantially lower rate of CIN2+ compared with those with a negative cytology result. Overall, these results together support the finding of a high sensitivity and high NPV of HPV testing, which consequently would allow extension of the screening interval to at least five years [68].

In A Randomised Trial in Screening to Improve Cytology (ARTISTIC) study, 24,510 women ages 20-64 were randomized to either LBC or combined LBC and HPV testing (HC 2) for cervical cancer screening. After the initial round of screening, a smaller proportion of cases of CIN2+ cases were diagnosed in HPV positive women with negative cytology. Additionally, after three rounds of screening or approximately six years, the cumulative CIN2+ rate in HPV negative women was less compared to cytology-negative women [69]. In their further analysis, the cost-effectiveness ratio to detect additional cervical precancers by adding HPV DNA testing to LBC was 38,771 pounds. The difference in the age-adjusted mean cost for LBC with HPV triage compared to HPV testing with LBC triage was only 9 pounds (39 vs. 48 pounds, respectively). Therefore, the investigators would not recommend LBC combined with HPV DNA testing based on the cost-effectiveness, but HPV DNA testing could be considered as primary screening with cytology triage [70]. Their findings provide further evidence that initial HPV testing provides a longer duration of protection when compared with LBC, which is important when determining screening intervals [43].

The Netherlands

In another large trial called POBASCAM (POpulation-BAsed SCreening study AMsterdam), 22,420 women ages 29-56 years were randomized to combined HPV DNA (HC 2) and CC or CC alone. At the second screening five years later, HPV DNA and cytology co-

testing was again performed in both groups. At baseline, HPV testing detected an additional 79 cases of CIN2 per 100,000 women and 30 additional cancers per 100,000 women compared with cytology alone. After five years, there were 24 fewer cases of CIN2+ per 100,000 women per year and 10 fewer cancers per 100,000 women per year due to the improved detection of CIN3 at baseline. Among the women in the co-testing group, negative cytology added minimal additional reassurance against a diagnosis of CIN3 and cancer in the second round of screening compared with HPV DNA testing alone.

The low estimated cancer risk (2.2 per 100,000 women per year) for women 30 years and older who tested HPV-negative with normal cytology supports the safe extension of screening intervals to five years. Therefore, the use of HPV DNA testing can lead to earlier detection of CIN2+, while also significantly reduces the detection of invasive precancers in subsequent screening rounds relative to CC [44].

Finland

Leinonen and colleagues conducted a randomized study in 54,207 women ages 25–65 years to compare cervical cancer screening by HPV DNA testing (HC2) to CC in Finland [42]. HPV DNA testing with cytology triage did increase detection of CIN2+ compared with cytology alone. And interestingly, the specificity of the HPV DNA test with cytology triage for detection of CIN2+ was equal to that of conventional screening for all age groups (99.2% vs. 99.1%). While numbers of immediate colposcopy referrals were similar between HPV screening (0.9%) and cytology alone (1.0%), retesting was recommended for slightly more women who received HPV testing than who received cytology (7.2% vs. 6.6%). During extended follow-up with a maximum of five years, a single round of HPV screening detected significantly increased numbers of CIN3+ compared to cytology alone. Furthermore, women with negative results on initial HPV DNA testing had a lower cumulative five-year CIN3+ rate than women with negative results on initial cytology.

Overall, in women ages 35 years or older, HPV DNA testing with cytology triage was more sensitive and more specific than conventional screening. Compared to HPV DNA testing alone, the addition of cytology as triage for HPV positive cases increased the PPV for CIN 2+ and CIN 3+ by more than six-fold. This highlights the valuable use of cytology as a triage test for HPV positive women. Overall, these results support the use of HPV DNA testing with cytology triage as a primary cervical screening method with high sensitivity and specificity for women aged 35 years or older.

Sweden

The comprehensive SwedeScreen study of 12,527 women [45] was used to compare the efficacy of 11 different screening strategies for cervical cancer in 6,257 women ages 32-38 [71]. These strategies were based on HPV DNA testing alone, cytology alone, and HPV DNA testing combined with cytology.

Compared to cytology alone, the strategy of a single HPV DNA test increased the sensitivity of detecting CIN2+ and CIN 3+ by about 30%. However, screening with a HPV DNA test decreased the PPV of detecting CIN2+ and CIN3+ by about 55% compared to cytology alone. Screening with a single HPV DNA test did not increase the total number of tests performed and actually reduced the number of screening tests needed to detect one case of CIN2+ and CIN3+ by about 25% compared to cytology alone.

Another strategy included HPV DNA testing followed by cytological triage and retesting of HPV DNA–positive women with normal cytology at least one year later to screen for persistence of HPV. Compared to cytology alone, this strategy increased the sensitivity of detection of CIN 2+ and CIN3+ by approximately 30% while not significantly decreasing the PPV. This screening method only increased the total number of tests performed by 12%, and also reduced the number of screening tests required per detected case of CIN2+ or CIN3+ by about 15%.

The investigators also looked at the strategy of screening for HPV types 16, 31, and 33 with cytological triage of women who were infected with other HPV types (not HPV 16, 31, and 33) and repeat HPV DNA testing for HPV DNA–positive women with normal cytology. Compared to cytology alone, this method resulted in an approximate 30% increase in the sensitivity to detect CIN2+ and CIN3+, but also led to a decrease in the PPVs.

Notably, there was no difference between screening with cytology alone compared to primary screening for HPV types 16, 31, and 33 in the sensitivity of detecting CIN2+ or CIN3+ lesions. Furthermore, when using this strategy, the PPVs for primary screening for HPV types 16, 31, and 33 were decreased for detection of CIN2+ and CIN3+.

Based on the overall evaluation, the investigators concluded that the most effective screening strategy was primary screening with an HPV DNA test (the test used in this trial included HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) followed by cytological triage and rescreening for persistent infection at least one year later in cytology-negative women. They found HPV DNA screening resulted in an increased sensitivity to detect CIN2+ and CIN3+ lesions, maintained a high PPV, and only modestly increased (by 12%) the number of screening tests and referrals compared with CC [71].

Canada

In Canada, cytology-based screening programs have been effective in dramatically reducing the rates of cervical cancer. In 2008, there were only 1,419 new cases of cervical cancer and 544 deaths from cervical cancer [54].

The Canadian Cervical Cancer Screening Trial randomized 9,667 women ages 30-69 to either receive CC followed by HPV DNA testing (HC-2), or HPV DNA testing followed by cytology. Consistent with previously mentioned trials, the sensitivity of the HPV test was significantly higher than cytology (94.6% vs. 55.4%), and the specificity of the HPV test was lower than the Pap test (94.1% vs. 96.8%). While 12% of the high-grade cervical lesions were found in HPV-negative women, 68% of the high-grade cervical lesions were found in women with negative Pap smear tests. This study confirms the higher sensitivity and higher NPV of HPV DNA testing compared to cytology for cervical cancer screening [46, 47].

In an ongoing trial called HPV-FOCAL, approximately 33,000 women aged 25-65 in British Columbia will be followed for four years for the detection of advanced cervical precancers. The goal is to evaluate the use of HPV DNA testing as a stand-alone screening test followed by LBC triage compared to LBC followed by HPV triage for cervical cancer screening [72]. The results of round one with a total of 18,648 women either randomized to the control arm or HPV arms have been published recently. At baseline, the rates of detection of CIN2+ were not significantly different between the two arms. However, in the subsequent screening round 12 months later, HPV DNA testing detected more CIN2 or greater lesions than cytology alone [72]. Further study results from long-term follow-up will be important in determining the efficacy of HPV DNA testing in the earlier detection of cervical precancers.

United States

Although cytology-based screening has been successful in the United States, the low sensitivity of cytology has led to an interest in more cost-effective and efficient screening methods. ATHENA (Addressing THE Need for Advanced HPV diagnostics) is the largest United States trial comparing HPV DNA testing with individual genotyping for HPV 16 and HPV 18 to LBC. In this trial, 40,901 women received both screening with LBC and two first-generation HPV DNA assays (Amplicor HPV test and Linear Array HPV genotyping test) and a second-generation cobas HPV test (HPV 16- and HPV 18-specific). The cobas HPV test was more sensitive than LBC for the detection of pre-invasive cancer (92% vs. 53%), but less specific (57% vs. 73%). The addition of LBC to HPV DNA testing only increased sensitivity for CIN3+ by about 5% (from 92% to 97%), and it also increased the number of screen-positives by 35% compared with HPV testing alone. Because co-testing provides little additional benefit over HPV testing alone, we recommend HPV testing as the primary screening test, and use of LBC to triage HPV positive women [73].

Ongoing Challenges for Screening in High Resource Countries

Trials in Europe and North America contribute to the growing evidence of the increased sensitivity of HPV testing compared to cervical cytology for the detection of cervical precancers. The high NPV also supports the extension of screening intervals to at least five years in women. The addition of cytology triage to HPV testing is a way to maintain a high specificity and increased PPV of the screening method. Therefore, HPV testing as primary screening in high-resource countries could replace cytologic testing, and cytology could be reserved as triage testing for HPV-positive women. This would result in an overall more cost-effective screening method. In order to make a transition from frequent cytologic testing to less frequent HPV testing, an HPV test that is accessible, affordable, and high quality will be necessary. Screening programs will also need to establish correct screening intervals and protocols for follow up of positively screened women to avoid overtreatment [59].

Co-testing has been introduced in the United States as an option in low-risk women over 30 [74], and could be considered a bridge to implementing HPV testing as a primary screen in the future. Studies have evaluated provider and patient preferences toward co-testing and acceptability of reduced frequency of screening. In one academic center using pathology data, the proportion of HPV co-testing with Pap smears among patients 30 and older was 7.8%. It was the highest at 15% in 2006, and then remained stable around 13% [75]. In a 2002 telephone survey of 360 women aged 40 years or older with no cancer history, the majority (75%) preferred screening at least annually by a Pap smear, and about 70% of women reported that they would try to continue being screened annually despite physician recommendations. The reason for this reluctance to reduce the frequency of screening was a belief that these changes were based on cost [76]. Also, as HPV is a sexually transmitted illness, changing to HPV as a primary screening method has the potential for increased psychosocial distress. More studies will need to be done on provider and patient acceptance of a less frequent HPV-based method of screening and its emotional impact.

Furthermore, for countries with established cervical cancer screening programs, there are a number of barriers to adopting new technologies. Change is a significant barrier. There are also the additional barriers of the investment of patients, clinicians, health care payers, and

professional organizations to an existing current system. As mentioned above, some women prefer to see a clinician once a year for a Pap smear. In turn, some clinicians are also more comfortable with this routine process, which not only allows them patient care continuity, but also provides them with reliable revenue. In countries with a cytology-based screening system, if the decision is made to move away from cytology to another detection method, such as HPV testing, cytologists, professional cytology organizations, and companies linked to cytology services may be required to adapt or perish. There is also the concern of generalizing screening for different resource settings: what may be useful in a low-resource setting might be seen as unacceptable in a high-resource setting. Further, just as patients perceive generic medicine with less confidence, health care decision-makers may perceive less expensive screening methods as less accurate and helpful. Overcoming these barriers requires time, persistence, and focused attention on evidence and not expert opinions. The evidence should be based on the outcome of reduced morbidity and mortality for cervical cancer, not just the performance characteristics of a test. Future studies should also focus on patient and provider preferences in different resource settings, low and high.

A summary of cervical cancer screening guidelines and organizational recommendations in low and high resource settings is provided in Table 3.

Table 3. Overview of International Screening Guidelines.

Country/Organization	Age Range	Interval	Primary Screening Modality
American College of Obstetricians and Gynecologists	≥21 y	Every 2–3 y	Cytology, optional HPV co-testing at >30 y
European Guidelines for Quality Assurance in Cervical Cancer Screening	Beginning between ages 20 and 30 y until 60 y	Every 3–5 y	Cytology
World Health Organization (WHO) Guidelines for Developing Countries	25–49 y, 3-y interval if not resource-limited >30 y, at least 1–3 times lifetime if resource limited		Cytology, other modalities also acceptable
South Africa (Department of Health)	≥30 y	3 tests, lifetime	Cytology
India (Government of India/WHO collaboration)	30–59 y	Every 5 y	VIA
Peru	25–59 y	Every 2 y	Cytology or VIA
Thailand	35–54 y	Every 5 y	Cytology nationally, VIA regionally

Adapted from reference [48]

HPV CO-TESTING FOR REGULAR RISK WOMEN

The natural history of HPV infection has shown decreased incidence in women over the age of 30. Because the prevalence of high-risk HPV infection declines with age, the likelihood increases that HPV positivity represents persistent disease (Sherman et al. 2002). While Pap smears are standard of care for primary cervical cancer screening, the sensitivity of conventional and LBC for CIN2 remains low at approximately 55-57%. The use of HPV co-testing, Pap smears plus HPV DNA testing, increases sensitivity for CIN2+ to 92-100% with a NPV for CIN3+ of 99-100% [12, 44, 77].

Given this high NPV, combining cytology with high-risk HPV testing allows for extended screening intervals if both tests are negative [41]. In the United States, providers are moving towards a model that includes co-testing as a cost effective and well-accepted option for screening regular risk women over 30. In 2006, co-testing was approved by the United States Food and Drug Administration for cervical cancer screening in women over 30 in the United States.

Recent guidelines issued from several major United States organizations, including the US Preventive Services Task Force (www.uspstf.org 2012) [78], American Society of Colposcopy and Cervical Pathology (www.asccp.org 2012), American Cancer Society (www.acs.org), and American College of Obstetrics and Gynecology (www.acog.org) recommend Pap test alone starting at age 21 years, with reflex HPV testing for Pap tests showing atypical squamous cells of undetermined significance (ASCUS). These organizations vary in their opinions regarding routine primary screening of low-risk women starting at age 30 years by co-testing with Pap and HPV test. However, they do all agree that if both tests are negative, no further testing is needed for five years.

HPV TESTING AS TRIAGE FOR ABNORMAL CYTOLOGY

The methods of triaging abnormal cytology found by primary screening vary internationally. This next step in secondary prevention of cervical cancer depends on the burden of cost, follow-up rate, and similar socioeconomic barriers that existed for primary screening. Once screened positive by cytology, patients are commonly triaged to either repeat cytology, reflex testing, or colposcopy [79].

Colposcopy involves visualization of abnormal cervical lesions under magnification, with or without direct biopsy for histologic diagnosis. Independently, colposcopy has a varied sensitivity reported between 44-77%, and a specificity reported between 85-90% [80]. Colposcopy can be impractical in many resource settings, requiring costly equipment, available pathology services to interpret biopsies, and a system to relay results and arrange return visits for treatment or reassessment.

Reflex testing (HPV testing when cytology is abnormal) was first studied and found to be a viable option for screening in the atypical squamous cells of uncertain significance (ASCUS)/low grade squamous intraepithelial lesion (LSIL) Triage Study (ALTS) trial. The ALTS trial found that HPV DNA testing after an ASCUS Pap smear was a sensitive and cost effective strategy. HPV DNA testing detected CIN3 with a sensitivity of 96% and it decreased the number of colposcopies by 50% [81]. Several other trials have also indicated that reflex HPV DNA testing provides the same or greater life expectancy benefits and is more cost-effective than other management strategies for women diagnosed as having ASC-US and LSIL, though not for high-grade intraepithelial lesions (HSIL) [82, 83]. More recent studies have also looked into HPV mRNA assays in triage of ASCUS and demonstrated higher specificity with predictive values comparable with those of repeat cytology [84, 85].

HPV TESTING FOR POST-TREATMENT SURVEILLANCE

Patients with cervical cancer precursors such as high-grade lesions (CIN 2/3) are treated with ablative or excisional procedures such as cryotherapy or excision of the transformation zone, called a loop electrosurgical excision procedure (LEEP). Approximately 4-18% of patients have persistent or recurrent CIN2+ after LEEP, and HPV testing can be used for surveillance in these patients [83]. Early three-month follow-up testing after LEEP can offer timely information about residual/recurrent disease and alleviate patient anxiety early about treatment failure [86]. It has similarly been found to predict treatment failure post-conization [87].

Both methods of LEEP and conization require highly trained personnel as well as expensive equipment. Cryotherapy of the cervix is a less invasive and less costly alternative treatment for CIN2+ that is well accepted by participants and commonly used in low-resource settings. Cryotherapy has demonstrated a significant decrease in immediate post-treatment detection of HPV [15, 88]. However, it has also been observed that up to 30% of CIN 2/3 lesions are not eliminated by cryotherapy during post-treatment surveillance colposcopy at 6 and 12 months [15].

The common follow-up surveillance strategy after treatment is cytologic and frequent colposcopic assessments. With the newer HPV testing technologies, post-screening surveillance using HPV testing in patients treated with cryotherapy is promising, particularly if the samples are clinician-collected vs. self-collected [89]. Both in the United States and Europe, HPV DNA or mRNA-based testing for high-risk types of HPV after treatment of CIN is now considered an acceptable approach to post-treatment follow-up [90, 91].

In a review evaluating the use of HPV testing after treatment for CIN 2/3, five studies across the United States, Europe, South America, and Asia were included to compare HC 2 to cytology for the detection of CIN2+. They found that HPV DNA testing by HC 2 detected residual or recurrent CIN2+ in 90.7% of women compared to only 76.7% women who received cytologic testing. HPV testing did have a lower specificity compared to cytology (74.6% vs. 89.7%, respectively). The longest follow up time after treatment was approximately 30 months [92]. However, there has been one study that has assessed the predictive value of surveillance testing over a period of 10 years. In a study of 435 Dutch women previously treated for CIN 2 or 3, three consecutive negative cytology results (6, 12, 24 months) resulted in 0.7% risk of CIN3+ and two negative co-tests (6, 12 months) in a 0.0% risk of CIN3+ at 5- and 10-year follow-up. Based on this level of risk, these women could be returned to regular population-based screening, which is currently recommended in Dutch guidelines [93]. Based on the higher sensitivity and higher NPV of HPV testing, the most recent guidelines by the American College of Obstetricians and Gynecologists and from the American Society for Colposcopy and Cervical Pathology recommend HPV testing alone at 6 and 12 months as follow up for high-grade dysplasia and surveillance for at least 20 years following initial treatment [94].

CONCLUSION

Ideally, all countries would adopt and implement a high quality, systematic strategy to eliminate cervical cancer. But countries do not operate in ideal and identical circumstances. Consideration needs to be given to cultural acceptance, medical infrastructure, the resources available, and the opportunity cost of timely results. The governing bodies of each country will need to define the path to more accurate and efficient cervical cancer detection methods, which will save lives and ultimately reduce the cost of long-term care for cancer patients. In Table 4, some of the key aspects of the possible screening strategies are summarized.

In communities without access to cervical cancer screening, we can see the burden of cervical cancer. In the United States, for example, the incidence of cervical cancer is highest in the Appalachian region, a geographic region that stretches from southern New York to northern Georgia, Alabama, and Mississippi [95, 96]. It is not an issue of a lack of screening system in place—women simply cannot afford to access it. In the United States, HPV-associated cancers had the largest relative contribution to mortality-related burden in women ages 30-34 [97]. In contrast, countries in the Asia Oceania region which lack an organized cervical cancer system, account for just more than 50% of all cases and 50% of all deaths from the disease worldwide [98]. However, there is significant variation in the incidence and mortality between regions in Asia Oceania region which appear linked to the presence of an organized screening system [98]. The same appears to be true in Europe [99].

Table 4. Cervical Cancer Screening Methods: Summary of Characteristics of Screening Methods for Cervical Cancer.

	Training	Feasible with Limited Resources/ Infrastructure	Immediacy of Results	Evidence of Decreased Mortality****
VIA	Limited*	Yes	Yes	No
HPV Self-Collection	Limited*	No***	No***	No
HPV Health care worker collected	Extensive**	No***	No***	Yes
Cytology Self-Collected	Limited	No	No	No
Cytology Health care worker collected	Extensive	No	No	Yes

* Limited implies that lay persons can be trained to perform the task.

** Extensive implies that only trained health care works can perform the task which may also include processing and interpreting the results such as cytology.

*** All HPV tests are not feasible in setting of limited resources or infrastructure and do not provide same day results except CareHPV.

**** Evidence is available that demonstrates that use of the screening method reduces death from cervical cancer.

Despite the known benefits of cytologic screening in reducing cervical cancer incidence and mortality, there are drawbacks to cytology-based screening programs. Cytology is subjective and requires cytotechnicians and pathologists for interpretation, as well as an infrastructure for sample collection, storage, and processing. The various steps in the process require quality control and quality assurance to maintain accuracy and reproducibility of

results. In resource-limited settings, primary screening with HPV testing has advantages over cervical cytology. HPV testing has greater objectivity and reproducibility in interpretation, high sensitivity, and high NPV. HPV testing is appropriate particularly for settings in which women have few lifetime examinations. Furthermore, HPV tests have a higher PPV in these settings because of the higher incidence of cervical cancer [94]. As demonstrated in the Finland study, the addition of cytology triage to HPV DNA testing increases the specificity and PPV of the screening method [42]. The use of the APTIMA assay for HPV testing also achieves both high sensitivity and specificity as shown in China and Mexico [29, 58]. And although either VIA or HPV DNA testing can be utilized in screen-and-treat settings, the study in India demonstrates that HPV DNA testing is superior in detecting more pre-invasive cancers and in reducing cervical cancer-related mortality compared to VIA [16]. Future studies will have to assess the cost-effectiveness of HPV testing compared to VIA as the individual HPV test becomes more affordable. Currently, the use of the careHPV test appears to be the most promising for use in low-resource countries. Also, the option of self-collected HPV samples is a further strategy for reducing the costs in these areas [48].

In high-resource countries, HPV testing has the benefit of a lower cost along with improved detection of pre-invasive and invasive cancers when compared to cytology alone. Studies also show that HPV testing is superior to cytology in the ability to provide long-term risk stratification given its high NPV [42-44]. There is little additional benefit of adding HPV DNA testing to cytology compared to HPV DNA testing alone. However, in countries with a lower incidence of cervical cancer, HPV DNA testing will have a lower PPV. Therefore, further studies will need to determine the age-specific prevalence of HPV infection and age-specific incidence of CIN 2/3 in these countries as this will be important for determining a cut off of HPV testing [42].

Policy and public health efforts to reduce the suffering from cervical cancer need to include vaccination programs to prevent infection with high-risk HPV genotypes. There are currently two approved HPV vaccines and more forthcoming. Details of HPV vaccine strategy and prevention are addressed in Chapter ZZ of this book. As HPV vaccination becomes more widely available, this cohort of women will reach screening age. The reduced prevalence of abnormalities will affect the PPV of any screening test, particularly cervical cytology, given that its interpretation is subjective [94]. There are ongoing studies to evaluate the use of alternative HPV-based biomarkers, such as testing for the highest-risk HPV types and measuring p16 or viral E6 expression, to further triage women with abnormalities [100]. Ultimately, the use of an automated objective test such as the HPV test for primary screening may be preferable to cytology in the era of HPV vaccination. While the search for more accurate and affordable screening tests continues, there also must be ongoing efforts to create quality systematic screening programs. Without an organized screening system, countries will suffer the burden of premature death of women, which could have been prevented. While we have the means to eradicate cervical cancer, whether or not we have the will remains to be determined.

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Chapter 5

HPV INFECTION IN FEMALES: EPIDEMIOLOGY AND RISK FOR CERVICAL CANCER

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ABSTRACT

Human papillomaviruses (HPV) are small, double-stranded DNA viruses that primarily infect basal epithelial cells of skin and mucosal body membrane. Of 120 HPV genotypes which have been identified, at least 40 infect the genital region. Genital HPV can be classified as low-risk HPV (LR-HPV) and high-risk HPV (HR-HPV) based on their oncogenic potential. LR-HPV cause benign lesions, while HR-HPV cause precancerous and cancerous lesions of lower part of female genital tract.

HR-HPV DNA is detected in virtually all squamous cell carcinomas, which represent 90% of cervical cancer. In adenocarcinomas, the HPV prevalence is lower. HPV16 and HPV18 are the most frequent and the most aggressive HPV genotypes, contributing for at least 70% of all cervical cancer. The distribution of the other HPV genotypes varies by geographical regions and histological types of cervical cancer.

Genital HPV infection is the most common sexually transmitted disease and the main factor in cervical cancer development. The lifetime risk of genital HPV infection for sexually active women is more than 80%. Most of these infections clear spontaneously within a year or two. In 10–20% of women, infection remains persistent, giving the risk of progression to grade 2/3 cervical intraepithelial neoplasm (CIN). About 30–40% of CIN3 progress to invasive cervical cancer. The time period from the detection of HR-HPV to cervical cancer is 10–20 years, approximately.

Integration of HR-HPV genotypes in the DNA of host cell is the key event in progression of neoplastic lesions to cancer. It results in uncontrolled and higher expression of E6 and E7 virus oncoproteins. These oncoproteins inhibit the function of P53 and RB tumor-suppressor and other cellular proteins and lead to cell proliferation, disruption of DNA repair, differentiation, and apoptosis.

Cervical cancer is the third most common malignancy in females worldwide with more than 85% of cases in middle and low-resource settings of Eastern, Western, and Southern Africa, as well as South-Central Asia and South America. The HPV prevalence

in women with normal cervical cytology is also higher in less-developed than more-developed region. These differences arise from socio-economical, cultural, lifestyle and biological factors. The meta-analysis of one million women with normal cytological findings was estimated that global HPV prevalence was 11.7%. Sub-Saharan Africa (24.0%) had the highest HPV prevalence, followed by Latin America and Caribbean (16.1%).

In developed countries, cervical screening programmes dramatically reduce cervical cancer incidence. HR-HPV testing is part of these programmes. HPV testing has higher sensitivity and higher negative predictive value, but lower specificity than cytology for detecting CIN2 or worse.

Effective tool for prevention of cervical cancer is also prophylactic HPV vaccination, using two approved HPV vaccine: quadrivalent (protect against HPV6, 11, 16, 18)- Gardasil® and bivalent vaccine (protect against HPV16, 18)- Cervarix®.

Implementation of HPV vaccination will not only reduce cervical cancer, but also the other HPV-related disease. Distribution of HPV genotypes will be certainly changed, raising the future necessity for more specific screening and preventive strategies.

1. HUMAN PAPILLOMA VIRUSES

1.1. History of Papilloma Viruses Research

Papillomatous and verrucous skin and genital lesions have been described since ancient Greece and Rome (Bäfverstedt, 1967).

In 1842, Italian physician Rigoni-Stern noted a high frequency of cervical cancer in married women, widows and prostitutes, but their rare occurrence in virgins and nuns in the period from 1760 to 1839 in Verona. He concluded that this disease related to sexual contact (zur Hausen, 2009). In the second part of the 19th century, the rapid development of bacteriology linked the disease with sexually transmitted infection. On the end of the 1960s, the first candidate for cervical cancer etiology was Herpes simplex virus type 2 (Rawls et al., 1968; Naib et al., 1969), but the large prospective study failed to confirm these results (Vonka et al., 1984a; Vonka et al., 1984b).

Research on papillomavirus (PV) began in the early twentieth century. In 1933, the etiologic agent of cutaneous warts in rabbits was identified (Shope and Weston, 1933). In subsequent years, the carcinogenic potential of rabbit PV was demonstrated (Rous and Beard, 1935; Rous and Kidd, 1938).

The first visualization of PV particles in human warts by electron microscopy was reported in 1949 by Strauss et al. (1949). The structure of human papilloma viruses (HPV) genome was characterized in 1965 (Crawford, 1965; Klug and Finch, 1965).

Interest of HPV started to arise during 1970s. At that time, investigators described the various forms of warts caused by the *human wart virus* (Rowson and Mahy, 1967). Zur Hausen postulated and analyzed a possible role of HPV in cervical cancer development (zur Hausen, 1974; zur Hausen, 1976; zur Hausen, 1977). The researchers reported that the appearance of koilocytes in cervical smears indicates the presence of a PV infection (Meisels and Fortin, 1976; Purola and Savia, 1977). Studies in following years supported this idea by identification of typical PV particles in mild dysplastic lesions of the cervix (Della Torre et al., 1978; Laverty et al., 1978; Meisels et al., 1981). The first HPV types (HPV16 and

HPV18) were isolated directly from cancer biopsies of the cervix and were cloned in 1983 and 1984, respectively (Dürst et al., 1983; Boshart et al., 1984).

Within a few next years, the role of HPV in cervical cancer etiology had been described. A specific opening within the viral ring molecule and integration into host genome was shown in cervical cancer cell lines, as well as the expression of E6 and E7 viral genes (Boshart et al., 1984; Schwarz et al., 1985). The immortalization property of viral DNA and transformation property of E6 and E7 was also demonstrated (Dürst et al., 1987a; Pirisi at al., 1987; Münger et al., 1989).

The first epidemiological study on HPV infection in women with and without abnormal cervical cytology was published in 1987 (de Villiers et al., 1987). Since then, several studies have confirmed that cervical infection by high-risk HPV types is the main risk factor for cervical cancer development (Muñoz et al., 1992; Bosch et al., 1995). The discovery of the infection etiology of cervical cancer resulted in the Nobel Prize for Medicine that was awarded to Dr. Zur Hausen in 2008. Today, it is well known that HPV are important human carcinogens. They cause not only vast majority of cervical cancers, but substantial proportion of other anogenital and head and neck cancer (zur Hausen, 2009). In the last years, the knowledge about HPV has been translated into the clinic to allow the prevention and screening of cervical cancer and other HPV-related disease. It reflected in introduction of HPV testing in cervical screening programmes and development of HPV preventive vaccines.

1.2. Classification of HPV

Unlike other viruses, PV are not classified by serotypes, than by genome sequence to the taxonomic levels (family, genus, species, types, subtypes, and variants).

PV belong to the family *Papillomaviridae* (from Latin term **papilla**, which means nipple and **Greek suffix** –**oma**, which means **tumor**). This family consisted of large number of PV types which widespread among higher vertebrates and are species- and tissue specific. Different genera within the family share less than 60% nucleotide sequence identity in the L1 ORF (open reading frame). Different virus species within the same genus share about 60-70% similarity in L1 ORF. Species further were classified to types, subtypes and variants with no more than 90% homology, 90- 98% and more than 98% homology in L1 ORF sequence, respectively (de Villiers et al., 2004).

HPV are grouped into the following genera: *Alpha-*, *Beta-*, *Gamma-*, *Mu-* and *Nu-*papillomavirus (de Villiers et al., 2004) (Figure 1). To date, 120 different types of HPV have been fully characterized (Bernard et al., 2010). Considering their tissue tropism, HPV are grouped into cutaneous and mucosal types.

Cutaneous types of HPV are targeting the skin of the hands and feet. Mucosal types infect the lining of the mouth, throat, respiratory tract, or anogenital epithelium.

HPV have maintained their basic genomic organization for a millions of years. Furthermore, the spectrum of diseases associated with HPV infections (anogenital cancer and warty lesions) has accompanied humans throughout evolution (Mariani and Venuti, 2010).

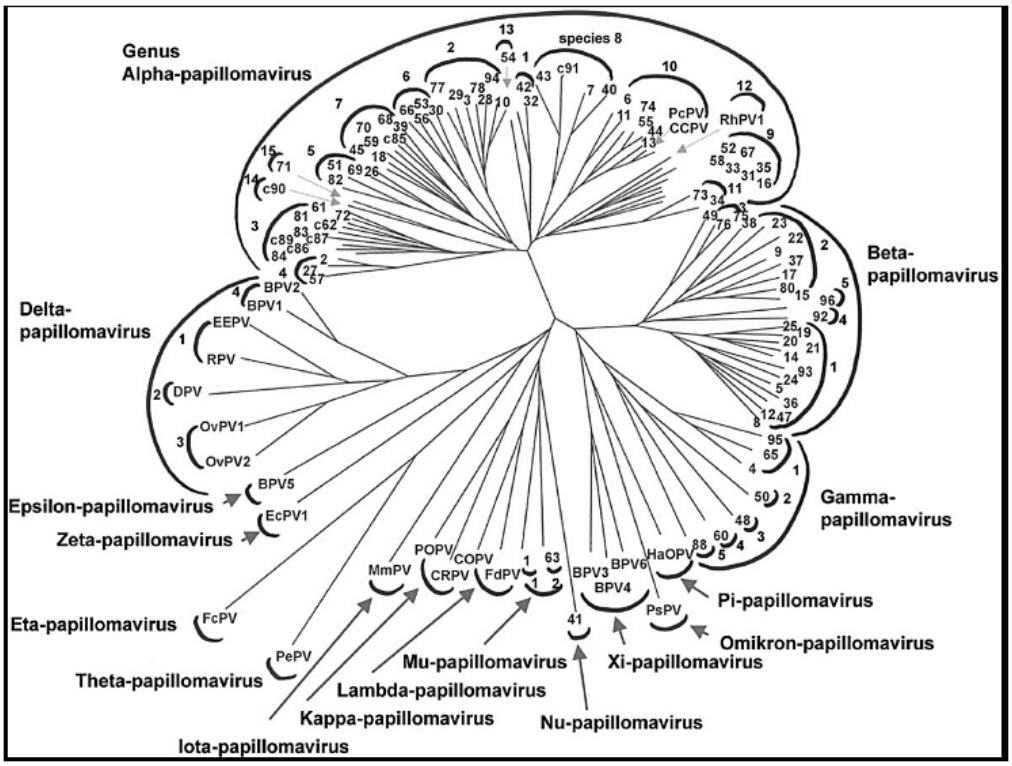


Figure 1. Phylogenetic tree containing the sequences of 118 PV types. The numbers at the ends of each of the branches identify an HPV type; c-numbers refer to candidate HPV types. All other abbreviations refer to animal PV types (Source: de Villiers et al., 2004).

It was postulated that the origin of the evolution of some HPV types should point to Africa, since humans evolved from nonhuman primates in this continent. In addition, the phylogeny of HPV variants (3 lineages: European, Asian American and African) reflects the migration patterns of Homo sapiens (Ong et al., 1993).

1.3. Characteristics of HPV and Organisation of Virus Genome

HPV are small (50-55 nm in diameter), non-enveloped, DNA viruses which exhibit tropism to epithelial cells, infecting the skin and mucous membranes (Leto et al., 2011).

HPV are consisted of icosahedral capsid composed of 72 capsomers, with virus genome inside it. The capsid contains two structural proteins: major capsid protein- L1 (55 kDa in size; 80% of total viral protein) and minor capsid protein- L2 (70 kDa). Each capsomer is a pentamer of the major capsid protein (IARC Monographs, 2007). The virus resembles a golf ball when viewed by electron microscopy (Figure 2).

The HPV genome consists of a single molecule of double-stranded, circular DNA, approximately 8 000 bp in length and associated with histones (Burd, 2003). The HPV genome has partially overlapping 8-10 ORF protein-coding sequences, restricted to one DNA strand (Prendiville and Davies, 2004) (Figure 3). These ORF encode a variety of proteins and

have been classed as early (E) or late (L) depending on their time of expression in infected cells (Dell and Gaston, 2001). The genome can be functionally divided into three regions:

- The first one is the *long control region (LCR)* or upstream regulatory region (URR). This is noncoding region, mostly situated between the start of the E6 ORF and the end of the L1 ORF (Figure 3). It contains core promoter along with enhancer and silencer sequences that are necessary for the replication and transcription of viral DNA, as well as the origin of replication (Dell and Gaston, 2001). This region has the highest degree of variation in the viral genome (Burd, 2003).
- The second one is the *early region* (ORF E1 - E8). These ORF encode early proteins which are expressed in the basal and suprabasal layers of the epithelium. The early proteins are involved in viral replication, regulation of transcription and oncogenesis (von Knebel Doeberitz, 2002; Burd, 2003).
- The third one is the *late region* (ORF L1 and L2). These ORF encode the late proteins, L1 and L2, which are expressed late in the HPV lifecycle in the upper layer of the epithelium. They are structural capsid proteins required for virus assembly (Burd, 2003).

1.4. The HPV Life Cycle

HPV infect the basal cells of the epithelium, usually at sites of microtrauma or where the anatomical architecture provides easy access. In uterine cervix, it is the transformation zone between the stratified squamous epithelium of the ectocervix and the columnar epithelium of the endocervix (von Knebel Doeberitz, 2002).

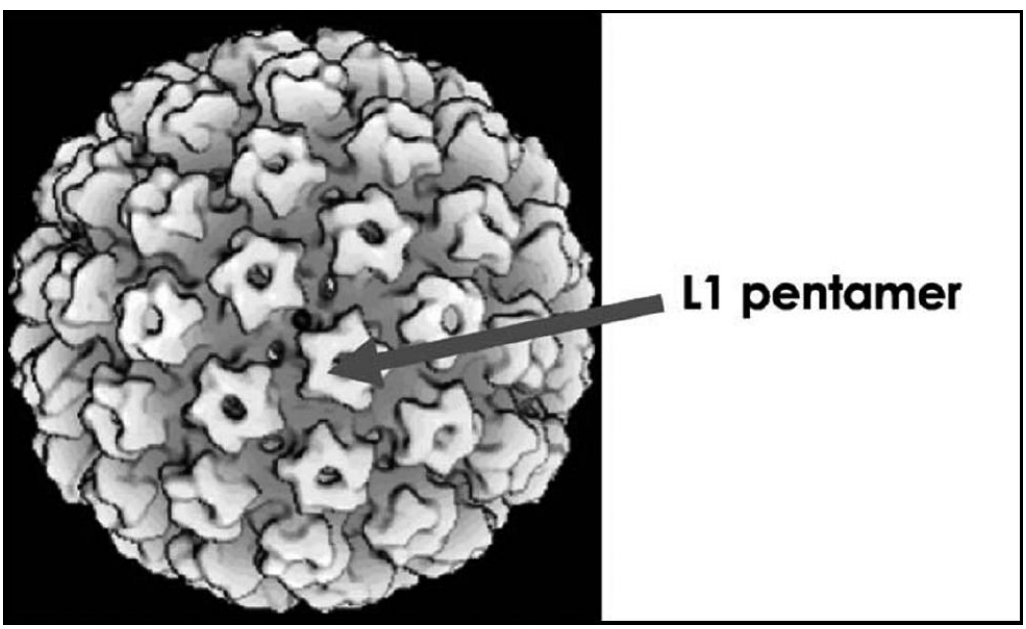


Figure 2. The model of virus capsid (Modified from: Stanley et al., 2006).

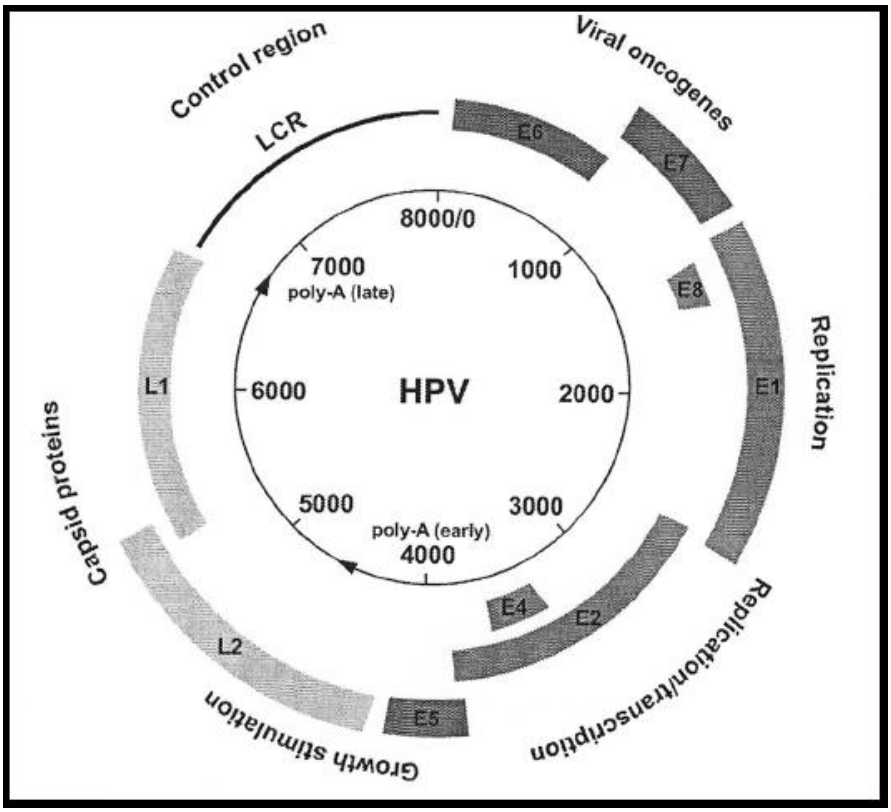


Figure 3. The organisation of HPV genome (Source: Prendiville and Davies, 2004).

The entry of viral particles in the basal cells is mediated by cell surface receptors - heparin sulfate proteoglycan and alpha-6 integrin (Giroglou et al., 2001; Evander et al., 1997). After entering the host cell, viruses are released from capsid and viral genomes are moved into the nucleus where they persist in the extrachromosomal episomal form (about 50 copies per cell) (Fehrmann and Laimins, 2003).

Upon infection, the virus begins to replicate in the infected cells and advances into the upper layers of the epithelium as the infected cells mature. The virus utilizes the host cell DNA transcription and replication machinery for their propagation.

At the beginning of infection, the early transcripts are activated primarily by cellular transcription factors that bind to sequences in the LCR. E2 dimer binds to E2BS (E2 binding site) sequences in the LCR, located close to the binding sites of cellular transcription factors. At low concentrations, E2 still activate early gene expression; while with increasing concentration prevent it by blocking cellular transcription factors binding to LCR (Steger and Corbach, 1997). HPV ORF expression is regulated by the two major promoters - early (upstream of the E6 ORF) and late (within the E7 ORF). HPV messenger RNAs (mRNA) are mostly polycistronic and include several ORF. Expression of early viral gene is partially regulated by a mechanism of alternative splicing of mRNA. In the basal layer, the expression of early HPV transcripts encoding E1, E2, E6 and E7 viral proteins are occurring (Stubenrauch and Laimins, 1999).

The replication of viral episomes in the basal layer of the epithelium is synchronized with the host nucleus DNA replication and requires E1 and E2 proteins, as well as the replication

machinery of the host cell. E1 protein in complex with the E2 dimer binds to AT rich sequences at the origin of replication in LCR (Frattini and Laimins, 1994). E1 proteins have DNA binding activity, DNA helicase activity, and DNA-dependent ATPase activity. After binding to DNA, E1 proteins form hexamers (Sedman and Stenlund, 1998) involved in the separation of double-stranded viral DNA, linking host cell DNA polymerase α (Masterson et al., 1998) and the assembly of additional proteins that mediate in the replication.

Upon cell division, the infected epithelial cells leave the basal layer and migrate to the intermediate and superficial epithelial zone. Normal epithelial cells, after separation from the basal membrane are differentiated and gradually lose their nuclei. Unlike them, the infected cells re-enter the S phase of the cell cycle. E6 and E7 proteins help entry into S phase and proliferation of infected cells (Longworth and Laimins, 2004). Along with migration of cells to the upper layers of the epithelium, E2 protein blocks early promoter and further expression of E6 and E7 genes. At the same time, E2 protein activates the late promoter and consequently increases synthesis of E1 protein as well as E4, E5, L1 and L2 proteins (Stubenrauch and Laimins, 1999).

The increase of E1 protein leads to intensive amplification of viral genomes, up to one thousand copies per cell (Fehrmann and Laimins, 2003).L1 and L2 proteins, in the upper layer of the epithelium, form the viral capsid with the viral genome is packaged in it. Viral E4 protein facilitates the release of viral particles by disruption of cytoskeletal organization (Doorbar et al., 1991). In the superficial zone, the mature virus particles are release through epithelial desquamation.

Apart from this function, E4 may cause a G_2 arrest by binding cyclin dependent kinaze/cyclin complexes (Davy et al., 2002; Davy et al., 2006). Also, by interacting with E2 mediate its relocation from nucleus to cytoplasm, thus influence on E2 activity (Davy et al., 2009). E5, E6 and E7 are oncogenic proteins. In the normal HPV life cycle expression of E5, E6 and E7 is regulated within cells. Their overexpression leads to development of malignant phenotype.

Table 1. Human papillomavirus proteins functions

Viral protein	Function
E1	Helicase function; essential for viral replication and control of gene transcription
E2	Viral transcription factor; essential for viral replication and control of gene transcription
E3	Unknown (only present in a minority of HPV)
E4	Binding to cytoskeletal proteins and breakdown the cytoskeletal network
E5	Interaction with EGF/PDGF-receptors
E6	Interaction with several cellular proteins; degradation of P53 and activation of telomerase
E7	Interaction with several cellular proteins; interaction with PRB
E8	Unknown (only present in a minority of HPV)
L1	Major capsid protein
L2	Minor capsid protein

In addition to mentioned viral proteins, the existence of E8 and E3 proteins is revealed. E8 protein with part of E2 protein suppresses viral replication and transcription and it is believed that play an important role in the maintenance of viral latency in infected basal epithelial cells (Stubenrauch et al., 2001). E3 protein was detected only in few HPV and it is of unknown function (Prendiville and Davies, 2004). The main functions of HPV proteins are summarized in Table 1.

1.5. HPV-Related Diseases

HPV is associated with a variety of clinical manifestations that range from benign lesions to cancer (Table 2). Globally, HPV accounts for about 5.2% of the total cancer burden and the highest among all infectious agents (Tota et al., 2012). HPV infection is necessary event for cervical cancer development and it is present in almost all of cervical cancer cases.

HPV16 and 18 types induce up to 70% of cases (Li et al., 2011a). Besides cervical cancer, HPV is related to other anogenital cancers (vaginal, vulval, penil, anal) as well as oropharyngeal cancers. HPV was found in 40% of vaginal, 70% of vulval (De Vuyst et al., 2009), 47% of penil (Miralles-Guri et al., 2009) and 84% of anal cancer (De Vuyst et al., 2009). In head and neck cancers, HPV was found in 22% (Dayyani et al., 2010). HPV16 is more frequent (>75%) while HPV18 is less frequent (<10%) in HPV-positive vulvar, vaginal and anal carcinomas than in cervical carcinomas (De Vuyst et al., 2009).

Table 2. The most prevalent HPV types and HPV-associated lesions*

HPV-related disease	HPV type**
Plantar warts	**1, 2**, 4, 63
Common warts	**2, 1**, 7, 4, 26, 27, 29, 41, 57, 65, 77, 3, 10, 28
Flat warts	**3, 10**, 26, 27, 28, 38, 41, 49, 75, 76
Other cutaneous lesions	6, 11, 16, 30, 33, 36, 37, 38, 41, 48, 60, 72, 73
Epidermodysplasia verruciformis	**2, 3, 10, 5, 8, 9, 12, 14, 15, 17**, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 50
Recurrent respiratory papillomatosis	**6, 11**
Focal epithelial hyperplasia de Heck	13, 22
Conjunctival papillomas/carcinomas	**6, 11, 16**
Condyloma acuminatum	**6, 11**, 30, 42, 43, 45, 51, 54, 55, 70
LR-CIN	**6, 11**, 16, 18, 31, 33, 42, 43, 44, 45, 51, 52, 74
HR-CIN	**16, 18**, 6, 11, 31, 34, 33, 35, 39, 42, 44, 45, 51, 52, 56, 58, 66
Cervical cancer	**16, 18**, 31, 45, 33, 35, 39, 51, 52, 56, 58, 66, 68, 70
Other genital carcinomas	**16, 18**, 31, 45, 33, 35, 39. 51, 52, 56, 58, 66, 68, 70

Note: LR-CIN- Low-risk cervical intraepithelial neoplasia; HR-CIN- High-risk cervical intraepithelial neoplasia. The most prevalent HPV types in specified genital lesion are bolded.

*Data from reference Gómez and Santos, 2007.

**Order of HPV type indicates relative frequency.

HPV16 type accounts for 87% of HPV-positive head and neck cancer (Dayyani et al., 2010).

The data from meta-analysis suggest that HPV has a role in the development of several other cancers: esophageal (Liyanage et al., 2012), lung (Syrjänen, 2012), colon (Lorenzon et al., 2011), breast (Simões et al., 2012).

Also, there are same evidences of presence of HPV in ovary (Malisic et al., 2012), prostate (Lin et al., 2011), urinary bladder (Li et al., 2011b), but current epidemiological data do not yet support a causal role for HPV in the etiology of these cancers. Presence of HPV in tumors could also affect to prognosis and response to anticancer therapy (Malisic et al., 2011; Psyrri et al., 2012).

This chapter is focused on genital HPV infection in females, its epidemiology and risk for cervical cancer. Also, the strategies for prevention of cervical cancer will be discussed.

1.6. Genital HPV Infections: Transmition and Clinical Manifestations

At least 40 types of HPV infect the genital region (Steben and Duarte-Franco, 2007). Genital HPV are part of *Alpha-papillomavirus* group and based on their oncogenic potential, can be classified as low-risk or non-oncogenic HPV (LR-HPV) and high-risk or oncogenic HPV (HR-HPV) (Klingelhutz and Roman, 2012).

LR-HPV include types 6, 11, 40, 42, 43, 44 (Muñoz et al., 2003), while HR-HPV include types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. HPV 26, 53, 66, 67, 68, 70, 73, and 82 are recognized as potential high-risk genotypes (Bouvard et al., 2009).

Genital HPV infection is one of the most common sexually transmitted infections. The lifetime risk of genital HPV infection for sexually active women is more than 80% (Heley, 2003).

Genital HPV is transmitted through contact with infected genital skin or mucosa. The primary way of transmission is sexual intercourse. Non-sexual transmission via fomites (inanimate objects such as contaminated underwear or bath towels) is also possible, because HPV is very resistant to heat and desiccation (Roden et al., 1997). Genital HPV leads to one of three possible outcomes and largely depends on which HPV type is involved:

- The first one is the appearance of *anogenital warts* (*condyloma acuminatum*) on or around the genitals and anus in both men and women. Anogenital warts are generally associated with LR-HPV, and do not lead to cancer. Types 6 and 11 of HPV account for about 90% of all cases (Stanley, 2012). Most of these epithelial changes are asymptomatic and may spontaneously resolve in 3 to 4 months, remain the same, or increase in size and number (Burd, 2003).
- The second one is the *latent or inactive infection*, in which few people know they are infected since noticeable symptoms rarely exist and the infected area remains cytologically normal (Burd, 2003). HPV DNA is present in approximately 12% of women with cytologically normal cervical epithelium (Bruni et al., 2010).
- The third one is the active infection, associated mainly with HR-HPV types which cause changes in infected cells which may results in *anogenital intraepithelial neoplasia and cancer*.

1.7. Epidemiology of HPV Infection: HPV Infection in Women with Normal Cervical Cytology

The meta-analysis was performed to assess HPV prevalence in women with normal cervical cytology worldwide. The analysis includes studies published between 1995 and 2009 that used polymerase chain reaction or Hybrid Capture 2 methods for HPV detection in women with normal cytological findings. Thus, a total of one million women (n=1,016,719) were analyzed (Bruni et al., 2010).

The estimated global HPV prevalence among women with normal cytological findings was 11.7%. African and Latin American regions showed higher average HPV prevalence than European, Northern American, and Asian regions (Bruni et al., 2010). These differences arise from socio-economical, cultural, lifestyle and biological factors. HPV prevalence for Sub-Saharan Africa was 24.0%, while for Latin America and Caribbean was 16.1%. In Asia and Europe, the highest prevalence had Southeastern Asia (14.0%), and Eastern Europe (21.4%), respectively. The remarkable differences were perceived between countries and among studies within the same region (Bruni et al., 2010).

Age-specific HPV prevalence is present as either a bimodal or a unimodal distribution. In all regions, a peak in HPV infection was found at younger ages (<25 years), declining to a lower prevalence plateau in middle age. In some regions, a second peak of HPV prevalence was observed at age >45 years (Figure 4). The first peak is related to start of sexual intercourse and it is mostly transient HPV infections that clear rapidly. A less intense second peak that has been observed in some population could be caused by immunosenescence, changes in sexual behavior during middle age (both for men and women), or population specific period/cohort effects (de Sanjosé et al., 2007). Also, there are some indications that this perimenopausal increase may be mostly due to higher rates of HPV persistence at older ages rather than new HPV acquisition (Castle et al., 2005). Other studies have suggested that HPV prevalence implying some hormonal interaction with the HPV life cycle or that geographical variability in this second peak may be partially explained by indirect indicators of menopausal hormonal patterns, such as body mass index and ethnicity, not only the age (Althoff et al., 2009).

Data about type-specific HPV prevalence were taken from studies which analyzed specific HPV types in women with normal cervical cytology (n=215,568). Oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 were accounted for 70% of HPV infections among women with cytological normal cervical findings. Among HPV-positive women, HPV16 accounted for >22% of HPV infections. A significant inverse correlation was observed between overall HPV prevalence and the contribution of HPV16, with the lowest HPV16 proportions in the regions with the highest HPV prevalence (Bruni et al., 2010).

The five most common types worldwide were HPV16 (3.2%), HPV18 (1.4%), HPV52 (0.9%), HPV31 (0.8%), and HPV58 (0.7%). Compared with other types, HPV31 was especially frequent in Europe (2.3%), while HPV52 was more frequent in Northern America (2.1%), Africa (2.4%), and Asia (0.7%) (Figure 5).

Approximately 3.2% of HPV tested women had infections with multiple HPV types (Bruni et al., 2010).

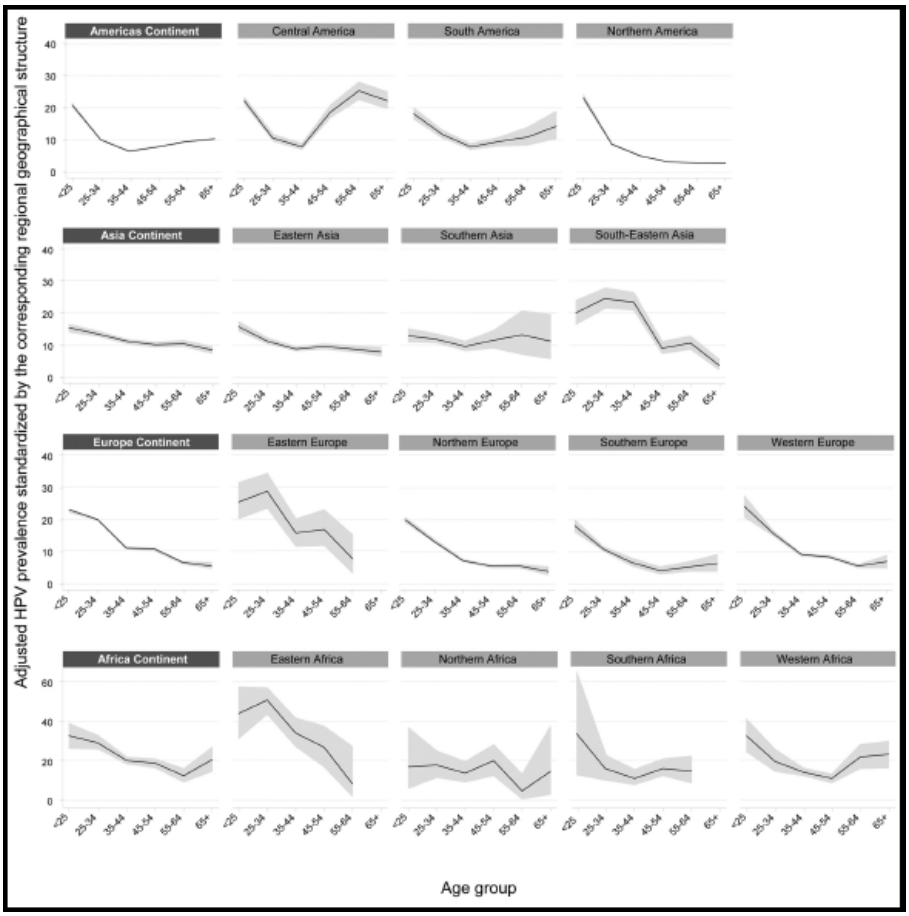


Figure 4. Age-specific HPV prevalence in women with normal cervical cytology from five world regions. (Shaded areas represent 95% confidence intervals. Source: Bruni et al., 2010).

2. HPV AND CERVICAL CANCER

2.1. Cervical Cancer: Ethiology, Prognosis and Epidemiology

Cervical cancer is malignant neoplasm of cervix uteri. Almost all cancers of cervix uteri are of the epithelial origin. The epithelial tumors of cervix uteri include range of invasive squamous and glandular intraepithelial carcinoma and its precursor lesions.

85-90% of cervical cancers are squamous cell carcinomas arising from malignant transformed squamous epithelial cell of the cervix. The remaining 10-15% are adenocarcinomas, arising from transformed columnar epithelial cell that covers the inside of the cervix and the cervical glands (Tjiong et al., 2001). These cancers arise from precursor dysplastic lesions that are classified on the basis of cell abnormalities. Squamous cell carcinomas arise from low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) (Bethesda system of cervical cytology classification, 2001), while adenocarcinomas arise from adenocarcinomas *in situ*. LSIL correspond to mild dysplasia (CIN1), while HSIL correspond to moderate (CIN2) or severe dysplasia (CIN3).

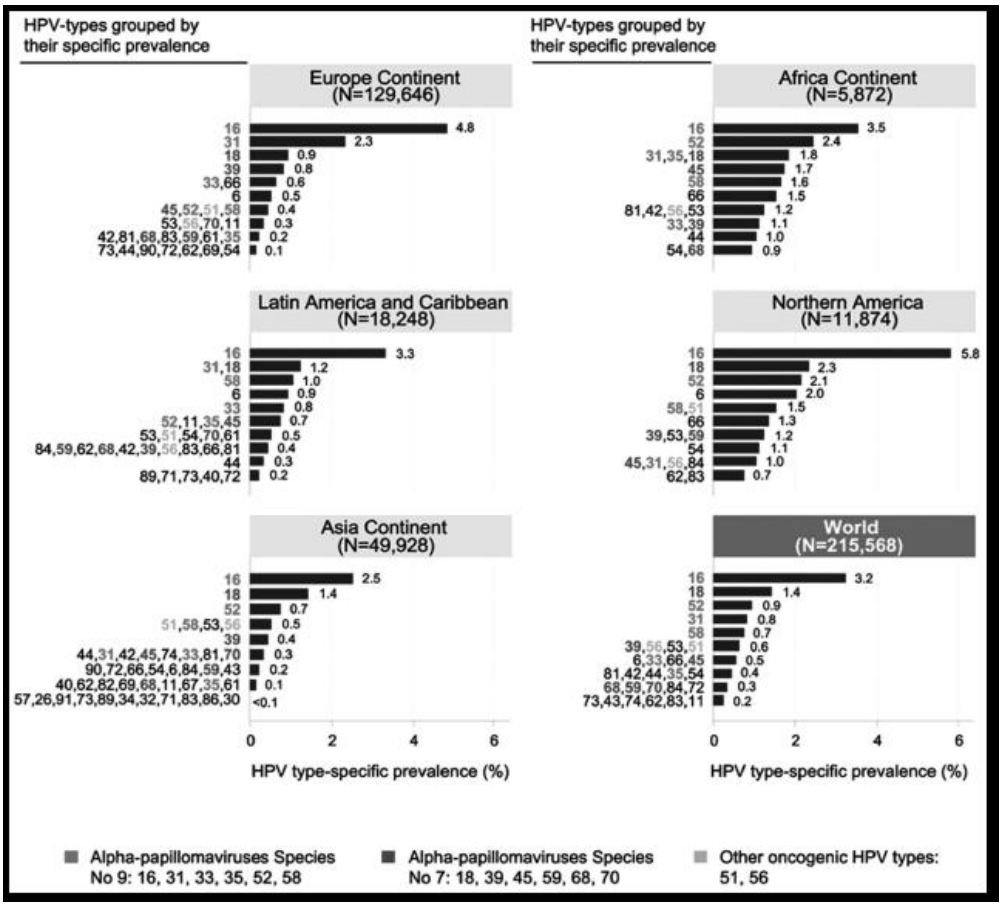


Figure 5. Worldwide HPV prevalence, by type, among females with normal cytological findings. Type-specific HPV prevalence is weighted by study size and standardized by the world's geographical structure (Source: Bruni et al., 2010).

The main risk factor for cervical cancer development is infection of cervix with HPV. This infection is presented in almost all cervical cancer, and in substantial percentage of CIN (73- 93%). Additionally, in more than half of atypical squamous cells of uncertain significance (ASCUS) HPV infection was found (Guan et al., 2012).

Cervical cancer is preventable and curable disease if diagnosed at an early stage. Today, it is well-known the risk factors and causes of this malignancy and developed the methods for early detection of the disease (cytology, colposcopy and detection of oncogenic HPV types). Also, by effective treatments (surgery, radiotherapy, and chemotherapy) the cure rate in the early stages is very high. Thus, the five-year survival rate for cancer confined to the cervix (Stage I) is 80-90%, for cancer extends outside the cervix but still within the pelvis (Stage II) is 60-75%. When cancer extends throughout the pelvis (Stage III) or has metastasized beyond the pelvis (Stage IV) the five-year survival rate is only 30-40% or less than 15%, respectively (Porter and Kaplan, 2008). Despite the mentioned, cervical cancer is the third most common malignancy and the fourth leading cause of cancer death in female worldwide. Every year, about 530,000 women are diagnosed and 275,000 die from cervical cancer. More than 85% of the cases and deaths occur in developing countries. The highest incidence rates are in Eastern

and Western Africa, where the age standardized incidence rates are over 30 per 100,000. Other high-risk regions with age standardized incidence rates of over 20 per 100,000 include South Africa, South-Central Asia, South America, Melanesia, Middle Africa, Central America and the Caribbean. Rates are lowest in Western Asia, Australia and New Zealand, and Northern America, where the age standardized incidence rates are less than 6 per 100,000 (Figure 6). India, the second most populous country in the world, accounts for 27% of all cervical cancer deaths globally (Jemal et al., 2011).

Socio-economic status, inadequate education of women and lack of screening are the main cause of the high incidence and mortality from cervical cancer in low-income countries. Periodical screening is very important, because the early stages of the disease are generally asymptomatic and may go unnoticed.

2.2. The Role of HPV in Cervical Cancerogenesis

Infection with some of the HR-HPV, so-called oncogenic HPV types is a major factor in the development of cervical cancer.

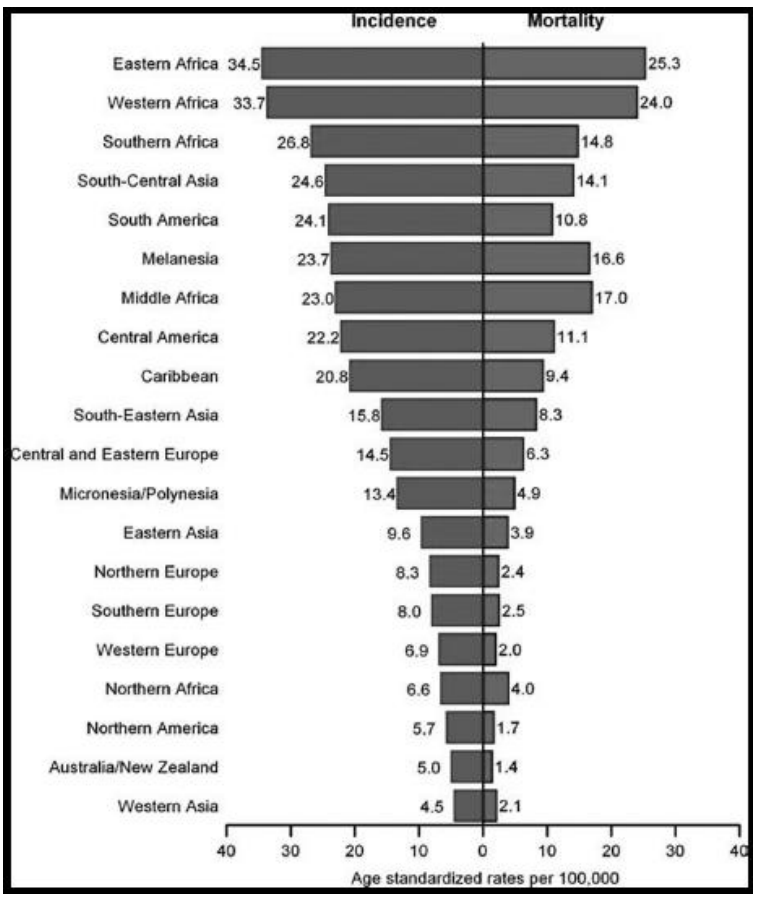


Figure 6. Age-Standardized Cervical Cancer Incidence and Mortality Rates by World Area (Source: Jemal et al., 2011).

The most common oncogenic types in cervical cancer are 16 and 18. HPV16 and HPV18 are also the most aggressive types in cervical carcinogenesis. Namely, it was shown that HPV16 or HPV18 positive women with ASCUS had a greater risk of CIN 2 or worse (CIN2+) compared with pooled HR-HPV positive and HR-HPV negative women (Castle et al., 2011; Stoler et al., 2011).

Contrary to LR-HPV types, genomes of HR-HPV types are integrated into the DNA of host cell. It is the key event in progression of neoplastic lesions to cancer. Integration is random throughout the host genome with higher specificity for integration in common fragile sites (CFS), specific chromosomal loci that are prone to forming double strand breaks (Wentzensen et al., 2004). Integration of HPV DNA may contribute to the malignant phenotype by activation of cellular oncogenes (Dürst et al., 1987b; Peter et al., 2006).

The mechanisms by which HPV integrates its DNA into the human genome are not fully understood. The question whether integration precedes genetic instability or integration arises due to genetic instability remains opens (Raybould et al., 2011). Although number of studies has observed that HPV DNA integration preferentially occurs near CFS, there is also speculation that HPV oncoproteins E6 and E7 induce DNA damage (Duensing and Münger, 2002) and increase the risk of HPV DNA integration (Kessis et al., 1996).

Circular viral DNA usually opens within the E2 ORF and part of ORF E2 with E4, E5 and part of L2 is lost (Figure 7) (zur Hausen, 2002). The loss of E2 ORF leads to uncontrolled and enhanced expression of E6 and E7 proteins.

The main role of E6 protein in oncogenesis is the inhibition of P53 protein function. P53 protein is essential for the prevention of inappropriate cell proliferation and maintenance of genome integrity. Under exposure to cellular stress, P53 activates transcription of target genes and causes a variety of cellular responses such as cell cycle arrest, induction of apoptosis, DNA damage repair, senescence, inhibition of angiogenesis etc (Vousden and Lu, 2002; Vogelstein et al., 2000).

E6 protein of HPV binds to the cellular ubiquitin ligase, termed E6-associated protein (E6-AP). This dimer protein complex binds P53 protein and induces the multi-ubiquitination of P53 in the presence of enzyme complex and the degradation of P53 in the proteasome (Scheffner et al., 1993) (Figure 8).

This drastically reduces the half-life of P53 protein from several hours to less than 20 minutes, and also reduces the level of P53 protein on up to half of the level which present in the normal epithelial cells (Prendiville and Davies, 2004). In addition, the binding of E6 to P53 block interaction with P53 transcription co-activator CBP/p300, and inhibits P53-dependent transcription (Zimmermann et al., 1999).

Besides the effect on P53 function, E6 protein exhibits the other activities. E6 protein interrupts apoptosis and DNA repair mechanisms and induces immortalization of HPV-infected cells.

Thus, HPV E6 abrogates function of proapoptotic protein Bak, by targeting and promoting its proteolytic degradation (Jackson et al., 2000). This reveals a survival mechanism of virally infected cells. E6 protein of some types of HPV interacts with components of the DNA single-strand breaks repairs systems and inhibits them (Iftner et al., 2002). Expression of the gene for the catalytic subunit of enzyme telomerase (hTERT) is activated by E6 protein (Oh et al., 2001).

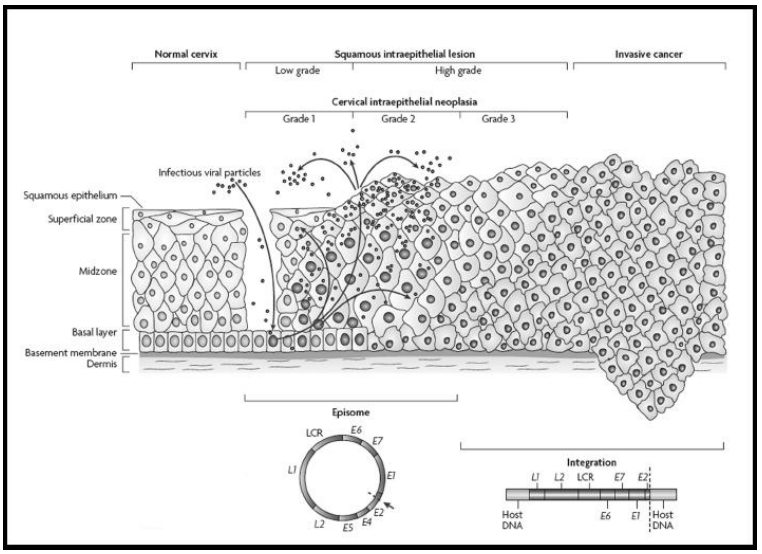


Figure 7. HPV-mediated progression to cervical cancer (Modified from: Woodman et al., 2007).

This leads to the addition of hexamer repeats to the ends of chromosomes in differentiated cells and prolonged life of HPV-infected cells. E7 protein achieves its oncogenic potential by binding to the members of the retinoblastoma (RB) family of proteins and stimulates their proteasomal degradation.

PRB are tumor suppressor proteins that prevent excessive cell growth by inhibiting cell cycle progression. PRB proteins interact with E2F family of transcription factor repressing the transcription of genes required for the S phase of the cell cycle (Gordon and Du, 2011).

Binding of E7 HPV protein to PRB leads to the release of E2F transcription factors from the inhibitory effect of RB protein, thereby stimulating entry into S phase of the cell cycle and DNA replication (Figure 8).

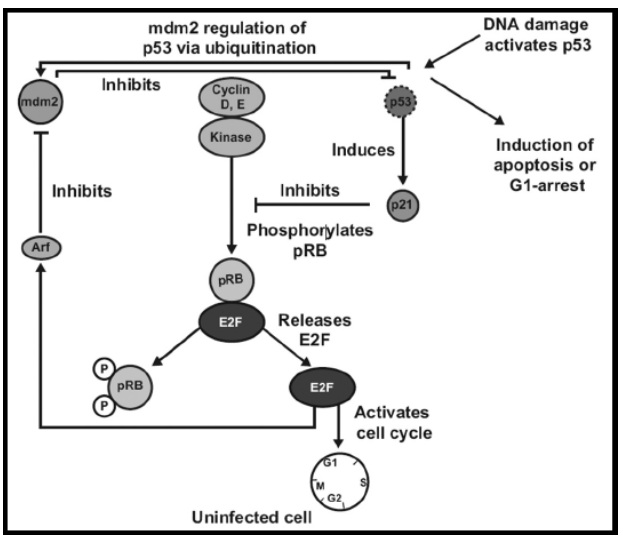


Figure 8. The role of E6 and E7 viral proteins in the cell proliferation (Source: Prendiville and Davies, 2004).

The E7 protein stimulates the cyclins involved in the S-phase of the cell cycles (cyclin A and E) (Zerfass et al., 1995), and simultaneously blocks the function of cyclin-dependent kinase inhibitors P21 and P27 (Funk et al., 1997; Zerfass-Thome et al., 1996). E7 induce centromeric amplification, which results in the appearance of abnormal mitoses and aneuploidy (Duensing et al., 2001). E6 and E7 proteins react with a number of other cellular proteins and impair their function. Thus, the continuous overexpression of E6 and E7 viral proteins results in increased proliferation, impairment of DNA damage repair process, differentiation, and apoptosis, increased genomic instability and chromosomal abnormalities.

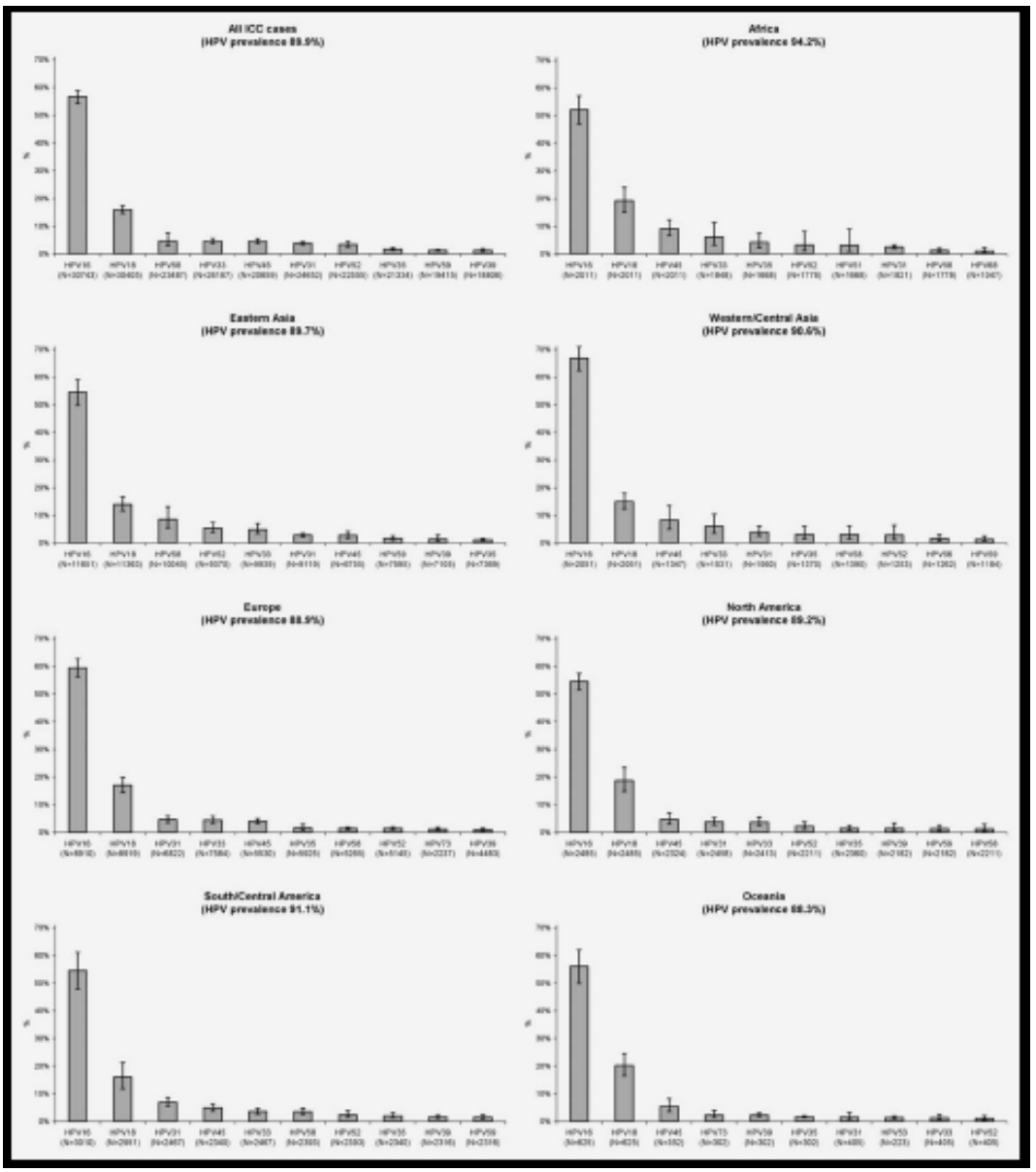
Viral E5 protein enhances oncogenic potential of E6 and E7. E5 proteins form complexes with EGFR (epidermal-growth-factor receptor), PDGFR–beta (platelet-derived growth-factor-β receptor) and CSF1R (colony-stimulating factor-1 receptor) and stimulate cell proliferation. E5 proteins are small hydrophobic proteins located in the membranes of endosome, Golgi complex and plasma membrane (Fehrmann and Laimins, 2003; zur Hausen, 2002). E5 protein also binds to the 16kDa subunit of vacuolar proton-ATPase and inhibits acidification of endosomes. In this way, E5 inhibits the degradation of the EGFR in endosomal compartments after ligand-stimulated endocytosis. It results in returning of EGFR on the plasma membrane and prolonged mitogen signaling (Straight et al., 1995).

2.3. Worldwide Distribution of HPV in Cervical Cancer and Its Precursor Lesions

The meta-analysis, performed on 115,789 HPV-positive women among a total of 369,186 eligible women, gave the data about overall HPV and HR HPV-type distribution in women with different cervical disease grade and geographical region. Overall HPV prevalence increased with increasing severity of cervical disease from 12% in normal cytology to 89% in ICC. The overall HPV prevalence was 52%, 73%, 86%, 93% for ASCUS, CIN1, CIN2 and CIN3 respectively. HPV16 positivity increased through normal cytology (20%), ASCUS (23%), CIN1 (28%), CIN2 (40%), CIN3 (58%) to reach 63% in ICC. HPV18 positivity varied very little between normal cytology and CIN3 (7–10%), but increased to 16% in ICC. HPV16, 18 and 45 were the only HR types found more frequently in ICC than normal cytology samples (Guan et al., 2012). The other meta-analysis was performed on 30,848 invasive cervical cancers (ICC) to estimate the HPV type-specific prevalence. It was shown that the distribution of HPV genotypes varies by geographical regions and histological types of cervical cancer.

Overall prevalence of any HPV type in ICC was 89.9%. Overall HPV prevalence ranged from 88.3% in studies from Oceania to 94.2% in those from Africa. Single and multiple infections were present in 79.0% and 11.2% of cases, respectively. The proportion of ICC associated with HPV16 and/or 18 (HPV16/18) was between 70 and 76% in all world regions except Asia. In Western/Central Asia, 82% of ICC was HPV16/18-associated compared to only 68% in Eastern Asia. The two most common HPV types were HPV16 (57%) and 18 (16%), following by 58 (4.7%), 33 (4.6), 45 (4.5), 31 (3.8), 52 (3.4), 35 (1.7), 59 (1.3), 39 (1.3), 51 (1.0). The prevalence of other types, belonging to the high-risk clade, ranged from <0.1% for HPV85 to 0.8% for HPV56. HPV6 and 11 were detected in 0.4% and 0.5% of ICC, respectively (Li et al., 2011a). HPV16 was the most common type in all regions (ranging

from 52% in Africa to 67% in Western/Central Asia), followed by HPV18 as the second most common type (ranging from 14% in Eastern Asia to 20% in Oceania).



Abbreviation: N: number of cases tested for the given HPV type. (Source: Li et al., 2011a)

Figure 9. The ten most frequently detected HPV types in invasive cervical cancer 1990–2010, by region.

The other most common HPV types were almost always HPV31, 33, 35, 45, 52 and 58, although their relative importance slightly differed by region (Figure 9) (Li et al., 2011a).

With respect to the histology, overall HPV positivity was higher among squamous cell carcinoma (SCC)/unspecified histology (90.9%) than in adeno/adenosquamous carcinoma (ADC) (82.0%). HPV18 was over-represented in ADC (36.8%) compared to SCC (13.2%). Conversely, HPV16 was under-represented in ADC (36.3%) compared to SCC (59.3%) (Li et al., 2011a).

2.4. Persistance of HPV Infection and Malignanant Progression

HPV are weak immunogens. They have developed many mechanisms to escape from the immune response. The viral antigen synthesis, in the basal epithelial layers, is limited and occurred only during the desquamation of mature epithelial cell when large number of HPV is shed. Therefore, there is a little exposure of HPV antigens to the immune system. Also, there is no cell lysis, no local inflammation, little or no release of local cytokines.

Antigen-presenting cell activation is limited and consistently no cell-mediated immune response.

Also, HPV have mechanisms for inhibiting interferon synthesis. Thus, HPV can be present for long period of time (Bodily and Laimins, 2011; Mariani and Venuti, 2010). These mechanisms enabled HPV to become one of the most common sexually transmitted infections worldwide (Einstein et al., 2009a).

Despite the ability of HPV to evade the host's immune system, a primary HPV-infection is cleared in mostly (~ 90%) of cases within two years (Bosch et al., 2008). Persistence of HPV infection increases the risk of progression to precancerous lesions.

The persistent HPV infection with high-risk type is a key event in malignant process (Figure 10). CIN 1 and CIN 2 regress spontaneously in 60-90% and 50% of cases, respectively (Horn and Klostermann, 2011). About 30–40% of CIN3 progress to invasive cervical cancer.

The time from the detection of HR-HPV to the development of CIN3 is 3–5 years, but the progression to invasive cancer takes a further 10–20 years (Stanley, 2010).

The peak prevalence of transient infections with HR-HPV occurs among women during their teens and 20s, after the initiation of sexual activity. The peak prevalence of cervical precancerous conditions occur approximately 10 years later and the peak prevalence of invasive cancers at 40 to 50 years of age (Schiffman and Castle, 2005).

2.5. Factors That Contribute to HPV-Induced Malignant Progression

High-risk HPV infection is necessary although not sufficient for the development of cervical cancer. HPV infection clears in many cases. Additionally, in case of persistence HPV, there is a latency of a few to more than 10 years from the infection to the development of the disease. This suggests the existence of other factors and co-factors that lead to malignant transformation (Motoyama et al., 2004).

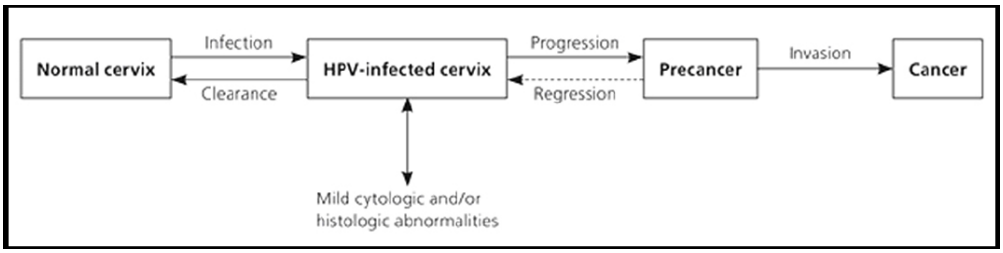


Figure 10. Model of cervical carcinogenesis. The persistence of oncogenic HPV types is necessary for progression and invasion. HPV infection may be associated with cytologic and histologic abnormalities.

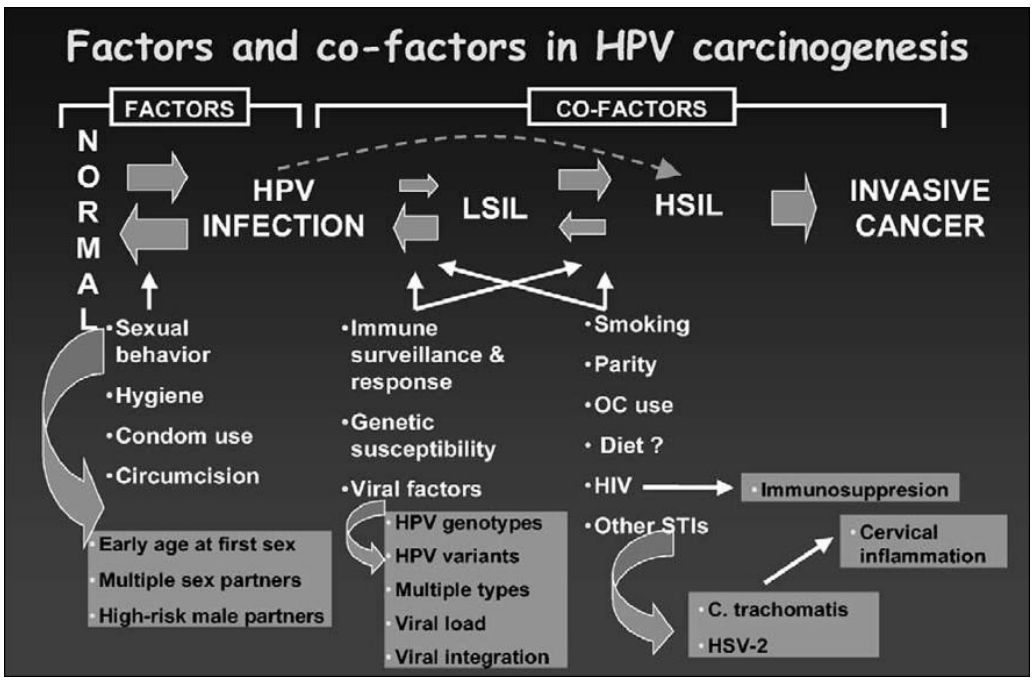


Figure 11. Factors and co-factors in cervical carcinogenesis (Source: Castellsagué et al., 2002).

Epidemiological studies have established a number of factors that contribute to the development of cervical cancer (Figure 11):

- Sexual behavior

Sexual activity starting at a young age (<16 years), a high total number of sexual partners (more than four), and personal history of genital warts or sexually transmitted diseases (including her sexual partners) are risk factors for cervical cancer development (Waggoner, 2003).

- Hormonal factors

Among HPV positive women, the risk of cervical cancer increases significantly with the use of oral contraceptives longer than five years, and the number of pregnancies (Moreno et

al., 2002; Monsonego et al., 2004). Hormone-related mechanisms may promote integration of HPV DNA into the genome of the epithelial cervical cells (Castellsagué et al., 2002). Experimental studies have shown that estrogen can stimulate the transcription of HPV16 E6 and E7 genes in cell lines containing integrated HPV16 (Mitrani-Rosenbaum et al., 1989).

- Tobacco smoking

Tobacco-specific carcinogens and polycyclic aromatic hydrocarbons have been identified in the cervical mucus or epithelium of smokers (Prokopczyk et al., 1997; Melikian et al., 1999). These compounds bind to cellular DNA and damaged it.

Besides the direct effect of tobacco metabolites, tobacco smoking induce immunosuppression and reduction of dietary antioxidants (Bosch and Muňoz, 2002), and contributes to the malignant phenotype.

- Other sexually transmitted diseases

Certain herpes viruses have been detected in some women with cervical cancer. Some studies showed that human herpesvirus 6 activates oncogenes of papillomavirus (Chen et al., 1994).

Infection with *Chlamydia trachomatis*, especially G serotype increases the risk of cervical cancer development (Anttila et al., 2001). Chlamydia trachomatis inhibits apoptosis in infected cells by blocking the release of cytochrome c from mitochondria and activation of caspase (Fan et al., 1998).

Infection with HIV (human immunodeficiency virus) contributes to increased risk for cervical cancer, probably due to immunosupression (Denny et al., 2012).

- Dietary factors

Diet rich in β-carotene, vitamins A, C, E, B and folate reduces the risk of cervical neoplasia. Vitamins C, vitamin E and carotenoids could act as scavengers of free radicals and oxidants.

These substances, which are produced during normal metabolism and the inflammatory process, could cause damages of DNA, proteins and lipids. Vitamins C and vitamin E have a protective effect of persistent HPV infection by enhancing of immune function and modulating of inflammatory response to infection. They also could inhibit the DNA adducts formation, which are induced by tobacco products and other chemicals.

Antioxidants decrease the virus replication and gene expression. Folate, vitamins B6 and B12 are involved in the synthesis and repair of DNA and DNA/RNA methylation, which may play a role in the integration of viral DNA and gene stability (García-Closas et al., 2005).

- Host factors

The HLA (human leukocyte antigen) genes, particularly the class II HLA alleles, are the primary mediators of cell mediated immune system responses to exogenous pathogens,

including viruses. Certain polymorphic variants of HLA genes are associated with an increased risk of cervical neoplasia (Ferenczy and Franco, 2002).

- Virus factors

The sequence variation in the *E6/E7* oncogenes and LCR, which controls their transcription, has functional importance. There may be differences in transcription activity, oncogenic potential of E6/E7, its interactions with P53 and RB, and thereby to formation of cervical neoplasia (Ferenczy and Franco, 2002). Thus, non-European variants (Asian, Asian-American, African 1 and African 2) compared with the European HPV16 variant have 2-9 times greater risk of HSIL and cervical cancer (Hildesheim and Wang, 2002).

- Hereditary predisposition

Some studies have indicated familial history of cervical cancer and the potential hereditary basis of this malignancy (Hemminki et al., 1999; Magnusson et al., 2000; Zoodsma et al., 2004; Hemminki and Chen, 2006).

- Genetic alterations

In addition to numerous structural and numerical chromosomal aberrations, cervical cancer and precursor lesions are associated by gene amplification (*MYC*), increased expression (*MYC*, *ERBB2*, *EGFR*) and point mutations (in the *RAS* gene family) (Whang, 1997; Busmanis, 1998; Lazo, 1999).

3. HPV AND PREVENTION OF CERVICAL CANCER: SCREENING AND VACCINATION

3.1. Cervical Cancer Screening Programmes

Although a very few HPV infections lead to cervical cancer, it is of great importance to find pre-cancer cells before the cancer is occurred by regular screening. Thus, in countries where screening programmes are existed, the incidence of cervical cancer is dramatically reduced. The screening programmes are based on cytology (conventional Pap test or liquid based cytology) and HPV testing.

Current scientific evidence supports initiating cervical cancer screening for immunocompetent women at age 21. It well known that HPV cervical infections are highly prevalent among females under 21 years, yet this age group has a low incidence of cervical cancer (Schwaiger et al., 2012). Recommended screening for women ages 21-29 years includes cytology every 3 year, while for women 30-65 years includes cytology and HPV testing (cotesting) every 5 years or cytology alone every 3 years. Screening in women older 65 years can be stopped if she has had 3 consecutive negative cytology results or 2 consecutive negative cotests results within the 10 years and had no history of CIN2+ within the last 20 years (American Cancer Society Guidelines) (Saslow et al., 2012). The European Guidelines currently recommend Pap test every 3-5 years starting at age 22-30 and stopping

at age 60-65. HPV testing is used to triage of borderline lesions and to post-CIN follow up (Rossi and Ronco, 2012), or recommended as method for primary screening (Habbema et al., 2012).

3.1.1. Cervical Cytology Testing- Adventages and Disadventages

The conventional cytological test for detection of cervical abnormalities is Papanicolaou-stained (Pap) smear. This method was named after pathologist George Papanicolaou, who introduced the method in 1949, before the cause of cervical cancer was known (Papanicolaou, 1949). The Pap smear is a screening tool able to recognize the changes in cells of the transformation zone of the cervix. Scoring criteria include the assessment of cell size/nuclear size, the nuclear shape and nuclear staining intensity, nuclear and chromatin architecture, shape of the nuclear membrane and the ratio between cytoplasm and nuclear volume (Koss, 1989).

The Pap test has high specificity in the detection of cytological abnormalities, but also has its limitations. Up to 8% from the samples are inadequate and false negative rates rise to 20-30%. Some of the reasons for the false negative results may be: other contents of the cervical specimen such as blood, bacteria or yeast which contaminate the sample and prevent the detection of abnormal cells; exposure to air too long before being fixed on the slide can distorted cervical cells; the non-uniform spreading of the cells on the slide; a low number of abnormal cells in the sample; human error in interpretation; etc (Burd, 2003). Low-grade changes are often prone to misinterpretation.

Liquid-based cytology, introduced in the mid 1990s, has some advantages in relation to conventional cytology. It may reduce unsatisfactory specimens and can be suitable for HR-HPV testing from the same sample (Ronco et al., 2007). However, specificity and sensitivity in detection of cervical cytological abnormalities are similar for both methods (Arbyn et al., 2008).

The disclosure of molecular pathogenesis of cervical cancer has been opened field of new screening strategies based on HPV testing.

3.1.2. Introduction of Hpv Testing in Cervical Screening Programmes

The fact that practically all of cervical cancer are caused by HR-HPV has led to the development of screening tests that detect HR types. The HR-HPV testing is more sensitive than cytology in primary screening for CIN2/3 and cervical cancers. Randomized trial, performed on ten thousand women ages 30 to 69 years, was shown that the sensitivity of HPV testing for CIN of grade 2 or 3 is 95%, whereas the sensitivity of Pap testing is 55%. The specificity was similar (94% for HPV testing and 97% for Pap testing). The sensitivity of both tests used together was 100%, and the specificity was 92% (Mayrand et al., 2007).

HR-HPV test has a high negative predictive value (from 97% to more than 99%) (Bhatla et al., 2012). Even more important, cytologically normal women who test HPV negative will have reassurance at lest five years that they are at low risk of CIN3 and cervical cancer (Dillner et al., 2008; Schiffman et al., 2011a). Introduction of HR-HPV testing allows increased length of screening interval.

Detection of HR-HPV DNA is now used in primary screening for cervical cancer (alone or HPV and Pap co-testing), but also in triage of women with equivocal or low-grade cytologic abnormalities (Bhatla et al., 2012) and prediction of the therapeutic outcome after treatment CIN lesions (Kocken et al., 2012).

Colposcopy examination and biopsy result determine how screen-positive women (whether by a single test, cotesting or triage) are managed and treated (Schiffman et al., 2011b).

3.1.2.1. Methods for HPV Detection

HPV cannot be propagated in tissue culture. Other direct virological diagnostic techniques, such as electron microscopy and immunohistochemistry, lack the sensitivity as well as specificity for the routine detection of HPV (Poljak and Kocjan, 2010). Serology is not suitable for distinguishing present and past infections, because antibodies against the major capsid protein remaining detectable for many years (Dillner, 1999). Consequently, the diagnosis of HPV infection is based on molecular biology techniques for detection of viral nucleic acid in infected cells (Poljak and Kocjan, 2010). They are many available commercial assays for HPV detection: HR-HPV-DNA-based screening assays, HR-HPV E6/E7 mRNA-based screening assays and *in situ* hybridization assays (Poljak and Kocjan, 2010). HR-HPV-DNA-based screening assays with HPV16 and HPV18 genotyping belong to group of novel HPV assays. They are based on results of long-term HPV natural history study which was shown that HPV16 and HPV18 give a greatest risk of CIN3 than other HR-HPV types. It may permit less aggressive management of women with other HR-HPV infections (Khan et al., 2005).

Among the most commonly used HPV tests for detecting clinically significant levels of HR-HPV types are: the Digene HC2 High-Risk HPV DNA test (Qiagen), the Cervista™ HPV HR test (Hologics), and the cobas 4800 HPV test (Roche Molecular Systems). Cervista™ High-Risk and HC2 High-Risk tests indicate the presence of one or more of the HR types but does not indicate a specific type. The cobas test provides individual detection of HPV16 and 18, as does another test, the Cervista™ 16/18 HPV test (Hologics). None of the HPV tests are approved for use in men, adolescents, or detection of infection in partners. HPV testing is not used for screening of HPV associated lesions in anatomic sites other than the cervix, and it is not useful in diagnosis or clinical management of cancer, cancer precursors, or warts (Hariri et al., 2011).

3.1.3. Screening in Low-Resource Areas

In low-resource countries, Pap smear cytology, from one part it could be to expensive and even more important it could not be easily done. Namely, cytology-based screening requires complex and expensive infrastructure and well-trained medical personal. Visual inspection method and molecular testing for HR-HPV represent an alternative for cervical cancer screening in these areas.

Visual inspection allows an immediate result and if it is possible could be immediately followed by cryotherapy (so called "screen-and-treat" approach) done by trained nurses and midwives (Saxena et al., 2012).

Evidence shows that visual inspection method using diluted acetic acid (VIA) is more sensitive test than the Pap-smear screening test. In the pooled analysis estimated sensitivity, specificity, positive predictive value and negative predictive value to detect CIN 2+ lesions were 80%, 92%, 10% and 99%, respectively (Sauvaget et al., 2011). A high degree of variability in sensitivity and specificity in detection of CIN2+ is found in studies from different countries (from 55% and 75% for sensitivity and specificity, respectively) (Wright and Kuhn, 2012).

A new HR-HPV test, specifically designed for use in low-resource settings, is developed by Qiagen. This test is called *care*HPV. Test showed sensitivity and specificity for CIN2+ of 90% and 84%, respectively (Qiao et al., 2008). It does not require reagent refrigeration, can be carried out by a healthcare worker with minimal lab training, and takes only 2.5 h or less to complete it (Wright and Kuhn, 2012). Also, it is of great importance that cervical cell samples can be self-collected giving advantage where cultural barriers discourage gynecological exams.

Two-stage screening approach, where HR-HPV used to screen and then HPV positive women undergo VIA, reduce the over-treatment of women without CIN 2 and 3 (Wright and Kuhn, 2012).

3.2. HPV Vaccination

There are two types of cancer vaccines: preventive (or prophylactic) vaccines - intended to prevent cancer development in healthy people, and treatment (or therapeutic) vaccines – intended to treat established lesion, either precancerous or invasive (Radulovic et al., 2009).

Currently, the Food and Drug Administration (FDA) has approved two preventive HPV vaccines: a quadrivalent vaccine (HPV4; Gardasil, produced by Merck) and a bivalent vaccine (HPV2; Cervarix, produced by GlaxoSmithKline). HPV2 is directed against two oncogenic types (HPV16 and -18). HPV4 is directed against two oncogenic types (HPV 16 and -18) and two non-oncogenic types (HPV 6 and 11). Neither vaccine is a live vaccine; both vaccines are composed of virus-like particles (VLPs) prepared from recombinant L1 capsid protein of the targeted HPV types (Hariri et al., 2011). They are produced by expressing the viral L1 protein in yeast (for Gardasil) or insect cells (for Cervarix). The L1 protein has the ability to efficiently self-assemble into VLP, which are highly immunogenic (Lowy and Schiller, 2012).

Clinical trial data have demonstrated that both vaccines induced high protection against persistent infection and premalignant anogenital disease associated with HPV16 and -18 (and against HPV6 and HPV11-associated genital warts for Gardasil) (Muñoz et al., 2010; Lehtinen et al., 2012). The vaccines also induced some cross-protection against other non-vaccine oncogenic HPV types (Brown et al., 2009; Wheeler et al., 2012). Regarding to immunogenicity, clinical trials have shown that Cervarix induces higher antibody titers than Gardasil (Einstein et al., 2009b).

Also, it seems that is more efficacious against non-vaccine HPV types 31, 33, and 45 than Gardasil (Malagón et al., 2012). Smaller number trials of Gardasil in males have shown protection against genital warts and premalignant anal neoplasia (Giuliano et al., 2011; Palefsky et al., 2011). In United States and many other countries, the Cervarix is approved for females aged 10 to 25 years, and Gardasil is approved for females and males aged 9 to 26 years.

The computer-based model predict that vaccination of adolescents against HPV types 16 and 18 associated with screening every 3-year, could reduce the incidence of cervical cancer by 94% (Goldie et al., 2004).

Nine-valent HPV vaccine, V503 (produced by Merck), is designed to protect against HPV6, -11, -16, -18 and further five types of HPV (31, 33, 45, 52 and 58) that cause cervical cancer. Currently, it is in Phase III of clinical trials.

FUTURE PERSPECTIVES

The knowledge about HPV has been translated into the clinic by production of preventive HPV vaccines and introduction of HPV testing in cervical screening programmes, with goal to reduce the incidence of cervical cancer.

Preventive vaccination will decrease the current prevalence of HPV types 16 and 18, but also 6 and 11, and in less extent related HPV types. It will change HPV type-specific prevalence worldwide, and incidence of HPV-related diseases. High vaccine coverage, could practically diminished HPV types that the vaccine made against it. Adequate monitoring will be required to estimate global and local prevalence of HPV genotypes in women with no cytological abnormality, and those with cervical precancerous lesions and cancer. These changes in distribution of HPV types will require more specific screening and preventive programmes in the post-vaccination era.

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Chapter 6

HPV INFECTION IN PREGNANT WOMEN: REVIEW

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ABSTRACT

Human Papillomavirus (HPV) is a common sexually transmitted infection (STI). It affects genital and oral mucosa and is associated to cellular lesions which can progress to cervical cancer and some head and neck cancers respectively. HPV prevalence, genotypes and associated risk factors varies from one region to another. Effectively, risk factors are linked to sexual behavior, health history, hygienic habits and some cultural considerations, that differ across geographical regions. Pregnancy is also considered, by some authors, as a risk factor of HPV infection. However, this infection in pregnant women and its outcomes are not largely studied, in spite of the risk of vertical transmission (during pregnancy or delivery) to newborns.

In this review, we tried to summarize pubmed available data on genital HPV infection in pregnant women. Data on HPV worldwide distribution, associated risk factors and rates of transmission to newborns were reported and compared according to geographical regions. Also, comparison between HPV prevalence in pregnant and non pregnant women was made to discuss the association of this event to HPV infection.

Pregnant women show high rate of infection comparing to others that can, certainly, be associated to the variation in the microenvironment of the female reproductive tract. The rate of HPV infection in pregnant women varies between 4.5% and 77.7% according to geographical regions and seems to be associated, among others, to primiparity. Therefore, rate of vertical transmission shows very large variability. As in non-pregnant women, the HPV infection prevalence remains relatively low in developed countries compared to developing countries.

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This review underlines the association between HPV infection and pregnancy, but it shows some limits. These limits were linked to different used methodology in different included studies. A meta-analysis could be an alternative to verify our conclusions.

INTRODUCTION

Human papillomavirus (HPV) harbor many types that can be classified into mucosal or epidermic HPV. Mucosal types are specific of infections located on mucosal epithelia whereas epidermic ones are responsible for infections of the skin (Segondy, 2008). Both mucosal and epidermic HPV have been shown to be involved in cellular lesions that can evolve to warts or cancers in the infected sites (Segondy, 2008; Karly et al., 2008). Mucosal HPV infect human aero-digestive and ano-genital regions causing oral or genital warts respectively. They were shown to be responsible for approximately all cervical and some oro-pharyngeal cancers. However, cutaneous types are less frequently involved in oncogenesis and require genetic predispositions or other co-factors (Karly et al., 2008; Torbjörn et al., 2010).

HPV infection concerns every age and gender. Genital infections are so common that 10% of women without apparent symptoms are infected at any given point in time (Bosch et al., 2008). Even if the prevalence of infection and genotype distribution vary according to geographic area, the highest prevalence values are generally observed in pregnant women and in age group 20 - 30 years regardless the geographic location (Bosch et al., 2008; Smith et al., 2008).

Epidemiologic studies have identified many risk factors associated to HPV infection with controversies for some of them. Generally, smoking habit, number of sexual partners, number of term births, histories of sexually transmitted infections and histories of warts or abnormal cytology were identified as the promoters of HPV infection (Bosch et al., 2008; Trottier et al., 2006; Bennani et al., 2012). Pregnancy appears to be a risk factor for cervical HPV infection. This infection seems to be determinant in pregnancy outcomes and also in oral HPV acquisition for newborns (LaCour et al., 2012; Zuo et al., 2011; Gomez et al., 2008; Rosi et al., 2005; Hermonat et al., 1998).

The aims of this report were: i) to determine and to compare the distribution of HPV infection in pregnant and non pregnant women according to geographical area; ii) to elucidate the role of pregnancy (cervico-vaginal micro-environment changes) as a risk factor of HPV infection; iii) to assemble vertical transmission rates iv) to determine the impact of infection on pregnancy outcomes and on newborns, in order to draw out conclusions concerning the management of HPV infection during pregnancy.

HPV PREVALENCE

HPV prevalence in pregnant women was taken from data available between 1997 and 2012. Generally, a large variation in HPV prevalence in pregnant women from one country to another and sometimes from one study to another in the same country (like in Poland) is observed (Tarka et al., 2011; Nowak et al., 2007; Biernat et al., 2003). This variability can, at least in part, be attributed to the size of studied population (sampling size), the selection

criteria and the differences of HPV diagnosis techniques. It's well known that cytological techniques under-estimate HPV prevalence; it only detects infections that lead to observable lesions when molecular techniques allow DNA detection before lesions installation (Eren et al., 2010). This could explain the lower prevalence values observed in some countries, notably in South Africa, in Thailand and also in HIV positive French pregnant women (Khaengkhor et al., 2011; Faucher et al., 2001; O'Farrell et al., 1989). So, in this report, HPV prevalence in pregnant and non pregnant women are reported, compared and discussed on the basis of results obtained using similar diagnosis techniques and according to geographical distribution (Table1).

Table 1. HPV prevalence in pregnant and non pregnant women

	HPV prevalence in pregnant women (n)	HPV prevalence in non pregnant women (n)
Africa (22.1% in healthy non pregnant women*)		
Senegal	24% (n = 72)# (Chabaud et al., 1996)	18% (n = 2065)# (Xi et al., 2003)
South Africa	5.7% (n = 193)▼ (O'Farrell et al., 1989)	20.7% (n = 8050)# (McDonald et al., 2012)
Uganda	60% (n = 987)# (Banura et al., 2008)	-
Zimbabwe	13.71% (n = 175)▼ (Latif et al., 1984)	24.5% (n = 2040)# (Fukuchi et al., 2009)
Asia (8% in healthy non pregnant women*)		
China	**36.21% (n = 152)#** (Deng et al., 2005)	11.14% (n = 1100)# (Zhang et al., 2012)
Hong Kong	10.1% (n = 308)# (Chan et al., 2002)	11.4% (n = 308)# (Chan et al., 2002)
India (Kolkata)	28.1% (n = 135)# (Bandyopadhyay et al., 2003)	-
India (Kolkata)	68% (n = 25)# (Bandyopadhyay et al., 2006)	25% (n = 24)# (Bandyopadhyay et al., 2006)
Japan	12.5% (n=1183)# (Takakuwa et al., 2006)	-
South Korea	18.9% (n = 291)# (Park et al., 2012)	7% (n = 13386)■ (Kim et al., 2012)
Thailand	7% (n = 145)▼ (Khaengkhor et al., 2011)	8.23% (n = 14747)■ (Sukumarn et al., 2010)
Europe (8.1% in healthy non pregnant women*)		
Austria	36.6% (n = 153)# (Worda et al., 2005)	-
Bulgaria	17.7% (n = 166) (Zlatkov et al., 2003)	13.1% (n = 84)▼ (Zlatkov et al., 2003)
Finland	16.3% (n = 304)# (Luvanto et al., 2010)	-

Table 1. HPV prevalence in pregnant and non pregnant women

	HPV prevalence in pregnant women (n)	HPV prevalence in non pregnant women (n)
Europe (8.1% in healthy non pregnant women[*])		
Italy	5.4% (n = 752)[#] (Tenti et al., 1997)	11.3% (n = 504)[#] (Tenti et al., 1997)
Lithuania	17.8% (n = 230)[#] (Gintautas et al., 2011)	25.1% (n = 1120)[#] (Mindaugas et al., 2006)
Poland (Krakovia)	13.1% (n = 145)[■] (Biernat et al., 2003)	9.6% (n = 145)[■] (Biernat et al., 2003)
Poland (Lodzi)	4.5% (n = 400)[#] (Nowak et al., 2007)	-
Poland (Poznań)	29.7% (n = 185)[#] (Tarka et al., 2011)	-
Spain	6.5% (n = 828)[#] (Castellsagué et al., 2009)	14.3% (n = 3261)[■] (Castellsagué et al., 2012)
Turkey	29.9% (n = 164)[#] (Aydin et al., 2010)	19.6% (n = 153)[#] (Aydin et al., 2010)
North America (11.3% in healthy non pregnant women[*])		
USA (Indiana)	31% (n = 245)[■] (Fife et al., 1996)	17.7% (n = 248)[■] (Fife et al., 1996)
USA (Iowa)	29% (n = 577)[#] (Smith et al., 2004)	-
USA (New Orleans)	28% (n = 2597)[×] (Hagensee et al., 1999)	-
USA (New York)	27.3 (n = 114)[•] (Morrison et al., 1996)	18% (n = 74)[•] (Morrison et al., 1996)
Latin America (12.3-20.4% in healthy non pregnant women[*])		
Brazil (Rio Grande do Sul)	77.8 (n = 63)[#] (Rombaldi et al., 2009)	11% (n = 399)[#] (Miranda et al., 2012)
Brazil (Espirito Santo)	25 (n = 40)[#] (Freitas et al., 2009)	20% (n = 60)[#] (Freitas et al., 2009)
Mexico (Colima)	75 (n = 72)[#] (Uribarren-Berrueta et al., 2012)	9.1% (n = 929)[#] (Lopez et al., 2012)
Mexico (Cuernavaca)	37.1 (n = 274)[■] (Hernández-Girón et al., 2005)	14.2% (n = 1060)[■] (Hernández-Girón et al., 2005)

[*] Bosch et al. 2008[#]; PCR Diagnosis[▼]; Cytology Diagnosis[■]; Hybrid capture Diagnosis[•]; RFLP Diagnosis[×]; Serological approach using HPV16 antigens.

HPV prevalence in African pregnant women ranged from 5.7% in South Africa to 60% in Kampala, region of Uganda (Banura et al., 2008; O'Farrell et al., 1989). In non pregnant women the prevalence varies from 18% in Senegal to 24.5% in Zimbabwe (Fukuchi et al., 2009; Xi et al., 2003). According to a meta-analysis conducted in non pregnant women, Africa presents the highest prevalence of HPV infection (22.1%) (Bosch et al. 2008). HPV prevalence on pregnant and non pregnant women can be compared only in Senegal where the

studies (in both pregnant and non pregnant women) were made with the same technique. This comparison lead to conclude that HPV prevalence was higher in pregnant women (Xi et al., 2003; Chabaud et al., 1996). Studies on pregnant women in South Africa and Zimbabwe were performed using cytological approach underestimating by the way the real HPV prevalence (O'Farrell et al., 1989; Latif et al., 1984). Therefore, the determined rates could not be compared to those in non pregnant women which were obtained by molecular approach (McDonald et al., 2012; Fukuchi et al., 2009). In non pregnant women, HPV 16, 52, 18 and 58 were reported as the most prevalent types (Bosch et al. 2008). However, Ugandan pregnant women were infected most frequently by HPV 51 and 52 followed by HPV16 and 18 while HPV16 and 18 were the most prevalent genotypes in Senegal (Banura et al., 2008; Chabaud et al., 1996).

In Asia, pregnant women also present high HPV prevalence values compared to non pregnant women except for one study on 308 pregnant women in Hong Kong where no significant difference was observed (Chan et al., 2002). HPV prevalence in pregnant women ranged from 7% in Thailand to 68% in India whereas the mean prevalence in general female population of the continent was about 8% (Khaengkhor et al., 2011; Bosch et al. 2008; Deng et al., 2005). The low prevalence of 7% determined in Thailand must be taken with precaution because it was determined using cytological exam (Khaengkhor et al., 2011). However, a large difference in the rate of HPV infection was observed in pregnant Indian women belonging to the same geographical area. This difference was explained by the differences on sampling time. The lowest value (28.1%) was observed among pregnant women 10-15 days prior to delivery, whereas the highest value (68%) was obtained (in later study conducted by the same group) at the early third trimester of pregnancy (Bandyopadhyay et al., 2003; Bandyopadhyay et al., 2006). The leading genotypes detected in Asian non pregnant women were HPV 16, 52 and 58 (Park et al., 2012; Bosch et al., 2008). Thus, HPV 18 and 16 remains the most prevalent types in pregnant women as determined in India and Hong Kong (Bandyopadhyay et al., 2003; Chan et al., 2002).

In Latin America HPV prevalence, in pregnant women was higher than the prevalence in non pregnant ones. The mean prevalence for healthy women ranged between 12.3% and 20.4% in South and Central Americas, respectively with HPV16 and 18 as the most prevalent types (Bosch et al., 2008; Smith et al., 2008). A variable value of infection has been detected in Mexican pregnant (75%-37.1%) and non pregnant women (14.2%-9.1%) (Uribarren-Berrueta et al., 2012; Lopez et al., 2012; Hernandez-Giron et al., 2005). This variability has been also observed in Brazilian pregnant women, where the prevalence runs from 25% in "Espirito Santo state" to 77.8% in "Rio Grande do Sul state" (Freitas et al., 2009; Rombaldi et al., 2009). It is of interest to take into account the fact that the highest value of 77.8% was obtained in pregnant women with history of HPV infection, abnormal Pap smear or genital warts. So, it didn't reflect the real prevalence in pregnant women. Remarkably, most prevalent types detected in this country were HPV 6/11 followed by 16 and 42 while HPV 18 was found to be the most prevalent in Mexican pregnant women (Uribarren-Berrueta et al., 2012; Rombaldi et al., 2009).

In North America, prevalence of HPV infection in pregnant women seems to be relatively constant with rates ranging from 27.3% to 31% (Fife et al., 1999; Morrison et al., 1996). These values are higher than the prevalence determined in non pregnant women which was about 18% (Fife et al., 1999; Morrison et al., 1996) and mean value of 11.3% (Bosch et al., 2008). The increased prevalence of HPV infection in pregnant women was also confirmed in

a group of pregnant women with cytological abnormalities (88.6%) compared to non pregnant women (83.8%) (Lu et al., 2003). The most prevalent types determined in USA were HPV 16, 53 and 52 for non pregnant women while HPV 52, 16 and 39 were the most predominant types in pregnant women (Bosch et al., 2008; Lu et al., 2003).

In Europe and particularly in Spain, Italy and Lithuania the HPV prevalence in pregnant women was curiously lower than the prevalence in non pregnant ones (Castellsagué et al., 2009; Bosch et al., 2008; Nowak et al., 2007; Tenti et al., 1997). The highest infection rate observed in European pregnant women was 36.6% as reported in Austrian study (Worda et al., 2005). However, a large difference on HPV rates was observed in Polish pregnant women according to geographical area. The HPV prevalence varies from 4.5% (in Lodzi region), which is the lowest value observed worldwide, to 29.7% (in Poznan) that consist of only HR-HPV types (Tarka et al., 2011; Nowak et al., 2007). Like in non pregnant women, HPV 16 and 18 were the most prevalent genotypes (Luvanto et al., 2010; Castellsagué et al., 2009; Bosch et al., 2008; Tenti et al., 1997).

Generally, the HPV prevalence tends to be higher in pregnant women when compared to non pregnant ones in most geographic regions (Table 1). Exceptions were noted in Italy, Spain and Lithuania where lower prevalence values were observed in pregnant women (Guintautas et al., 2011; Castellsagué et al., 2009; Mindaugas et al., 2006; Tenti et al., 1997). The highest HPV prevalence in pregnant women (77.8%) was reported in Brazilian women with histories of cervical abnormality and the lowest prevalence (4.5%) was reported in Poland (Rombaldi et al., 2009; Nowak et al., 2007). In non pregnant women, HPV prevalence ranged from 7% in South Korea to 25% in Lithuania (Kim et al., 2012; Mindaugas et al., 2006). In spite of limit associated to study design (pregnancy age at the sampling time) and to diagnosis techniques, as observed in non pregnant women, HPV prevalence in pregnant women varies widely between regions of the same country.

Genotype distribution studies were either focused on few or extended to a wide range of genotypes using HC2 or SPF10 Line Probe Assay genotyping approaches (Castellsagué et al., 2009; Bandyopadhyay et al., 2003; Hagensee et al., 1999). Generally, high risk types were found to be predominant and also associated to high persistence in pregnant women (Banura et al., 2008; Aydin et al., 2010; Bandyopadhyay et al., 2003; Tenti et al., 1997; Chabaud et al., 1996). In most studies, types 16 and 18 were found to be the leading ones but in Uganda, types 51 and 52 were the most prevalent (Banura et al., 2008). HPV types 45 and 58 were found with high rates in oral mucosa of Finnish pregnant women (Rautava et al., 2012). However, in Brazil and especially in 'Rio Grande do sul' (region with the highest HPV prevalence (in spite of inclusion criteria)), low risk HPV 6 and 11 taken together counted for a high proportion of HPV infected pregnant women (Rombaldi et al., 2009; Rombaldi et al., 2008). So, HPV distribution genotypes vary also from one region to another.

HPV Infection Risk Factors in Pregnant Women

Risk factors associated to HPV infection vary according to studies. Age, age at the first intercourse, number of sexual partners, number of term births and histories of abnormal cytology and warts or other sexually transmitted infections are generally associated to increased HPV prevalence in both pregnant and non pregnant women (Bennani et al., 2012;

Bosch et al., 2008; Trottier et al., 2006; Hernandez-Giron et al., 2005; Morrison et al., 1996). In other way, the outcome of exposure to sexually transmitted virus seems to be conditioned by several endogenous and exogenous biological factors. The microenvironment of genital tract is critical in initiation of infection and the immune responses that clear infection. It can be modified by vaginal pH, Lactobacilli and ovarian hormones status in pregnant and non pregnant women. In this report we will discuss factors directly associated to genital tract micro-environment changes that can lead and explain the increase of HPV infection rate especially in pregnant women.

Ovarian Hormones

Studies on animal models concluded that progesterone treatment (not estradiol) increases the risk of sexually transmitted infection (STI). It acts by both increasing susceptibility and decreasing mucosal immune response (Gillgrass et al., 2005 a, b; Baeten et al., 2005; Gillgrass et al., 2003; Marx et al., 1996). Effectively, this hormone plays an important role in regulating number of immunologic pathways: inhibition of cytotoxic T cells (CTL) and natural killer cells, decreasing the production and altering glycosylation of IgG and IgA (Hel et al., 2009; Baeten et al., 2007; Fish, 2008; Hao et al., 2007). *In vitro*, sex hormones treatments (progesterone and 17-βestradiol) decreased the levels of IFNα and IL2 and increased the level of IL10 synthesis in responses to HPV16-VLPs (Marks et al., 2010). These modulations of immune response (associated to progesterone) lead to increased chronic inflammation and thereby to viral persistence. High rate of HPV infection associated to oral contraception has also been reported (Marks et al., 2011) and can support this idea.

During the first trimester of pregnancy the level of circulating sex hormones, especially progesterone is very high. It becomes stable in the second and third trimesters and declines after delivery (Zou et al., 2009). Those data let us to suppose that the high progesterone rates produced in this stage may predispose women to virus acquisition and the fact that this hormone inhibits rhythmic contraction of uterine muscles can help to viral installation. The effect of sex steroid in pregnant women is not limited to increasing infection rates but it may also be related to an increased risk of malignancy as reported for sex cord stromal tumors (Chen et al., 2011).

The parity appears in many studies as a factor associated to HPV infections but the conclusions are mitigated. Some authors conclude to protective effect of high parity (Rautava et al., 2012; Hernandez-Giron et al., 2005; Fife et al., 1996; Morrison et al., 1996) and to increased risk of HPV acquisition in primiparous women (Park et al., 2012; Banura et al., 2008). This data has been explained by the progressive acquisition of the immunity that can also been associated to progesterone effects. The fact that the level of progesterone detected in the first full term pregnancy were higher than that in multiparous women (Garcia-Closas et al., 2002; Musey et al.,1987; Bernstein et al., 1985) explains the differences in HPV rates observed between primigravid and multigravid women and confirm the hypothesis of progesterone as a risk factor.

Cervico-vaginal pH

The cervico-vaginal acid-base environment is tightly regulated and determined by cohabitating Doderlein Lactobacilli (Weinstein et al., 1939). At menopause, cervico-vaginal pH increases and the female genital tract flora is perturbed (Moller et al., 1991). The elevated pH value (6.5/7.0) has been associated with urinary tract infections, vaginal atrophy and dyspareunia (Raz et al., 1993; Pandit et al., 1997; Bernan et al., 1999). This can also explain the increasing rates of HPV observed in menauposed women. Effectively, a recent study has demonstrated that elevated vaginal pH is associated with 30% greater risk infection with multiple HPV types, especially in women younger than 35 and older than 65 years (Clarke et al., 2012). So we think that both factors (immune response and pH) may increases the risk of HPV reactivation or re-infection. In pregnant women, inflammation associated to high progesterone levels will influence the cervico-vaginal pH. The increasing pH is known to be positively associated to the risk of STI and thereby of HPV infection.

Sexual Behavior

Sexual behavior is also considered a risk factor in many ways: age at first sexual intercourse, number and lifetime sexual partners. More than five lifetime sexual partners and lower age at first sexual intercourse are associated with increased HPV prevalence (Khaengkhor et al., 2011; Guintautas et al., 2011; Hernandez-Giron et al., 2005). In fact, sexual behavior is a factor that should be considered when studying factors related to cervico-vaginal micro-environment change. Effectively, HPV infection is considered the first viral sexually transmissible infection and several studies have shown that the rate and risk of infection is higher in sexually active women with multiple partners. There is no doubt that HPV is sexually transmissible but the question about the route of increasing the risk of infection installation and persistence must be discussed. It's evident that the high number of partners increases the risk of exposition to virus and infection. It's also of interest to know that high number of sexual intercourses increased the vaginal pH values and causes breach in this tract. This affects the balance of the genital tract microenvironment (notably lactobacillus activity) and also permits the access to basal cells (via breach) that can facilitate infection installation and also persistence. Probably, the risk of HPV infection is also associated to the frequency of sexual intercourses. Hence, multiple sexual partners and the frequency of sexual intercourses do not only increase the risk of infection but also make changes in the vaginal tract micro-environment that help to virus installation and persistence. This hypothesis must be verified by conducting studies on HPV infection according to the frequency of sexual intercourses in pregnant and non pregnant women.

Age

Epidemiologic studies have reported that the prevalence of HPV infection shows generally two peaks: the first below 30 years and the second after the age of 45 or 50 years, coinciding with the postmenopausal years (Bosch et al., 2008; De Sanjosé et al., 2007). The first peak of HPV prevalence in healthy women corresponds also to the reproductive age. So,

the high prevalence of HPV infection observed during pregnancy must be considered a simple component of normal HPV prevalence profile when adjusted to the age or as a consequence of micro-environment changes? It will be more reasonable to consider the increased prevalence of HPV infection in pregnant women as a result of the age and changes (caused by pregnancy) association or as synergic effect of these risk factors. Successfully, study conducted by Takakuwa et al., on large pregnant women sampling, showed a significant association of HPV infection and age. Pregnant women aged less than 25 years were at high rate of infection (22.6%) than those over the age 25 years (11.3%) (Takakuwa et al., 2006). The involvement of pregnancy as a risk factor is also confirmed by the decreasing HPV prevalence in the post-partum. Furthermore, even in pregnant women, the age remains a risk factor (Khaengkhor et al., 2011; Castellsagué et al., 2009; Hernandez-Giron et al., 2005; Chan et al., 2002).

History of Sexually Transmissible Infections

Both bacterial and viral sexually transmitted infections can have an impact on the susceptibility to HPV. Recent studies have shown that bacterial infections like *syphilis*, *N. gonorrhea* or C*hlamydia trachomatis* (CT) promote both the acquisition and persistence of HPV infection (King et al., 2011; Lehtinen et al., 2011; Silins et al., 2005). In effect, the asymptomatic nature of CT infection, its long-term persistence in the absence of treatment and its capability of inducing metaplasia and chronic inflammation lead to consider this bacteria as a potential cofactor for high-risk persistent HPV infection and oncogenesis promotion (Smith *et al*., 2004; Simonetti *et al*., 2009; Silins *et al*., 2005). High rate of simultaneous CT and HPV infections in pregnant women has been detected in Brazil conducting authors to recommend the screening of pregnant women for both CT and HPV (Pereira et *al*., 2010). More attention must be given to this group of women.

A history of herpes simplex virus (HSV) and human immunodeficiency virus (HIV) infections were also reported as risk factors of HPV infection (Hessol et al., 2004; Berrébi et al., 2008; Denny et al., 2008). Effectively, a considerable increased prevalence of HPV infection (mostly high risk genotypes) has been observed in all studies conducted on HIV infected populations. In Uganda, 72.2% of HIV positive pregnant women were also HPV positives with multiple types in 45.8% of cases (Banura et al., 2008). The rate of infection varies from 62.7% to 94.11% in Brazilian HIV+ pregnant women (Meyrelles et al., 2013; Jalil et al., 2009; Brandão et al., 2009). In France, a cytological evaluation of HPV infection in HIV infected pregnant women showed a low value of 13% (Faucher et al., 2001) but this rate must be taken with precaution because of the limitation associated to diagnosis used techniques. The high rate of infection generally detected in HIV pregnant women has been explained by the tendency of HPV to persist, thus differing from what occurs among immune-competent women (Nappi *et al.*, 2005; Hawes et al., 2006; Palefsky et al., 2006; Berrébi et al., 2008; Denny et al., 2008). In gestational period, a reduction in immunity occurs and specifically in genital organs for reasons cited above (vaginal flora imbalance, hormonal factors….). Anatomical modifications as: hypertrophy, congestion and ectopy were increased and followed by metaplasia. The area of immature squamous metaplasia is more susceptible to the development of preneoplastic lesions and HPV infections (Mota et al., 2002; Correia et al., 2007). All these factors predispose women to the acquisition and development of lesions

induced by HPV (Armbruster-Moraes et al., 2000; Costa et al., 2000; Correia et al., 2007). Recently, it has also been reported that HSV 2 infection or co-infection with HPV might be involved in cervical cancer development (Zhao et al., 2012). However, no significant correlation has been determined between HPV and AAV in spite of their occurrence in co-infections (mostly in pregnant women) (Freitas et al., 2009; Grce et al., 2004).

Therefore, the increased infection rate in pregnant women is multifactorial or the result of natural events that produce a series of cascading changes in cervico-vaginal microenvironment. During the first trimester of pregnancy, there is a low immune response to HPV, which explains the higher frequency of persistence of the virus during pregnancy. However, this deficient response undergoes an intense recovery at the start of the third trimester, with reinforcement during the postpartum period and consequent regression of the infection (Puig-Tintoré et al. 2002; Fife et al., 1999; Fife et al., 1996).

CONSEQUENCES OF PREGNANT WOMEN INFECTION

HPV Infection Outcomes in Pregnant Women

As in non pregnant women, most part of HPV infections are self-limited and spontaneously clear within several-years as a result of immune response development. Contradictory opinions exist on the moment of clearance onset. Some authors concluded that there is an increasing HPV clearance rate during the third trimester and at the post partum period (Nobbenhuis et al., 2002). Other authors concluded to an increased rate of infection throughout pregnancy and high clearance only at post partum period (Fife et al., 1999). It has also been shown, in Kampala, an overall constant prevalence value during pregnancy that was associated to a high rate of clearance with an equal rate of new infections (Banura et al., 2008). Thereby, HPV clearance at post partum period has been well shown and would be the result of restoring the immune response at this period. However, HPV persistence occurs mostly in pregnant women infected with high-risk genotypes and increases the risk of cervical cancer development (Gintautas et al., 2011). Effectively, many studies have shown that pregnancy might worsen the outcome of HPV infection allowing the progression of intraepithelial lesions to more invasive ones (Bandyopadhyay et al., 2006; Armbruster-Moraes et al., 2000; Nevin et *al.*, 1995). Moreover, in pregnant women with cervical intraepithelial lesions, the risk of postpartum persistence and progression of lesions seem to be associated to the age, lesion grade and also to the infecting genotype (Cubo-Abert et al., 2012).

HPV Infection and Pregnancy Outcome

Lower genital tract infections are recognized as main causes of preterm birth (Goldenberg, 2000). Few studies are focused on the outcomes of HPV infections on pregnancy and most of them concluded on negative effect of the virus. Effectively, all conducted studies on this subject have indicated that placental infection with HPV may be risk factor for spontaneous abortion or preterm delivery (Gomez et al., 2008; Hermonat et al., 1998). Gomez

et al. have demonstrated that HPV infection can impair extra-villous trophoblast invasion into the maternal uterine wall, probably by causing increased rates of trophoblast, resulting in placental dysfunction. This dysfunction may be the reason of preterm delivery or spontaneous abortion (Lopez et al., 2012; Gomez et al., 2008; Kulvachev et al., 2007; Manavi et al., 1992). This has been confirmed by a recent study conducted in large patient cohort that has demonstrated the association of HR-HPV cervical infection with placental abnormalities and also with preterm birth (Zuo et al., 2011). A significant relationship between HPV infection and abortion has also been reported in previous study conducted by our group and where the rate of infection in women with past history of abortion was 3.76 times higher than control group (without history of abortion) (Bennani et al., 2012).

Preterm births remain one of the top causes of infants deaths worldwide (Damus, 2008; Lawn et al., 2010). It has short term impact on infant health and poses sever threats to children long term development and physical and psychological health (Sayres, 2010; Huddy et al., 2001). To prevent the risk of preterm birth, some authors recommend HPV screening for pregnant patients, especially those with LSIL or HSIL (Zuo et al., 2011).

HPV Vertical Transmission

It is well established that HPV is one of the most prevalent sexually transmissible agents. Non-sexual routes of transmission have also been confirmed; they consist of direct contact with mucosa or skin or trough contaminated objects or during the perinatal period. Many studies concluded that the perinatal transmission occurs in many ways but in different proportions. These ways include i) passage of the fetus trough birth canal and on coming into contact with infected maternal secretions (Puranen et al., 1997; Tseng et al., 1998) ii) ascending infection from the vaginal canal after a premature rupture of the amniotic membrane (Tenti et al., 1999; Eppel et al., 2000); iii) during vaginal delivery from contaminated objects; iv) intrauterine transmission at the time of fertilization, from sperm carrying latent HPV or during the egg implantation (Lai et al., 1996; Rombaldi et al., 2008).

Transmissions via placenta or cord blood have been confirmed in many recent studies and seem to be the most common ways (Sarkola et al., 2008; Eppel et al., 2000). Brazilian study has demonstrated that 54.5% of vertical transmission rate was transplacental (Rombaldi et al., 2008). The association of delivery method to vertical transmission is not clearly defined. In South Korea, association between vaginal delivery and high vertical transmission of HPV DNA from mother to neonates has been reported (Park et al., 2012). However, cesarean sections were also found to be associated with high rates of prenatal transmissions in India (Bandyopadhyay et al., 2003). Even so, mother-to child HPV transmission was higher after vaginal delivery than cesarean section according to a systematic quantitative review of Medeiros et al. (2005). In addition, association of labor duration and premature rupture of the membrane to the transmission of the virus to newborns has been reported but remains controversial (Park et al., 2012; Tenti et al., 1999). For these reasons, some authors suppose that cesarean delivery could reduce transmission; it avoids ingestion of infected maternal secretions or blood during fetal passage through the birth canal and also prevents labor duration (Tseng et al., 1998; Minkoff et al., 2003).

Table 2. HPV vertical transmission rates

	N (HPV positive mothers)	**Newborns transmission rates (%)**
Brazil (Rombaldi et al., 2009)	49	22.4
Finland (Koskimaa et al., 2012**)**	329	17.9
India (Bandiopadhyay et al., 2003)	38	18.42
Italy (Tenti et al., 1999)	711	30
South Korea (Park et al., 2012)	55	18.2
Spain (Castellsagué et al., 2009)	66	19.7
USA (Watts et al., 1998)	80	4

HPV vertical transmission has been confirmed by concordance of HPV types detected in neonate and mothers. Effectively, the types of HPV identified in cervical samples of mothers during pregnancy were the same detected in oral samples of newborns after delivery (Tenti et al., 1999; Rombaldi et al., 2009). Variable rates of vertical transmission were noted from one study to another and ranged from 4% to 30% as reported in Table 2 (Koskimaa et al., 2012; Park et al., 2012; Rombaldi et al., 2009; Castellsagué et al., 2009; Bandyopadhyay et al., 2003; Tenti et al., 1999; Watts et al., 1998). The very low rates were reported in USA whereas this rate is about 22.4% in Brazil (Rombaldi et al., 2009; Heather et al., 1998). Strangely, high rates of vertical transmission were found in Italy (30%) and Spain (19.7%) regardless of the low HPV prevalence in pregnant women (Castellsagué et al., 2009; Tenti et al., 1999). Type specific investigations lead to the conclusion that high transmission rates including transmission to newborns are associated to high risk HPV and multiple genotypes infections (Koskimaa et al., 2012; Rombaldi et al., 2009; Castellsagué et al., 2009; Banura et al., 2008). The rate of transmission can also been influenced by immunological status of the mother. Thus, Rombaldi et al. have reported that maternal immune-depression caused by HIV infections increases the rate of HPV transmission to newborns (Rombaldi et al., 2009).

Therefore, most of these newborns infections appear to be transient and it is unlikely that the majority have adverse clinical consequences. HPV infections in newborns are followed by high rates of clearance. In effect, it was noticed a total clearance of infected newborns 6 months after their birth in South Korea and Brazil and after one year in India (Park et al., 2012; Rombaldi et al., 2009; Bandyopadhyay et al., 2003). Other studies concluded that the persistent oral carriage in the infant was associated to persistent oral HR-HPV in the parents and the presence of hand warts in the mother (Rintala et al., 2005). The persistence of HPV in children can lead to some cytological lesions. Oral papilloma and hyperplasic oral changes were the most frequent lesions reported in infants (Puranen et al., 1996, He et al. 2002; Liu et al., 2003.). A case of giant condyloma acuminata was described in a Turkish infant of 18 months old (Altinay-Kirli et al., 2011).

HPV VACCINE AND PREGNANCY

Both bivalent and tetravalent vaccines used against HPV are recombinant proteins of the viral capsid. Quadrivalent HPV vaccine has been shown to elicit a strong antibody response

and to engender immune memory upon re-exposure to HPV vaccine (Olsson et al., 2007). Additionally, transplacental transport of vaccine-induced antibodies has been reported and seems to be able to prevent newborns infection (Matys et al., 2012; Heim et al., 2007). Furthermore, HPV vaccination seems to reduce the risk of preterm birth (Watson et al., 2012).

However, women who received the bivalent vaccine during pregnancy showed a high proportion of spontaneous abortion compared with a control group but this association still not significant or demonstrated (Cervarix product monograph, 2011). Thus, and according to clinical trial and registry data, the HPV vaccine, when administered during pregnancy, has not been associated with adverse pregnancy outcomes, including major malformations (Narducci et al., 2012). However, the Food and Drug Administration (FDA) classifies HPV vaccines pregnancy in category B medication and vaccines still not recommended for pregnant women (Garland et al., 2009; Narducci et al., 2012). More studies are needed to determine its effect. Nonetheless, inadvertently administration of HPV vaccine during pregnancy will not justify its termination; however, it's recommended to delay remaining doses until delivery (Narducci et al., 2012).

CONCLUSION

Pregnancy is a natural biological phenomenon; it brings about complex and shifting physical, physiological, immunological and emotional changes. The cervico-vaginal microenvironment changes lead to weakness of immunity that makes pregnant women a population at risk for several infections including HPV. HPV infection risk factors are the same for pregnant and non pregnant women. In spite of limits linked to different used methodology in different studies (study design, pregnancy age at the sampling time, diagnosis techniques and sampling size), we can conclude that the risk of HPV infection is generally higher in pregnant women. In other way pregnancy may be considered an indirect risk factor of HPV infection. In pregnant women as in non-pregnant ones, the HPV infection prevalence remains relatively low in developed countries compared to developing countries. A meta-analysis could be an alternative to verify our conclusions.

HPV infection during pregnancy exposes newborns to HPV infection in variable rates according to regions. The vertical transmission seems to be of reduced importance because HPV positive neonates will almost totally be cleared after one year approximately. Many adverse effects such as spontaneous abortion, preterm birth or newborns HPV infection can result from HPV infection during pregnancy. So, it will be of interest to perform HPV diagnosis on pregnant women to prevent these events. HPV positive pregnant women should be treated with special attention because they are more likely infected by high risk genotypes exposing them to high risk of cervical cancer development. Vaccination before pregnancy of women at reproductive age could help to prevent mother and neonates infections (at least with genotypes 6, 11, 16 and 18) and also to reduce the risk of spontaneous abortion and preterm births.

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Chapter 7

IMMUNOGENICITY OF HPV VACCINATION

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ABSTRACT

Naturally induced humoral immunity after human papillomavirus (HPV) infection is relatively low and often inconclusive. The main reason could be the local character of HPV disease because the immune system obtains little or no information about the on-going infection. Conversely, this is altered by HPV vaccination because the robust immune response is observed in most vaccines compared to naturally infected individuals.

Although at present there is no immune correlate of protection, it is becoming evident that neutralizing IgG antibodies play one of the most crucial roles. Moreover, vaccine induced antibodies can transudate from the serum to the oral or vaginal mucosa where they take a part in virus elimination.

Seroconversion rates and antibody levels are limited by the different specificity of detection antigens, cut-offs and the type of method. Current various serological assays measure not only total type-specific IgG antibodies but also functional neutralizing ones. Only a part of the total or neutralizing antibodies most likely can contribute to real protection against HPV. Even less than 1% of vaccine-induced antibodies is eligible to cross-react with other human papillomaviruses highly related to vaccine ones and neutralize them.

While the antibody levels peak and subsequently decay within the first 6 months after vaccination, the matured antibody levels slightly but significantly increase to reach a plateau. Total type-specific IgG and neutralizing antibodies for all vaccine HPV types persist in lower levels related to their peak for at least 48 months following immunization regardless of the HPV vaccine used. Finally, long-lasting protection can be sustained with the vaccine generated immune memory independent of the presence or the absence of antibodies.

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Antibody response inversely correlates with age and the highest was observed in subjects younger than 12 years old regardless of gender. Interestingly, Black men had higher titers of neutralizing antibodies than did both Caucasian and Asian men.

The post-vaccination response becomes stronger if the interval of the first two doses is extended over 6 months. Therefore, an alternative 2-dose regimen could eventually replace currently recommended 3-dose vaccination.

A higher antigen content formulation of vaccines or a combination more than one antigen in multivalent vaccines did not alter immune response compared to licensed vaccines or monovalent candidate vaccines, respectively.

The result of HPV vaccination is unaffected by concomitant immunization with other inactivated vaccines.

Maternal antibodies of immunized women undergo transplacental transport during pregnancy and they can protect new-born infants at least in the short-term. In spite of poor response to HPV vaccination, there is a humoral and specific cell-mediated immunity confirmed in immunocompromised individuals.

INTRODUCTION

Human papillomaviruses are highly successful contagium because they induce persistent infections with no apparent systemic sequelae and only occasionally kill the host.

Attempts to prevent these infections with the most prevalent high-risk oncogenic HPV types (HPV-16 and -18) and low-risk non-oncogenic HPV types (HPV-6 and -11) led to the development of two highly efficacious HPV vaccines: the bivalent HPV-16/18 vaccine (Cervarix; GlaxoSmithKline, Brentford, UK) and quadrivalent HPV-6/11/16/18 vaccine (Gardasil; Merck, Whitehouse Station, NJ, USA).

Both vaccines comprise virus-like particles (VLPs) of appropriate HPV types assembled from the major capsid protein L1 exhibiting genotype specificity and a much higher immunogenicity compared to the minor capsid protein L2 which is largely an internal protein, about 30-fold less abundant than L1.

A serum neutralizing antibody to the major capsid protein L1 and specific cell-mediated immunity are elicited after the vaccination or natural infection and they together generate protection against subsequent viral challenge, i.e. prevents the genital HPV associated disease.

Although there is no currently established immune correlate of protection, the humoral immune response is considered as the primary endpoint of protection. In view of this, this review is aimed at elucidating the definitive particulars of serological response after HPV immunization.

PROTECTION MECHANISM

Human papillomavirus infection triggers no inflammation and papillomavirus replication signals no warning to the immunity system. Also, papillomaviruses down-regulate secretion of type I interferons act as a bridge between adaptive and innate immunity (Stanley M. 2006).

The existence of persistent HPV infection is revealed by delayed humoral response with type specific antibodies to the major viral coat protein L1. This seroconversion appears between 6 and 18 months after the first detection of HPV DNA (Stanley M. 2009).

However, not all infected individuals have specific antibodies because only about 50% or 70% of women infected with HPV-16 or -6, respectively, seroconverted (Carter et al. 2000). Moreover, a relatively lower sensitivity of serological assays could contribute to these results. Post-infection seroconversion, including its inception, is dependent on the HPV genotype (high- or low-risk) and viral load.

This modest humoral response is not surprising since there are residual antigen particles captured by Langerhans cells that transport them to draining lymph nodes where immune responses are initiated.

Naturally induced genotype-specific HPV antibodies are able to reduce the risk for subsequent infection with not only the same type but also its genetically related types. It was demonstrated that the risk of HPV reinfection was reduced to about 50% in women with post-infection IgG antibodies to HPV-16 (Ho et al. 2002). The evidence that these antibodies are crucial for protection was confirmed by experimental challenge with high doses of infectious virus in animals immunized with serum or purified antibodies specific to animal papillomavirus (Ghim et al. 2000, Breitburd et al. 1995).

Finally, cell-mediated immunity as well as antibodies directed against HPVs contributes to the protection against reinfection because patients with antibody immunodeficiency are no more susceptible to reinfection with cutaneous HPVs than healthy individuals (Stanley M. et al. 2006).

It has been shown that HPV infection is associated with an inhibition of gamma interferon secretion and a reduction in the expression of different innate immune receptors, including Toll-like receptor 9 and natural-killer receptors, restrict the cytotoxic activity of the NK cells (Arreygue-Garcia et al. 2008, Garcia-Iglesias et al. 2009). Ultimately, HPVs are able to evade with a different immune mechanism, including inhibition of receptor's expression on T-cells, and induction of IL-10 interferes with the activation of Th1 lymphocytes (Wang et al. 2001). However, the evasion of NK cell activity may play an important role in the progress of the infection. Immunization with quadrivalent vaccine induced the expression of NK-receptors (primarily NKG2D, NKp30 and NKp46) and immunoglobulin-like transcript 2 (ILT2) receptor on NK, NKT cells and monocytes. This receptor may solely be relevant in the modulation of the innate and adaptive immune responses (Colmenares et al. 2012). NK and NKT cells strengthened by vaccination are vigilant against the HPV infection.

SEROLOGICAL ASSAYS

Measurements of serum IgG anti-HPV L1-VLP antibodies offer fundamental information about the post-vaccination immune response. The competitive radioimmunoassay cRIA (Palker et al. 2001), used within early immunogenicity studies after vaccination with quadrivalent HPV vaccine, was subsequently replaced by more suitable competitive Luminex immunoassay cLIA (Opalka et al. 2003).

The cLIA assay uses L1-VLPs antigens of all vaccination genotypes (HPV-6, -11, -16 and -18) that have been covalently conjugated to Luminex microspheres. They are identified

by their fluorescent dye spectral properties. The principal of the immunoassay is that type-specific phycoerythrin labeled monoclonal antibodies competes with serum antibodies for binding to the appropriate neutralizing epitopes on the VLPs. The chosen monoclonal antibodies (H6.M48 for HPV-6, K11.B2 for HPV-11, H16.V5 for HPV-16 and H18.J4 for HPV-18) are HPV type-specific; they bind to neutralizing epitopes and do not cross-react with other HPV genotypes. The detection of monoclonal antibodies is inversely proportional to the serum neutralizing antibody titer. This competitive assay allows for simultaneously measuring monoepitope neutralizing antibodies.

These same antibodies were determined with single epitope-based inhibition enzyme-linked immunosorbent assay (SEBI-ELISA) after vaccination with bivalent HPV vaccine. The inhibition assay is based on the indirect measure of serum antibodies specifically directed either against neutralizing V5 or J4 epitopes (Giannini et al. 2006). Both methods determine type-specific antibodies to neutralizing epitopes on HPV, but they do not predict the entire neutralizing antibody response.

A study assessment of immunogenicity induced by bivalent vaccine was alternatively performed using a conventional direct VLP ELISA (Harper et al. 2004). HPV-16 or HPV-18 antibodies of serially diluted sera are measured separately by adsorbing either HPV-16 or HPV-18 L1 VLPs as coating antigens onto well microtiter plates. The color intensity quantitated by optical density determines the amount of total IgG specific to appropriate HPV. The fraction of serum IgG attributable to the neutralizing antibody cannot be specifically quantitated although a high correlation between them has been suggested (Dessy et al. 2008).

Total IgG antibodies specific to HPV genotype were assessed after quadrivalent vaccine immunization using Luminex immunoassay (LIA) with no competition of monoclonal antibodies (Brown et al. 2011).

Both non-competitive methods, VLP ELISA and LIA, are sensitive to capturing not only neutralizing but also non-neutralizing conformational antibodies exhibiting cross-reactivity to epitopes shared on the surface of the genetic related HPV. Post-vaccination antibodies demonstrated a high affinity to the HPV-31 and -45 homologous to vaccine HPV types (Einstein et al. 2011, Smith et al. 2007). The variability of the broad reacting epitopes of L1-VLP detection antigens contributes to the lower specificity of these assays (Eklund et al. 2012).

Neutralization of HPV viruses can result from an antibody directly binding on viral neutralizing epitopes or an antibody binding other epitopes which might sterically prevent linkage of the virus to the target cell. Therefore, the results of non-competitive methods based on multiple-epitopes correlate better than those of competitive methods with results of pseudovirion neutralization. Most of the discordances were observed at lower monoepitope antibodies titers determined by competitive assays (cLIA).

In vitro neutralization assay is based on pseudovirions (PsV) that contain two capsid proteins L1 and L2 enveloping plasmid DNA carrying a sign as a reporter or marker gene (Buck et al. 2005). Synthetized pseudoviruses are able to infect human cells and transport marker genes, such as alkaline phosphatase (Pastrana et al. 2004) or red fluorescent protein (Krajden et al. 2009). Antibody-mediated pseudovirion neutralization is detected by a reduction in activity of the secreted sign. Serum neutralization titers are defined as the reciprocal of the dilution that caused 50% reduction in activity pseudovirus when compared to the control without serum.

The sensitivity of this method can be influenced by antibody non-specific inhibition at low dilution of sera because the antibodies block cell attachment by steric interference with the virus receptor site or bind across the groove between the pentamers, thus stabilizing the particle and preventing virus entry (Trus et al. 1997). Lower assay preciseness is also demonstrated by coefficient variability reported at 33.2% and 46.2% for anti-HPV-16 and -18, respectively (Dessy et al. 2008, Roberts et al. 2012).

Women classified as HPV-naïve can be misleading when different serology assays were used. Because competitive methods can detect only serum antibodies exclusively biding to one specific epitope, serostatus refers to this one epitope of the appropriate HPV genotype. In contrast, as the ELISA assay measures total serum IgG antibodies exhibiting cross-reactivity to other HPV types, such seronegativity is more comprehensive, not only related to one target HPV type (Safaeian et al. 2012). Cross-protection of HPV vaccines were assessed in cohorts of HPV-naïve women classified with unlike serological methods (Paavonen et al. 2009, Joura et al. 2008). Therefore, cleaner HPV-naïve populations have been identified using ELISA serology than cLIA.

INTERNATIONAL STANDARD AND ASSAY CUT-OFFS

Currently, there is still no definition of seroprotection limits correlating with real protection after HPV vaccination.

The WHO Expert Committee on Biological Standardization in 2009 established 05/134 as the first international standard for antibodies to HPV-16, human serum, with an assigned potency of 10 IU/mL (Ferguson et al. 2011). The standard was obtained from three women naturally infected only with HPV-16. The women were willing to donate blood in an amount of 450 ml every third month over a 3-year period. The serum samples were pooled, filled into ampoules in 0.5 ml and freeze-dried.

A common methodology for serology based on VLP ELISA has been evaluated in an international HPV serology proficiency study based on a blinded panel consisting of serum samples from women with PCR-verified HPV-16 infection, control serum samples from virginal women and the international standard (05/134) (Eklund et al. 2012). There was a strong correlation between the potency obtained by the VLP ELISA and the neutralizing titers of serum samples.

It was found that a mean value of the results from the negative control sera plus three standard deviations was the cut-off criterion that gives the most robust results in different settings of laboratories. The cut-off values ranged between 0.53 IU/mL and 2.84 IU/mL for different laboratories.

In spite of absence of standards for other HPV antibodies, the current international standard of anti-HPV-16 can help standardize serological methods and allows for comparing their performance such as the conversion factors to international units: 1 IU = 6.1 ELISA U/mL and 1 IU= 13.28 mMU/mL (Safaeian et al. 2012).

Seropositivity reported in immunogenicity studies was established as an antibody titer greater or equal than the assay cut-off. The cut-offs of VLP ELISA, inhibition ELISA or LIA were established as the assay threshold for the appropriate HPV type (Dias et al. 2005, Harper et al. 2004, Harper et al. 2006, Giannini et al. 2006, Dessey et al. 2008, Brown et al. 2011). In

contrast, dilution corrected cut-offs of cLIA were defined as the lowest titer of positive sera for appropriate HPV type, i.e., 2- to 8.3-fold higher than a threshold limit of the cLIA assay (Villa et al. 2006).

The geometric mean concentration of naturally induced antibodies against HPV-16 and HPV-18 were 50 ELISA U/ml (95% Cl: 40.9-60.4) and 41 ELISA U/ml (95% Cl: 34.2-49.0), respectively, i.e., 5.9- to 6.3-fold higher than ELISA cut-offs (Harper et al. 2004).

It is clear that the post-vaccination and post-infection seroconversion rates can be affected by an arbitrarily chosen serostatus cut-off. Comparing VLP ELISA and cLIA seropositivity to HPV-16 based on the standard cut-offs have shown a significant discordance in an unvaccinated population. With a higher VLP ELISA cut-off of 54 ELISA U/ml and, keeping cLIA cut-off at 20 mMU/ml, the overall agreement increased to 97%, i.e., both assays consistently resulted in 91% and 6% seronegative and seropositive samples, respectively (Safaeian et al. 2012).

Table 1. Standard cut-offs of different assays

Method	Cut-off	HPV6	HPV11	HPV16	HPV18
cLIA*	the lowest titer of positive sera	20 mMU/ml	16 mMU/ml	20 mMU/ml	24 mMU/ml
LIA*	assay threshold	3,4 mMU/ml	2,2 mMU/ml	8 mMU/ml	5 mMU/ml
VLP ELISA*	assay threshold	ND	ND	8 EU/ml	7 EU/ml
SEBI ELISA*	assay threshold	ND	ND	41 EU/ml	110 EU/ml

* cLIA: competitive Luminex immunoassay; LIA: Luminex immunoassay; VLP ELISA: virus-like particle enzyme-linked immunoassay; SEBI ELISA: single epitope-based inhibition ELISA enzyme-linked immunoassay.

POST-VACCINATION ANTIBODIES

HPV L1-VLPs immunized individuals had an anti-VLP antibody response significantly greater than that identified in natural infections (Stanley M. 2006). Current HPV vaccines are able to induce a robust humoral immune response that is represented by up to 100-fold higher antibody levels than those observed in naturally infected woman (Harper DM et al. 2006, Mao et al. 2006, Villa et al. 2006). The humoral immunity is comprised of neutralizing and non-neutralizing antibodies with main immunoglobulin G of three isotypes IgG1-3 from which there is predominant IgG1 response (Harro CD. et al. 2001). Immunodominant neutralizing antibodies are type-specific against epitopes exposed on the surface loops of L1 proteins of the papillomaviruses.

The presence of IgG antibodies induced by immunization was observed in oral and cervicovaginal secretions which most likely resulted from their transudation from the serum to the mucosa (Weinberg et al. 2012, Rowhani-Rahbar et al. 2012, Fife et al. 2004, Einstein et al. 2009, Einstein et al. 2011). Although HPV immunization also induced the local production of oligomeric secretory immunoglobulin A, the predominant immunoglobulin in female genital mucosal secretions and oral fluids was monomeric IgG. Antibodies in secretions were detected at least in 70% of individuals one month after vaccination and the mucosal and serum IgG antibody concentration were highly correlated. It can be assumed that serum IgG

transudes across mucosa bind virus particles and prevents infection. Furthermore, epithelial microtrauma should increase the penetration of systemic IgG into the site of damage by additional exudation of serum antibodies. Anti-HPV IgG levels in cervicovaginal secretions were relatively constant throughout contraceptive pill use. In contrast, their concentrations were varied during the menstrual cycle, i.e. the highest concentrations were observed during the proliferative phase and the lowest during the ovulation (Nardelli-Haefliger et al. 2003). Although IgG detection in secretions declined with extending time, there was no wane of post-vaccination protection (Barr and Koutsky 2004, Einstein et al. 2011).

The maturation of affine antibodies induced by immunization was characterized by their avidity growth. This increase in avidity occurred during the time when the antibody titers were decreasing, i.e. within the first 6 months after complete vaccination (Dauner et al. 2010, Kemp et al. 2012). This suggests that the quality of the response might increase while the quantity contracted. Finally, antibody avidity remained almost unchanged up to 40 months after the HPV immunization (Petráš et al. 2012, Kemp et al. 2012). Index avidity was higher after the immunization with quadrivalent than bivalent vaccine (Petráš et al. 2012) and after the bivalent vaccine immunization of seronegative than seropositive women (Kemp et al. 2012).

The available evidence is that the neutralizing antibodies generated by vaccines exhibit a restricted cross-neutralization of highly homologous genotypes (Giroglou et al. 2001). Virus-like particle proteins of vaccine and non-vaccine HPV types such as HPV6/11 (Orozco et al. 2005), HPV31/33, HPV18/45 (Giroglou et al. 2001, Smith et al. 2007) or HPV16/31 (Fleury et al. 2006) share one or more non type-specific neutralization epitopes.

Neutralizing antibodies generated by linear epitopes within L1 protein show less than 1% of the cross-neutralizing activity against the dominant conformational epitopes of other HPV types (Combita et al. 2002). Antibodies induced by bivalent vaccine were able to cross-reactively bind with HPV-31, HPV-33, HPV-35 and HPV-45, but its amounts were considerably low, i.e., <1% of related vaccine type-specific titers (Draper et al. 2011). Interestingly, the cross-neutralizing antibodies against HPV-31 and -45 were not detected before the complete 3-dose immunization with bivalent vaccine (Kemp et al. 2012). Earlier, i.e. within few months after vaccination, there was neutralization detected most frequently for HPV-31, -33, -45 in 42% to 87% individuals (Draper et al. 2011, Einstein et al. 2011 A). Cross-reactive properties of neutralizing antibodies persisted for at least 3 years (Kemp et al. 2012) but the percentage of cross-seropositive individuals most likely declines much earlier and more substantially (Einstein et al. 2011 A).

ANTIBODY IMMUNE RESPONSE TO HPV VACCINES

Only one dose of HPV vaccine induces a relatively low post-vaccination response specific to representatives neutralizing epitopes of vaccine genotypes, i.e., monoepitope or single-epitope antibodies against H6.M48 (HPV6), K11.B2 (HPV11), H16.V5 (HPV16) and H18.J4 (HPV18), measured exclusively by competitive immunoassays (GlaxoSmithKline Biologicals 2012, Brown et al. 2011). About 60-90% of adolescent females and young women had a monoepitope antibody response against HPV-16 and HPV-6, -11, -16 after one dose of bivalent and quadrivalent vaccine immunization, respectively, while only 40%

individuals against HPV-18, regardless of the used vaccine. The second dose of quadrivalent vaccine administered in a 2-month interval increased seroconversion to 95-100% for all vaccine HPV genotypes. However, slightly lower seroconversion rates of 78% and 51% to the HPV-16 and -18, respectively, were reached by the second dose of the bivalent vaccine, most likely the result of the one-month interval between doses. Partial seroconversion insufficiency was compensated with the last third dose of both vaccines that increased by at least 50-fold geometric mean antibody concentrations (Villa et al. 2006).

Both vaccines were able to elicit a similar amount of neutralizing antibodies measured with PsV assays in 93-100% young women against HPV-16 and -18 after the administration of two doses (Einstein et al. 2009). Despite the high levels of these antibodies in all fully-immunised women regardless of the used vaccine, there were significantly higher titers measured in bivalent vaccines than quadrivalent ones. This discordance might arise from the different conformity between L1-VLPs of vaccine and assay.

One month after complete HPV vaccination, there was 100- to 500-fold growth of total IgG antibodies specific to vaccine genotypes related to assays cut-offs (Brown et al. 2011, Harper et al. 2006).

Total genotype-specific IgG antibodies concentrations rapidly decreased up to 6-fold over the first year after the administration of the third dose of bivalent or quadrivalent vaccine. The early waning of antibodies is most likely accompanied by their positive selection, i.e., antibody maturation.

Post-vaccination immune response is dependent on the interval between the first and second dose of HPV vaccine (Neuzil et al. 2011). If the standard 2-month interval was extended to 6 or 12 months, the levels of neutralizing monoepitope antibodies for all 4 HPV types increased 1.4 to 1.8-fold or 2.4 to 3.4-fold, respectively.

Just this extended interval could play a role in replacement of 3-dose regimen with a reduced 2-dose one. Two doses of quadrivalent vaccine at 0 and 6 months elicited antibody concentrations that were equivalent to 3 doses at 0, 2 and 6 months in girls 9-13 years of age, i.e., response ratios between both regimens ranged from 0.7 to 1.17 for all HPV genotypes (Dobson et al. 2010). Although the mean concentration of neutralizing anti-HPV-18 were approximately 1.4-fold lower in young girls immunized with 2 doses than 3 doses, the levels of these antibodies for both regimens were in young girls 2- to 2.5-fold higher than those for standard 3-dose vaccination of women aged 16-26 years (Krajden et al. 2011). Likewise, 2 doses of bivalent vaccine administered at 0 and 6 months to girls aged 9-14 years elicited 1.1- and 1.3-fold higher response of the total IgG antibodies against HPV-16 and -18, respectively, than those elicited by the recommended 3-dose schedule in young women aged 15-25 years (Romanowski et al. 2011). The double antigen content formulation of bivalent vaccine given in two doses of over a 6-month dosing interval induced similar antibody concentrations in girls 9–14 years of age to the recommended 3-dose schedule and HPV-16 and -18 antibody responses were superior to those induced by the licensed 3-dose schedule in young women 15–25 years of age.

Alternative 3-dose schedules compared with a standard one do not result in inferior antibody concentrations if all three doses are administered within 12 months, i.e., at 0, 3, and 9 months or 0, 6, and 12 months or 0, 1, and 12 months or 0, 2, and 12 months (Brown et al. 2012, Esposito et al. 2011, Neuzil et al. 2011, Zimmerman et al. 2010). Immunization with a dosing schedule at 0, 12, and 24 months surprisingly elicited lowered antibody response to HPV-16 and -6 (Neuzil et al. 2011).

The higher antigen content of the HPV vaccines does not fundamentally induce higher antibody levels. The double or triple antigen content formulation of bivalent vaccine compared with the licensed vaccine marginally increased the immunogenicity by 1.1 to 1.2-fold for both HPV types (EMA. Cervarix 2007, Romanowski et al. 2011). Interim analysis of three formulations of quadrivalent vaccine with 40 mcg or 80 mcg of HPV-6, -11 and -18 L1-VLPs did not result in a significant difference of immunogenicity (Mao et al. 2006, Villa et al. 2006).

The immunogenicity of multivalent HPV vaccines is no worse than that induced by monovalent vaccines, i.e., different HPV L1-VLPs combined in one dose do not act antagonistically to each other. Peak anti-HPV-16 concentrations after immunization with monovalent HPV-16 vaccine and quadrivalent vaccine were approximately 40- and 100-fold higher, respectively, than those observed in women following natural HPV-16 infection (Villa et al. 2006).

The immune response in both 10- to 15-years old girls and boys was similar but higher than those that were observed in women aged 16-23 years. More than 99% of boys aged 10-15 years seroconverted to all 4 HPV types and had 1.8- to 2.7-fold higher geometric mean concentrations of neutralizing antibodies compared to young women (Block et al. 2006). In more than 92% of 9- to 15-year-old adolescents, regardless of gender, the quadrivalent vaccine induced anti-HPV serologic responses persistent for at least 12 months following complete immunization (Reisinger et al. 2007). The antibody levels in the boys aged 10 to 14 years fully immunized by the bivalent vaccine were higher than those reported in girls of the same age (Petäjä et al. 2009). Compared with women aged 15 to 25 years, the bivalent vaccine elicited 2.5- to 3.1-fold higher concentrations of total IgG specific to HPV-16 and -18 in boys and adolescent males 10-18 years of age. The majority, 97.4% of adolescent males and young men 16-26 years of age had seroconversion for all vaccine HPV types one month after the administration of the third dose of quadrivalent vaccine. Only 0.9% of them did not undergo seroconversion to any of the four HPV types (Giuliano et al. 2011). Black men had significantly higher neutralizing antibody concentrations than did both Caucasian and Asian men after complete quadrivalent vaccine immunization (Hillman et al. 2012).

Although the seroconversion rates for each of the four vaccine HPV types are not affected by the age of vaccinated, the magnitude of the post-vaccination antibody response inversely correlated with age and the most robust was achieved in those younger than 12 years. One month after the 3-dose vaccination, the type-specific immune responses to the quadrivalent vaccine were approximately 2-fold stronger in preadolescent girls and boys than in 16- to 23-year-old females (Block et al. 2006). Much lower levels of neutralizing antibodies specific to HPV-16 and -18 were induced by both vaccines in women aged 27-45 years than younger women (Einstein et al. 2011). All women younger than 56 years responded to complete immunization with bivalent vaccine. The total IgG antibodies specific to HPV-16 and -18 were approximately 3-fold and 2-fold lower in women 46-55 years and 26-45 years of age, respectively, than those in women aged 15-25 years (Schwarz et al. 2009). Almost all fully immunized women aged 25-45 years seroconverted for all 4 vaccine HPV types. If they were stratified by age, then there was an expected trend towards slightly reduced immunogenicity in the older cohorts of women (Li et al. 2012, Muñoz et al. 2009). Women 35-45 years of age compared to younger than 26 years had about 0.7- to 0.9-fold lower levels of monoepitope neutralizing antibodies one month after vaccination (Muñoz et al. 2009).

HPV vaccination of seropositive individuals who had been previously infected with the relevant vaccine HPV type mounts an antibody response compared to seronegative ones. Especially the first dose of quadrivalent vaccine induced in seropositive individuals was a much stronger response than those in seronegative because it acted as a challenge dose eliciting a classic anamnestic response (Villa et al. 2006). However, the quality of post-vaccination response in seropositive individuals could be worse because lower antibody avidity was found (Kemp et al. 2012).

New-born infants, especially prematurely born, have an immature immune system that is not fully capable of actively protecting against HPV infection. Therefore, future mothers previously immunized have a chance to protect their newborns against HPV infections such as recurrent respiratory papillomatosis or condyloma because the vaccine-induced antibodies undergo transplacental transport during pregnancy. For women who that were immunized with quadrivalent vaccine at least one year before delivery, maternal antibodies were found in the cord blood of their infants.

The concentrations of total IgG and neutralizing antibodies for all vaccine HPV types in blood cord were highly correlated with those in maternal serum (Matys et al. 2012).

Co-administration of vaccines against human papillomavirus infections does not alter immunogenicity of inactivated or subunit vaccines. The immunogenicity of the HPV vaccines is unaffected by concomitant immunization with other inactivated vaccines. Simultaneous immunization with HPV vaccines and hepatitis A and/or hepatitis B vaccines induced seroconversion rates and antibody levels in women 9-25 years of age are equivalent to those induced by single vaccines (Wheeler et al. 2008, Pedersen et al. 2012, Leroux-Roels et al. 2011, Schmeink et al. 2011). At least 99% of preadolescent and adolescent girls seroconverted for all vaccine HPV types when the bivalent or quadrivalent HPV vaccines were given concomitantly with one dose of quadrivalent meningococcal conjugate vaccine and/or one dose of tetanus toxoid, reduced diphtheria toxoid, acellular pertussis vaccine and eventually poliomyelitis vaccine (Wheeler et al. 2011, Arguedas et al. 2010, Reisinger et al. 2010, Vesikari et al. 2010).

IMMUNOGENICITY IN IMMUNOCOMPROMISED INDIVIDUALS

The immunologic response to HPV vaccine is dependent on the quality of the immune system function. If it is impaired by immunosuppressive therapy or even reduced by immunodeficiency resulting from disease, vaccination can induce a much weaker humoral or cell-mediated immune response compared to that of healthy individuals.

The standard immunization regimen with quadrivalent vaccine seroconverted to all vaccine HPV types 97-100% HIV positive children aged 7-12 years that had a CD4 T-lymphocytes nadir equal or higher than 15%, irrespective of previous highly active antiretroviral therapy before vaccination (Levin et al. 2010, Weinberg et al. 2012). There were significant correlations of higher HPV type-specific antibody level with lower entry HIV viral load and lower CD8 T-lymphocyte counts (Levin et al. 2010). The CD4 counts and HIV viral loads were not altered by quadrivalent vaccine immunization. The anti-HPV-6 and -18 levels achieved by vaccination of HIV-positive individuals were 30-50% lower than those achieved in healthy children of the same age (Levin et al. 2010). Type-specific monoepitope

neutralizing antibody levels rapidly declined 2.2- to 6.3-fold for all vaccine genotypes over the first year after the complete immunization and 94-99% HIV-infected children remained seropositive for HPV-6, -11 and -16 while only 76% for HPV-18 (Weinberg et al. 2012). A booster dose administered one year later increased antibody concentrations and provided 96-100% seropositivity for all vaccine genotypes. Because the immune response to the fourth vaccine dose was significantly stronger than that to the first dose, it should be considered as an anamnestic response. Mucosal antibodies against HPV-16 and -18 were detected in the oral fluid of 69% and 35% HIV positive children, respectively, in contrast to 100% immunocompetent vaccines (Weinberg et al. 2012, Rowhani-Rahbar et al. 2012). Cytotoxic T lymphocyte response for HPV-16 was developed in 60% HIV-infected vaccines and 72% immunized with a booster dose.

Patients with systemic lupus erythematosus (SLE) are at higher risk of cervical HPV infections which are prevalent 1.5- to 4.3-fold more frequently in these patients than healthy women (Tam et al. 2004). The seroconversion rates in patients with SLE, irrespective of immunosuppressive therapy before vaccination, were lower for all vaccine HPV types compared to healthy subjects but they exceeded 74% and 76% at 1 and 6 months, respectively, after complete immunization with quadrivalent HPV vaccine (Mok et al. 2012). Lowered immune response was observed in patients in particular receiving mycophenolate mofetil combined with low-dose prednisolone. Therefore, the optimal timing vaccination for patients with SLE should not be during the stage of active disease that requires high doses of immunosuppressive medications. In the following 12 months after HPV immunization, there was no increase in SLE disease activity or flares and no alterations of anti-dsDNA titers and complement, including anti-C1q.

WHIM-syndrome (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis syndrome) is a rare congenital immunodeficiency disorder characterized by chronic noncyclic neutropenia with abnormal susceptibility to bacterial and viral infections, especially human papillomaviruses, resulting in warts. Therefore, these patients may crucially benefit from HPV vaccination. Neutralizing antibodies for all vaccine HPV types were induced by the quadrivalent vaccine immunization of a 12-year-old girl with WHIM syndrome accompanied by T- and B-cell lymphopenia, suffering from recurrent bacterial infections (Handisurya et al. 2010). In contrast to healthy girls, the fully-immunized WHIM patient had poorer but detectable humoral response for all 4 HPV types and comparable delayed lymphoproliferative response.

PERSISTENCE OF HUMORAL IMMUNITY

Monoepitope neutralizing antibodies wane faster than total IgG specific to HPV vaccine genotypes. More than 90% immunized with bivalent or quadrivalent vaccine had monoepitope or single-epitope neutralizing antibodies against HPV-16 or HPV-6, -11, -16, respectively, in sufficient levels through 48 months, while only about half of the vaccinees retained these antibodies against HPV-18 (GlaxoSmithKline Biologicals. 2012, Brown et al. 2011, Joura et al. 2008, Villa et al. 2007). Moreover, the monoepitope antibodies specific to HPV-6, -11, and -16 persisted in 3.2- to 18.4-fold higher levels than minimal those of naturally positive women. In contrast, anti-HPV-18.J4 antibody concentrations approached

naturally induced levels throughout 48 months after initiation of bivalent or quadrivalent vaccination. Why monoepitope HPV-18.J4 antibody levels further declined in relation to peak levels contrasted with those for the other genotypes may be explained by the non-identical immune response in all vaccines and the non-type specificity of the most immunodominant epitopes on a VLP (Brown et al. 2011). Furthermore, it was found that the sera of subjects vaccinated with quadrivalent vaccine before 48 months exhibited neutralization of HPV-18 although they might not be detectable for monoepitope antibody against HPV-18.J4 (Roberts et al. 2012).

Compared to monoepitope antibodies, total IgG antibody specific to vaccine HPV types persisted in nearly all women through 48 months in much higher levels than serological cut-offs, i.e. 29- to 51-fold and 9- to 56-fold higher in immunized with bivalent and quadrivalent vaccine, respectively (Harper et al. 2006, David et al. 2009, Brown et al. 2011).

A few years after immunization, there were less than 1% vaccine-induced antibodies that maintained the capability to neutralize other human papillomaviruses but genetically highly related to vaccine types, such as HPV-31, -45, and -58 (Kemp et al. 2012). Nevertheless, the percentage of women seropositive against HPV-31 and -45, immunized with bivalent vaccine declined on about one third and one tenth, respectively, during the 2 years after vaccination (Einstein et al. 2011).

Further maturation of antibodies characterized for early post-vaccination period was later not observed because the avidity of IgG was unaltered through the 3-4 years (Petráš et al. 2012, Kemp et al. 2012).

The persistence of humoral immunity is not dependent on age of the vaccinated. Women up to 55 years of age, as well as young women and adolescent females, maintained the seropositivity for all vaccine HPV types for 4 years (Castellsagué et al. 2011, Schwarz et al. 2011).

Besides antibodies, an immune memory is generated by HPV vaccination. The proportion of women aged 18-45 years with detectable circulating memory B-cells was 67-83% and 53-76% for HPV-16 and -18, respectively, at 24 months following immunization with bivalent and quadrivalent vaccine (Einstein et al. 2011).

The development of immune memory was also confirmed by HPV re-exposure simulated with one dose of quadrivalent vaccine administered 5 years after vaccination (Olsson et al. 2007). This challenge rapidly mounted concentrations of monoepitope neutralizing antibodies for all vaccine HPV types in the great majority of women, i.e., 1 week after challenge, they reached levels comparable with that one month following primary immunization. These levels further increased to 1.3- to 4.2-fold one month following challenge. Similarly, a strong anamnestic response was triggered by bivalent vaccine as a challenge dose administered 7 years following primary immunization (Moscicki et al. 2012). Compared to pre-challenge, the geometric mean of total IgG antibody concentrations for HPV-16 and -18 were increased respectively 9.3- and 8.7-fold at 1 week and 22.7- and 17.2-fold at 1 month after challenge.

Up to 9.6 years, 90% of women immunized with monovalent HPV-16 vaccine maintained seropositivity (Rowhani-Rahbar et al. 2012). Application of one dose of quadrivalent vaccine quickly restored and amplified primary immune response in all women and the level of monoepitope neutralizing antibodies against HPV-16 was 68.3-fold higher than that pre-dose.

Table 2. Seropositivity determined by cut-offs of total IgG (Harper et al. 2006, Brown et al. 2011)

months	qHPV				bHPV	
	HPV6	HPV11	HPV16	HPV18	HPV16	HPV18
7	99.7	99.7	99.7	99.7	100	100
24	100	100	99.7	97.2	99	99
48	100	100	100	96.7	100	100

Table 3. Seropositivity determined by cut-offs of monoepitope neutralizing IgG (GlaxoSmithKline Biologicals. 2012, Brown et al. 2011)

months	qHPV				bHPV	
	HPV6	HPV11	HPV16	HPV18	HPV16	HPV18
7	99.7	99.5	99.7	99.2	100	99.5
24	95.5	99.0	99.7	75.9	97.6	61.8
48	90.2	95.5	98.5	64.8	92.3	45.8

Long-term immunogenicity of bivalent vaccine was documented up to 8.4 years after initial vaccination. Total IgG antibodies for both HPV-16 and -18 were at least 10-fold higher than antibody levels of the natural infected (Roteli-Martins et al. 2012).

Post-vaccination and post-infection humoral immunity maybe persists as well. Naturally induced antibodies were generally stable over several years of follow-up (af Geijersstam et al. 1998), and at least 25% women remained seropositive over 10 years after the last detection of HPV DNA (Stanley et al. 2006).

Mathematical modeling of antibody decay suggests that 50% of vaccinees could have detectable monoepitope neutralizing antibodies against HPV-16.V5 for 32 years following primary immunization (Fraser et al. 2007). Even total IgG type-specific antibodies elicited by bivalent vaccine might persist above levels associated with natural infection for at least 20 years (David et al. 2009).

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Chapter 8

HUMAN PAPILLOMAVIRUS INFECTION IN SYSTEMIC RHEUMATIC DISORDERS: CURRENT CONCEPTS, CHALLENGES AND EXPECTATIONS

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ABSTRACT

There is strong evidence that women with systemic rheumatic disorders, particularly systemic lupus erythematosus (SLE) have an increased risk for developing cervical cancer. Several epidemiological studies have shown causal relationship between human papilloma virus (HPV) infection and cervical neoplasia in general population, nevertheless; information about the impact of HPV infection in women with rheumatic

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disorders treated with immunosuppressive drugs or biologic therapy is still evolving. HPV infection is more frequently observed in some rheumatic systemic diseases mainly in SLE with relevant data available. Prevalence figures in SLE for HPV infection vary from 11.8% to 54%. Recently, our group reported in Mexican women with SLE, HPV infection is 14.7%, a figure that is important for Latin America where cervical cancer is highly prevalent. Longitudinal designs have been rarely performed; one study reported an increase in frequency of HPV infection from 12.5% to 25% after 3 years of follow-up. In SLE there is a high frequency of infection with several type of virus (around 17%), most of them considered as high-risk virus for cervical neoplasia. Data in African American women with SLE show that around 3% have high-grade squamous intraepithelial lesions (HSIL) and 1.2% cervical cancer. Epidemiological studies of HPV infection in other rheumatic diseases are infrequent; our group reported HPV infection in 31% of the rheumatoid arthritis (RA) patients, whereas others reported 3% in Sjögren syndrome. Although common risk factors such as age, occupation, lifetime sexual partners, other sexually transmitted co-infections, or early age at first intercourse, are related with HPV; in rheumatic disorders the association between HPV infection with utilization of immunosuppressive drugs or with longer duration of treatment with corticosteroids but with no disease activity is relevant. Some immune abnormalities induced by the treatment have been associated with an increased frequency of HPV, where lower levels of B lymphocytes and NK cells in peripheral blood are observed in SLE under treatment with immunosuppressive drugs. TNF-α is involved in signaling apoptosis in infected cells, participating in the inhibition of viral replication, therefore TNF inhibitors may theoretically increase the risk for persistent infection but data supporting this hypothesis are still insufficient. HPV immunization with recombinant vaccine is useful to prevent precancerous cervical lesions, although; some autoimmune adverse events have been developed after the vaccine application including Guillain-Barré, transverse myelitis, optic neuritis, multiple sclerosis or myasthenia gravis, and there are some rare case-reports of RA, SLE, mixed connective tissue disease, Sjogren syndrome, dermatomyositis and scleroderma developed after the vaccination. On counterpart, vaccination is safe in patients with inactive RA or SLE. No significant differences in seroconversion rates have been observed between users and non users of immunosuppressive drugs except for mycophenolate mofetil. In summary, HPV infection in rheumatic disorders is an exciting area for research and a future task is to design a clinical guide for preventive measures in these patients, as well as a strategy for follow-up and treatment in those patients who have HPV infection; particularly for those who receive immunosuppressive therapy or anti-TNF agents.

Systemic autoimmune rheumatic diseases constitute a complex group of disorders that have in common a dysfunction in the immune system leading to abnormalities in the immune response with inflammation and organ damage mediated by cells, auto-antibodies and pro-inflammatory cytokines. Systemic rheumatic diseases are associated with high morbidity and mortality. A multiplicity of diseases are included in this group, the most representative of these are systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, autoimmune inflammatory myopathies (such as polymyositis and dermatomyositis), Sjögren syndrome, and systemic vasculitis (such as poliarteritis nodosa, Wegener granulomatosis, temporal arteritis, Takayasu disease, etc). Most of these diseases have a recognized decrease in life-expectancy and many of them are associated with the development of neoplasia, including in some cases, cervical cancer. There is new information regarding the human Papillomavirus

infection in these patients that shares some similarities with observations in the general population, but also there are some differences that we will discuss in this chapter.

RELATIONSHIP BETWEEN PAPILLOMAVIRUS INFECTION AND CERVICAL NEOPLASIA IN GENERAL POPULATION

Cervical cancer constitutes one of the most prevalent types of cancer in women worldwide. [1] Squamous cell carcinoma, and in the last two decades cervical adenocarcinoma, have constituted the main types of cervical cancer. [2] Human papilloma virus (HPV) specially types 16 and 18 are considered the most identified etiologic agent implicated in cervical cancer [3, 4]. Although there is a multiplicity of HPV types most cases of cervical cancer are related to HPV 16, 18 and 45 types [5]. In the general population most often the HPV infections are usually transient [6]. These viruses are detected in most of the cases intermittently; with certain frequency there is a detection of different HPV types when the samples are taken in the same population sequentially [6]. In the general population factors associated with an increase for HPV infection are early sexual activity, multiple sexual partners, parity, genital warts, sexually transmitted disease, genital tract abnormalities, age, smoking, passive smoke, poor nutrition, immunodeficiency and malnutrition, where condoms may not prevent this infection [7]. Factors associated with development of cervical cancer in individuals infected by HPV are: smoke, co-infection with other sexually transmitted infections (example herpes simplex virus (HSV) and C. trachomatis infection), and persistence of HPV infection [8].

PREVALENCE, INCIDENCE AND RISK OF CERVICAL DYSPLASIA AND CERVICAL CANCER IN SYSTEMIC RHEUMATIC DISORDERS

Frequent cytology screening is recommended for patients with rheumatic disorders because some of these diseases are related with an increase in the risk for cervical neoplasia. Some guidelines for cervical cancer screening should be adapted for patients with systemic rheumatic disorders. For instance the American College of Obstetricians and Gynecologists recommends yearly cervical cancer screening in immunosuppressed persons, regardless of age [9], and emphasizes close surveillance with Pap testing in all women exposed to immunosuppressive therapies in special cyclophosphamide. [10].

Most of the information about the increase in risk for cervical neoplasia is derived from studies performed in women with systemic lupus erythematosus (SLE). Cibere et al. evaluated 297 patients with SLE in a cohort performed in Canada with a mean follow-up of 12 years; they observed an above-average number of cases of cancer of the cervix in comparison to what is expected for the general population [11]. Abdull Gaffar et al. reported a 25.4% of squamous epithelial abnormalities in women with SLE compared to 3.5% of women without SLE with similar age and demographic background [12]. Dhar et al. reported that 2.8% of women with SLE had high-grade squamous intraepithelial lesions (HGSIL) in cervical cytologies and cervical cancer in 1.2% [13]. Our group reported that around 5.9% of Mexican patients with SLE have HGSIL in cervical cytology [14]. In a cohort study designed

with the aim to identify the incidence of cervical intraepithelial neoplasia (CIN) a group of patients with SLE and normal cytology at baseline was followed by a mean of 3 years, patients with SLE had an overall 3-year incidence of CIN of 9.8% [15] being this increases the risk associated with the use of cyclophosphamide. Table 1 shows the prevalence of squamous intraepithelial lesions and other abnormalities in the Pap in patients with SLE. The prevalence of HGSIL goes from 3.5 to 11%. Table 2 shows the risk factors for squamous intraepithelial lesions in SLE. These factors are mostly related with HPV infection and include high-risk HPV infection, multiple types of HPV, and persistence of HPV infection.

Hemminki K et al., analyzed in a Swedish national dataset the standardized incidence ratios (SIRs) for incident cancers in 200,000 female patients diagnosed with any of 33 different autoimmune diseases observing that for cervical cancer, the risk was increased in comparison with the general population in patients with discoid lupus erythematosus and systemic sclerosis [16].

Table 1. Frequency of squamous intraepithelial lesion (SIL) and other abnormalities in the Pap observed in patients with systemic lupus eryrtematosus (SLE)

Author, year (reference)	Country	Number of patients	Prevalence cervical cancer
Nyberg G, 1981 [56]	Sweden	80	19 / 80 (24%) atypical cervical cytology
Blumenfeld Z, 1994 [8]	Israel	39	14 /39 CIN (35.9%)
Dhar JP, 2001 [59]	USA	29	7 cases (1 HGSIL and 6 LGSIL)
Ognenovski VM, 2004 [15]	USA	61	9.8% CIN
Tam LS, 2004 [60]	China	85	11% SIL, 8.2% LGSIL, 3.5% HGSIL
Dhar JP, 2005 [13]	USA	321	13 cases HGSIL
Nath R, 2007 [24]	UK	30	8/30 (27%) SIL
Febronio MV, 2007 [61]	Brazil	52 (juvenile SLE)	25/52 Inflammatory cervicovaginal cytology, 1 case LGSIL
Mercado U, 2009 [62]	Mexico	62	13 cases SIL (8 LGSIL and 5 HGSIL)
Tam LS, 2011 [63]	China	137	12 cases of SIL (8.8%)
Rojo-Contreras, 2012 [14]	Mexico	34	5.9% HGSIL, one cervical cancer

Abbreviations: SIL: squamous intraepithelial lesion, LGSIL: low-grade squamous intraepithelial lesion; HGSIL: high-grade squamous intraepithelial lesion; CIN: cervical intraepithelial neoplasia.

Table 2. Risk factors associated with squamous intraepithelial lesion (SIL) in patients with systemic lupus erythematosus (LES)

Risk Factors:
- Low education level
- > 3 sexual partners
- History of sexually transmitted diseases (including other than HPV)
- HPV infections
- Multiple types of HPV
- High risk HPV infections
- Persistent HPV infections
- Previous treatment of cervical lesions
- Use of azathioprine
- Use of cyclophosphamide

Abbreviations: HPV = Human Papillomavirus.

Although SLE is the disease most studied for the relationship with cervical cancer, in systemic sclerosis only a few studies evaluate the association with cervical cancer. Bernatsky et al., analyzed the frequency of abnormal Pap tests in 320 women with systemic sclerosis (SSc) observing that according to self-report the prevalence of an abnormal Pap test was 25.4% significantly higher that the reported by general population 13.8% [17]. Nevertheless, et al. in the Scleroderma Registry, evaluating 538 patients (436 of them females) only 3 cases of cervical cancer were observed and no significant differences were found as compared with the rates in the local general population [18]. Patients with rheumatoid arthritis (RA) have been observed to have an increase in the frequency of abnormal Pap smear testing in comparison with controls (9.3% vs. 1.7% respectively) [19]. In patients with inflammatory myopathies there are some cohort studies identifying an excess of risk for cervical cancer. Chen et al., in a nationwide cohort study of 1,012 patients with dermatomyositis (DM) and 643 patients with polymyositis (PM), found an increase in the number of cervical cancer cases observed in comparison to controls in DM (SIR 3.28, 95%CI 2.93 to 3.68) [20].

PREVALENCE OF HUMAN PAPILLOMAVIRUS (HPV) INFECTION IN SYSTEMIC RHEUMATIC DISORDERS

Prevalence of HPV infection in systemic rheumatic diseases is variable depending on the specific type of disease, population studied, demographic characteristics of women included, and known factors for HPV infection presented in these patients.

Only in SLE have different countries shown a wide variability of prevalence of HPV and the HPV types found. Table 3 shows the prevalence of HPV infection in SLE observed in different studies.

In Latin America, the information reported about the prevalence is dependent on the region or country. Our group found HPV infection prevalence in only 14.7% of the Mexican women evaluated with SLE [14] whereas, Klumb et al., reported that Brazilian women with SLE had HPV in 20.2% of their patients [21]. In Asian patients with SLE the prevalence is variable. Lee, in Korean women, identified a prevalence of 24.6% of HPV infection in their patients with SLE [22]. Instead, Tam et al. reported a prevalence of 12.5% in Chinese women with SLE [23]. Nath et al., reported a prevalence of 54% of HPV infection in their patients with SLE in the United Kingdom [24].

Table 3. Prevalence HPV infection in patients with systemic lupus erytematosus (SLE)

Author, year (reference)	Country	Number of patients	Prevalence HPV infection
Tham LS, 2004 [60]	Hong –Kong (China)	85	11.8%
Nath R, 2007 [24]	United Kingdom	30	54%
Klumb EM, 2010 [21]	Brazil	173	20%
Tham LS, 2010 [23]	China	144	25%
Lee Y , 2010 [22]	Korea	134	24.6%
Rojo-Contreras, 2012 [14]	Mexico	34	14.7%

Abbreviation: HPV: Human Papillomavirus infection.

As observed in Table 3, there are different prevalence levels of HPV infection across countries. These studies show a wide variability in the prevalence of HPV infection; therefore, the risk of an infection in these patients should be considered as highly influenced by the country where these individuals originate.

RISK FACTORS ASSOCIATED WITH HPV INFECTION IN SYSTEMIC RHEUMATIC DISORDERS

There is a multiplicity of factors that can be associated with HPV infection in systemic rheumatic diseases, most of the information proceeds from studies performed in SLE, where there is an extensive list of factors related with the infection. These factors can be classified into three groups: a) general factors that can be observed in the general population (such as, number of sexual partners, age at time of first sexual intercourse or low level of education), b) factors associated with disease characteristics (such as, disease activity, organs involved) and c) factors associated with the treatments (such as use of cyclophosphamide or azathioprine). Table 4 summarizes the factors associated with HPV infection in different studies.

Table 4. Risk Factors associated with HPV infection in Systemic Lupus Erythematosus

Characteristics associated with HPV infection in SLE
General Factors
Multiple sexual partners [22]
Abnormal smears [22]
Sexually transmitted disease [21]
Disease related factors
Major organ involvement [22]
Associated to immunosuppressive therapy
Use of immunosuppressive drugs [21]
Methotrexate utilization [14]
Use azathioprine [14]

INCIDENCE OF HPV INFECTION IN RHEUMATIC DISORDERS

There are few studies evaluating the incidence of HPV infection in rheumatic diseases. All of them have been performed in SLE, therefore some of their results are extrapolated to other diseases until new information can be generated.

Tam et al., [23] performed a prospective cohort evaluating the presence of HPV DNA infection by polymerase chain reaction (PCR) every 6 months for up to 3 years in 144 women with SLE. These authors observed an increase in the cumulative prevalence of HPV infection from 12.5% at the baseline to 25% after 3 years of follow-up [23]. These authors identified an incidence of HPV infection in their cohort of 17 per 1,000 patient-months [23]. They also observed that the prevalence of high-risk HPV infection increases from 11.1% at baseline to 20.8% after 3 years, and increases in prevalence of multiple virus infection from 6.9% at baseline to 16.7% after 3 years [23]. These data indicate that an increased risk exists for new

HPV infection in patients with SLE and these patients must be followed, searching for the development of high-risk infection. Virus persistence: Tam et al., also investigated the time to clear the HPV infection in SLE; these authors observed that from preexistent HPV infection 68.8% were cleared at 12 months and 84.4% were cleared at 24 months [23].

Nevertheless, these authors also reported that for new infections acquired during the follow-up only 13.2% were cleared [23]. Overall, it is considered that patients with SLE may have a predisposition for persistent HPV infection, being this persistence associated with the high frequency of squamous intraepithelial lesions observed in these patients.

Potential factors associated with persistent infection in SLE include a number of lifetime sexual partners ≥ 3, multiple virus types, and infection with high-risk HPV types [23].

Relationship between Immunosuppressive Status and HPV Infection

Immunosuppressed patients, including patients with systemic rheumatic disorders or HIV positive individuals, have a high risk of HPV infection. In patients infected by HIV the high HIV RNA levels and lower counts of CD4 (<200 cells/mm^3) are associated with persistent HPV infection as well as incident infection [25].

Among patients with HIV, those women infected with oncogenic types of HPV the presence of low CD4 cell counts are more likely to develop into squamous intraepithelial lesions (SIL) than women or HIV-positive women with high counts of CD4. [25]. In patients with immunosuppression due to other causes, such as patients with systemic rheumatic diseases, there are a number of abnormalities that we will describe briefly.

Immunological Mechanisms of Immune response to HPV Infection

HPV infection produces only a mild stimulation for the activation of immune response. This poor effect on the immune system can be explained by several mechanisms: 1) Virus replication is developed in the basal cells in the outermost epithelia layers, that are located far from the presenting cells and therefore, these cells are limited in their capacity to present the viral antigens to the effectors cells. 2) A second mechanism is the viral proliferation without cytolysis being a poor stimuli for the inflammatory response, 3) a third factor is that nuclear proteins produced in the infected cells are unable to produce immune system activation because they are not released outside the cells [26,27].

HPV has also a number of mechanisms for evading the hosts immune response: 1) Langerhans cells located in the epidermis are not activated by the type 16 of HPV particles (virus that is associated with risk of cervical cancer) and therefore, these cells are relatively unable to take up the viral particles and to present them to immunocompetent cells. 2) Under normal conditions, there is an interaction between Langerhans cells and epithelial cells necessary for the immune response, this interaction can be inhibited by the oncoprotein E6 of HPV, with the subsequent decrease in the cytokines production leading to dendritic cell depletion in the ephitelia infected [28], 3) HPV 16 oncoprotein E7 causes a reduction in class I heavy chain promoter activity of the biodirectional promoter leading to repression of components of the class I antigen of the major histocompatibility complex (MHC), presentation pathway representing a way to escape from immune surveillance. [29]. HPV 16

also modulates the expression of the toll-like receptors [30]. 4) Tumor necrosis alpha (TNF-α) is one of the main mediators of inflammation in the skin and mucosa; this cytokine constitutes a relevant mechanism for response to the epitheliotropic virus including HPV [31].

As TNF-α binds to the TNF receptor superfamily this process leads to signaling pathways for blocking viral proliferation [32]. This cytokine is also an activator of Langerhans cells and is expressed constitutively by basal keratinocytes in the normal cervix; nevertheless, some authors have observed that TNF-α expression is absent in samples of patients with cervical intraepithelial neoplasia (CIN) [33].

All these factors cause immunological tolerance for HPV infection, and the clearance of infection may take several months even in patients with normal immune response, therefore, in immunocompromised patients, such as individuals with systemic rheumatic disorders or those taking immunosuppressive drugs, there may be an increased disability of the immune system to clear this virus.

With certain frequency a number of immunocompetent individuals experience persistent HPV infection (lasting several years) with viral replication within the cervical epithelia but without viremia. [27].

Cell-mediated immune responses participate in the clearance of HPV in these individuals. Among these cells, particularly cytotoxic CD8+ T-cells, the reaction against HPV proteins associated to Class I of the MHC molecules and the transporter associated with antigen processing subunit 1 (TAP 1) leads to eliminate the infected cells [29]. An impairment in the T cell responses directed against cells expressing early HPV16 protein in those patients with premalignant or malignant lesions has also been observed [35]. Additionally, HPV induces an overexpression of IL-10 and an underexpression of IL-12 subunit p40, which contribute to the decreased immune response developed against the virus. [36]. Finally, there is a lack of type-specific neutralizing antibodies formation directed to HPV where only low titers are observed in a subgroup of patients. [37].

Abnormalities in Immunological Mechanisms Observed in Systemic Rheumatic Diseases and Their Relation to HPV Infection

Patients with systemic rheumatic disorders have different abnormalities in immune response. These patients also frequently receive drugs that may affect some of the mechanisms implied in this immune response against HPV infection. Systemic lupus erythematosus (SLE) has deficiencies in immune cell function that affects the virus clearance independently from the use of immunosuppressive drugs that produce suppression of the immune system [21]. Additionally, the use of immunosuppressive drugs increases the risk for persistent HPV infection and the subsequent development of cervical cancer. [21]. Many patients with systemic rheumatic disorders eventually, during the evolution of its disease, receive immunosuppressive therapy including corticosteroids, synthetic drugs and biological therapy. Briefly we review some of the information generated about these agents in relationship to HPV.

Immunosuppressive therapy is a predisposing factor in the occurrence of a gynecological cancer in lupus patients. [15].

CORTICOSTEROIDS AND HPV INFECTION

There is a lack of information about the effects of glucorticoids utilization on HPV infection and cervical cancer. In vitro studies have observed that dexamethosone induces a decrease in the HPV16 E7 oncoprotein and nuclear matrix (NM) protein fractions and possibly down-regulate the growth of cervical cancer cells [38]. Nevertheless, there are no well-designed follow-up studies evaluating the effects of glucocorticoids in the reactivation of latent HPV infection. There are some case reports of reactivation of latent HPV infection in patients treated with topical glucorticoids for lichen sclerosus (39). Patients with systemic rheumatic disorders frequently receive long-term glucocorticoids therapy and there is a wide variability in the doses, depending on disease severity and the organs involved. Therefore, studies about the effects of corticosteroids in patients with HPV infection and systemic rheumatic diseases receiving different schemas are required.

ANTI-MALARIALS

Chloroquine and hydroxychloroquine are two anti-malarials frequently used in systemic rheumatic diseases principally in rheumatoid arthritis and systemic lupus erytematosus. These drugs are also used to treat some cutaneous manifestations in dermatomyositis. In patients infected by HPV one of the mechanisms implied in the virus clearance is the innate immune response.

For the development of an adequate immune response it is necessary to recognize viral nucleic acid and subsequent activation of the nucleic acid-specific Toll-like receptors (TLRs) that trigger the induction of cytokines for the innate immune response. Activation of TLR 7 and TLR9 are implied in the immune response necessary for the virus clearance and a decrease in the activation of these receptors may lead to persistence of the HPV infection. Yu SL et al., observed in a cross-sectional study performed in patients with SLE that a decrease in TLR9 levels correlated with the cumulative dose of hydroxychloroquine as well as with the duration of azathioprine treatment [40]. These data suggest that the use of hydroxychlorquine in patients with rheumatic disease infected by HPV may down-regulate TLR9 levels favoring viral persistence and this information must be taken into account mainly in patients with high-risk types of HPV that are associated with cervical cancer. Therefore, a prospective follow-up study to evaluate if patients receiving anti-malarials have an increased viral persistence is needed.

CYTOTOXIC DRUGS AND IMMUNOMODULATORS DRUGS

Two immunosuppressive drugs are related with the increase in risk for development of gynecological cancer: these are cyclophosphamide and azathioprine [15].

Cytotoxic drugs are capable of inhibiting the synthesis of nucleic acids required for desoxyribonucleic acid (DNA).

Cyclophosphamide

Cyclophosphamide (CYC) is a nitrogen mustard-alkylating agent used frequently in the management of severe systemic lupus erythematosus, especially in patients with major organ involvement such as kidney or lung. Cyclophosphamide is also used in the treatment of necrotizing vasculitis, such as; Wegener granulomatosis, poliarteritis nodosa or other necrotizing vasculitis and is used also in systemic sclerosis for the treatment of interstitial lung disease. One of their metabolites, phosphoramide, is associated with effects on the immune system, but also with hematological toxicity. CYC decreases the immune response in the inflammatory process produced in systemic rheumatic diseases, either by inhibiting the immune cell proliferation or causing cell death. CYC has a potential role in the development of CIN because some of its metabolites are associated with direct mutagenic actions, as well as with potent immunosuppressive effects that decrease the cellular response required to attack tumors. Ognenovski et al., followed prospectively 61 women with SLE in order to identify in cervical smears the incidence of abnormalities in the cytology included cervical intraepithelial neoplasia I-III (CIN I-III) [15]. After 7 years of follow-up, CIN was observed in 15% of the group treated with intravenous cyclophosphamide. Associated with this increase in the incidence of CIN with accumulative doses of this immunosuppressor agent, these authors observed that each increase of 1 g of intravenous CYC corresponded with 13% of increased risk for the development of CIN. [15]. There is a lack of controlled follow-up studies evaluating if CYC can increase the risk of cervical cancer when it is associated with an HPV infection. A case report observed the development of bladder squamous cell carcinoma in a patient receiving CYC over four years, identifying HPV in the bladder [41], but an isolated finding is insufficient to establish conclusions. There is also interest in evaluating if cyclophosphamide utilization could be associated with persistence of HPV infection. Our group in a cross-sectional study did not find differences in the frequency of CYC utilization between patients with HPV infection and not infected in patients with SLE and RA, neither the current doses or cumulative doses were associated with this infection [14]. Tam et al., in a prospective follow-up study, identified an association between incident HPV infections and receiving cyclophosphamide in a univariate analysis [23]. Cao et al. identified in one study that a low-dose of CYC may prevent the recurrence of condylomata acuminata after treatment with laser therapy through depletion of Treg cells and enhancing NK and T-cells function in response to HPV [42].

Azathioprine

Azathioprine is an immunosuppressive anti-metabolite agent, classified as purine analogue, used in systemic lupus erythematosus, rheumatoid arthritis, inflammatory myopathies and vasculitis. Its active metabolite is 6-mercaptopurine.

Very limited information exists about the effects of azathioprine in patients rheumatic disorders infected with HPV. In a prospective cohort of 230 patients with inflammatory bowel disease Seksik et al. observed the appearance or worsening of warts in 17.2% of their patients receiving azathioprine compared with only 3.3% of their patients without this immunosuppressive drug ($p=0.004$) [43] Our group reported no differences in the prevalence of HPV infection according to accumulated doses of azathioprine between patients with RA

or SLE, although a lower frequency of azatiophrine utilization was observed in patients with HPV infection compared with patients without infection (p=0.027) [14]. To date, there is insufficient data to conclude the true effect of azathioprine in patients with systemic rheumatic diseases who have persistent HPV infection.

Nevertheless, in inflammatory bowel disease it has been recommended that azathioprine should be discontinued in cases of persistent HPV infection [44]. With this insufficient information the extrapolation of this recommendation to systemic rheumatic diseases is discussable, and needs to be supported by further evidence in controlled prospective follow-up studies.

Methotrexate

Methotrexate (MTX) is a synthetic agent with anti-inflammatory properties mediated by adenosin metabolism modulation as well as immunosuppressor properties attributed to the inhibition of the dihydrotofolate reductase enzyme necessary for the synthesis of tetrahydrofolate, required for nucleic acids synthesis. There is a lack of studies evaluating the effects of MTX on patients infected with HPV. Rojo-Contreras et al., reported that patients with SLE or RA who had HPV infection had a higher frequency of MTX use (72%) in comparison with patients without HPV infection (44%, p=0.036) [14]. It is well described that in patients with MTX some viral infections can be increased. There are some case reports identifying development of molluscum contagiosum in patients with systemic rheumatic disorders treated with MTX.

In one case of mixed connective tissue disease treated with low doses of MTX (7.5mg per week), the appearance of molluscum contagiosum was observed [45]. Being that MTX is one of the drugs most frequently prescribed in patients with RA and other systemic rheumatic diseases, it is necessary to perform controlled studies to evaluate the effect on the persistence of HPV infection.

Mycophenolate Mofetil

Mycophenolate mofetil (MMF) is an immunosuppressor used with frequency in patients with glomerulonephritis secondary to SLE, is also used in some vasculitis. This is a prodrug of mycophenolic acid, an inhibitor of inosine monophosphate dehydrogenase implied in the purine biosynthesis, mainly guanine, necessary for the growth of T cells and B cells that are participants in the inflammatory process in systemic rheumatic disorders. Very few studies have evaluated the association between HPV and the use of mycophenolate mofetil (MMF). Abud-Mendoza et al., in a cross sectional study compared 16 patients with SLE infected with HPV with 20 patients with SLE without infection. They observed that mean cumulative doses of MMF increased in patients with HPV in comparison to those non-infected [46]. These authors also observed a decrease in the levels of B and NK cells in their patients treated with MMF [46]; an observation that is interesting and should be further explored in the context of follow-up studies evaluating the persistence of HPV infection in SLE.

Leflunomide

Leflunomide (LEF) is an immunomodulatory drug that inhibits mitochondrial enzyme dihydroorotate dehydrogenase, which is an enzyme involved in the novo pyrimidine synthesis. This enzyme plays a relevant role in the synthesis of pyrimidine ribonucleotide uridine monophospate. Tam et al., observed an association between the incident HPV infection and the current use of LEF in patients with SLE [23]. To date, additional information derived from other cohorts is still required to identify if LEF is a factor associated with persistence of HPV, but the findings of this study described by Tam have clinical relevance and should be taken into account in patients with systemic rheumatic diseases with high risk HPV infection.

Anti-TNF Therapies

TNF-α is known to exert antitumor and antiviral effects and to participate in the regulation of the immune response, which also restricts the expression of E6/E7 oncoproteins in the cells infected by HPV 16 or 18. However, during tumor progression HPV infected cells become insensitive to TNF-α. [47]. E6 oncoprotein of HPV 16 binds directly to the TNF-α receptor inhibiting TNF-induced apoptosis of the host cell. Case reports show that some patients with anti-TNF agents may develop condylomata lesions. Georgala et al., reported a case series of three patients with psoriasis treated with infliximab, who developed HPV infection (2 cases) or molluscum contagiosum (1 case); these lesions were developed during their treatment. [48].

Anti-B-Cell Therapies

B cells are implied in the pathogenesis of SLE with the development to plasmocyte cells. They synthesize auto-antibodies in SLE and other rheumatic disorders. Rituximab is an anti-CD20 monoclonal antibody used for the treatment of some systemic diseases where CD-20 cells are implied, including SLE and RA depleting B cells autoreactive reducing autoantibody production. Meta-analysis of different studies has shown that in SLE, rituximab has favorable results in refractory SLE [49]. To date, there is no information about the effects of rituximab on patients with HPV infection in RA or SLE. This interesting aspect should be evaluated in future studies. Nevertheless, it likely that rituximab may reduce the titers of anti-viral autoantibodies in patients receiving several vaccines. This issue should be considered before the application of a vaccine.

VACCINATION AGAINST HPV IN SYSTEMIC RHEUMATIC DISEASES

Currently there are two vaccines: a bivalent vaccine (Cervarix®, Glaxo Smith Kline) that protects against HPV types 16 and 18 and a quadrivalent vaccine (Gardasil ®, Sanofi Pasteur

MSD) that protects against HPV types 6, 11, 16, and 18. Both vaccines are effective in preventing HPV16 and HPV18 infections associated with the development of cervical cancer but only the quadrivalent vaccine protects against the development of condylomata because this is associated with the HPV types 6 and 11. Some issues are relevant about these vaccines and systemic rheumatic diseases: First, are the vaccines against HPV related to the development of autoimmune diseases? Some cases of systemic lupus erythematosus have been reported following vaccination against HPV [50]. Chao et al. performed an observational study of surveillance of the quadrivalent HPV vaccine, following the subjects for 180 days after each dose of vaccination, searching for new diagnoses of autoimmune conditions [51].

Chao et al. sampled 347 new cases of autoimmune diseases that appeared after vaccination (80 of them rheumatologic⁄autoimmune, 167 endocrine, and 100 neurological ⁄ophthalmic) obtained from 149,306 women that met the 12-month follow-up criteria. From 80 cases of rheumatic diseases, 25 corresponded to probable SLE but the Committee confirmed only 8; similarly 17 of 80 corresponded to probable RA but only 3 cases were confirmed by this Committee, and 13 of 80 cases with probable juvenile chronic arthritis confirming 3 cases [51].

Overall, these authors observed that the incidence of new cases of systemic rheumatic diseases is not different from those expected in the general population, and the only significantly elevated IRR in comparison with that expected for the general population was for Hashimoto's disease [IRR = 1.29, 95% confidence interval: 1.08–1.56] [51]. These data support that vaccination with HPV quadrivalent is safe and only a few cases of rheumatic diseases can likely be expected with no difference from what is expected for the general population. Yet, more surveillance studies should be performed to establish definite conclusions.

A second issue is, if the vaccination in patients diagnosed with systemic rheumatic diseases is safe or can a relapse or flare-up of the disease be expected. Soldevilla et al. reported one case of a woman with SLE previously in long-time remission that developed a severe renal relapse, with thrombocytopenia, anemia, transaminitis and cutaneous activity, who unfortunately died during this event; this relapse was developed after receiving two doses of HPV vaccine [50].

The third issue is, are the vaccines effective in patients receiving immunosuppressive drugs? Canadian guidelines for the pharmacological management of rheumatoid arthritis with traditional and biologic disease-modifying antirheumatic drugs recommend that wherever feasible, patients with RA should be immunized when a maximum immune response can be anticipated; if possible before the initiation of immunosuppressive treatment [52]. This concept is valid in most of the systemic rheumatic diseases. Nevertheless, in most cases this is not possible and a vaccination must be utilized in a patient receiving immunosuppressive drugs, and a suspension of immunosuppressive drugs in order to administer a vaccination increases the risk of a relapse. VPH vaccine may be administered during the regular treatment of RA including immunosuppressive drugs or biologic agents [52], and this concept seems to be valid for other rheumatic diseases.

Vaccination guidelines described by the European League Against Rheumatism (EULAR) in 2011 for autoimmune rheumatic diseases makes reference that any vaccination ideally should be considered when the disease is stable and can be administered during the use of antirheumatic drugs including methotrexate and anti-TNFα agents, but should ideally

be administered before starting B cell depleting biological therapy with rituximab [53]. In the particular case of HPV, these guidelines conclude that there is insufficient information about the efficacy of HPV vaccination in autoimmune inflammatory rheumatic diseases [53] but these guidelines also recommend considering HPV vaccination in women with SLE until the age of 25 years. In summary, some autoimmune adverse events have been developed after the vaccine application including Guillain-Barré, transverse myelitis, optic neuritis, multiple sclerosis or myasthenia gravis. There are also some rare case-reports of RA, SLE, mixed connective tissue disease, Sjogren syndrome, dermatomyositis and scleroderma developed after the vaccination but these events are rare and in most of the cases the incidence of these diseases are similar to those expected for the general population.

Nevertheless, new studies about the risk of HPV vaccination for the development of autoimmune diseases are required, including the identification of events in populations with high risk for autoimmune diseases, such as in patients with family antecedents of autoimmune systemic diseases.

TREATMENT OF HPV IN INFLAMMATORY DISEASES

General criteria for the treatment of HPV are applicable for rheumatic diseases. Treatment of external genital warts: The choice of treatment depends on several factors including status of disease activity, immunosuppression, characteristics of the patients, location of warts as well as the number, size, nature of the lesions and patient preferences. Psychological support is required for all patients, condom utilization during sexual intercourse should be recommended and in some cases circumcision in males may help. Previous to starting any treatment, a structured assessment of the genital tract should be performed especially to exclude cervical lesions and malignancy, if required (for example in case of atypical appearance) a biopsy of the warts should be taken.

Although there is a lack of studies evaluating the effects of warts treatment in patients with active disease in systemic rheumatic diseases, it is advisable to delay the treatment until the disease becomes inactive. Since spontaneous regression of warts has a low frequency, all patients should be offered treatment.

For patients with rheumatic diseases the therapeutic options are similar to those offered to the general population: physical or chemical destruction, and immunomodulating drugs [26]. Recurrences are frequently observed in immunosuppressed patients. Aynaud et al. reported a recurrence rate of condylomata lesions of 25% after receiving CO2 laser treatment in patients with therapeutic immunosuppression, which was similar to the observed in the group of patients infected with human immunodeficiency virus [54]. Interestingly, Cao et al. observed that a low-dose of cyclophosphamide might prevent recurrence of condyloma accuminata lesions because it depletes Treg cells and enhances the function of T-cells and NK cells [42]. This observation is too preliminary to establish definite conclusions but deserve further investigation.

TREATMENT OF CERVICAL DYSPLASIA OR CANCER IN SYSTEMIC RHEUMATIC DISEASES

Similar recommendations used for immunocompetent patients are applicable to patients with systemic rheumatic diseases; colposcopy and biopsies are frequently required. In patients with low-grade lesion and the squamous-columnar junction visible, a destruction of the lesions, using laser vaporization; and a new Pap smear and colposcopy after 6 months is a regular conduct [26]. Most of the LGSIL resolve spontaneously within 2 years. Nevertheless, the risk of lesion progression to high-grade squamous intraepithelial lesions (HGSIL) is related with the viral load. Therefore, viral load determination should help to identify patients at risk for progression to HGSIL. In patients with high-grade intraepithelial lesions conization is the standard treatment. [26].

One of the barriers for the early treatment is the low frequency of cervical cancer screening in patients with rheumatic diseases compared with that observed in the general population. Bernatsky et al. reported in a survey that only 33% of Canadian patients with SLE, aged less than 30 years, had Pap tests in the past 12 months, compared with 56% in women of similar age from the general population [55].

CONCLUSION

In summary, HPV infection in rheumatic disorders is an exciting area for clinical and epidemiological research. To date most of the information is generated from studies evaluating patients with SLE, although some observational works have evaluated Sjögren, RA, as well as other rheumatic disorders. These complex diseases have abnormalities in the immune response that may make the virus clearance difficult and they may receive immunosuppressive therapy causing an additional decrease in the immune mechanisms involved in the response to HPV. These two aspects increase the concern in the development of future cervical neoplasia. A future task is to design clinical guidelines for preventive measures in patients with systemic rheumatic disorders as well as a strategy for follow-up and treatment in those patients who have high-risk types of HPV infection, particularly for those patients who receive immunosuppressive therapy or anti-TNF agents. HPV vaccination and its effects on patients with established systemic rheumatic disorders are considered interesting topics that deserve further investigation. Future guidelines for HPV vaccination must consider the patients with systemic rheumatic diseases as a high risk group for cervical neoplasia; this group represents a challenge for clinicians in order to achieve the objective of decreasing the high-rates of HPV infection observed in these patients but without increasing the possibility of disease reactivations. Clinicians should be aware that these patients with systemic rheumatic diseases require an appropriate long-term evaluation combining HPV infection detection and cervical cytology and other diagnostic procedures in order to detect and to treat early SIL and avoid the development of most severe complications.

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Chapter 9

CLINICAL VALUE OF HPV TESTING IN CERVICAL CANCER

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ABSTRACT

Worldwide, cervical cancer is among the most common cancers in women. Human papillomavirus (HPV) has a strong association with cervical cancer. Since HPV plays an important etiological role in cervical cancer, it is logical to use HPV as a marker for early detection of cervical cancer and precancer. Recent advances in technology enable the development of high-throughput HPV assays of different formats, including DNA-based, mRNA-based, high-risk group-specific and type-specific methods. These assays are to improve the accuracy and cost-effectiveness of cervical cancer screening programs. While conventional Pap smear or liquid-based cytology is still the standard for care in many parts of the world, the intrinsic drawbacks of cytology-based screening call for replacement by HPV testing or the addition of adjunct markers. However, HPV test results are bound to have a low positive predictive value that may subject women to unnecessary follow-up investigations. A fine balance has to be established between the sensitivity and specificity of the HPV test to achieve a clinically useful predictive value, thus maximizing the efficacy of screening.

INTRODUCTION

Globally, a decent number of infections play a causal role of human cancers. In 2008, it is estimated that 16.1% of 12.7 million new cancer cases were attributed to infectious agents; human papillomavirus(HPV), hepatitis B and C viruses, human herpes virus type 8, and etc.

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Among them, HPV is the second most common infectious agent resulting in cancers; cervical cancer, penile cancer, anal cancer, vulvar cancer, vaginal cancer, and oropharyngeal cancer are attributed to HPV [1]. To date, more than 120 HPV types have been identified and all known types are currently classified by the similarity of their genome into five genera (alpha, beta, gamma, mu, and nu) [2]. Forty different types from genus alpha, clinically the most important HPV genus, exclusively infect the mucosal epithelium, resulting in variety of clinical conditions that range from innocuous lesions to cancer [3, 4].

The strong association between cervical cancer and HPV is recognized. Over the decades, immense development of molecular technology led to the evolution of HPV detection tests. The assays yielded improved accuracy and cost-effectiveness of cervical cancer screening programs [5].

In this chapter, we will update and discuss about the clinical value of HPV testing in cervical cancer.

CLINICALLY IMPORTANT BASIC VIROLOGY

1. Viral Genome

Papillomaviruses have a double-stranded DNA genome of about 8 kb in length. The key proteins encoded by different regions of the HPV genome are the targets for designing detection assays. The HPV genome has 8 open reading frames (ORFs) and they are functionally divided into 3 regions (Figure 1); long control region (LCR), the early (E) region, and the late (L) region.

LCR is a non-coding region that regulates DNA replication by controlling the transcription of the ORFs. Early region proteins include E1, E2, E4, E5, E6, and E7 and late region proteins include L1 and L2 [6].

E6 and E7 proteins, that place adjacent to LCR, are the most important HPV proteins in the tumor progression. Infection by high-risk HPV types causes E6/ E7-mediated proliferation of basal and parabasal cells, which facilitates an expansion of lesion size. Mediated by the E6-associated proteins, high-risk E6 proteins bind to the tumor suppressor protein p53, inactivating its function. When E6 overexpression occurs, degradation of p53 protein, anti-apoptosis, chromosomal destabilization, enhancement of foreign DNA integration, and activation of telomerase take place. High-risk E7 proteins bind to retinoblastoma (Rb) protein, an important negative regulator of cell growth, inactivating its function. Apart from its interaction with Rb protein, E7 interacts with a wide variety of cellular proteins, thus promoting unregulated cell cycle and division, cell survival, evasion of immune surveillance, and anti-apoptosis [6, 7].

Although, the precise role of E1 and E2 proteins during the HPV infection needs further clarification, they are considered to take essential roles in the initial amplification phase. E2 protein regulates viral transcription and forms a complex with E1, thus initiating viral replication. Moreover, E2 influences the expression of E6 and E7 proteins. In addition to E1 and E2 proteins, E4 and E5 proteins are considered to contribute to genome amplification indirectly [8, 9].

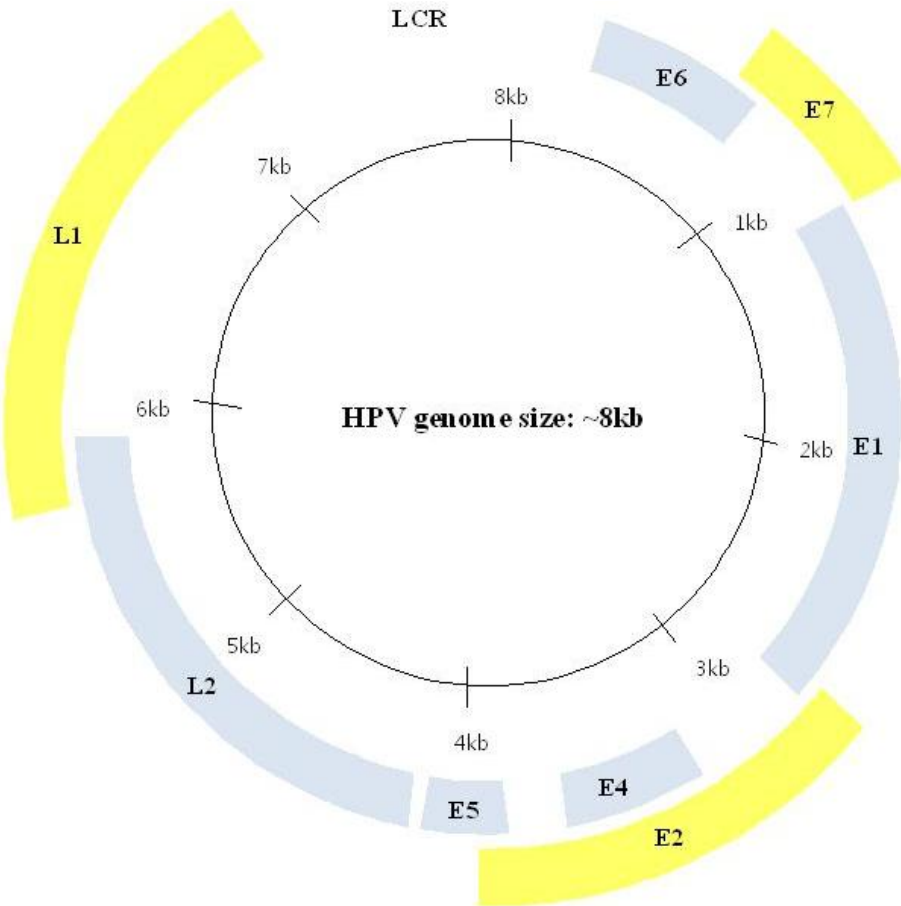

Figure 1. Open reading frames of human papillomavirus(HPV); key targets of HPV genome detection.

L1 and L2 proteins protect the viral genome inside; L1 open reading frame encodes the "major capsid protein" and L2 open reading frame encodes the" minor capsid protein". Under the appropriate in vitro condition, L1 protein can assemble itself into empty capsid-like structure which immunogenicity is similar to that of the infectious virions. Therefore, it is considered to be the main constituent of current prophylactic vaccines and the prime target as antigens for serological assays [10, 11]. L2 protein helps the process of viral entry into cells, the localization of viral components to the nucleus, the binding of DNA, and the formation and stabilization of the capsid. It evokes a broad spectrum of neutralizing antibodies against different types of HPV. Because it elicits antibodies that are more cross-reactive between HPV types than does L1, it is considered to be the component of the future vaccine [12].

2. HPV Types

HPV types refer to "genotypes" that are classified based on sequence similarity; L1 ORF is a key region for the classification. More than 10% difference in DNA sequence of the L1 ORF is noticed between one HPV type and the other. When the difference in sequence is

within 2-5%, it is regarded as subtypes and when it is below 2%, it is referred as variants [2, 13, 14].

The HPV type classification has strong clinical significances. First of all, the phylogenetic classification of HPV types reflects the tissue tropism observed in clinical infections. HPV types that belong to alpha genus infect the mucosal and cutaneous areas whereas types that belong to beta genus does not infect mucosal areas [14]. Secondly, HPV infection-induced immune system is type-specific, thus type-specific prophylactic vaccines have been produced; one is bivalent with HPV 16 and 18, and the other is quadrivalent with HPV6, 11, 16, and 18 [11]. Thirdly, cancer development is HPV type related. HPV is grouped into high risk and low risk. There are 12 high-risk or class I HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) according to International Agency for Research on Caner. Well known low-risk HPV genotypes are 6 and 11; yet, low-risk or unknown risk HPV genotypes have not been clearly defined [2, 15-17].

HPV DETECTION TESTS

Immune system recognizes foreign agents and forbid them from invading the body; however, HPV successfully evades the system despite its long lasting infection. HPV infects and multiplies in keratinocytes that have short-life span. Progeny viruses are released in a natural way without inducing cell-lysis, thus avoiding the triggering immune responses associated with cellular damage. In addition, HPV down regulates the synthesis of interferon. For this reason, robust antibody response does not occur although the immune system plays a role in clearing the infection [18-20]. Serologic diagnosis has limited clinical value; only about 50-70% of women with persistent cervical HPV infection show detectable antibody response and the women with transient infection do not show any. Therefore, cervical sampling is mainly used in order to detect HPV infection that amplifies the viral genome or mRNA or viral proteins [21].

ORFs work as targets for amplifying HPV genome; as mentioned above, L1 region is the most frequently used target. Yet, the design of consensus primers well conserved to amplify a broad spectrum of HPV types by using a single set of degenerated primers. The commonly used primer sets are MY09/11, PGMY09/11, GP5+/6+, and SPF [22, 23]. While L1 is recognized as the best target for HPV detection, it is often not found in invasive cervical cancer samples; viral genome integration might have caused the disruption. When such occasion occurs, E6 or E7 can be amplified to verify the presence of HPV infection [24]. E6 and E7 has diverse nucleotide sequence between HPV types, therefore the type specific primers are necessitated. Unlike L1 region, both E6 and E7 genes are expected to be retained regardless of viral integration, because E6 and E7 gene expression is requisite for the maintenance of the transformed phenotype of infected cells [25, 26].

E2 gene is considered to be a surrogate marker to indicate the status of viral integration. It is because when HPV-mediated cervical carcinogenesis proceeds via the integration of viral genome and disruption of the E2 ORF, suppressive control of E2 exerts the expression of E6 and E7 oncogenes. Therefore, measuring the ratio of E2 and E6 (or E7) gene copy numbers is the estimating the proportion of integrated viral genome present in a clinical sample [9].

1. DNA-Based Assays

HPV assays that are currently in use mostly rely on the detection of viral nucleic acids. They can be divided into signal amplification methods (ex: hybridization and invader assay), target amplification methods (ex: real time PCR, microarray, and reverse line-blot Hybridization on PCR), and direct hybridization methods (ex: Southern blot) [27-29]. The choice of HPV test depends on the application (table 1). For molecular epidemiological studies and evaluating vaccine efficacy, the tests with high analytical sensitivity are appropriate. HPV typing assays with high analytical sensitivity and specificity are the keys in virological surveillance. And when applied in the clinical situation for cervical cancer screening and post treatment follow up, lower analytical sensitivity may produce a better positive predictive value [30-32]. Few of the commercially used HPV detecting methods are described below.

1.1. hrHPV DNA Tests

hrHPV DNA tests detect oncogenic HPV types. These tests do not allow type-specific classification [33]. Two HPV tests from this group are approved by United States (US) Food and Drug Administration (FDA) and two additional tests are considered to be clinically validated [31].

1.1.1. Hybrid Capture®2(HC2) HPV DNA Test(Quiagen Inc, Gaithersbur, MD)

Hybrid Capture®2(HC2) HPV DNA test(Quiagen Inc, Gaithersbur, MD) is the most frequently used test in the world and it is approved by the US FDA in 2003 for i) triage in cases of equivocal cytology results showing the presence of atypical squamous cells of undetermined significance (ASCUS), to determine which patients should be referred for a colposcopy and ii) as a screening test for use in addition to cytology screening for women 30 years of age and older [31]. It is based on liquid phase hybridization using long synthetic RNA probes complementary to the genomic sequences of 13 high risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 5 low risk types (6, 11, 42, 43, and 44); they are used to prepare high risk (B) and low risk (A) probe cocktails that are used in two separate reactions.

In practice, high risk probes are used mostly, but low risk probes are not. DNA-present in the biological specimen is hybridized in solution phase with each of the probe cocktails and forms specific HPV DNA-RNA hybrids. After removal of excess antibodies and non hybridized probes, the immobilized hybrids are detected by a series of reactions that give rise to a luminescent product that is detected by a luminometer. The intensity of emitted light provides a semi-quantitative measure of the viral load. The HC2 is currently available in a 96-well microplate format and it is easy to perform in clinical settings and suitable for automation [34, 35].

Unlike PCR protocols, HC2 does not require special facilities to avoid cross contamination because it does not rely on target amplification to achieve high sensitivity. US FDA-recommended cut-off value for test-positive results is 1.0 RLU (equivalent to 1pg HPV DNA per 1mL of sampling buffer) [36].

Table1. Most widely used commercial HPV tests

	Molecular Target	Principle	Test name	Manufacture	Current status
DNA based assays					
hrHPV DNA tests	Full genome	Hybridization	Hybrid Capture®2(HC2) HPV DNA test	Quiagen Inc, Gaithersbur, MD	US FDA-approved (2003)
			Care HPV test™	Quiagen Inc, Gaithersbur, MD	Clinically validated*
	L1 ORF	Invader assay	Cervista® HPV HR test	Hologic ,Madison, WI	US FDA-approved (2009)
		Real Time PCR	EIA kit HPV GP HR	Diassay, Rijswijk, The Netherlands	Clinically validated*
hrHPV DNA tests with concurrent or reflex partial genotyping for the main hrHPV types	L1 ORF	Real Time PCR	Cobas®4800 HPV test	Roche Moledcular System Inc., Alabama, CA	US FDA-approved (2011)
			RealTime High Risk HPV test	Abbott Molecular, Des Plaines, IL	Clinically validated*
		Hybridization	Cervista® HPV 16/18 test	Hologic ,Madison, WI	US FDA-approved (2009)
HPV DNA full genotyping	L1 ORF	Reverse line-blot Hybridization on PCR	Linear Array® HPV Genotyping Test	Roche Moledcular System Inc., Alabama, CA	
			INNO-LiPa HPV Genotyping Test	Innogenetics NV, Gent, Belgium	

	Molecular Target	Principle	Test name	Manufacture	Current status
			Digene HPV genotyping RH Test	Quiagen, Hilden, Germany	
		Microarray	Clart® HPV2-Papillomavirus Clinical Arrays	Genomica, Coslada, Spain	
	E1 ORF	Microarray	PapilloCheck® HPV-Screening Test/ High-risk Test	Greiner Bio-One, Frickenhausen, Germany	Clinically validated*
mRNA based assays					
hrHPV mRNA Test	E6/E7	TMA[1]	APTIMA® HPV TEST	Gen-Probe Inc, San D, CA iego	US FDA-approved (2011)
Partial genotyping-HPV 16/18/31/33/45	E6/E7	NASBA[2]	NucliSENS EasyQ® HPV[3]	Biomerieux, Marcy l'Etoile, France	
			PreTect HPV-Proofer[3]	NorChip, Klokkarstua, Norway	

[1]TMA=transcription-mediated amplification.

[2] NASBA=Nucleic acid sequence–based amplification.

[3] Same technology marketed under different names in different countries.

* According to the international Guidelines for HPV DNA test requirements for primary cervical cancer screening in women 30 years and older [31].

A number of studies have noted that the high-risk probe cocktail in HC2 cross reacts with HPV types that do not exist in the probe mix. HPV types 53, 66, 67, 37, and other undefined types have been detected at a 1.0 pg/mL cut off; yet, raising the cut-off to 10.0 pg/mL does not fully eliminate the cross reactivity.

The cross reaction with high risk HPV types is considered to be beneficial for the detection of malignancy; however the cross reaction with low risk HPV types causes false positive results, decreasing the specificity and positive predictive value [37].

To date, clinical validation of HC2 has been approved through number of randomized, controlled and cohort studies [34, 38-40]; therefore new HPV tests are recommended to show that their clinical characteristics are non-inferior to HC2 before they are used for cervical cancer screening [30].

1.1.2. Care HPV Test™ (Quiagen Inc, Gaithersbur, MD)

It is a clinically validated, simple, and rapid test, detecting hrHPV in 2.5 hrs. It is based on simplified HC2 technology, presenting a promising result as a primary screening method for cervical cancer prevention in low resource regions [41].

1.1.3. Cervista® HPV HR Test (Hologic ,Madison, WI)

It is based on signal amplification invader chemistry and a US FDA-approved HPV detecting method. US FDA approved Cervista® HPV HR test(Hologic ,Madison, WI) for 2 indications; i) to triage patients with ASCUS cervical cytology to determine the need for referral to colposcopy and ii) for adjuvant use with cervical cytology to screen women with 30 years and older [40, 42, 43].

1.2. hrHPV DNA Tests with Concurrent or Reflex Partial Genotyping for the Main hrHPV Types

1.2.1. Cobas®4800 HPV Test (Roche Molecular System Inc., Alabama, CA)

The test is based on two processes; one is automated specimen preparation to simultaneously extract HPV and cellular DNA and the other is PCR amplification and real-time detection of target DNA sequences using both HPV and β-globin specific complementary primer pairs and probes. It is the only US FDA-approved test that allows concurrent screening for hrHPV and partial individual typing for HPV 16 and HPV 18. HPV testing with separate HPV 16 and HPV 18 detection are considered to provide more sensitive and efficient strategy for cervical cancer screening than other methods that are based solely on cytology.

US FDA approved the method for 4 indications; i) to triage women 21 years and older with ASCUS to determine the need for referral to colposcopy; ii) for the usage in patients 21 years and older with ASCUS cervical cytology results, to assess the presence or absence of HPV 16 and HPV 19; iii) in women 30 years and older, the test can be used with cervical cytology adjunctively to screen to assess the presence or absence of hrHPV types; iv) in women 30 years and older, the test can be used to assess the presence or absence of HPV 16 and HPV 18 [44-46].

1.2.2. Cervista® HPV 16/18 Test (Hologic ,Madison, WI)

Cervista® HPV 16/18 test(Hologic ,Madison, WI) was approved by the US FDA in 2009 and was intended to be used as a reflex test for Cervista® HPV HR test(Hologic ,Madison, WI). The test was approved for 2 indications; i) to assess the presence or absence of specific hrHPV types in women 30 years and older in combination with cervical cytology, and ii) to triage in patients with ASCUS cervical cytology results [43].

1.3. HPV DNA Full Genotyping Test

The tests allow individual determination of several alpha-HPV types and they account for the largest group of currently available HPV commercial tests.

Yet, the clinical value of HPV DNA based full genotyping tests has not been fully determined [38].

1.4. HPV DNA Type- or Group Specific Genotyping Tests

HPV detection systems that identify high risk or low risk HPV types as a group are referred as "group-specific" and that identify individual HPV types are referred as "type-specific".

The tests that belong to this group allow individual determination of only a limited number of clinically important alpha-HPV types. Similarly to HPV DNA based full genotyping tests, the clinical value of HPV DNA-based type- or group-specific genotyping tests has not been finally determined [47].

2. E6/E7 mRNA Based Assays

E6 and E7 are oncoproteins and their persistent expression could serve as indicators of progression from intraepithelial neoplasia to invasive cancer [48]. Several recent studies have shown that testing for HPV mRNA instead of HPV DNA can be clinically useful, due to its higher clinical specificity [49, 50]. Recently, a few commercial assays have been developed (Table 1). Among them, APTIMA® HPV TEST(Gen-Probe Inc, San Diego, CA) is an US FDA-approved test; the indications are i)to triage women 21 years and older with ASCUS to determine the need for referral to colposcopy and ii) for screening women of 30 years and older in combination with cytology. CLEAR study and FASE study support the approved indication [51, 52]. Arbyn et al. showed that in triage settings, APTIMA is as sensitive but more specific than HC2 for detecting cervical precancer lesions [45].

3. E6/E7 Protein Based Assays

It is suggested that measuring the E6 or E7 proteins may provide a better predictive value than detecting viral DNA alone. However, its clinical evaluation has not been done yet [53].

Persistent infection with hrHPV is a necessary etiological factor in the development of cervical carcinoma (Figure 2). As a general rule, HPV testing must be performed in appropriate, evidence-based contexts to maximize the benefit and reduce over diagnosis.

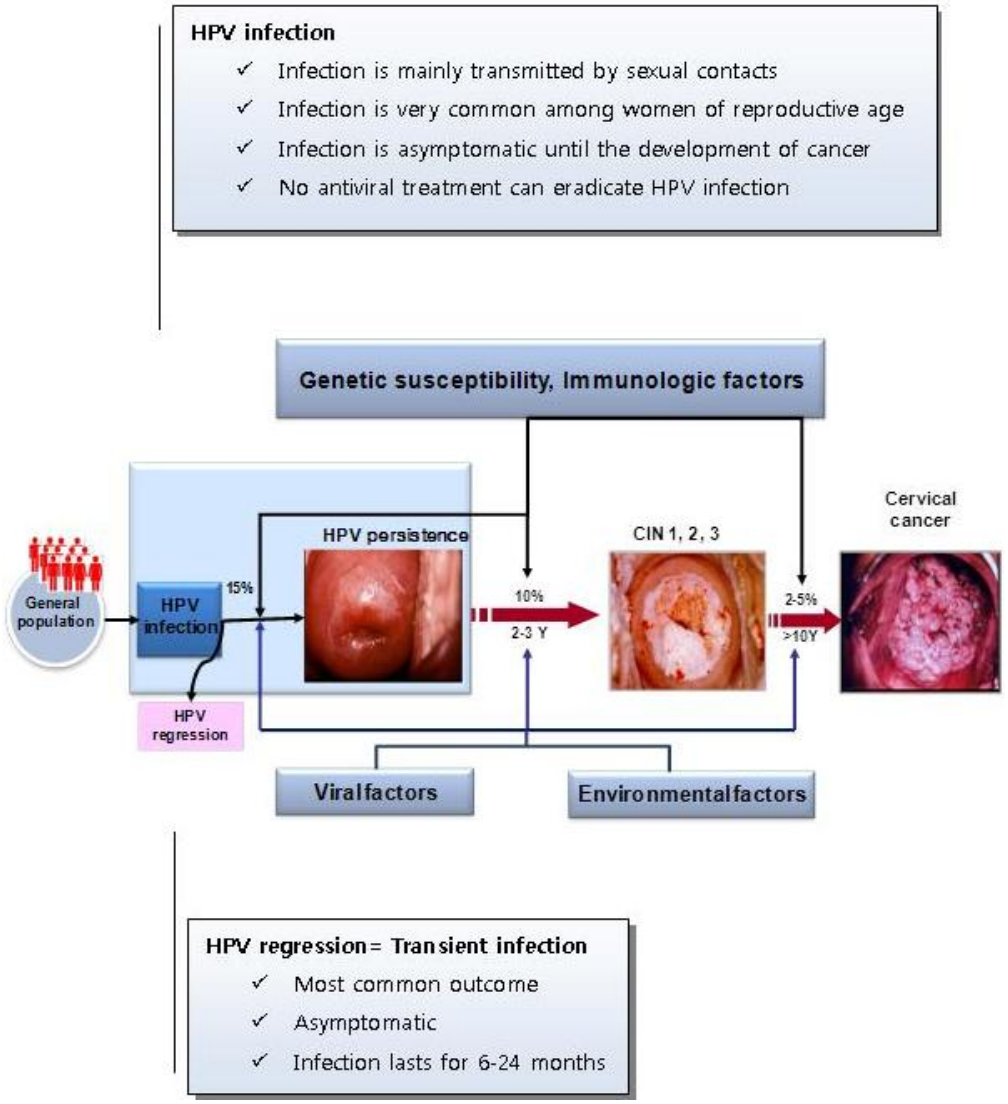

Figure 2. Natural history of HPV infection.

CLINICAL APPLICATIONS OF HPV TESTS

1. Primary Screening

Cervical cancer progresses slowly by HPV infection, therefore different pathological stages are examined from exfoliated cervical cells (Figure 2). Cervical screening has been based on cytological examination of exfoliated cervical cells and its benefits are well known. The regions where cytology based programs have been launched effectively, annual rates of cervical cancer have been reduced by 50-90%; there are marked differences in cervical cancer incidence rates between countries with and without organized screening programs [54, 55]. However, there are several drawbacks of cytology-based screening that call for replacement

by HPV testing or the adjunctive testing. The cytology screening requires training, the development of laboratory infrastructure, standardization, and quality control measures. Number of HPV testing has increased and so has its availability (Table 1) [33]. Therefore, a large number of studies have been conducted to investigate the value of using HPV DNA detection as a primary or adjunctive tool for cervical screening. Moreover, European community and some advanced countries are now including the recommendation to use HPV as the sole primary screening test and it accelerates the more widespread implementation of this important new tool for cancer prevention.

The advantages of HPV testing over cytology are feasibility for high throughput, greater objectivity in result interpretation, high sensitivity, high negative predictive value and ability to provide long term risk stratification [56-58]. HPV DNA detection has 20-45% greater one-time test sensitivity for precancer than cytology-based test. Importantly, it is reported that a single round of HPV based screening reduces the incidence of cervical cancer within 4-5 years and caner-related mortality within 8 years [5, 56, 57, 59-61]. Moreover, the performance of HPV assay is less dependent on centers. When the same series of studies across the continents were investigated, the sensitivity of cytology ranges from 33.8% to 94.0% where as that of the HPV detection test (HC2) varies from 84.9% to 97.6% [62-66].

However, HPV detecting tests have their own drawbacks in clinical practice. HPV infections are transient in most women and the prevalence of high grade cervical intraepithelial neoplasia or cervical cancer is low (Figure 2). Therefore, they present low specificity and low positive predictive value. The approaches to improve positive predictive value are described below.

1.1. Target Population Set-Up

Many studies suggested that HPV test be performed to women over 30 years of age. The reason for this is that the women under 30 years of age commonly have transient infection, lowering the positive predictive value. Therefore, the target population to whom HPV detection test is performed should be kept as the ones over 30 years of age [57, 67].

1.2. Limitation of High-Risk HPV Types

According to International Agency for Research on Caner, there are at least 12 high-risk or class I HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) that are considered to be linked to cervical cancer [2, 13, 14, 16]. Most HPV assays are designed to detect as many high-risk types as possible; however covering small risk may jeopardize the overall positive predictive value of the test. HPV type 16 and 18 are the ones that should be included as they are the 2 most commonly detected high-risk types. In addition to these 2 types, HPV type 31, 33, 45, 52, and 58 are the next group. The types in detail vary depending on the geographical distribution, however types, such as HPV 58, 52, and 45, account for more than negligible portion of cervical malignancy, therefore a careful assessment to include additional high risk HPV is suggested [15, 68-70].

1.3. Reflex Follow-Up Test

“Reflex follow-up” test for HPV positive samples may improve the positive predictive value of HPV detecting test as a primary screening tool. Moreover, it can prevent an extra visit [9].

1.3.1. E6/E7 mRNA

First test is to detect direct indicators of HPV oncogenes expression, such as E6/E7 mRNA.

Molden et al. compared the detection of HPV DNA with detection of mRNA. The study resulted that for high grade-SIL, detection of E6/E7 transcripts from hrHPV types 16, 18, 31, 33, and 45 are present to the same degree as DNA; and for normal, ASCUS, or low grade SIL, only a small portion of hrHPV DNA was detected. The study indicates the mRNA test has a higher specificity compared to the HPV DNA test [71]. Moreover, few other studies support the high specificity of the test [72, 73].

1.3.2. Biomarkers for Transforming HPV Infection

In HPV associated tumors, the inactivation of Rb by E7 leads to a marked overexpression of the cycling-dependent kinase inhibitor, $p16^{INK4a}$, as a result of the loss of negative feedback regulation that depends on Rb activity. Thus $p16^{INK4a}$ works as a surrogate marker for the transforming activity of hrHPV oncoproteins that are essential for the initiation and maintenance of the carcinogenesis [74, 75]. Many clinical research studies have presented that immunochemical staining for $p16^{INK4a}$ is a diagnostic adjunct in the evaluation of cervical histology and cytology specimens. Moreover, Zhang Q et al. demonstrated that when the biomarker is used with HPV test, the sensitivity and specificity profiles are substantially improved [76].

Moreover, the combination of antibodies detecting $p16^{INK4a}$ and the cell cycle progression marker Ki67 is considered to be a promising reflex test. The $p16^{INK4a}$/ Ki67 dual stain provided a high sensitivity for the detection CIN2+ lesion in women with ASCUS or LSIL Pap cytology results. It was comparable to the results reported for HPV test and $p16^{INK4a}$ cytology. However, the specificity of the $p16^{INK4a}$/ Ki67 dual stain test was substantially improved compared with the earlier $p16^{INK4a}$ cytology assay [77, 78]. Some studies are in use of the dual test to help determine the primary cervical screening [79].

1.3.3. Markers for Aberrant S-Phase Induction

Topoisomerase IIA (TOP2) and minicoromosome maintenance proteins (MCMP) are proteins that are expressed in cells with aberrant phases. It is reported that primary hrHPV screening followed by an antibody cocktail test that highlights the MCMP and TOP2 leads to significant increase in sensitivity (ratio: 1.30) and positive predictive value (ratio: 2.89)[80].

1.4. Usage of Viral Genome Characterization

Viral integration is thought to be a consequence of chromosomal instability, which takes place during the early course of infection. Although it can be detected in normal and low grade lesions, cervical samples from invasive cancer harbor purely the episomal form of viral genome. Therefore, it is suggested the viral integration be a reflex approach [81-83].

DNA methylation is an epigenetic event that is linked to cancer development. A number of studies have reported the association of either elevated or reduced levels of DNA methylation in ORFs or hrHPV with cervical cancer and CIN 2+ [84, 85]. As with viral integration, there is much to study about DNA methylation in order to achieve a clinical value.

2. Co-Test with Cytology

Co-test refers to the use of both HPV and cytology tests in parallel as first-line screening. The main advantage of co-test is the improvement in sensitivity for CIN 2+ lesions [86]. The most recent guidelines from the United States recommend HPV and cytology co-testing every 5 years for women aged 30-65 years[87]. The management of women with normal cytology but an HPV positive result has been an issue. Kjaer et al. presented that for women with normal cytology who were concurrently various hrHPV positive, the estimated probability of developing CIN 3+ within 12 years follow-up was up to 26.7% [88]. According to the recent guidelines for the United States, co-test 12 months later is one of the options for women with HPV positive and cytology negative screening results [87].

3. HPV as a Triage for Abnormal Cytology

The triage of patients presented with ASCUS on Pap smear was the first clinical application of HPV test; and it still is the most common application. Patients who were HPV positive would be referred for colposcopy whilst those who were HPV negative could be followed by repeating the Pap smear 12 months later[89, 90]. HPV detection test is in use to triage women with ASCUS, however, to triage of LSIL by HPV test is not recommended; about 80% of patients presenting with LSIL were HPV positive[39]. Yet, the use of HPV test to triage women with LSIL depends on the context. US FDA has approved the use of HPV test in post menopausal women presenting with LSIL since the prevalence rate of HPV is low in this subset of patients. Prevalence of HPV infection among the population tested is the main variable. Therefore, using HPV test only for the target population may increase the specificity; reassuring the patients with HPV negative results [91, 92]. In addition to the screening malignancy, the triage is used to analyze the progression rate of ASCUS or LSIL according to HPV type-specific infection. Lee et al. designed a 5 year multicentre prospective cohort study in women who are with either ASCUS or LSIL and HPV-positive [79].

4. Post-Treatment Surveillance

Women who have been treated for cervical precancer must be followed carefully in order to monitor possible recurrence. The rate of residual or recurrent CIN2+, evaluated over 2 years or less, varied in numerous studies from 4-18 %, with an average of 8%. When compared to conventional cytology, HPV test is more sensitive, carries a higher negative predictive value for recurrent or residual lesions. In addition, post-treatment follow-up is recommended by the American Congress of Obstetricians and Gynecologists [93, 94].

CONCLUSION

To date, cytology–based screening is the standard of care, thus being used popularly. However, HPV tests have presented satisfactory results with high sensitivity. The assays are

now being introduced into some countries as primary screening tests, co-tests, post-treatment surveillance or the triage in cases with equivocal findings. Yet, HPV detection tests have low specificity and positive predictive value. If the approaches to surmount the drawbacks, such as setting up the target population or limiting high-risk HPV types, are practiced properly, the HPV test will achieve a clinically useful predictive value, thus maximizing the efficacy of screening.

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The authors have disclosed no potential conflicts of interest and no financial or consulting relations with any company.

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Chapter 10

HUMAN PAPILLOMAVIRUS DETECTION: INDICATIONS AND AVAILABLE METHODOLOGY

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ABSTRACT

Human Papillomavirus is a double stranded DNA virus that is now well established as a causative agent in human cervical cancer. Although many women who contract high risk HPV strains have an immune response which successfully eliminates it, the virus is the root of the overwhelming majority of both squamous cell carcinoma and adenocarcinoma histologies. Though strains 16 and 18 are the root of up to 70% of cervical cancers, there are now at least 14 identified strains of HPV which are considered high risk including 33, 45, 51, and 52, with increasing evidence that the epidemiology of HPV infection may differ across women of different races. Recent advances have allowed for early detection of HPV concurrent with pap smears, prior to the onset of advanced cervical dysplasia, making its detection a dramatic step in prevention of cervical cancer. This is now becoming standard of care, allowing women to have less frequent screening with the more sensitive HPV DNA test in combination with cytology versus with cytology alone. Additionally, there are new developments in the detection of HPV DNA in which patients may self-swab, or undergo urine screening with potentially equal sensitivity, which may lead to better patient compliance with screening. This chapter will detail the various assays for HPV detection including the details of standard detection methods, new assays, and the potential for HPV testing to be used as primary cervical cancer screening.

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INTRODUCTION

The detection of cervical cancer was revolutionized by the work of a Georgios Papanikolaou, for whom the Pap smear was named. His work, published in 1943 showed that squamous cells collected from the cervix could be utilized to detect cervical cancer and precancerous lesions (Wells, 1943). Since that time cervical cytology screening has brought about an approximately 75% decrease in the burden of cervical cancer (National Cancer Institute, 2005), and has been implemented as standard of care screening for reproductive age women.

Further research in the development of cervical cancer has revealed that human papillomavirus (HPV), a double-stranded DNA virus is the causative agent for the overwhelming majority of cervical cancers (Munoz, 2006). There are more than 30 recognized oncogenic strains of HPV, of which strains 16 and 18 are known to account for 70% of cervix cancers. After infection with HPV, viral DNA integrates into native cellular DNA, and early proteins products E6 and E7 lead to dysplasia and malignant transformation of the cervical cells. Though HPV is a ubiquitous infection with approximately 50% of women acquiring infection after 4 years of sexual activity, the prevalence of cervical dysplasia is much lower at 5-15%, showing that HPV infection is necessary but not sufficient for the development of cervical dysplasia. As such, the detection of HPV DNA as screening for cervical dysplasia has emerged as an important topic, for which new technologies and guidelines are being developed.

Though there have been many updates to Pap smear guidelines, the US Preventive Services Task Force (USPSTF) guidelines which are the most familiar to clinicians have been updated in 2012 to recommend the age of screening for cervical cancer to be 21, regardless of sexual history. The previous recommendation was to begin screening 3 years after sexual debut, but adequate evidence has shown screening in women younger than 21 does not reduce cervical cancer incidence and mortality (Moyer, 2012). This is secondary to the natural course of cervical cancer being a slow progression, and women under the age of 21 are exceedingly unlikely to have high grade dysplasia or overt malignancy, regardless of age of onset of intercourse. Additionally, many young women are likely to have complete resolution of low grade cervical intraepithelial neoplasia (CIN) and have higher rates of spontaneous resolution of even high grade dysplasia. Consequently fewer invasive excisional procedures are being performed in favor of continued surveillance to rule out progression. The current USPSTF recommendation for screening for cervical cancer in women ages 21 to 65 years is with cytology (Pap smear) every 3 years or, for women ages 30 to 65 years who want to lengthen the screening interval, screening with a combination of cytology and human papillomavirus (HPV) testing every 5 years with a grade A Recommendation (Moyer 2012).

There currently is as USPSTF recommendation against screening for cervical cancer using HPV DNA tests in women younger than age 30, commenting that “there is adequate evidence that the harms of HPV testing (alone or in combination with cytology) in woman younger than age 30 are moderate”. Referral for colposcopy is universally agreed upon for pap results of high-grade squamous intraepithelial lesions (HSIL) as well as atypical squamous cells , cannot rule out high grade (ASC-H) and atypical glandular cells (AGC) Pap test results. However it was not as apparent for low-grade squamous intraepithelial lesions

(LSIL) or atypical squamous cells of undetermined significance (ASCUS) whether colposcopy was indicated.

Population	Women ages 21 to 65	Women ages 30 to 65	Women younger than age 21	Women older than age 65 who have had adequate prior screening and are not high risk	Women after hysterectomy with removal of the cervix and with no history of high-grade precancer or cervical cancer	Women younger than age 30
Recommendation	Screen with cytology (Pap smear) every 3 years. Grade: A	Screen with cytology every 3 years or co-testing (cytology/HPV testing) every 5 years Grade: A	Do not screen. Grade: D	Do not screen. Grade: D	Do not screen. Grade: D	Do not screen with HPV testing (alone or with cytology) Grade: D

Figure 1. Current USPSTF recommendations for cervical cancer screening.

The National Cancer Institute initiated the ASCUS-LSIL Triage Study (ALTS) trial, the results of which provided evidence of a benefit to the use of HPV testing in patients with equivocal cytology (Solomon, 2003).Women were referred to 1 of 4 centers if they were greater than 18 years old and had either an ASCUS or LSIL pap smear result, and were subsequently triaged to immediate referral to colposcopy at enrollment, testing for HPV DNA (and cytology at an HSIL threshold) to triage to colposcopy, or follow-up with cytology only, using a referral threshold of HSIL. All women were followed up every 6 months for 2 years with pelvic examinations, cytology, masked HPV testing, and masked cervicography. It was found that HPV DNA testing for LSIL pap smears was not useful secondary to the overwhelming positivity in this group. However testing for cancer-associated HPV DNA in women with ASCUS Pap smears identified 96% (95% confidence interval [CI] = 92%–99%) of women with CIN 3+, while referring only 56% of the population to colposcopy, making this a effective triage tool to prevent unnecessary costs and morbidity associated with colposcopy. Currently the American Society for Colposcopy and Cervical Pathology (ASCCP) does make recommendation that “reflex” testing of the initial liquid based sample for high risk strains of HPV be utilized when cytology result is atypical squamous cells of undetermined significance (Wright et al., 2006). Women with ASCUS Pap tests and who are positive for high risk HPV should subsequently be triaged to colposcopy in addition to all women with LSIL Pap tests. Since this recommendation was made, the technology and options for HPV DNA detection have exploded with many new tests available. In fact, most clinicians do not know exactly which tests their labs are using and what the differences are between these methodologies. These differences may ultimately affect how the patient is counseled and treated after a positive high risk HPV result.

HPV Detection Methodologies

According to the College of American Pathologists, there are currently five FDA-approved assays to detect high-risk HPV. The first of the HPV DNA tests which became

approved in the United States for co-screening with cytology was the Hybrid Capture 2 test (HC2: QUIAGEN, In; Valencia, CA). This uses a pooled probe set for 13 oncogenic HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) but did not distinguish the individual HPV type present.

The technology utilizes nucleic acid hybridization, where the specimens containing the target DNA are mixed with a specific HPV RNA probe for the above types. The resultant DNA:RNA hybrids are captured on a microplate coated with antibodies specific to the hybrids. After signal detection with antibodies conjugated with alkaline phosphatase and substrate, the emitted light is measured in a luminomoteter as relative light units (RLU). A positive HR HPV classification is an RLU reading greater than 1.0, which is equivalent to 1 pg HPV DNA/mL per the manufacturer (Soderlund-Strand et al., 2005). As is true with other screening tests, the sensitivity and specificity may be altered by the cut-off value for which the test is considered to be positive, however generally the RLU reading of 1.0 is used to determine positivity. The test can be run from PreservCyt solution specimens in which a liquid based pap smear is collected, which can be stored for up to three months at temperatures of 2–30°C, allowing for testing after cytological diagnosis is able to be completed from the same sample.

HPV DNA detection methods have been expanded to utilize PCR, most often using the primer pairs GP5+/GP6+, which consists of a fixed nucleotide sequence for each primer. This method detects a wide range of HPV types by using a lowered annealing temperature during PCR. Additionally, MY09/11 and their derivatives PGMY09/11, which are synthesized with several degenerate nucleotides in each primer and is thus a mixture of 25 primers, are capable of amplifying a wide spectrum of HPV types. In a comparison of clinical sensitivity for post-treatment follow-up of cervical intraepithelial neoplasia, Soderlund-Strand et al. (2005) found substantial concordance between PCR methods using GP5+/GP6+ primers and the HCII. Patients were tested using both methods for HPV after having atypical vaginal smears. High grade disease was treated with excision and patients were retested for HPV 4-6 months after conization. The pretreated correlation of HCII and PCR gave a kappa value of 0.70, post-treatment kappa value of 0.72, which correlates to good or moderate agreement. It should be noted that specific HPV DNA strains may be better detected by the different primers respectively, the most marked being the ability of MY-PCR to amplify HPV-35 and GP1-PCR to amplify HPV types 53 and 61 (Qu, 1997). These small differences may have impacts for the tests which are utilized geographically relative to the epidemiology of HPV strains.

In a comparison of the MY09/11 and GP+ primers, the authors concluded that though both primer sets had very high reproducibility, the MY-PCR primer set was more robust than the GP+-PCR primer set in the amplification of multiple HPV DNA types within a given sample, giving higher utility in detection of multiple HPV strain infections.

The Cervista HPV HR test kit (Hologic, In; Marlborough, MA) is an in vitro diagnostic test for the qualitative detection of high-risk HPV DNA from 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 - a genotype not detected using HCII, and 68). It is used for similar indications with equivocal pap smears or in women over age 30. It is a signal amplification method for detecting specific nucleic acid sequences in which a secondary reaction producing fluorescence yields a positive result. Due to the utilization of only common laboratory equipment, this method requires less laboratory space than a PCR-based method, which requires separate preamplification and postamplification areas.

Additionally the Cervista HPV HR has an internal control and software to measure technician reproducibility, meant to minimize contamination and allow for improved reliability. Cervista requires a 2mL sample volume for test performance, reducing the potential of insufficient sample and false negative results. This method was clinically validated in a multicenter trial in which samples were tested from PreservCyt medium (Einstein et al., 2010).

Screening was performed using traditional cytology and those with ASCUS pap smears then underwent testing with Cervista and colposcopy with biopsy.

There were 1347 subjects for whom cytology, colposcopy, HR HPV, and histology were completed. The sensitivity and negative predictive value for detecting CIN2 or greater was 92.8% and 99.1% respectively. For detection of CIN3 or greater this improved to 100% and 100% respectively. Similarly, Zhao et al. (2012) used the Cervista HPV HR test kit for cervical cancer screening in a Chinese population and concluded its clinical sensitivity and negative predictive value are consistent with that of PCR/sequencing using GP5+/GP6+ primers. The Cervista was used for similar indications in equivocal pap smears and was found to have a 98.5% sensitivity, 68.21% specificity, 30.68% positive predictive value, and 99.69% negative predictive value for detection of CIN2 or greater.

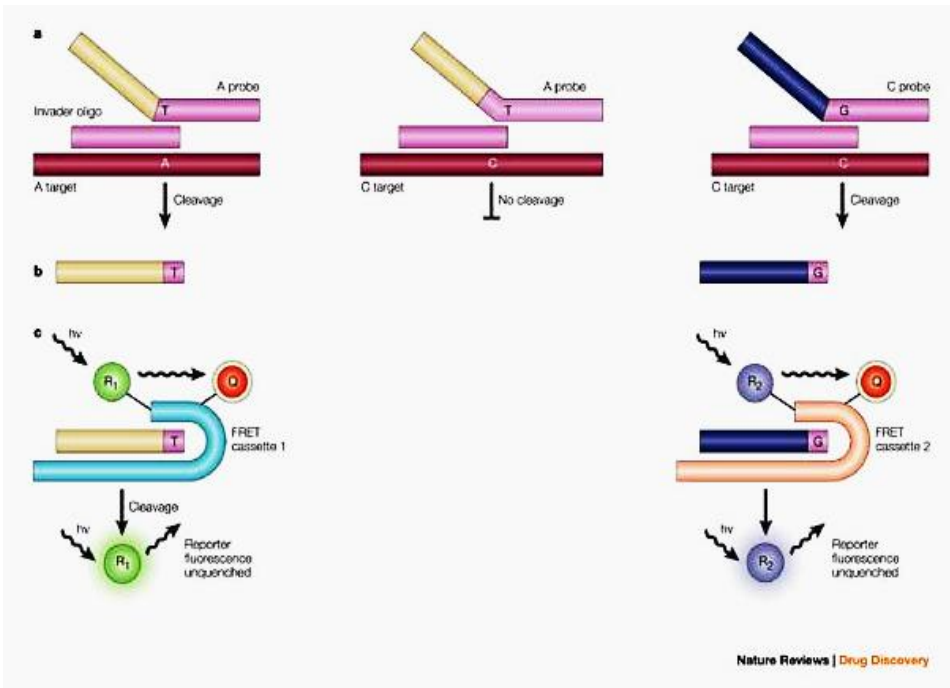

Figure 2. Schematic representation of single-base discrimination and detection with the Invader DNA assay, used in Cervista. a | Hybridization between the Invader oligo and the probe to complementary target DNA forms an invasive structure from the single-base overlap generated. No invasive structure is formed when a mismatch between the probe and target DNA prevents formation of the single-base overlap. b | For biplex detection, the 5' flaps cleaved in a primary reaction serve as Invader oligos in a secondary fluorescence resonance energy transfer (FRET) reaction, in which specific cleavage of multiple FRET cassettes results in the generation of fluorescence signal (c). The primary and secondary Invader reactions occur simultaneously.

This high negative predictive value should give the clinician comfort to not miss true high grade cervical disease if using Cervista for screening. Cervista also has the unique capacity to further test for the specific 16/18 strains of high risk HPV using the Cervista HPV 16/18 tests, similarly using invader chemistry. This has the advantage of identifying those strains responsible for the majority of cervical dysplasia, yielding increased specificity. For detection of CIN 2 or greater, Einstein et al. (2010) found a sensitivity of 68.8% (56.6-78.8) and specificity of 69.3% (65.7-72.6). The manufacturers comment that Cervista HPV 16/18 is not meant to be primary screening and should be interpreted in the context of cytology and Cervista HPV HR results.

In effort to make HPV DNA testing more targeted toward detecting persistent infection and dysplasia, a new method of testing has been developed to recognize messenger ribonucleic acids from the viral oncoproteins E6 and E7.

These proteins are responsible for the integration of the HPV DNA with the native DNA and subsequent malignant transformation. This test, called the APTIMA HPV assay (Gen-Probe Inc, San Diego, CA) can be done similarly from PreservCyt liquid based screening medium. Using an isothermal target amplification assay using transcription-mediated amplification, the mRNA of 14 high risk HPV genotypes can be detected.

In a prospective, multicenter US clinical study known as the CLEAR (Clinical Evaluation of APTIMA mRNA) study, Stoler et al. (2013) compared the APTIMA with HC2 to assess sensitivity and specificity for detecting CIN2 or greater. 865 women with ASCUS pap smears underwent testing with both the APTIMA and HC2 methods, and colposcopy was performed with random biopsies in 4 quadrants as well as endocervical curettage.

The results showed that for all specimens, the sensitivity of AHPV and HC2 was not statistically significantly different for detection of CIN2 or greater (86.5% vs 88.8%, $p>0.05$) or CIN3 or greater (89.7% vs 92.3%).

The specificity, however, was significantly higher for AHPV vs HC2 for detection of CIN2 or worse (62.6% vs 55.8%, $p<0.0001$) and CIN3 or worse (59.8% vs 53.3%, $p<0.0001$). The authors conclude that this increased specificity would translate into sparing 70 women out of 1000 with an ASCUS pap smear from having an unnecessary colposcopy and biopsy if AHPV were used in place of the standard HC2.

The most recent US FDA approved test for high risk HPV currently in use is the cobas 4800 test (c4800; Roche Molecular Diagnostics, Mississauga, Ontario, Canada). This test utilizes DNA extraction, PCR amplification, and real-time detection in an automated fashion. Detection of the human β-globin gene is used as a control, to monitor the specimen's cellularity. Cobas offers the advantage of detecting multiple types of HR HPV and indicating whether genotypes 16 or 18 are present. In a comparison with HC2 in 466 samples, it was found that cobas 4800 agreement with HC2 was 93.8% (kappa = 0.87, CI 0.83-0.92) (Wong et al., 2011).

All samples were also tested with linear array for comparison, which showed that cobas 4800 sensitivity for identifying genotypes 16 and 18 was decreased when there was co-infection with many strains. Fourteen samples were identified to have HR HPV strains other than 16 and 18 by the cobas 4800 but were found to have co-infection with 16/18 when tested by linear array.

This finding, may not carry clinical significance given the majority of referrals for colposcopy are based qualitatively only on presence or absence of any HR HPV strain

without specificity to genotype, but in rare clinical situations, has potential to influence triage of a patient to colposcopy or observation.

An additional new HPV-DNA test has been designed for use in low-resource settings, utilizing signal-amplification technology but has not been implemented in the US. Designated the *care*HPV test (QIAGEN, Gaithersburg, MD, US), this test detects target HPV-DNA from 14 carcinogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). It requires only 25x50 cm bench-top work space, does not require electricity or running water, and can be done by a technologist without significant training, taking approximately 2.5 hours to yield results (Qiao et al., 2008). The clinical validation of this test is discussed elsewhere in the chapter.

	HC2 (SZAREWSKI ET AL, 2012)	Cervista (EINSTEIN ET AL, 2010)	Cobas (SZAREWSKI ET AL, 2012)	Aptima (STOLER ET AL, 2013)	CareHPV (QIAO ET AL, 2008)
Technique	Nucleic acid hybridization	Invader technology	PCR amplification	Target amplification assay for mRNA	Signal-amplification
Sensitivity ≥ for CIN2	96.3% (93.8-98.0)	92.8% (84.1-96.9)	95.2% (92.5-97.2)	93.3% (84.1–97.4%)	84.3% (75.8-92.8)
Specificity ≥ for CIN2	19.5% (16.7-22.6)	44.2% (41.5-46.9)	24.0 (20.9-27.2)	61.5% (58.3-64.7%)	87.5% (86.1-88.8)
Approximate Cost	$100	$50	$50	$75	$4
HPV 16/18 testing available	No	Yes	Yes	No	No

Figure 3. Showing various methods of HPV detection with sensitivity and specificity for detecting high grade cervical disease, cost, and capacity to test for HPV 16 and 18 strains.

HPV IN PRIMARY SCREENING

There are promising studies that HPV testing can be used as effective primary screening methods in detection of cervical dysplasia and cancer. One such study, performed in a Canadian population by Mayrand et al. (2007) randomized over 10,000 women for primary screening using Pap or HPV as initial screening and referral for colposcopy with positive screening. The sensitivity of HPV was noted to be 94.6 (84.2-100.0) vs 55.4 (33.6-77.2) for cytology in detecting high grade disease, CIN2 or greater, with similar specificity of 94.1% (93.4-94.8) vs 96.8% (96.3-97.3%) for Pap smear. Naucler et al. (2007) tested 12,527 Swedish women, 32 to 38 years of age who were randomly assigned to HPV and Pap vs Pap testing alone with colposcopy for abnormal pap results. Women with positive HPV testing and normal pap were rescreened at 1 year with HPV test and those with persistent high risk HPV underwent colposcopy and subsequent treatment. At 4 years of follow up it was noted that women who had the addition of HPV testing to Pap smears had reduced incidence of high

grade CIN or cancer detected by subsequent screening, giving credence to longer testing intervals with HPV co-testing. A similar study in the Netherlands of 8575 women ages 29-56 years of age showed a 70% increase in the baseline detection of CIN3 or greater lesions (68/8575 vs 40/8580) and subsequent 55% decrease in number of CIN3 or greater lesions during average 6.5 years follow up (24/8413 vs 54/8456) (Bulkmans et al., 2007). These findings agree with the assertion that co-testing can be utilized to lengthen the interval between screening for cervical cancer. In addition, this improves the lead time for diagnosis with more preinvasive disease diagnosed prior to progression to cancer.

Primary screening for cervical dysplasia using HPV DNA does pose dilemmas. Firstly, it is well known that HPV infection is transient in most women, giving HPV positivity a low specificity in most populations.

In the currently accepted US triage system, primary screening with cytology without HPV testing prevents the unnecessary invasive and expensive biopsies which would result from colposcopy for transient HPV infection. For this reason, proponents of HPV testing as primary screening for cervical cancer advocate for screening only women above the age of 30 or 35, in which a positive result is more likely to signify persistent infection. Colposcopy in all women who are confirmed HPV positive would also be a poor allocation of resources, given the poor positive predictive value of positive HPV testing. As shown in the studies above, a triage method in which a patient with a positive HPV test is then triaged to have the cytology specimen analyzed would result in fewer colposcopy referrals than with the current system of pap only or pap with HPV co-testing. New markers are also being tested as secondary testing to identify those women who require colposcopy.

HPV TESTING IN THE DEVELOPING WORLD

While improved detection of HPV in the developed world does allow for more sensitive means to prevent cervical cancer deaths in an appropriately screened population, the majority of cervical cancers arise in populations that do not have access to proper screening. While screening with cytology has proved beneficial in a consistently screened population, in a population without access to care, a one-time cytology screen is very unlikely to have benefit.

Additionally, interpreting cytology is a time-intensive process which requires a trained clinician andrarely is available in these settings. There have been attempts to implement visual inspection with acetic acid (VIA) in many rural areas both in the US and around the world as one-time screening with poor results. Generally, the process is very time consuming with poor sensitivity overall for detection of cancer and dysplasia.

Recently attention is being shifted to evaluate screening for HPV using molecular methods as primary cervical cancer screening in populations who do not have access to traditional Pap smear screening, with some promising results.

There is significant evidence that in developing countries, screening using HPV DNA can significantly impact mortality from cervical cancer (Sankaranarayanan, 2009). In a study of previously unscreened women in the Osmanabad district in the state of Maharashtra, India, women were randomized to screening by HPV testing using HC2, cytologic testing, VIA, or standard care, which consisted of education regarding causes of cervical cancer, but no offering of screening.

A total of 131,746 women were included between January 2000 and April 2003. Incidence of CIN2 or greater were noted to be similar for all groups, but the cumulative incidence of advanced cervical cancer and the cumulative risk of death were lower in the HPV-testing group than in the control group with a death rate hazard ratio of 0.52 (0.33-0.83). By contrast, there was no significant reduction in the mortality rate in either the cytologic-testing group or the VIA group, as compared with the control group, which the authors attribute to an increased sensitivity of HPV testing to detect lesions with high potential for malignant transformation.

In a comparison of the *care*HPV test, a signal amplification test for HPV DNA in an area of rural China, Qiao et al. (2008) demonstrated that similar predictive value can be achieved from self-swab and provider obtained cervical swabs.

The *care*HPV test is unique in that it provides results within 2.5 hours, costs approximately one tenth the cost of HC2 testing, and does not require significant training for a technician to become competent using the test. A total of 2388 women between the ages of 30 and 54 were tested using both self-obtained vaginal swabs, provider obtained swabs, HC2 cervical swabs, liquid-based cytology, visual inspection with acetic acid and colposcopy with directed biopsy and endocervical curettage as appropriate by a gynecologist. *care*HPV is similar to HC2 in that relative light units (RLU) are arbitrarily set to designate the threshold of positivity. The authors found sensitivity for detection of CIN2 or greater on histology when a 0.5 RLU cut-off ratio was used to be 81.4% (72.3-90.5, 95% CI), with a negative predictive value of 99.3% (99.0-99.7).

This is similar to the values for sensitivity from provider collected cervical specimens of the *care*HPV; sensitivity 90.0 (83.0-97.0, 95% CI), negative predictive value 99.6 (99.4-99.9, 95% CI). However, the sensitivity was lower than for matched HC2 cervical specimens (97.1 [93.2-100.0, 95% CI]).

The main thrust of the study was to show performance of *care*HPV in self-obtained swabs for optimizing screening high risk populations in low-resource public-health settings, given its known advantages over other HPV self-testing. The tests were done by inexperienced, newly trained, minimally educated technicians on site under suboptimum conditions of temperature, humidity, lighting, and space.

However the negative predictive value for high grade dysplasia as determined by colposcopy directed biopsy by a trained gynecologist remained very high, lending validity to self-swab of *care*HPV test as primary screening for cervical cancer.

ALTERNATIVE TESTING METHODS

Due to the increased sensitivity of HPV DNA testing, it may not be required for clinicians to collect the samples from patients through the traditional speculum exam. To this end alternative collection methods including urine-based testing, self swab or cervico-vaginal lavage have been tested. These alternative methods may spare patients an invasive exam, but additionally have the advantage of allowing increased sample collection, widening the screening base due to requiring only very limited ancillary instrumentation and access to providers.

Urine based PCR testing has become established as standard in detection of sexually transmitted infections including *Neisseria gonorrhea* and *Chlamydia trachomatis* due to its high sensitivity. Similarly, a first voided sample can be used for urine testing for high risk HPV strains. Recent testing of urine screening of HIV-infected women with PCR-based methods for HPV detection showed good correlation with cervical specimens (Tanzi, 2013). First voided urine specimens as well as paired cervical specimens and were tested with PCR-based methods utilizing the MY09/11 primers in 107 HIV-infected woman. HPV DNA was detected in 64.5% of both specimens, with a very high concordance rate (k=0.96; 95% CI 0.90-1.0).

While this represents only a small sample size in which prevalence of HPV is higher than general population, this represents promise of future direction of screening.

Of note, urine specimens do require processing using centrifugation, amplification and electrophoresis, requiring equipment costs and technicians trained in their use. Despite notable obstacles, these are fewer than with traditional cytological screening.

Other methods for which screening for cervical dysplasia have been proposed include testing for high risk HPV DNA with self-inserted tampon, vaginal Dacron or cotton swab, cytobrush, or self-administered cervico-vaginal lavage.

These would have the benefit of not requiring an uncomfortable provider performed examination and allowing for screening a population outside the clinical setting and samples which could be preserved for later testing. A recent meta-analysis of 18 studies in which self-sampling was compared to physician sampling in a prospective, observer-blinded manner, the average detection rate was 27.4% (95% CI, 26.2-28.6) for self-sampling and 28.0% (95% CI, 26.8-29.1) for physician collected samples (Petignat, 2007).

Overall agreement between sampling was good for detection of HR HPV (kappa=0.66 (95% CI, 0.50 -0.82)). Notably, the lowest detection rates were noted with cervicovaginal lavage. Of these studies, six included patient preference and showed, not surprisingly, the vast majority of women preferred self-sampling.

In a large study in China, self-sampling of HPV DNA using the HC2 test was compared to liquid based cytology and visual inspection with acetic acid to determine its clinical parameters (Zhao et al., 2012). Greater than 13,000 women were tested using self-swab, physician collected swab, liquid based cytology, visual inspection with acetic acid, and colposcopy if indicated by a positive screening test.

The sensitivity in detection of CIN2 or greater was lower with self-HPV compared with physician-HPV, 86.2% (82.9 to 89.1%) versus 97.0% (95.2 to 98.3%), however was noted to be greater than the sensitivity of liquid based cytology of 80.7% (77.0 to 84.0%). The specificity of self-HPV was lower than physician-HPV or liquid based cytology, 80.7% (75.6 to 85.8) versus 82.7% (78.4 to 87.0%) versus 94.0% (92.2 to 95.8%) respectively.

Notable in this study was the decrease of specificity of HPV testing with patient age, which differs from prior studies performed in a US population. This is attributed to variance in HPV prevalence, peaking in young- and middle-aged women in China contrasted with peaking only in young women in the United States. This could be extrapolated to an increased specificity in older women screened with HPV DNA testing in the US.

Also notable in this study was the limitation that participants were instructed by medical professionals on self-sampling technique prior to collecting samples. Though there were virtually no women in whom the self-collected sample was insufficient, without instruction these results may vary.

Conflicting data have been published regarding whether unsupervised sample collection with only written instruction may yield similar reliability.

NEW TECHNOLOGIES

A newly investigated marker which has been identified as being over-expressed in cervical cancer cell lines is p16-INK4A. This cyclin-dependent kinase inhibitor is usually expressed in low concentrations in healthy cells, but is overexpressed through mechanisms involving the expression of high-risk HPV E7 oncoprotein (Carozzi et al., 2008).

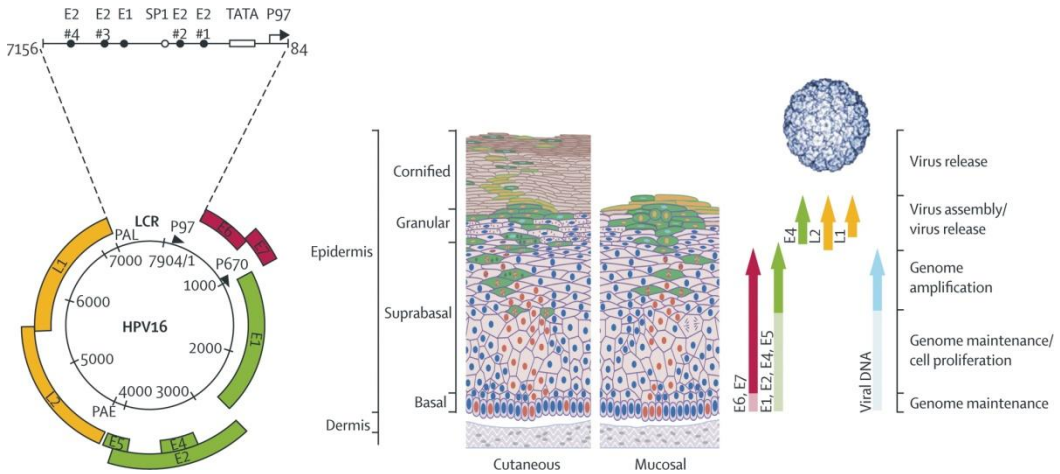

Figure 4. Showing the HPV genome and its expression within the epithelium. The viral genome is maintained at the basal layer of the epithelium, where HPV infection is established. Early proteins are expressed at low levels for genome maintenance (raising the possibility of a latent state) and cell proliferation. As the basal epithelial cells differentiate, the viral life cycle goes through success stages of genome amplification, virus assembly, and virus release, with a concomitant shift in expression patterns from early genes to late genes.

The theory of increasing specificity of screening by incorporating p16 testing was evaluated in a nested substudy of the New Technologies for Cervical Cancer screening (NTCC) working group. In this multicenter Italian trial, women who were screened positive for HPV were further testing with p16 immunohistochemistry. Residual thin-prep fluid was prepared using centrifugation, fixed and immunostained with p16-INK4A-specific monoclonal antibodies. A total of 1137 women had valid p16 testing, and using differing definitions of positive testing, the sensitivity and specificity for CIN2 or greater was calculated to be 88% (80-94, 95%CI) and 61% (57-64, 95% CI) respectively when greater than 1 cell stained was taken as positive, 61% (50-71, 95%CI) and 79% (76-83%, 95%CI) when equal to or greater than 5 cells stained was taken as positive, and 38% (28-49%, 95%CI) and 91% (89-94%, 95%CI) when equal to or greater than 10 cells stained was taken as positive. Importantly, this was able to be further subcategorized by age, and it was noted in the 25-34 yo age group to maintain similar clinical utility with sensitivity and specificity of 36% (23-50%, 95%CI) and 94% (91-97%, 95%CI) when equal or greater than 10 cells stained was taken to be positive. Given this high specificity in an age group in which HPV

infection may be transient or persistent, the utilization of p16 screening may have significant benefit as adjunct testing to HPV testing as primary screening. This may avoid colposcopy and biopsy in many young patients who may be better served with only retesting of HPV to evaluate for persistence of infection.

CONCLUSION

While there have been many improvements of detection of HPV DNA infection, there is much remains to be validated in clinical practice. Currently it is agreed that persistence of high-risk HPV strains in women over 30 warrants further testing and closer screening in the US population. Women between age 21 and 30, HPV DNA testing is recommended only as secondary screening given its poor specificity in this population. New technologies which have been developed to aid in cervical cancer screening in resource-poor areas have shown promise, including with self-collecting samples. Though the sensitivity may not be as high as provider collected samples in resource rich areas, for women who may have only one opportunity for screening, these options are much better than being unscreened. Many questions remain regarding best practice and new tests to improve the specificity of HPV testing, making recommendations likely to be continually updated.

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Chapter 11

THE TRIAD HPV-ORAL SEX-ORAL CANCER: LAY PUBLIC AND DENTAL PROFESSIONALS' UNDERSTANDING

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ABSTRACT

Human papillomavirus (HPV) remains a risk factor for ano-genital and cervical cancers and for more than a decade, emphasis has also been placed on the links between oral sex, HPV infection, and head and neck malignancies. However, the level of knowledge that dental professionals and lay people have in terms of HPV transmission through oral sex and oral cancer development remains unknown. In this chapter, we summarize the results from two major studies we conducted in the past five years in Vancouver, Canada: one in a format of a consensus-building community forum gathering researchers, clinicians, and the community to discuss these issues, and another in a format of a knowledge-based survey questionnaire distributed at not-for-profit health organizations and dental schools. Although recent attention has been given to the potential links between HPV infection and oral cancer, such links remain mostly unknown by the public, and HPV vaccines are yet to be proven efficacious in also preventing oral malignancies. Physicians and dentists could discuss oral sex practices to raise awareness with their patients.

1. INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide, and much like Human Immunodeficiency virus (HIV), the spread of HPV and its impact on individuals and populations has been influenced by social, economic and political factors as well as biological conditions [1]. Conservative projections estimate that about 75% of all sexually active men and women are infected, and although asymptomatic, less than one-

third of them have heard about HPV [2]. Although there are more than 100 different strains, HPV 16 and 18 have been linked to anogenital carcinomas and have also been associated with oropharyngeal carcinomas for more than a decade [3, 4, 5, 6]. Since the HPV virus is easily transmitted through skin/mucosal contact, oral sex has been identified as a potential vehicle for transmission from the ano-genital area to the mouth [6, 7, 8]. Although practiced by people of all genders and sexual orientations and considered a low risk sexual activity when compared to unprotected vaginal and anal intercourse [9, 10], oral sex can also transmit pathogens of gonorrhoea and Chlamydia [11, 12]. It remains difficult to demonstrate, however, the extent to which oral sex might constitute a route for spreading HPV [13, 14] and be a potential risk factor for the development of multifactorial diseases such as oral cancer [15, 16]. There is some evidence suggesting that individuals with frequent oral sexual encounters, a greater number of different sexual partners, and earlier sexual experiences are at higher risk for oral cancer development [9, 10]. In fact, patients who have had prior HPV infection are 32 times more likely to develop oral malignancies than those who have not. This likelihood is much higher than other well know and frequently discussed risk factors: chronic alcohol consumption and tobacco smoking increase the risk of oral cancer by about 2.5 and 3 times, respectively [7]. Oral cancer has high mortality and morbidity rates and its progress and surgical invasive treatment significantly impacts the quality of life both physically and psychosocially [18]. Although in the U.S. between 12,000 and 15,000 cases of oral cancer are diagnosed every year and over 3,000 are diagnosed in Canada in the same period, oral cancer has a low prevalence (~2%) when compared to all types of cancer and yet, is a major cause of premature death in Canada [3], Australia [5], and the U.S. [17] More than half of the 650,000 patients who receive a diagnosis of head and neck cancer each year worldwide will die within 12 months of initial diagnosis (Table 1) [18].

Table 1. Types of oral cancer by numbers and survival rates in Canada*

Types of oral cancer	Number of new cases (total of 3.061)	5-Year survival rate (in percentage)
Lip and tongue	1.107	53 (tongue) to 94 (lip)
Gums	672	60
Floor of the mouth	345	53
Tonsil and Oropharynx	499	50
Salivary Gland	438	74

*Adopted from British Columbia Cancer Agency in 2008.

When considered in general terms, the overall ratio of males to females over 40 years with oral malignancies is 1:1 [19], however, the incidence of oral cancer is rising among young individuals between the ages of 20 and 39 [2, 5]. Since HPV16 and 18 are the most predominant strain in anogenital carcinomas, its transmission to the oropharyngeal region is likely to occur through the mouth (e.g., tongue and lips), particularly during oral sexual practices [20]. However, such supposition does not exist without controversy among the scientific, dental professional and lay communities [21, 22]. Moreover, the level of knowledge that these communities hold in regards to the links between HPV inflectional and oral cancer development through oral sex remains unknown at best, and ignored at worse. In this chapter we summarise the results from two major studies conducted in the past five years

in Vancouver, Canada, while refering to the following publications to support our arguments: 1) Brondani MA, Cruz-Cabrera MA, Colombe C. Oral sex and oral cancer in the context of Human Papillomavirus infection: lay public understanding. Oncology Reviews 2010; 4(3): 171-176; 2) Brondani MA. Cafe discussions on oral sex, oral cancer and HPV infection: Summative report. Archives of Sexual Behavior 2010, 39: 1453-1455; and 3) Brondani MA. HPV, oral sex and the risk for oral cancer: food for thought. Special Care in Dentistry 2008; 28(5):183-4.

The studies we present were conducted in the format of two knowledge-based survey questionnaires distributed at not-for-profit health organizations (Study 1A) [23] and at a dental school (Study 1B), and in the format of two consensus-building community forums emulating a 'Café Scintifique' gathering researchers, clinicians, and the community discussing these issues [16]. We conclude the chapter by highlighting the major points gathered from these studies.

1. Study 1A - Lay public's Perspectives and Knowledge

Ethical approval was obtained through UBC Researcher Information Services (RISe) H07-3121. A brief questionnaire was developed with multiple choice questions with inquiries including:

1) perceived risk of transmission of HPV, HIV and other STIs through oral sex ('no risk', 'low risk', 'moderate risk', 'high risk' and 'don't know');
2) frequency of oral sex activities for the past month ('daily', 'up to 3 times a week', 'once a week', 'a few times per month', 'once a month', 'rarely');
3) number of different oral sex partners for the past month[1] ('my partner only', 'a person other than my partner', 'two to three different persons', 'four to five different persons', and 'more than five different persons).

The questionnaire also contained YES/NO questions regarding the perceived risk of oral cancer development through oral sex; the use of protective measures while engaging in oral sex; and visits to a dentist and/or a physician over the past year. Two other questions investigated whether or not the respondent's physician and dentist have ever discussed the risks associated with oral sex practices. Age and sexual orientation (e.g., queer and straight) were asked for demographic purposes. The questionnaire was meant to be self-completed anonymously by participants who were 19 years and older, in Vancouver, Canada. Two questionnaire sets were distributed. The first set comprised of 150 copies distributed to three local organizations that expressed interest in this study. Fifty questionnaires were sent electronically as an email attachment to members of Gayway, while 50 hardcopies were distributed at AIDS Vancouver and 50 at PRIDE UBC. The hard copies of the questionnaires were distributed with envelopes so that respondents could seal their complete questionnaires

[1] High risk participants were defined as those having four or more different oral sex partners for the last 30 days, whereas low risk included those with up to three different partners for the same period. High frequency of oral sex (three to five times a week in the past 30 days) increases nine times the risk for developing oral cancer.

prior to dropping them off in boxes placed at the two organizations' information desks. After 2 weeks, all hardcopies of the questionnaires (completed or not) were collected from AIDS Vancouver and PRIDE UBC. The electronic survey from Gayway was closed after 14 days (e.g., replies with the questionnaire received after 14 days were not considered for data analysis).

The second set comprised of 100 hard copies of the questionnaire that was administered at a major event in Vancouver during the first Sunday of August, 2008: The Annual Vancouver Pride Parade. The attending public was invited to fill out the questionnaires voluntarily and anonymously as they passed by the assembled booth in which a research assistant was handing out the hard copies of the surveys. Surveys were gathered on the spot, as potential respondents filled them out and dropped them off in a box placed by the booth.

Since all the above locations are inclusive and do not limit their membership and services exclusively to queer men and women, two samples of respondents were sought: men and women who self-identified as queer and men and women who self-identified as straight. Data was analysed statistically for comparisons between age groups (19–30, 31–40, 41–50, >50) as younger generations, being more inclined to talk openly about sexual issues and having greater access to information from a variety of sources including peers, the Internet, news and the media would be more knowledgeable on the issues surveyed. We also compared gender (male, female) and sexual orientation (queer, straight) under the assumption that there would be some differences in the way people think and behave in regards to the issues in question.

2.1. Results

One hundred and sixty-one completed questionnaires were gathered: 61 electronically and 100 in hardcopy forms. One hundred and twenty-three respondents self-identified as gay (including gay, lesbian, bisexual and transgendered) and 38 as straight. Table 2 shows the 161 participants distributed according to age: between 19 and 30 years, 31 and 50 years, and 50 years and older. As per age distribution, PRIDE UBC had the highest percentage of younger participants (92% were between the ages of 19 and 30 years) compared to 56% at Gayway and 29% at AIDS Vancouver (data not shown).

Table 2. 161 participants according to age group and sexual identity [3]

Age Group	19-30	31-40	41-50	>50
Gay men	33	13	14	7
Gay women	17	2	2	2
Bisexual men/women	14	6	7	3
Transgendered mtf/ftn	2	0	0	1
Straight men	10	8	2	2
Straight women	10	4	0	2
	Number of Participants			
	<21-30	31-40	41-50	>50
	86 (55.5%)	33(19.2%)	25(14.1%)	17(11.5%)

* The large number of queer-identified participants was due to the selection of the venues rather than a representation of the sexual identity distribution in Vancouver, Canada.

Among the participants older than 51 years, 43% of queer-identified and 40% of straight-identified men reported having practiced oral sex exclusively with their current partners, whereas 83% of queer-identified and 90% of straight-identified women from all ages reported the same behaviour. For younger ages, these percentages tend to decrease, i.e., younger men and women of any sexual orientation reported having had more than one oral sex partner over the last 30 days.

The box below summarises the major findings of the two survey studies based on the age range of all participants, their perceived risk for the transmission of HIV, HPV and other pathogens, and for the development of oral cancer in the context of oral sex practices.

Results[iv] from 161 participants

- 105 participants accounted oral sex as an activity of *low* or *no-risk* for HIV transmission;
- 134 participants judged the same activity as *moderate* to *high risk* for other sexually transmitted infections including gonorrhoea and chlamydia;
- 44% of the respondents (71 participants in total and the majority being younger than 40 years-old) accounted oral sex as *no risk* whereas 45% *did not know* about the risks for oral cancer;
- 35% of the respondents believed that oral sex is an activity of either no risk or low risk for HPV transmission;
- 81% of the respondents were never asked about oral sex practices by a physician whereas 93% where never asked by a dentist;
- 65% of the respondents were at high risk for HPV infection.

[iv] No significant difference between the answers of the organizations or age group (tested separately) was found, which suggests that neither age nor organization influences the responses of the subjects.

When asked about the use of protection while engaging in oral sex activities (YES/NO answers), 89% of the participants reported that they did not use any. From the 12 participants (11%) who did use protective measures, seven stated that they avoided swallowing male or female fluids (sperm and vaginal secretion), three used condoms and rubber dams, and two did not specify the kind of protection used. All 161 participants reported having had a medical and a dental appointment at least once in the previous year. During these visits, 80% of the respondents were never asked about oral sex practices by their physicians. This percentage reaches 95% (152 participants) for those whose dentists never asked about the same practices.

Figure 1 (A and B) presents the beliefs of 161 participants about the risk of HPV infection through oral sex. Figure 1-A shows that 35% of all participants (57 respondents) regarded oral sex as an activity of low or no risk for HPV transmission, whereas 72% judged the same activity as moderate to high risk for HPV infection. None of the participants believed that oral sex was an activity of no risk for the transmission of other STIs (data not shown).

Figure 1-B takes a further look at the 57 participants who perceived oral sex as an activity of low or no risk for HPV transmission. Eighty-five percent (48 in total and the majority younger than 40 years) believed oral sex to be of no risk for oral cancer development whereas

15% did not know whether or not such risk existed. Among them, 74% perceived oral sex as a low or no risk activity for the transmission of HPV, yet these same respondents were mostly at high risk for HPV infection based on the number of different oral sexual partners and the frequency of engaging in oral sex during the past 30 days [9, 10]. None of the 57 participants reported using any form of protection while engaging in oral sex practices. ANOVA test showed that the answers for these questions in the four age categories did not vary significantly among the organizations and the Pride Parade event (p = 0.00085).

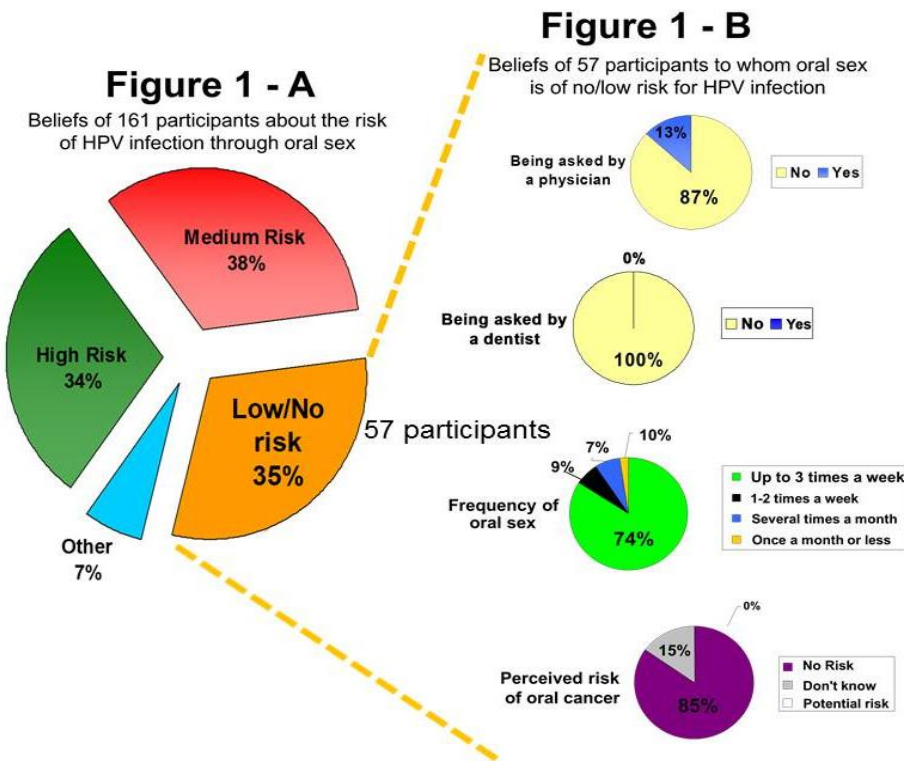

Figure 1. (A and B) – Beliefs of 161 participants about the risk of HPV infection through oral sex (A) and beliefs of 57 participants to whom oral sex was of no/low risk for HPV infection.

None of these 57 participants were asked by their dentists about oral sexual practices while only 13% were asked the same question by their physician. This is a small size study of 161 responses and the results should be interpreted with caution and not generalized at face value.

2.2. Discussion

From the 250 questionnaires distributed, 64% were returned fully completed (52 at Gayway, 31 at AIDS Vancouver, 27 at Pride UBC, and 50 during Vancouver Pride Parade). According to Bogen [24] and Edwards et al. [25] such a rate is considered good and might be explained by the relatively short length of the questionnaire and its easy administration

approach (e.g., electronic and dropped-off as opposed to mailed-in). Within the small sample size, the ANOVA test showed that the answers to most questions did not vary significantly between the organizations and the Pride event. This invariance did suggest that a shared general or common knowledge exists among these respondents who came from three different resource exchange and proactive health educational organizations. The predominance of younger participants might have biased the survey and a larger sample size is warranted to include middle-aged and older adults. The knowledge that oral sex is considered a low risk activity for the transmission of HIV and of moderate to high risk for other sexually transmitted infections found by Gair [26] was shared by 65% and 83% of all the respondents, respectively, independent of gender or sexual orientation; this disproved our assumption. However, more than one third of all respondents (35%) also believed oral sex to be of no or low risk for HPV transmission. Such a belief may be explained by the current lack of agreement as to whether or not HPV can be transmitted via oral sex even though, according to Mork et al. [13], such transmission might indeed occur. The fact that almost two-thirds of the respondents did not associate oral sex with oral cancer might be explained in part by the content of the question posed: 'For the development of oral cancer, do you think oral sex is a risk? (yes, no, don't know)', which did not mention HPV. Some respondents might have thought exclusively on the 'act' of oral sex in itself rather than on the possibility of HPV transmission. Either way, the belief that oral sex may not be a potential venue for HPV transmission and the fact that a quarter of the respondents did not know the risk of oral cancer development through oral sex underlines the gap between peer-reviewed information and lay public awareness at least for this limited number of participants. Knowledge transfer seems to fall short in terms of advising the community at large about studies linking HPV with some oral cancers genetically [3, 4, 20, 27]. It was only in 2008 that HPV became listed as a potential risk factor for oral malignancies on the websites of the Canadian Cancer Society [28], BC Cancer Agency [29], and the Australian Cancer Society [30], for example. The American Cancer Society has stated that 'the current view is that HPV may be a factor in the development of around 20–30% of oral and oropharyngeal cancers [31]'. HPV transmission through oral sex in the context of oral cancer appears to raise different views among different health professional organizations [38]. Eleven percent of all the participants took protective measures while engaging in oral sex, as was found by other studies [32]. For the majority of these respondents, the most common procedure described was to avoid swallowing male (sperm) or female fluids (vaginal secretion). However, such measures may not be adequate in preventing the spreading of HPV as skin-to-skin/mucosal contact still exists [15] since the virus may spread throughout the entire region between the legs and not necessarily only at the genital or anal area [8]. It seems that most people underestimate the potential risks associated with oral sex.

Data from this small study showed that for 35% of the respondents, there was a lack of awareness of the risk of HPV infection through oral sex independent of the age group, which again disproved our assumption even though the sample had a predominance of younger participants. This study among the lay public hoped to reinforce the need for educational awareness to the public who, once provided with consistent information, should be able to filter an array of available knowledge and be in better control of their health. Hence, this study also hoped to support earlier oral cancer screening and detection, and to bridge gaps between scientific and public knowledge. Since adults from any sexual orientation are sexually active in a variety of ways, including engaging in oral sex with multiple partners,

HPV infection and oral cancer remain a health concern. However, due to the convenient sampling strategy used and the relatively small sample size, the results of our study might be limited despite the great value of this emerging area of study. Further and more comprehensive survey studies are warranted. Moreover, the age distribution of this sample favoured younger participants which may further limit the inferences from the findings.

Given these results, we were interested in finding out what beliefs undergraduate dental students would hold about the triad HPV-Oral Sex-Oral cancer using the modified survey questionnaire. The main results are described below.

3. STUDY 1.B - DENTAL STUDENTS' PERSPECTIVES AND KNOWLEDGE

Between the winter of 2010 and the Spring of 2011, an 11-item questionnaire survey (Figure 2) was developed and applied to all four years of the University of British Columbia (UBC) undergraduate doctors of medicine and dentistry (DMD) curriculum. Ethical approval was obtained through UBC Researcher Information Services (RISe) H10-02516. Informed by the first study above, and the public forum below, and aware that as with any cancer, early detection and diagnosis is key, we believe that it remains important for patients to see an oral health care professional regularly, particularly if they are in the high-risk groups. However, we were also aware that these professionals might not be engaging with their patients to discuss risk factors for oral cancer beyond use tobacco and alcohol abuse. As a result, we developed this study in which the main objectives included:

- Identifying the knowledge of UBC undergraduate dental students;
- Comparing junior (years 1 and 2) versus senior (years 3 and 4) students' knowledge;
- Identifying comfort level of UBC dental students in talking to their patients about oral sex and oral cancer.

As health care practitioners, dentists are responsible to screen their patients for oral cancer and educate them on the associated risk factors. By surveying UBC dental students, we also intended to highlight the strengths and weaknesses of educating these future dental professionals regarding this topic, and thus possibly lead to changes in how their patients are educated about it.

Each DMD academic year, UBC enrols between 45 and 65 undergraduate dental students [33, 34]. We intended to survey all 4 years of DMD cohort voluntarily and anonymously. In order to increase enrolment, we distributed the survey at the end of the Professionalism and Community Service classes in which the author actively teaches. The author encouraged the students to fill out the survey and suggested they place the surveys on a corner desk at the lecture room, after which he left the room to avoid sentiments of cohersion. The author returned to the room after 30 minutes to collect the questionnaires.

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Awareness, education and comfort level of dental students regarding the association between oral sex and oral cancer in the context of Human Papillomavirus (HPV).

Dear Participant:

We are conducting a survey to gather information about knowledge and beliefs in the dental community regarding the association between oral sex and oral cancer in the context of HPV (human papillomavirus).

This survey is completely anonymous and will not take more than 10 minutes of your time to complete.

If you are willing to participate, we would ask you to complete the following questionnaire.
Thank you.

Your age:_____
Your gender:_____
Your year of Dental School:_____

Please choose one option only for each of the following questions:

1. For the transmission of HIV, do you think oral sex is an activity of:
( ) No risk
( ) Low risk
( ) Moderate risk
( ) High risk
( ) Other _____

2. For the transmission of other STIs (sexually transmitted infections) such as gonorrhoea, syphilis and chlamydia, do you think oral sex is an activity of:
( ) No risk
( ) Low risk
( ) Moderate risk
( ) High risk
( ) Other _____

3. For the transmission for Human Papillomavirus, do you think oral sex is an activity of:
( ) No risk
( ) Low risk
( ) Moderate risk
( ) High risk
( ) Other_____

4. Are you aware of the association between oral sex and oral cancer due to HPV transmission?
( ) No
( ) Yes
( ) Don't know

Figure 2. Continued on next page.

5. If you answered "yes" to question 4: Where did you get this knowledge from? (Choose all that apply):
( ) dental school lecture
( ) internet
( ) textbooks
( ) journal article
( ) other _____

6. Have you ever been asked about oral sex practices by your dentist?
( ) Yes
( ) No
( ) I don't go to the dentist

7. Have you ever been asked about oral sex practices by your doctor?
( ) Yes
( ) No
( ) I don't go to the doctor

8. As a dental student, how comfortable would you feel discussing the risks of oral sex, with respect to oral cancer, with your patients?
( ) very comfortable
( ) comfortable
( ) uncomfortable
( ) very uncomfortable
( ) don't know

9. Should dentists/dental students receive training regarding how to approach the topic of oral sex with their patients?
( ) yes
( ) no
( ) don't know

10. Do you think it's important for patients/community to be aware of oral sex as a risk factor for oral cancer?
( ) Yes
( ) No
( ) don't know
( ) other_____

11. What tools should be used to educate patients on this topic?
( ) discussion with physician
( ) discussion with dentist
( ) discussion with dental hygienist
( ) brochures
( ) no education necessary
( ) other _____

Thank you very much for your participation.

Figure 2. Survey questionnaire applied to DMD students at the University of British Columbia in 2010/11

3.1. Results and Discussion

One hundred and sixty-eight completed questionnaires were gathered: 32 from year one, 34 from year two, 51 from year three and 51 from year four of the UBC undergraduate dental cohort. All but three students self-identified as straight. As per age distribution given a

university undergraduate course, 92% of the participants were students between the ages of 19 and 30 years. Similarly to the survey involving lay participants, the majority of the students attributed oral sex as a low risk activity for the transmission of HIV (69% compared to 65% from the lay public), and moderate to high risk for the transmission of other STI's (87% compared to 83% from the lay public). Figure 3 shows the level of awareness about HPV as a risk factor for oral cancer development (question #4).

Awareness of the association between oral cancer and HPV infection via oral sexual activities tends to increase as students advance further in their dental education, yet cross-sectional observation. When asked about the source of information for such associations (question # 5), 36% of the students reported discussing this issue via lectures at the dental school, most of them being 4th year as when the issue of oral cancer is deeply addressed. In a much lower percentage compared to the lay public survey, less than 1% of the dental students were asked about their oral sexual activities by their dentist while 7% were asked about the same behaviour by their physicians.

Figure 4 shows the comfort level that students would feel in discussing oral sex as a risk factor for oral cancer development in regards to HPV transmission (question # 8). Female more than male students would feel 'uncomfortable' in talking about oral sexual practices as a risk factor for oral cancer development. When asked about question # 9 (proper training to discuss issues of sexual practices, including oral sex, with dental patients), the majority of dental students did believe that dentists should be trained accordingly in how to most effectively discuss this topic with patients. Judging from the comfort level expressed by the students, this training is lacking.

In fact, given the responses we got from the dental students, and the lack of awareness on the triad HPV-Oral Sex-Oral cancer we observed from the lay public survey, medical and dental professionals might have to find ways to discuss the potential risks of oral sex practices with their patients in the light of almost 20 years of investigation [35].

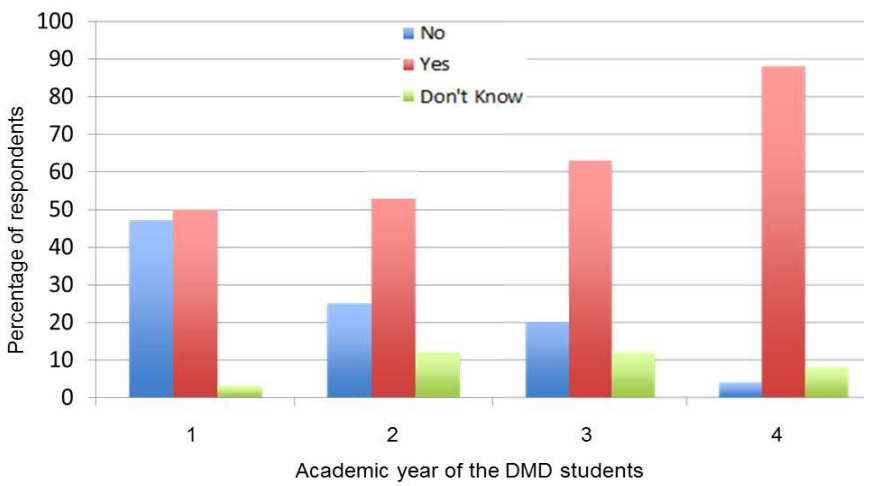

Figure 3. Awareness of the HPV infection via oral sex as a risk factor for the development of oral cancer.

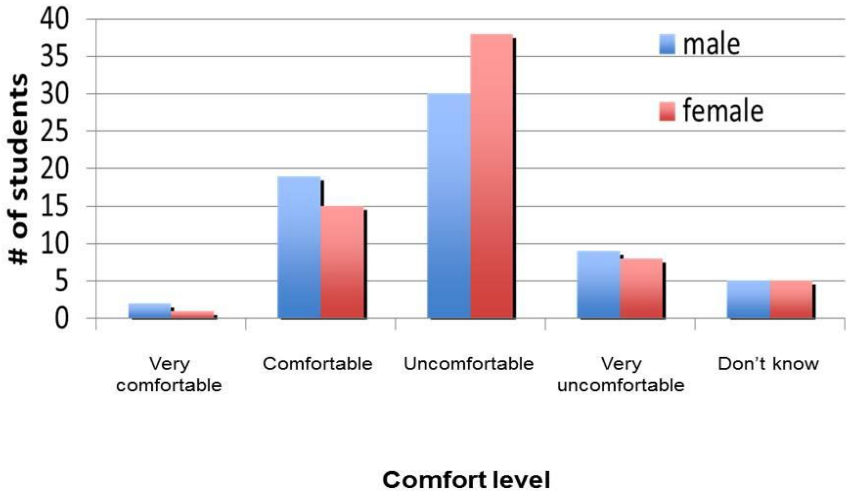

Figure 4. Comfortable level in discussing oral sexual practices as a risk factor for oral cancer development according to student gender.

There is reluctance from general health practitioners, as well as dental students, to ask their patients about sexual activities to the extent that only a few do inquire but they focus mostly in context of HIV prevention. This study does recognize that dentists may be reluctant to talk about oral sex practices for a variety of reasons: they may feel embarrassed, they may think that the issue is not relevant to 'their' patients, they may hold different beliefs as to 'whom they should address such a topic', or they may simply be unaware of the potential risks of oral sex. This study does not suggest that dentists, or any other health care professionals, should advise their patients to stop engaging in oral sexual activities.

4. PUBLIC FORUMS

Two Café Scientifique discussions sponsored by the Canadian Institutes of Health Research took place in Vancouver during the Fall of 2008, and gathered two panels of experts presenters with backgrounds in oral pathology, dentistry, oncology, social work, and community-based research who interacted with an audience of policy makers, health care administrators, sociologists, sexologists, pharmacists, clinical and social researchers, and graduate, undergraduate and high school students [36]. Both forums critically discussed four main areas related to the triad HPV-Oral Sex-Oral Cancer, including:

1) Oral malignancies: their incidence, morbidity, risk factors and screening programs. Within these broad areas, the following topics were discussed: transmission of HPV during oral sex and its potential association to oral cancer, as well as the roles of dental professionals on preventing HPV transmission.

2) Heterosexism (e.g., is a system of attitudes, bias, and discrimination in favor of opposite-sex sexuality and relationships) [37]. This topic was stressed as one of the potential reasons why health care providers avoid discussing sexuality/sexual practices and oral sex with their patients.
3) Belief systems. This topic was stressed under the current understanding of the triad HPV-oral sex-oral cancer from a community perspective through the results from the studies presented and discussed above.
4) Community-based research: This topic raised the values engaging the community in understanding sexual behavior, and its associated risks from their own perspectives.

After a summary of some of the available information on the HPV-related topics in general, presenters promoted a discussion to engage the audience. Each forum lasted for an average of 90 minutes and enrolled a total of 67 participants.

The forums stressed the need for fostering dialogue about the potential role of oral HPV infection to the oral health of individuals of all sexual orientations. Clearly there is a need for encouraging discussion between health professionals and the public about issues related to oral sex practices
and HPV transmission, which has been echoed in the above knowledge-based surveys. As we emphasized, the oral cavity is subject to harm from smoking, high sugar intake, and lack of oral hygiene and yet, we focus mainly on tobacco, dietary history and alcohol consumption when it comes to oral cancer risk factors, and avoid asking about oral sex practices. As such, oral sex can be a venue for HPV transmission and should be discussed accordingly since fewer than one-third of the sexually active individuals infected by HPV had actually heard about this virus [4], as we heard in our studies [16, 23].

The two forums outlined the need for opening our eyes about other potential risks for oral cancer beyond tobacco smoking and excessive alcohol consumption. Oral cancer has high mortality and morbidity, but still is a preventable disease [38].

The forum also briefly acknowledged the vaccination campaigns against HPV infection. In Canada, as well as elsewhere, public funded immunization strategies to prevent infection of four strains of HPV (e.g. Gardasil® from Merck Frosst Laboratories against strains 6, 11, 16 and 18, and CervarixTM from GlaxoSmithKline Laboratories against strains 16 and 18) have targeted females between the ages of 9 and 26 years, before the first sexual experience [39, 40]. The Canadian Guidelines on Sexually Transmitted Infections for prevention, diagnosis, treatment and management of STIs was updated in 2006 as an information resource for health care providers, educators and policy makers [41], which was followed by the 2008 Canadian Guidelines for Sexual Health Education, aimed at developing and improving sexual health education policies, programs and curricula to address the diverse needs of Canadians and to ensure that sexual health education is available to all Canadians [42]. It remains unknown the extent to which these guidelines can influence knowledge and enhance professional/patient communication.

5. Conclusion: From Here and Beyond

The study with the lay-public highlighted that:

- The general public seems to be unaware of the potential associations between oral sex practices and HPV infection for the development of oral malignancies;
- Physicians and dentists are not fully discussing general and oral health in relation to oral sex practices with their patients.

The study with the undergraduate dental students informed us that:

- Knowledge of the relationship between HPV transmission, oral sex, and oral cancer increases as students advance through the years of dental school;
- The majority of dental students are not comfortable discussing this topic with their patients but feel that they should be;
- Brochures are viewed as a useful tool to educate patients on this topic.

The two forums were successful for:

- Highlighting that although it remains difficult to prove that HPV can be transmitted through oral sex, such transmission is likely to occur;

Offering the opportunity for discussing scientific evidence in the realm of lay and professional public understanding.

In order for the ideas presented in this chapter to make a difference elsewhere, the points and issues brought up for discussion need to be disseminated widely and support further discussions, research, and knowledge transfer worldwide. This chapter emerges as another venue for dissemination, but it is up to the professionals and the public to determine and take the next steps to nurture open discussions of the potential implication of oral HPV infection and vaccine to sexually active individuals even though HPV-positive oral malignancies respond better to chemotherapy and chemoradiotherapy when compared to HPV-negative malignancies.

6. Support and Acknowledgment

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Chapter 12

PREVALENCE AND DISTRIBUTION OF HPV16, 18 AND 58 IN SOUTHEAST MEXICO

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ABSTRACT

HPV types 16 and 18 are considered responsible for 70% of cervical neoplasias worldwide. However, variations concerning the genotypes involved in cervical cancer and its precursor lesions in different geographic regions of the world have been reported. This fact could highly affect the impact of HPV vaccination, which mainly focuses on genotypes 16 and 18. Genotype 58 is the seventh most important in cervical cancer worldwide, but in the case of certain countries from Asia and Latin America, it is present with importantly higher frequencies. In Yucatán, southeast Mexico, our research group has found this genotype is highly prevalent in different populations, being as frequent as HPV16; contrary to this observation, HPV18 has been found with lower prevalence than reported worldwide.

This chapter describes the prevalence and distribution of genotypes 16, 18 and 58 in different populations, results from various projects carried out by our research group in Yucatan: women without cervical pathology attending for Pap smear, women with cervical lesions receiving attention in dysplasia clinics and anticancer center; obstetric patients attending for term pregnancies and for spontaneous abortion. In addition we have studied a population of incarcerated women from a social readaptation center prison. Finally, results from HPV58 and HPV16 genetic variability will be described, and perspectives for future research will be exposed.

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INTRODUCTION

Human papillomavirus are members of family Papillomaviridae. According to the International Committee of Virus Taxonomy (ICTV), genus and species within the family are denominated with the Greek alphabet and Arabic numerals respectively. (Fauquet et al., 2005)

However, virus types are the most used taxonomical reference, although not officially pronounced by the ICVT. This family of viruses comprises 189 viral types infecting vertebrates including humans. Types are denominated with letters referring to the host species followed by Arabic numerals. Accordingly, Human papillomavirus types are designed HPV followed by a numeral. Types classified according to nucleotide sequence similarity in the L1 gene, which encodes the major capsid protein, and is a relatively well conserved. (Bernard et al., 2010)

In addition to this classification clinicians have found useful to classify genital viral types in humans, according to their oncogenic potential. Accordingly, the so-called "high risk types" are known causative agents of cervical cancer and its precursor lesions, while "low risk types" are mostly associated to non-malignant lesions. The oncogenicity of high risk HPV types depend on two viral proteins called E6 and E7 and their specific interactions with cellular p53 and pRb proteins, respectively. As a result, the cell cycle is deregulated, leading to cell proliferation and eventually malignant transformation of the infected epithelium. Studies comparing the oncogenic potential of E6/E7 proteins from different types and their interactions cell cycle proteins have shed some light on the mechanisms defining the oncogenicity associated with high-risk types such as HPV 16 and 18, compared with low risk types such as HPV6 and 11 (Hiller et al., 2006)

Development of molecular biology was a key element for gaining knowledge on the epidemiology of HPVs. One of the most important methods implemented was the amplification of a 450 bp fragment from the L1 gene, which is well conserved amongst different genotypes, using universal primers MY09/MY11. This method became a tool applied worldwide. Afterwards, nested PCR methods were developed to increase the sensitivity of the detection of the genital infection. Many protocols have been developed since.

Particularly, genotype identification has been achieved throughout diverse techniques. Firstly developed methods were based in hybridization with specific probes, followed by restriction analysis and sequencing, which have been applied worldwide. In addition, the development of genotype-specific primers applied in single or multiplex, was a great advance. At present commercially available kits or genotype identification are widely used, based on the following methods: PCR –hybridization, end-point PCR and real time PCR.

These tests have been useful in determining the prevalence and distribution of the genotypes in different populations and geographic regions. At present, the available information on this topic is extensive: case-control studies were important for classifying "high risk" and "low risk" types; while cross sectional studies with cervical cancer samples and precursor lesions allowed to gain knowledge on the frequency and distributions of each genotype.

Worldwide the most prevalent genotype is HPV16, which is present in over 50% of cases of invasive cervical carcinoma (ICC) and high-grade squamous intraepithelial lesions (HSIL).

Type 18 is the second most prevalent worldwide, present in around 20% of all cervical carcinoma cases. Therefore, in a global context, HPV types 16 and 18 are considered responsible for 70% of cervical neoplasias (Muñoz et al., 2003; Clifford et al., 2003). Due to their importance these genotypes have been extensively studied worldwide, and are included in the two currently approved vaccines for cervical cancer prevention, Gardasil (by Merck) and Cevarix (by GlaxoSmithKline). Both vaccines are formulated with virus like particles (VLPs), the former is tetravalent (contains VLPs from types 16, 18, 6 and 11) the latter is divalent (contains VLPs from types 16 and 18). Both vaccines are applied in three doses. Their efficacy for preventing CIN2, CIN3 is above 90%. (Villa et al., 2005; Paavoneen et al., 2007; Kjaer et al., 2009)

Undoubtedly, the vaccines provide protection against the development of high grade lesions and cervical cancer caused by HPV16 and 18, however the presence of other genotypes involved in the etiology of such lesions worldwide has given elements to investigate which would be the real long-term efficacy of the vaccines. Some studies have evaluated the cross-immunity the vaccines may induce against other high-risk genotypes. The results show there is some cross-immunity, although the efficacies differ depending on the genotype involved (Brown et al. 2009). More studies are urged to determine if the current vaccines are able to induce an immune response sufficient to protect against other genotypes, or if it will be necessary to design new vaccines including other important genotypes.

Table 1. Worldwide distribution of HPV type 16, 18 and 58

Author	Diagnostic	N	Method	HPV 16	HPV 18	HPV58
Martin /Spain/ 2011	LSIL, HSIL	444	PCR, hybridization	30.6%	10.3%	9.6%
Muñoz /*/2003	HSIL, ICC	1918	PCR, hybridization	54.6%	11%	2%
Chan/Hong Kong/1999	LSIL, HSIL, ICC	158	PCR,RFLP	31%	8.2%	22.1%
Peto/England/2004	HSIL	266	PCR, hybridization	68.4%	6.8%	ND
Carozzi/Italy/ 2010	HSIL, ICC	722	PCR, hybridization	69.9%	8%	5.9%
Herrero/Costa Rica/ 2000	ICC	88	PCR, hybridization	47%	15%	12%
Sowjanya/India 2001	ICC	41	PCR, hybridization	66%	19.4%	2.8%
Bao/Asia/ 2007	ICC	5954	PCR	52.4%	14.5%	5.5%
Sughayer/Jordania/2009	ICC	41	PCR	28%	10%	2.4%
Santos/Peru/2001	ICC	198	PCR, hybridization	57.5%	5.5%	1.0%

LSIL: Low-grade squamous intraepithelial lesions; HSIL: High-grade squamous intraepithelial lesions, ICC: Invasive cervical cancer.

* Brazil, Mali, Morocco, Paraguay, Philippines, Thailand, Peru, Spain and Colombia.

Geographical variations in genotypes distribution have been reported. Meta-analyses have shown variations in genotype distribution, both geographically and in the frequency the genotypes are found in the range of lesions grade (Bao et al. 2007; Li 2011; Guan et al., 2012). Whether if these observations are merely circumstantial or if there is a biological component involved are important research questions. Little variation is found in prevalence of types 16 and 18 in CC, but other high risk types vary in importance when analyzing different countries. In particular HPV type 58, which in global context is seventh in importance, have been found in higher frequencies in Asia and in Latin America compared to Europe. (Chan 2012; Guan et al. 2012). As an example, documented differences in prevalence

and distribution of types 16, 18 and 58 are shown in Table 1. Some of the available reports include women without cervical lesions and women with condylomatosis, but for the means of this chapter, they were not analyzed.

In a meta-analysis performed for Latin America and the Caribbean, HPV16 is reported with a prevalence of 46.5% in HSIL and 53.2% in ICC, and HPV18 is reported with prevalence of 8.9% and 13.2% respectively. Focusing in Mexico only, the same work reported HPV16 with prevalence of 48.5% in HSIL and 54.9% in ICC, while HPV18 is reported with prevalence of 6% and 12.8% respectively. This lower prevalence of HPV18 in HSIL can be explained by other genotypes taking importance in some regions of Mexico (Peralta Rodriguez et al., 2012)

Mexico is a geographically diverse country, with an approximate territory of 1 964 375 km², with extremely different climates and landscapes; in addition important differences exist in productive activities, socioeconomical status, quality and availability of health services between regions. Cervical cancer is the first cause of death due to neoplasia in women from the South, while being the second cause in the North of Mexico, only displaced by breast cancer; social, economic and geographical explanations have been discussed (Palacio Mejia et al., 2003).

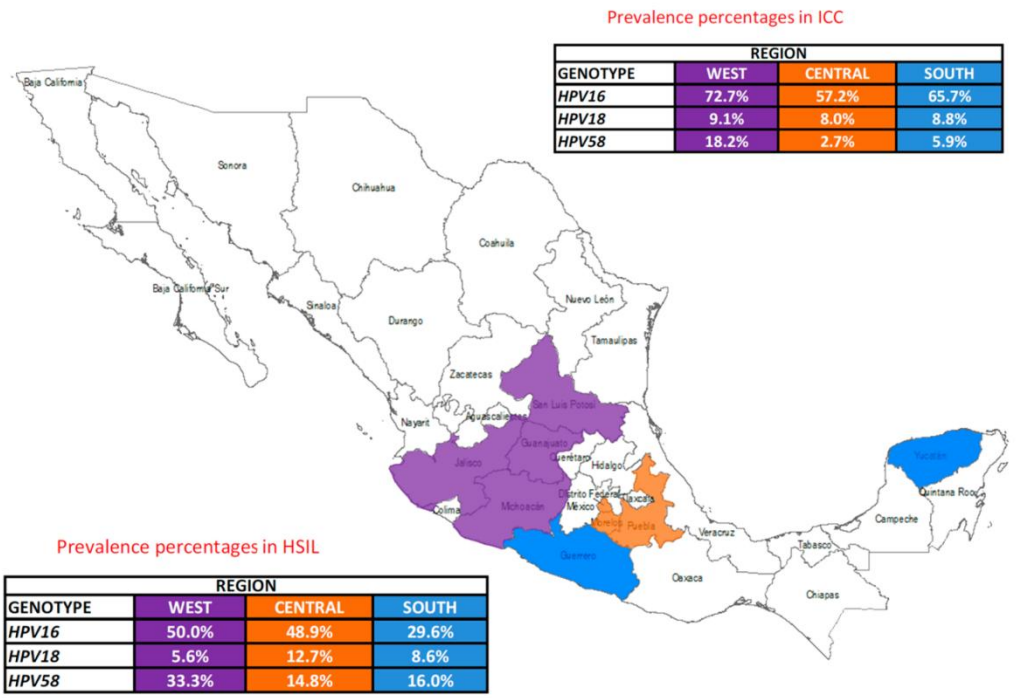

Figure 1. Frequency and distribution of HPV16, 18 and 58 in Mexico according to review by Peralta-Rodriguez et al. (2012).

HPV genotypes frequency and distribution in Mexico have been described in a recently published meta-analysis, which evidence the regional variations present, and show the importance of genotypes 58, 31 and 33, in addition to HPV16 and 18 (Peralta-Rodriguez et al., 2012). The paper analyses different geographical regions, conveniently organized as: “Central” (includes the Morelos, Mexico city and Puebla); “South” (includes: Guerrero and

Yucatán) and "West" (includes Jalisco, Michoacán, Guanajuato and San Luis Potosí). The results analyzed in the aforementioned work are included in figure 1.

The figure shows the differences observed in genotype distribution according to the regions defined by the authors, Central, West and South. The analysis show the importance of HPV16 and HPV58 that jointly account for 90% prevalence in the West region of Mexico, in which HPV58 is the second most prevalent. Interestingly, the authors included Yucatan located in the southeast region within their description of "south" but discussed the particular high prevalence of HPV58 observed in Yucatan. In fact it is the State with the highest prevalence of HPV 58 throughout the country, and is geographically distant from the other states included.

It is worth mentioning other interesting studies that were not included in this important meta-analysis, one of those was performed in northern Mexico (Sonora). In this work, HPV16 was the most frequent genotype found both in women with LSIL and HSIL, followed by HPV58, which was second most frequent in HSIL only, whilst in LSIL HPV39 and 59 were more frequent. According to lesion grade, HPV 16, 18 and 58 were found with the following prevalences: LSIL: 15%, 6% and 3%; HSIL: 27%, 14% and 0%, respectively. (Giuliano et al., 2001).

1.2 HPV 16, 18 AND 58 IN SOUTHEAST MEXICO

Women with CC and Precursor Lesions

Our group of research has studied the distribution and prevalence of types 16, 18 and 58 in diverse populations from southeast Mexico. One of the most relevant studies included 467 women without Social Security, who received attention from public dysplasia clinics from the Minister of Health. In this study, universal PCR with oligonucleotides MY09/MY11 was used for HPV detection in the first 186 patients, and in the following 211 patients in addition a nested PCR with GP5+/GP6+ was done to universal PCR-negative samples. In the first group genotyping was performed using Linear *Array HPV* Genotyping Test from Roche, while in the second group genotyping was performed using nested PCR with specific primers in multiplex (Sotlar et al., 2004). The studied women had LSIL (50.2%), HSIL (42.5%) and (ICC) 7.3%. Globally, HPV prevalence was 78.1%, corresponding to 94% in ICC, 96% in HSIL and 29% in LSIL (Fig 2) (Unpublished results). Similarly, in the meta-analysis performed in Mexico, the HPV prevalences found were 95% in ICC, however, in HSIL and LSIL the prevalences were 75.5% and 42% respectively (Peralta-Rodriguez et al., 2012). Interestingly, HPV58 was the most frequent type, it was present in 29% of all positive women, while HPV16 was found in 19% and HPV18 in 13.4%. Genotype distribution according lesion grade is shown in figure 2.

About multiple types, 13.5% of all analyzed women had multiple infections while 85.5% had single type infection. This rate is similar to reports from Latin America and the Caribbean, a meta-alnalysis showed 16.8% of multiple infections in HSIL and 12.6% in ICC (Ciapponi et al. 2012). It had been discussed the role of multiple infections in persistence, lesion development and prognosis, although this topic it is still in debate. (Gargiulo et al. 2007; Mejlhede et al., 2009)

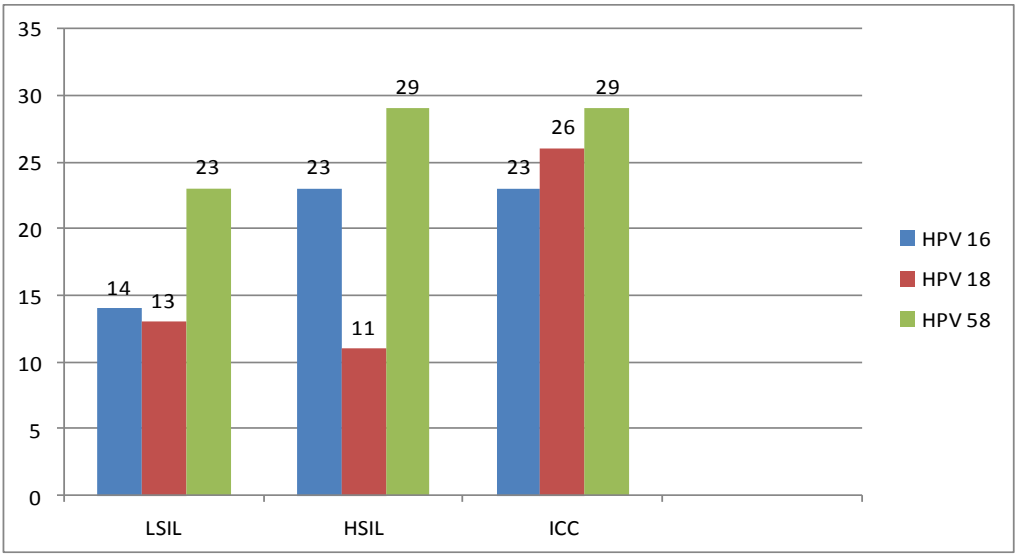

Figure 2. HPV type 16, 18 and 58 according to lesion grade

Our group of research performed a preliminary analysis to evaluate the presence of HR-HPV multiple infections, in women with normal Pap smear, LSIL, HSIL and ICC. The findings show that multiple infection frequency increases accordingly to lesion grade, and there is a statistically significant association between multiple infections and HSIL/ICC (Unpublished results)

According to our observations, HPV58 is undoubtedly the most frequent genotype found in ICC and precursor lesions, even with higher frequency than HPV16 and 18, in women receiving care in Health Minister public facilities from the state of Yucatan. These results are reproducible independently of the technique employed for genotyping, as previously mentioned two different methods were used, with similar results. Our observations differ from world literature in two aspects: first, HPV58 prevalence is higher and HPV16 prevalence is lower, even compared to Asian countries where HPV58 and 52 are of importance (Bao et al. 2007); second, HPV58 is important in every lesion grade and ICC. This last observation differs even when compared to Mexican meta-analisis in which HPV58 importance was limited to HSIL.

HPV IN WOMEN WITH NORMAL PAP SMEAR

Prevalence and distribution of HPV amongs women with normal Pap smear have been study in two groups: women living in rural area and incarcerated women.

The first was performed in a village located in the central area of Yucatan, Kantunil, in which 93 women without social security were included, all attended for cervical cancer detection program. Cervical samples were taken after sampling for Pap smear. HPV infection in cervical cells was detected using universal PCR, and typing with specific primers (Sotlar et al., 2004). HPV prevalence found was 14%, similarly, Velázquez-Márquez (2009) reported

previously same prevalence in women from a rural area from state of Puebla, located in central Mexico.

HPV16, 18 and 58 were detected amongst HPV positive women with 77, 38 and 31% frequencies respectively (unpublished results). Considering the total population studied the frequencies of these genotypes were 10.7 %(HPV16), 5.4% (HPV 18) 4.3% (HPV 58). The prevalence of HPV 16 and 18 reported by Velazquez-Marquez (2009) were similar to our findings (5.9% each one); however, HPV 58 was not studied.

Incarceration is a particular situation which includes exposure to a number of risk factors that can greatly affect the health in many ways. One of the frequent topics of study is sexually transmitted infections in prisoners. In Yucatan there are three social readaptation center (prisons): the main prison is located in Merida, capital city, the two others are located in Tekax (in the south of the state) and Valladolid (east of the state). Our study included a total of 82 incarcerated women at that time, as follows: 72 out of 120 in Merida, 5 out of 5 in Tekax, and 5 out of 5 in Valladolid, representing that 63% of all 130 incarcerated women accepted to participate. HPV was detected with universal PCR and genotyping was performed with specific primers (Soltar et al. 2004). Pap smears were simultaneously performed, and all resulted negative to ICC or precursor lesions. Results showed 17.1% of women had *Gardenella sp*, 14% bacterial vaginosis and 4.9% *Trichomonas vaginalis.*

HPV prevalence was 20.7%. Genotyping was successful in 94.1% of HPV positive samples, identifying fifteen genotypes. Low-risk types were the most frequent with a 60% prevalence, followed by High-risk types with 26.7%, and unclassified-risk types with 13.3% prevalence. Interesting to notice, 23.5% of positive samples had multiple types infections. The most frequent genotypes were low risk 6/11, and high risk types 58, 16 y 18 were found in 9.5%, 4.7% and 4.7% respectively. Other genotypes were represented with the same prevalence as HPV58 meaning that in this population HPV58 was not the most important high-risk type nor was 16 or 18. (Canche et al. 2011)

Prisoners are a highly vulnerable group as they are frequently exposed to several risk factors associated to STDs acquisition. This situation has been well documented in male prisoners, but unfortunately studies performed with women are scarce. In Mexico, population based studies, including women with normal cytology, report prevalences from 5% up to 14.5%; this is lower to what is found in incarcerated women, which fluctuates between 21 and 46%. (Bickell et al. 1991; De Sanjose et al. 2000). The results from our study show a relatively low prevalence (20,7%) given the women's conditions. One of the factors that could influence HPV high prevalence is concomitant HIV infection, which is generally more frequent in incarcerated populations. In our study however, only one woman was HIV-positive. More studies are urged to further analyze the risk factors associated to HPV infection in this special population.

HPV IN WOMEN AT REPRODUCTIVE AGE

Pregnancy is considered a special immunological state which could predispose to acquisition of certain infections, including STIs. HPV infection during pregnancy and its possible consequences for the mother and fetus are still not fully understood, and are interesting subjects of research.

We performed a study including 281 obstetric patients from an urban social security hospital. Patients were seeking medical care for delivery at term or spontaneous pregnancy loss at first or second trimester. We analyzed the cervical samples using MY09/MY11 universal PCR and genotyped the positive samples using specific primers (Sotlar et al. 2004). The prevalence found was 19.5% (55/281) which is relatively high in comparison to other reports from Mexico in women with normal cytology (Peralta-Rodriguez- 2012). A limitation is that at the time of the study the presence of lesions was unknown; a high percentage of the women (more than 43.8%) had never participated in cervical cancer screening (Conde-Ferraez et al., 2012). It has been reported that gestation may favor HPV. Evidence show HPV infection is more frequent in pregnant women compared to non-pregnant controls; the transformation zone is more exposed during pregnancy, and the high level of estrogens and progesterone have been shown to increase viral replication (Hernandez, et al. 2005, Chan et al. 2002; Coelho et al. 2004, Fife et al. 1999). However, HPV detection and cytology are not generally performed during pregnancy; treatment is discouraged in child-bearing aged women and is often believed to have more adverse effects than benefits (Kyrgiou et al., 2006).

Interestingly, in this group of patients HPV types 16 and 58 were similar in frequency (27.3% and 25.5% respectively), while HPV18 was less frequent (14.5%), ever lower than low risk types 6/11 (16.4%).

HPV16 AND HPV58 GENETIC VARIABILITY

Molecular variants of HPV are defined when a virus present more than 98% of similarity in L1 nucleotide sequence compared to the prototype.

In the 1990's the research on HPV16 genetic variability allowed to identify five branches corresponding to the geographical area in which the sampling was performed; and named them European (E) considered prototype, African 1 (Af1) and 2 (Af2), Asian-American (AA) and Asian (As) (Ho et al. 1993; Yamada et al. 1997) . Further studies have found that the variability is related to the pathogenicity. A good example is the evidence of epidemiological and functional studies showing a more oncogenic potential of AA HPV16 variants in comparison to E variants (Casas et al., 1999; Berumen et al., 2001). Therefore, in addition to the phylogenetic background the study of genetic variants is relevant to gain knowledge on their variation in oncogenicity.

In this context, a study was performed in Merida, Yucatan, analyzing 40 HPV16 positive samples from the Displasia Clinic in O'Horan public hospital, and from the National Cancer Institute from Mexico City. From these patients, 15 had LSIL and 25 ICC. The variants identified were E (42.5%), AA (27.5%) Af2 (2.5%), a variant of E called T-350-G (27%). Interestingly, according to diagnosis, 100% LSIL had E variant, and 44% of ICC had AA variant HPV16 AA variant and ICC showed significant association (p=0.01) CI 95% [1.07-26.56]. (Gonzalez-Losa 2004)

More recently, another study including women with normal Pap smear, LSIL, HSIL and ICC, showed that E variant and its related variants were found with 67.5% prevalence, T-350-G variant was found in 45% of all samples, from all groups. AA variants were found in 32.5%, but the frequency increased according to the lesion grade. However, when comparing the group of women with normal cytology + CIN1 vs. CIN2, CIN3 and ICC, it was not found

a significant association with any variant (unpublished results). It is necessary to perform a study incrementing the number of ICC cases in order to determine if there is an association between AA variants and CIN3/ICC.

HPV58 variants have also been studied, although not as extensively as HPV16. One of the problems is that the genomic regions analyzed are not always comparable. Similarly to HPV16 some studies on HPV58 have searched for variants in genes E6-E7 and its association with the risk for cervical carcinogenesis (Chan et al. 2002, Xin et al., 2001 Jeong-Hoon, 2009). Unlike HPV16 in which it has been described an important geographical association with the identification of certain variants, in the case of HPV58 it has not been a straightforward situation. L1 and LCR have been analyzed for genetic variation, and have been suggested to define "lineages" (Cento et al. 2011, Chen et al. 2011). Genome-wide analyses in order to classify the variants of HPV58, of samples from different countries have suggested a nomenclature of lineages (named A,B,C,D) and sublineages (termed A1, A2, A3, B1, B2, D1 and D2) according to phylogenetic relationships (Chen et al. 2011).

In an initial work we performed an analysis of variants in E6 gene similarly to what has been described for HPV16 (Canul-Canche et al. 2010). In this work we studied included women attending the Anticancer Center in Merida, Yucatan, the Colposcopy Clinic of the General Hospital in Chetumal, Quintana Roo and the General Hospital O'Horán in Mérida, Yucatan. Universal L1gene primers MY09 and MY11 were used to determine HPV infection. Specific PCR for HPV16, 18 and 58 DNA in HPV-positive samples was used (Sotlar et al. 2004). In total 145 samples were included. The general prevalence of specific types of HPV was as follows: HPV 16: 30.6% (23/75) and HPV 58: 24% (18/75). The presence of HPV 18 was not tested in CIN I samples, so the prevalence amongst the studied samples was 6.8% (3/44). The prevalence of specific types of HPV according to lesion grade was HPV 16, CIN I: 19.3% (6/31), CIN II: 28.5% (4/14), CIN III: 18.1% (2/11) and CC: 57.9% (11/19). HPV 58, CIN I: 29% (9/31), CIN II: 14% (2/14), CIN III: 27.2% (3/11) and CC: 21% (4/19). HPV 18, CIN II: 21.4% (3/14), CIN III: 0 and CC: 0.

The eighteen 274-bp amplicons obtained from the HPV 58 E6 gene were compared to the prototype sequence published by Kirii et al. in 1991, with the following findings: 94.4% (17/18) were identical to the prototype and 5.6% (1/18) were classified as variant. The variant found had a single change in nucleotide 418, from thymine to cytosine. However, this nucleotide change is silent, given that the codon in the variant and the prototype both code for cysteine. This work is the first analysis of HPV58 E6 variants in Mexico, and showed low genetic variability for the analyzed E6 region (Canul-Canche et al. 2010).

Preliminary results from another project analyzing a longer region of E6-E7 sequences have found higher genetic variability for E7 gene (Unpublished results). Although a high percentage of analyzed samples were identical to the prototype (33%) when analyzing E7 sequence, many variants were also present, including the previously reported amino acid changes T20I and G63S, 27% had both changes and 5% had only the first. Interestingly, some studies as reported by Chan et al. 2012 have found a positive association of CIN3-invasive cancer and the presence of amino acid changes in E7 protein (T20I and G63S, independently or in combination). These authors found that these changes are more frequently found in Asia and the Americas, accordingly to our recent findings. Whether if these variants represent a higher risk for ICC in our population is still under study. In addition, ongoing research on the phylogenetic relationships of these variants and those found around the world will enrich our current knowledge on HPV58.

CONCLUSION

In this chapter we summarized the findings about HPV 16, 18 and 58 prevalence and distribution in various populations.

HPV16 and 18 are present in all studied populations, with lower frequencies than reported internationally. HPV58 is always of importance in all our studied populations, with prevalences ranging from 4.5 to 29%. In women with normal cytology, this genotype was present with the lowest frequency; however, it is of notice that in incarcerated women the frequency was double than what is found in rural area.

Although HPV prevalence is lower in obstetric patients (19%) than in women with cervical lesions (78%), interestingly, the frequency of HPV type 58 amongst infected women was similar in both populations.

In the case of HPV16, the genetic variability and its relationship with the oncogenicity have been widely studied, and it has been determined that variant AA has the highest oncogenic potential. In the case of HPV58, some evidences have shown association of certain mutations with HSIL/ICC, however it is necessary to perform more in-depth studies to consistently confirm these results.

Further studies are needed to identify the determinant associated with the high prevalence of HPV58 in some regions of Mexico, and to understand the biological and epidemiological factors involved, in our highly susceptible population.

As mentioned through this chapter, HPV58 is present in women from Yucatan, particularly in HSIL and ICC. It is important to remark that in this group of women concurrent infection with other High-risk genotypes is common. Therefore, the expected efficacy of vaccination for cervical cancer prevention may be reconsidered in the particular case of Yucatan.

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Chapter 13

CERVICAL CANCER PREVENTION IN RESOURCE-LIMITED SETTINGS WITH SPECIAL EMPHASIS ON AREAS OF HIGH CERVICAL CANCER AND HUMAN IMMUNODEFICIENCY VIRUS ENDEMICITY

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ABSTRACT

The detection and treatment of pre-invasive cervical lesions are keys to the prevention of invasive cervical cancer (ICC) morbidity and mortality. The identification of human papillomaviruses (HPVs) as the main oncogens involved in anogenital as well as other HPV-induced cancers has revolutionized our understanding of the natural history of HPV-related pre-invasive and invasive cervical lesions. The availability of various methods to test HPV DNA has compelled us to reconsider the soundness of the traditional screening method based essentially on the practice of cytology.

Currently, a large number of screening algorithms based on cytology, HPV, and visual inspection (each alone or combined) have been and still are in the process of validation. Evidence is accumulating in favor of HPV testing as an essential first step. The traditional yearly Pap test follow-up is now challenged favoring HPV testing at longer time intervals (provided the last screening by cytology and/or HPV was negative).

The arguable inclusion of ICC amongst the AIDS-defining illnesses has added to uncertainties concerning screening algorithms for HIV-infected women. The developing world faces the double burden of high HIV and cervical cancer incidences. This is compounded by the difficult task of choosing between and prioritizing a range of other pressing medical conditions.

It is clear that cervical cancer primary prevention through vaccination and life-style changes is currently out of reach in resource-limited settings. Because of the lack of facilities, professionals, education, and high rates of women lost to follow-up, "first world style" secondary prevention is not yet feasible. Therefore, screening algorithms must be tailor-made to meet as best as is possible the need for ICC prevention in

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resource-limited settings. This may also require reconsidering some cancer prevention programs.

Keywords: Cervical cancer; primary prevention; secondary prevention; HPV; HIV; resource-limited settings; screening algorithms

KEY MESSAGE

Cervical cancer and HIV/AIDS incidences are highest where primary and secondary prevention are least available and affordable. Low cost and low technology HPV DNA testing for cervical pre-invasive lesions is a promising strategy since it has a better sensitivity and specificity than cytology and visual inspection.

INTRODUCTION

Cervical and breast cancer are the most common female cancers. In the industrialized world, breast cancer is the most common; effective large scale cytology screening has resulted in a significant decrease in the burden of disease resulting from cervical cancer [1]. Parallel to this evolution, the traditional Papanicolaou classification system has been replaced by the Bethesda system adding to it atypical cells of uncertain significance (ASCUS), atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASCH and HGSIL), low-grade squamous intraepithelial lesion (LGSIL), and atypical glandular cells of uncertain significance (AGUS) [2]. This went together with changes in the classical histopathological classification (mild, moderate, and severe dysplasia, and carcinoma in situ) into cervical intraepithelial lesions (CIN) 1, 2, and 3. Finally CIN2, CIN3, and CIS became grouped together in the CIN2+ class [3].

A major paradigm shift occurred with the discovery of the etiological role of human papillomavirus (HPV), especially the high-risk (HR) oncogenic subtypes, in anogenital pre-invasive and invasive lesions, and the discovery of a range of molecular techniques of HPV-DNA and RNA testing.

Finally, the manufacturing of and clinical trials with HPV vaccines have recently changed the approach to cervical cancer prevention by primary (i.e. life-style changes, vaccination), and secondary prevention (i.e. screening and treatment).

The proficiency of any screening strategy is gauged by its sensitivity and specificity. The most efficient one is the method with the highest negative predictive value (NPV) (i.e. when the test yields a negative result it is most likely correct in its assessment) [4].

Because of the high interobserver variability (i.e. false positives and negatives) that occurs both in cytology and histopathology, especially with low-grade lesions, alternatives to cervical cytology have been sought such as visual inspection of the cervix with acetic acid (VIA) or Lugol's iodine (VILI), and magnified cervical photographic images (cervicography, or cervigram) [5]. It should be borne in mind that the interpretation of any method's specificity depends on the cut-off levels of the disease's endpoint; the higher the cut-off the higher the sensitivity. Increased sensitivity results in prolongation of screening intervals,

lower cost, and better compliance with screening. In cytology the cut-off may be ASCUS, LGSIL, or HGSIL [4-5]. With molecular techniques cut-off levels of 1 or 2 pg/ml are used for Hybrid Capture II (HCII) [6]. With *care*HPV™ a cut-off ratio cut-point of 0.5 relative light units is recommended [7]. Table 1 illustrates reported values of sensitivity and specificity for various screening methods [8-21].

Because of variable sensitivities, specificities, and predictive values of cytology and naked eye inspection, there are now strong arguments favoring the use of molecular diagnostic testing methods of the HR-HPV as the primary screening test [8, 22-23]. Whatever the diagnostic tool algorithm used as primary screening, colposcopy-targeted biopsy remains "the" gold standard verification of any test. The rule is that any positive HR-HPV test or any lesion ASCUS or more should be biopsied and that any CIN2+ should be treated by ablation (large loop excision of the transformation zone, LLETZ, or loop electrosurgical excision procedure, LEEP) or destroyed (cryosurgery). The setback of cryotherapy is that it does not allow for histopathological diagnosis. The advantage is that it allows treatment on the spot, "see-and-treat", and avoids losses to follow-up. Now, because of the progress in the knowledge of the natural history of HPV, low-risk (LR) and HR-HPV, regression, persistence, or progression, the traditional concept of excision of the transformation zone "with therapeutic intent" (i.e. with margins free of lesion) has also been turned upside down because the characteristics of the margins are less important than the presence and persistence of HR-HPV [24-25].

Although much work has been done in the developing world in order to decrease the cervical cancer related morbidity and mortality, most studies were carried out in specific settings not reflecting the reality factor [5, 7, 26]. Lack of education and information result in underuse of screening facilities even where they exist to some extent [26]. Lack of understanding of cytology reports and recommendations by health professionals compound the problem [27-28].

Table 1. Overview of reported sensitivity, specificity, and negative predictive value of various screening tests

Endpoint	Test	Sensitivity	Specificity	NPV *	References
CIN 2+	Cytology	± 56.0%	± 96.0%	± 86.0%	5, 8-9
		(21.0 – 93.0) **			
CIN 3+	Cytology	± 87.0%	± 86.0%		10
CIN 2+	VIA	±73.0%	± 85.0%	±95.0%	5, 8, 11-12
		(41.0 – 82.0)	(64.0 – 95.0)		
CIN 2+	HCII DNA	± 87.0%	± 87.0%	99.0%	1, 8, 13
		(62.0 – 97.0)	(81.0 – 94.0)		
CIN 3+	HCII DNA	100.0%	90.6%		9
CIN 2+	careHPV™	± 87.0%	± 89.0%		7, 14-16
CIN 2+	PapilloCheck™	± 97.0%	± 97.0%		17-18
CIN2+	HPV + cytology	95.0%	± 75.0%	100.0%	10, 19-21

* Negative predictive value; ** Values are mean (range).

Shortages of equipment and of trained health professionals are endemic in such settings. Last but not least, cervical cancer and the acquired immunodeficiency syndrome (AIDS) are well known to be highly prevalent in the developing world where screening facilities are the least well established and where financial constraints as well as many other infectious diseases impose difficult choices on health priorities. For instance, in South Africa with its high prevalence of HIV/AIDS, as much as one third of the national health budget for laboratory services is allocated to HIV diagnostic procedures (ELISA, viral load, CD4+ T-cell count), while access to highly active antiretroviral treatment (HAART) requires a CD4 count of $<200/mm^3$. A National Cervical Screening Programme was launched in the early 2000, offering three free Pap screens at ten years interval starting at the age of 30 [29]. However, this opportunity is used only by less than 20 percent of women [26]. Moreover, it is felt that the time has come to move to HPV-based screening [30].

In 1987, the Centers for Disease Control and Prevention (CDC) created a list of clinical conditions called AIDS-defining illnesses. In 1993, three more were added, including invasive cervical cancer [31]. To qualify as an AIDS-defining cancer, the malignancy should be at increased risk in the HIV-positive population, and the risk should be inversely proportional to the degree of immunosuppression as expressed by the CD4+ T-cell count. In addition, partial immune reversal through highly active antiretroviral treatment (HAART) should decrease the incidence of AIDS-defining cancers [32]. The reality is that cervical cancer incidence among HIV-infected women in the US has been unchanged since the introduction of HAART [33-34]. As a whole, the incidence of HPV-induced diseases has increased since the introduction of HAART [35].

There is no convincing evidence that the HIV epidemic went parallel with an increase in ICC, neither in the US nor in sub-Saharan Africa [36]. In general, HPV-associated cancers are increased among persons with AIDS; however, the relative risk (RR) of invasive cervical cancer linked to immunodepression is only 1.32 (95% CI 0.96-1.80; $P = 0.077$) [37]. It has been speculated that the increased longevity of patients on HAART would result in an increase in ICC among HIV-infected women. Perhaps it is still difficult to witness this within a time-span of 10 years. However, the fact that the average age at diagnosis of ICC in HIV-infected women is at least ten years younger than in HIV-naïve ones does not support this hypothesis [38].

The role played by HIV infection and immunodepression on the natural history of pre-invasive and invasive cervical disease is complex and a matter of controversy. The reported rates of HR-HPV in HIV-infected women vary from around 20% (i.e. similar to HIV-naïve women) up to around 70% [39-42].

There is ample evidence that HIV-infected women tend to be more infected with multiple HPVs that are of HR type, to exhibit less clearance than their HIV-naïve counterparts, and to have HIV-related genotypes [43-44]. This has been attributed to low CD4 cell counts [45-46]. But this has not been confirmed by others [37, 47]. The role of immunosuppression on the persistence of HPV and the ensuing risk for CIN2+ is diversely appreciated. It has been reported that the odds ratio (OR) of progression was inversely proportional, and the clearance rate proportional to the CD4 count [45-46, 48-49]. At variance, others found no influence of immune competence or depression on the clearance/persistence rate of HPV and/or the progression from LGSIL to HGSIL or invasive cancer [34, 37, 39, 50-51]. The divergences can be attributed to the fact that the CD4 count is an imperfect surrogate of the degree of immunity in this regard because other additional immunologic factors are at play in HPV

suppression in the cervix [52]. Immunosuppression, as evidenced by CD4+ T-cell counts below 200/μL, influences the natural history of HPV-related anogenital lesions in general but not of invasive cervical cancer [35].

The influence of HAART is equally debatable. According to Palefsky and Holly [53], HAART does not influence the regression of CIN2+ because it has no effect on HPV-specific immunity. However, it has been shown that, in HIV-infected women with an immunological status of CD4 > 500/μL, undetectable viral load, and no cervical pathology at enrollment, HAART was associated with a reduction in SIL (not otherwise specified). A recent report from Soweto, South Africa, found that HAART increases the likelihood of regression of cervical lesions [54]. At variance, it has been found that on HAART a quarter of CIN1 still progress to CIN2+, and that CIN2+ do not regress because the lesions were already established when HAART was initiated [35]. Progression of LGSIL was shown to decrease if HAART was started before the occurrence of LGSIL [55].

The take home message seems that, especially in areas of high HIV and cervical cancer incidence, sexually active women should be tested for HIV and HR-HPV to estimate their risk of developing pre-invasive lesions. In addition, where the level of CD4 is used as an entry portal to HAART, it should be measured in all HIV-infected women. It has been shown that HIV-infected women with normal baseline cytology and a negative HPV-DNA test at enrollment have a 5-year cumulative incidence of CIN2+ that is similar to HIV-naïve women [56]. The South African guidelines for screening "immune compromised" (i.e. HIV-infected) women recommend normal screening intervals (i.e. after 5 or 10 years) if HPV DNA negative, and "increased surveillance (i.e. annually) or treatment" if HPV DNA positive [57].

The concepts of screening debut and screening intervals in general have evolved from the time of the annual Pap smear. Yearly cytology starting 3 years after sexual debut and no later than the age of 21 until the age of 30 is the current recommendation for HIV-positive and HIV-negative women [58]. HIV-infected women with normal baseline cytology and a CD4 count above 500 should be screened no differently from HIV-negative ones [59].

A strategy of co-testing with cytology and HPV, and screening every 3 years for women aged 30 to 65 with dually negative results is efficient compared to cytology-colposcopy [60]. Rapid HPV-DNA testing 3 times per lifetime is deemed more effective than traditional cytology [14].

PRIMARY PREVENTION

HPV being a sexually a sexually transmitted infection (STI), primary prevention entails life-style (mainly sexual habits) changes or choices such as age at first sexual contact, number of lifetime sexual partners, contraceptive barrier methods, and substance abuse [29, 33, 60]. In the sub-Saharan African context where herbs and cleansing ashes are traditionally inserted inside the vagina, it is also recommended to refrain from these practices because they may act as entry portal for HIV [29].

Anti-HPV vaccination in under-resourced countries entails a spectrum of difficulties. First, vaccination of 70 percent of young girls before coitarche to reach heard immunity would be hard to achieve, especially in countries that are still mainly rural and where school attendance by girls is limited. In addition, the cost would be hard to carry by governments

dealing with multiple vital priorities [61-64]. With the currently available bi-valent (anti-HPV 16/18) and quadri-valent (anti HPV 6/11/16/18) vaccines about one third of women would not be protected in view of the relatively high-rates of infection in sub-Saharan Africa by non-16/18 but oncogenic (i.e. 33, 35, 39, 45, 51, 52, 58, 59) or probably oncogenic HPV subtypes (i.e. 53) [65-67]. Even if there may be some degree of cross-immunity provided by the existing vaccines this would not achieve a substantial protection against non16/18 infections.

HPV16/18-induced precancerous lesions will virtually disappear in vaccinated cohorts. The 30% of cervical cancers caused by non-vaccine HPV types still need to be screened with HPV markers [30].

SECONDARY PREVENTION

Secondary prevention of cervical cancer can be summarized under the motto "screen and treat" (taken in a broad perspective). The screening modalities are many and depend on the available tools such as well-established cytology laboratories, HPV testing, and visual inspection (naked eye or colposcopy), treatment and follow-up. Therefore, the algorithms will differ between the developed and developing world. Even in the developed world various algorithms are still being tested. Currently, more than ten strategies are based on HPV-DNA testing alone, cytology alone, and HPV-DNA combined with cytology [1]. Five possible clinical applications of HPV DNA testing have been evaluated [8, 19].

A number of studies concur now that, in limited-resource settings, the most efficient and cost-effective primary screening should be HPV and cytology co-testing. With regards to cytology, it appears that liquid-based cytology is no better than conventional Pap smear cytology; furthermore, liquid-based cytology is hardly available in low-income countries [9, 26-27]. HPV testing can be done with HC II, *care*HPV, and more recent techniques. The advantage of *care*HPV is that the technique is easy, takes less time, is much cheaper than HC II, and has the same specificity but possibly a lower sensitivity [4]. Both methods cover the same spectrum of HPV, namely the 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 subtypes.

SCREENING ALGORITHMS

The aim is to enhance the linkage between screening and treatment through a reduced number of visits, improved follow-up, and reliance on less laboratory infrastructure than conventional cytology. The screening test depends on women's age. Because most HPV infections are transient it is recommended to wait till the age of 30 for HPV testing [58, 69-69]. Cytology screening debut is recommended 3 years after sexual debut, or the latest at the age of 21 [60].

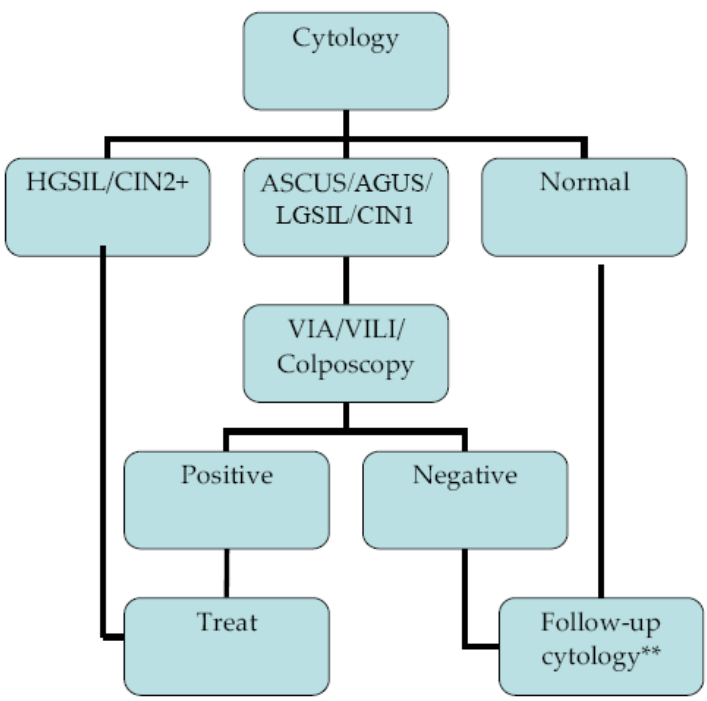
Secondary prevention strategies are many and still being validated. This means that the "best practice" still awaits for the final "best evidence". Because of the differences in availability of financial, technical, and human resources it is clear that the most efficient strategies will differ between developed countries and low/limited-resource settings.

Figure 1 shows a screening strategy for women below the age of 30 years that could be implemented in low-resource settings where opportunistic screening is practiced [70]. Algorithms should be flexible and adapt to local circumstances. Where colposcopy and histopathology services are available colposcopy-guided biopsies (followed by LLETZ/LEEP if diagnostic of CIN2+) would meet the gold standard. If not, VIA/VILI positive lesions are immediately destroyed by cryotherapy.

In cytology negative cases, recommended screening intervals vary from one to 3 or 5 years if the preceding screening was normal [27, 57, 71].

Figure 2 illustrates a cytology/HPV co-testing screening strategy for women aged 30 to 65 years where well-established cytology services are available. This flow chart would also be applicable in low-resource settings with opportunistic screening. The availability of a range of low-cost low-tech HPV DNA testing kits should no longer be an obstacle even in low-resource settings [7]. The same flexibility should apply as above mentioned. A one year screening interval is recommended if cytology is negative and HPV DNA positive [57]. If both are negative, a three year interval is recommended [68].

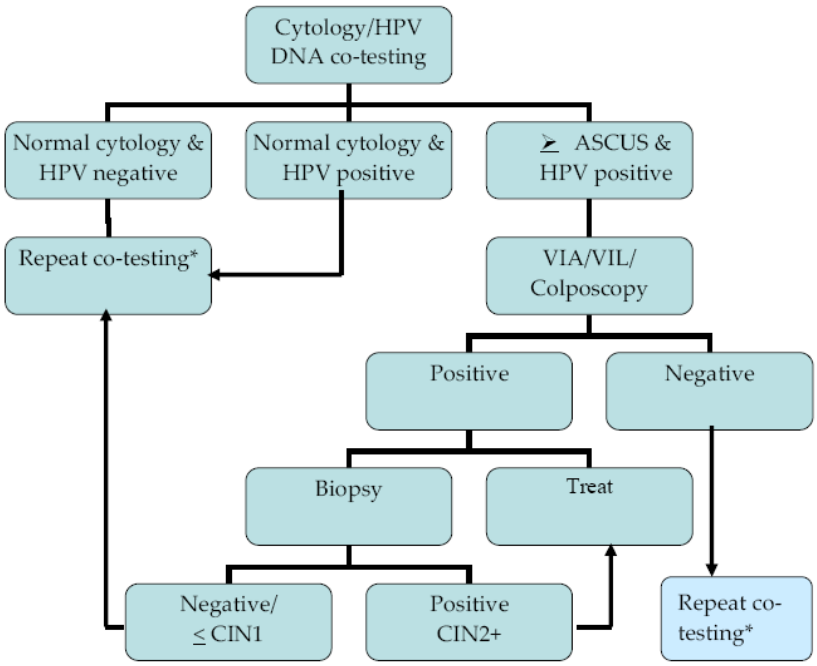
A HPV DNA screening strategy for women aged 30 to 65 is shown in Figure 3. In low-resource settings this strategy would be affordable since the cost of rapid HPV tests would vary between 3.5 and 8.2 US$, i.e. about 10 times less than HCII [14]. In the South African public health services this would be equivalent to the cost of a Pap smear, and would provide a much better negative predictive value than cytology [62].



* Modified from Palefsky, J. (2006) HPV infection and HPV-associated neoplasia in immunocompromised women. *Int .J. Gynecol. Obstet.* 94 (Supplement 1): 556-564 (Figure 1. Algorithm for screening for ASIL in HIV-positive women) [33].

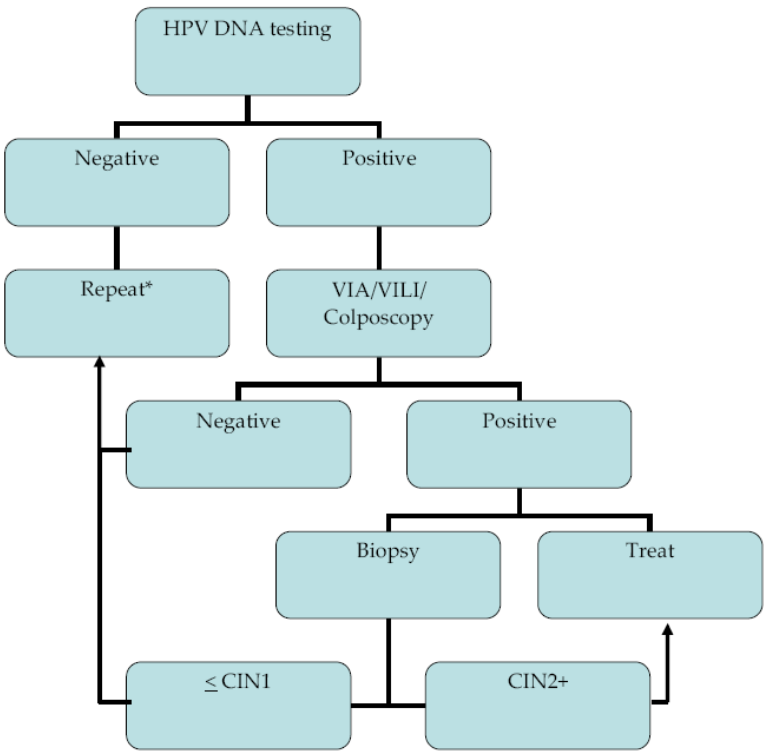
** Recommended screening intervals vary from one to 3, or 5 years.

Figure 1. Flow chart for screening women less than 30 years old *.



* Recommended screening intervals vary from 3 to 6 years.

Figure 2. Flow chart for women aged 30 to 65 years: well-established cytology services available.



* Recommended screening intervals: 3 to 6 years, or times 3 between the ages of 35 and 45.

Figure 3. Flow chart for women aged 30 to 65 years: HPV DNA based strategy.

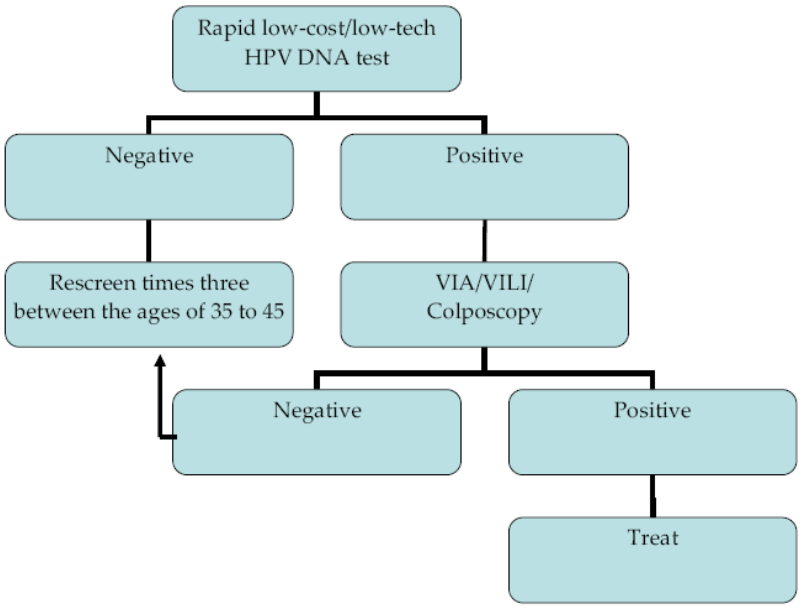


Figure 4. "See-and-treat" flow chart for women aged 30 to 65 years: low-resource setting without well-established cytology screening programmes.

Figure 4 illustrates a "see-and-treat" screening strategy for women aged 30 to 65 in a low-resource setting without well-established cytology screening program. It has the advantage to limit recalls to HPV DNA positive/visual inspection negative, and to HPV DNA negative cases to HPV DNA rescreening three times between the ages of 35 and 45.

Whether a screening strategy should be specifically designed for HIV-positive women is uncertain. However, since some VIA positive lesions are too extensive to be treated by cryotherapy on a "screen-and-treat" basis, provision should be made to establish referral and management systems using Excisional procedures [72].

CD4 cell counts are part of the assessment of the initial diagnosis of HIV/AIDS, and (in low-resource settings) the key to access to HAART. If the "see-and-treat" strategy is implemented it will prevent pre-invasive lesions from progressing to invasion, regardless of HIV infection or not.

CONCLUSION

The understanding of the essential role of the role of LR and HR HPV in the pathogenesis of HPV related anogenital (and others) a preinvasive and invasive lesion has revolutionized the approach to their screening methods. The availability of high/low tech and high/low cost HPV testing has increased the diagnostic sensitivity, specificity, positive and negative predictive values.

Although the traditional Pap smear is still valuable and associated to HPV testing in some algorithms, it tends now to be superseded by HPV testing. The argument that cytology will still be part of screening after prophylactic HPV vaccine is losing terrain in view of its low sensitivity and specificity, as it could be replaced by HPV testing.

In a world that is vastly unequal in terms of wealth, local resources and priorities, screening strategies are bound to depend on what would be the best available and feasible strategy to curb cervical cancer morbidity and mortality.

Evidence is accumulating that low tech/cost HPV testing would be the most efficient has the potential to achieve this goal in low-resource settings with the added advantage of a low number of follow-up sessions for HPV negative tests [14, 30, 61-62]. This implies a review of screening guidelines in high HIV and cervical cancer prevalence settings, keeping in mind that available data are insufficient to propose fully evidence-based protocols for low-resource settings [73-74].

Cervical cancer prevention remains an important goal in HIV-infected women [75]. However, no convincing evidence is available to consider cervical cancer as an AIDS-defining condition in areas of high cervical cancer and HIV prevalence. If that is the case, there is no compelling reason for specific guidelines for HIV-infected women. The promoting role of immunodepression in cervical carcinogenesis and the protecting effect of HAART in this regard are controversial.

It follows those HIV-infected women who test positive for HPV and/or exhibit an abnormal Pap smear may be safely managed by the same algorithm as their HIV-negative counterparts. It follows from the above that the screening policy of three Pap smears in a lifetime has become not only obsolete but counterproductive. The importance of the CD4 count relates to the HIV/AIDS condition and its management rather than to the cervical condition per se.

It has been stated recently "when a huge number of reports are published on the same subject in a relatively short period of time, with many variations in settings, study designs and applications, the result is often confusion and decreased comprehension by readers" [76]. There is, indeed, a huge number of publications concerning cervical cancer screening strategies for the developed and developing world. The contribution of HIV infection to the progression of pre-invasive cervical lesions into invasion remains elusive. This chapter is an attempt to contribute to the prevention of the morbidity and mortality of cervical cancer in low-resource settings where both HIV and cervical cancer are highly prevalent. For the time being, only pilot project have been implemented to test the best and affordable practice in this regard. These projects are very informative and indicate that the preventive policies need to adapt to new paradigms.

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Chapter 14

HUMAN PAPILLOMAVIRUS RELATED MALIGNANCIES OF THE REPRODUCTIVE TRACT

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ABSTRACT

Over 120 sub-types of small, non-enveloped, DNA human papillomavirus (HPV) selectively infect keratinocyte stem cells on the surface layer of human skin and mucus. Most of them are easily cleared up by immune system. However, HPV subtypes with oncogenic potential can induce cell proliferation and they origin benign warts or malign tumors. Over 5% of worldwide newly diagnosed malignancies are attributable to HPV. Pelvic and perineal structures are exposed to the virus through direct skin contact during the sexual practices, and can be place of persistent warts known as condylomata acuminata, premalignant lesions, and malignancies. Many vulvar, vaginal, penile, and anal cancers are also origin by HPVs. We review the role of HPV in all those malignancies of the human reproductive tract.

1. BACKGROUND

Human papillomavirus (HPV) take advantage of small microtraumas to infect the keratinocyte stem cells on the surface layer of epitheliums in the human genital skin and mucus, replicating later in the outermost layers of the skin. They are over 120 subtypes of non-enveloped DNA viruses [1, 2] with host- and tissue-tropism. Some HPV subtypes exhibit

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a selective affinity for specific tissues, being the human genital tract the target of many of them.

Symptoms of HPV infection are strongly widely. Most of them are cleared up by the immune system. However, HPV can origin warts, benign tumors or malignancies if remain and induce proliferation in the epithelium cells. HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are considered carcinogenic subtypes [3].

Peyton Rous first reported a HPV-related cancer in 1935. Actually, 5.2% of human malignancies are attributable to HPV. They origin over 561000 new cancers annually worldwide, that position HPV as the most important infectious cause of cancer [3]. Urogenital malignancies as cervix uterine, anal, vulvar, vaginal, and penile tumors have been classically related to a dozed high-risk oncogenic and sexually transmitted HPV subtypes. We review the epidemiology and management of HPV-related human malignancies of the genital tract.

2. Cervical Cancer and HPV

A HPV vaccine was approved by the US Food and Drug Administration in June 2006 to prevent cervical cancer. This announcement reflects the importance of the sexually transmitted HPV infection as a necessary cause for the development of this malignancy and positions the vaccine implementation as a major milestone to reduce the impact of the disease.

Over 525000 new cervical cancers are diagnosed every year worldwide. Cervical cancer is the seventh leading malignancy in the whole population, and the third among women, representing 9% of all female cancers. The incidence of cervical cancer is clearly high in the developing countries, in which cervical cancer is the second leading cause of female cancer death, and accounts 85% of the global burden of the disease [4]. On the basis of age-specific incidence and projected demographic changes, it is expected that the number of newly diagnosed cervical cancers rises from 530232 in year 2008 to 776327 in 2030 [5]. In Europe, the whole incidence and mortality of cervical cancer have progressively decreased to less than 10 new cases annually diagnosed every 100000 women along the last 50 years, concurrently to the introduction of Papanicolau (Pap) smear screening programs and to the fall of the fertility rates [6]. However, the incidence of cervical cancer is increasing among younger than 50 years women, probably due to a higher exposure to HPV infection.

The persistent infection by high risk oncogenic HPV sub-types seems to be a necessary cause for the development of cervical cancer. HPV DNA can be detected in 99.7% of cervical adenocarcinomas and squamous cell carcinomas [7], and the incidence of those cervical malignancies is proportional to the prevalence of HPV infection in the population. The prevalence of chronic HPV infection is 10-20% in countries with a high incidence of cervical cancer, whereas it decreases to 5-10% in countries with a low-incidence of the disease.

Over 40 HPV sub-types are capable to infect the human genital tract. They can be classified according to their tissue-tropism (some types have a predilection for cornified stratified squamous epithelium, and some others for moist mucous membranes) or to their oncogenic potential. Fifteen HPV sub-types are considered high risk because of their frequent association to cervical intraepithelial neoplasia (CIN) and invasive cervical, penile, anal, vulvar, and vaginal cancers [8], while the remaining genital HPV sub-types including HPV-6,

11, 42-44, with no or low oncogenic potential only cause subclinical infections or clinical visible benign lesions known as flat and condylomata acuminata, respectively.

Over 50% of people are infected with one or more genital HPV subtypes at some moment of life [9], but majority of cervical HPV infections are cleared to undetectable levels within 2 years of exposure. Only 10-20% HPV infections remain and origin progressive changes in the cervical tissues that range from mild (CIN1) or moderate dysplasia (CIN2) to carcinoma in situ (CIN3) or invasive malignancy. The age of the patients seems to be relevant for the HPV clearance. The CIN1 regression rate of adolescents and young women surpasses 90%, whereas it decreases to 70-80% only in adult women [10]. Almost CIN2 lesions can regress spontaneously if left untreated [11]. The 39% of untreated CIN2 lesions of young women disappear completely, and 92% revert to CIN1 within 3 years, progressing to cervical carcinoma 8% of cases only [12]. By the other hand, CIN2 regression rate in older women is 50-54% only, and progression rate rises to 20-30% of cases [13].

Most women are infected shortly after their first sexual relationship, and concurrent infections by multiple HPV subtypes are often found. The presence of a high viral load is common among young women and it is associated with a persistent infection, especially for HPV16 sub-type, the dominant form in premalignant diseases [14, 15]. The HPV16 can be considered the most carcinogenic HPV sub-type, and induces almost 12% of CIN3 or invasive malignancies after 3 years of persistent infection [15]. The HPV18 is the second most common subtype in cervical cancer. Both HPV16 and 18 sub-types origins over 70% of HPV-related cancers worldwide [16]. A second peak of relevant HPV infections is observed in postmenopausal women due to the reactivation of dormant infections in the immune senescence, or to newly HPV contagions associated to sexual practices in the patients or their partners [17].

3. Prophylaxis of Cervical Cancer

Cervical cancer can be preventing. Countries with prophylactic cervical screening programs have reduced the incidence and mortality of cervical cancers, while two of each three women that developed the disease still dye in the rest of them because of the lack of really curative treatment for the advanced disease. And the health implications are intense because cervical cancer affects women of social and economically relevant age, and can be prevented with a combination of vaccination, HPV DNA testing, and promising secondary prevention strategies [18].

Primary prophylaxis includes sexual education of the population, promotion of prophylactics, and the introduction of HPV vaccines. Two prophylactic HPV vaccines are available. The bivalent vaccine (Cervarix®) protects against HPV16 and 18, while the quadrivalent vaccine (Gardasil®) protects against HPV6, 11, 16, and 18. They both have been proven to be safe and virtually 100% effective to prevent the development of high-grade cervical intraepithelial lesions in previously uninfected women [19, 20], but only 44% effective to prevent them in the previously infected ones.

Both, the bivalent and quadrivalent vaccines, are given in three separate injections over a 6 month period and use virus-like L1 particles from the HPV capsid to induce immunity. The duration of immunity after vaccination is not completely clear but data suggest that the

quadrivalent vaccine could be effective for at least 5 years and up to 9.5 years [21]. Assuming a lifelong immunity and compared with the current screening practices, the cost-effectiveness ratio of HPV vaccination for a 12-year-old girl from the United States of America has been reported to be $43600 per quality-adjusted life-year gained [22]. It supports to recommend the universal vaccination of pre-teen girls in high-income countries but, unfortunately, vaccination currently remains too expensive for poorer nations where the incidence of HPV infection and cervical cancer is higher.

Besides, the pivotal multicentric HPV vaccine trials underrepresent Asian and African countries. Differences in the HPV genotype spectrum, immune response, sexual behaviours, age for sexual debut, and concurrent infections as human immunodeficiency virus one are evident between those countries and the industrialized ones. And it is difficult to extrapolate the current evidence of vaccine effectiveness to those poorer regions with a greater need for cervical cancer prevention.

Both bivalent and quadrivalent vaccines could also cross-protect against some nonvaccine HPV types. The efficacy to prevent cervical diseases in patients with 6 months persistent infection by HPV 31, 33, and 45 were 77%, 43%, and 79%, respectively, for the bivalent vaccine, and 46%, 29% and 8%, respectively, for the quadrivalent vaccine. The quadrivalent vaccine seems also to provide protection against the development of genital warts, and vulvar and vaginal cancers [24]. A nonavalent vaccine covering HPV16, 18, 31, 33, 34, 52, 58 6, and 11 sub-types that cause over 90% of cervical cancers worldwide is being currently tested in several clinical trials.

Nowadays, HPV vaccination is recommended in 9 to 26 years old pre-adolescents and young women who have not yet been exposed to the target HPV types. Furthermore, different questions about vaccine implementation and administration such as effectiveness of a minor number of doses, needing of booster doses, or timing according standard vaccine calendars still have to be fully answered.

Some other strategies for primary prevention include the use condom, which reduces HPV transmission by 70% but not always prevent skin to skin contagion [25]; male circumcision which reduces HPV contagion to women by reducing the HPV infection rate in men [26]; and the use of HPV inhibitory compounds such as topical microbicides [27].

Secondary prophylactic programs include the screening of cervical cancers in the female population. The Pap test, historic gold standard for cervical screening, reduces the incidence of cervical cancer from 50-80/100000 women in countries lacking of them to 4-10/100000 women in the developed countries. The success of Pap test detecting early stage cervical cancer and precancerous lesions is partially due to the natural history of the disease. Progression from HPV infection to cancer use to prolong by decades [28], allowing the detection of viral cytological alterations [29]. Current screening programs includes the screening of healthy women, the triage of equivocal results with another independent test; the histologic diagnosis of abnormal results; colposcopy follow-up if no lesion is found; resection of suspected lesions; and post-treatment follow up of pre-cancerous lesions [30]. Anyway, Pap test is far from perfect. Cytology depends on subjective interpretations of the morphologic alterations, and the high false negative rate is a severe limitation with important medical, financial, and legal implications. Liquid based cytology has improved the efficiency of smear processing, but does not address the limitation of poor test sensitivity. In order to increase the screening sensitivity, repeat Pap tests are currently recommended every 1 to 3 years.

4. CHALLENGES FOR THE FUTURE

In the last years of XX Century, the detection of HPV DNA using molecular-based technologies awoke interest for cervical cancer screening. HPV testing has 25- 35% higher sensitivity than cytology but 5-10% somewhat lower specificity detecting high grade lesions [31, 32]. The co-testing Pap and HPV tracts achieve a very high sensitivity and negative predictive value but seem to increase the number of patients that warrant colposcopies and further evaluations, raising the costs. The American Cancer Society and the American College of Obstetricians and Gynecologists recommend co-testing for women over 30 years of age [33] because HPV infection uses to be more transient in younger women [34].

We suppose that as while successive cohorts of women become vaccinated, the prevalence of cervical lesions will decrease and it will reduce the predictive capacity of Pap test. Besides, the reduction in the signal (squamous abnormalities) to noise (inflammation and reactive atypia) ratio could lead to fatigue and to a minor detection of conspicuous lesions. Maybe we should have to consider adopting HPV testing as the primary screening tool for cervical cancer in the future because they do not depend on subjective morphologic interpretations and it would also allow creating HPV infection and vaccination registries, an efficient and low-cost strategy to monitor the duration of immunity in vaccinated women. Pap cytology should be reserved for triage settings (i.e., assisting in the management of HPV positive cases) in which cervical lesions prevalence will be higher [35].

5. OTHER HPV-RELATED CANCER OF THE REPRODUCTIVE TRACT

Vulvar cancer accounts for 4% of all gynaecological malignancies. The most common histologic type of invasive vulvar cancer is the squamous cell carcinoma, and HPV can be detected in up to 71% biopsy samples. It develops from premalignant vulvar lesions (VIN I-III) that are similar to other anal, cervical, and penile lesions. HPV infection is thought to cause up to half of vulvar cancers, especially in younger women, being the HVP16, 18, and 31 the main related sub-types with this variant of cancer [36].

Vaginal cancers are really rare, but its incidence has been increasing during several decades as a that the diagnosis of this entity has increased over the past several decades, most likely as a consequence of heightened awareness, expanded cytological screening, and liberal use of colposcopy. The existence of precursor lesions is unknown, and many information has been extrapolated from the natural history of cervical and vulvar diseases. The HPV infection of other genital sites, the presence of vaginal condylomas, HIV infection, and immunosuppression (including HIV infection) have been related with the development of vaginal cancer.

Penile cancer appears in one every 100000 men in the United States of America, being HPV16 and 18 related to over 50% of cases. Male patients infected by high risk HPV sub-types and partners of women with cervical cancer have a higher incidence of penile carcinoma [37].

6. BREAST CANCER

Breast cancer (BC) is one of the most frequent malignancies worldwide [38]. Almost 30% of human BC samples are infected by HPV subtypes, and some authors have suggested an eventual association between HPV infection and BC pathogenesis. However, the relationship between HPV infection and BC development remains unproven.

Hereditary factors can be detected in 5-10% of BCs only. Most of them are sporadic. Endocrine and environmental exposures play the main role in a complex multistep process that finally lead to the uncontrolled cell growth [39, 40], but the specific role of viral infections in the development of BC remains controversial [41].

Over 12% of human cancers have been related to viral agents, and at least three different families of viruses (HPV, Epstein Barr virus [42] and Mouse mammary tumor virus [43]) could take part the development of BC. Attention to HPV as an eventual cause of human BC has been increased because of the eventual possibility of a primary prevention with HPV vaccines [44, 45]. The eventual relationship between HPV and BC is based on the identification of HPV genome in BC tissues, and *in vitro* immortalization of primary mammary epithelial cells by high-risk HPV. HPV is thought to be transmitted to breast tissues not only through sexual activities, but also through peripheral blood mononuclear cells. Infection of breast cancer cells through Id-1, a family of helix-loop-helix transcription factor, has been also suggested [46].

Di Lonardo [47] first investigated the presence of HPV in paraffin-embedded BC tissue sections using PCR and in situ hybridization in 1992. PCR identified high risk HPV16 in 29.4% of 17 samples, but hybridization did not detect any HPV track suggesting that this technique was less sensitive. Since then, many studies tried to confirm a relationship between HPV infection and BC development with different success. Several authors did not find [48-51] any association, some others reported less than 16% HPV infection rate in BC samples [52-54], and only a scarce number of works reports a higher 20-86% HPV infection rate [47, 55-69]. These confounding results reflect the controversial role of HPV in the BC development.

Case-control series does not clarify this item, mostly due to the presence of wide differences in the study designs, HPV detection techniques, and population of the sample. However, Li et al. [70] reported the analysis of ten case-control studies containing 447 BC cases and 275 healthy controls showing a higher risk of BC in HPV infected samples (OR= 3.63, 95% CI = 1.41–9.27). Only one of ten of these works showed a non-significant relationship between HPV infection and BC, and a significant 6.31 fold increased BC risk was reported among HPV infected women with this work was excluded. The analysis of 9 case-control studies reported by Simoes et al. [71] also revealed a higher incidence of BC in HPV infected women (OR, 5.9; 3.26-10.67; p= 0.0001).

The meta-analysis of Li et al. [70] included twenty publications and 1184 BC cases. They revealed 24.49% (95% CI = 22.07-27.05%) overall presence of HPV DNA in BC samples, especially by HPV33 (14.36%), HPV18 (7.13%), HPV16 (7.04%), and HPV35 (7.01%). The Simoes et al. meta-analysis [71] included twenty-nine primary studies, and samples from 1932 BC and 279 healthy controls, and revealed a higher overall HPV DNA presence in BC than in control samples (23% vs. 12.9%).

The prevalence of HPV infection in BC widely varies with geographic origin of the sample [72-80]. Yu et al. [55] reported the presence of HPV33 in 34.1% of 72 BC samples of Asian patients. In the meta-analysis of Li et al. [70], HPV infection rate rose from 12.91% (95% CI = 9.64-16.8) in Europe, to 32.42% (95% CI = 28.5-36.52%) in Asia, and to 42.11% (95% CI = 30.86-53.98%) in Oceania. Simoes [71] also reported a lower incidence of HPV infection in BC samples from Europe (3.4%, 95% CI, 10.2%-16%) than in North America and Australia (42.9%, 95% CI, 36.4%-49.4%). Geography also impacts the prevalence of HPV sub-types. HPV11, 16, and 18 seem to be more frequent in Europe, whereas HPV33 and 56 are more common in Chinese and Japanese woman.

Prevalence also seems be higher when HPV was analysed in fresh breast tissues instead from paraffin-embedded samples (OR= 1.73, 95% CI = 1.21 – 2.74), especially when they have been preserved for extended periods of time. Moreover, HPV virions could be destroyed during the fixation process. HPV infection rate is also higher in invasive and metastatic BC than in situ BC [46], and in BC samples from women with previously diagnosed with cervical cancer [81-83] also identified HPV DNA in BC tissues of patients that had been. They both postulated that HPV DNA might be transported from the original infection site to other organs via lymphatic or haematological being responsible of the development of cancer in other distance organs.

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Chapter 15

IN *SILICO* CHARACTERIZATION OF MAJOR CAPSID PROTEIN L1 OF HUMAN PAPILLOMAVIRUS TYPE 16 BY MOLECULAR DYNAMICS AND DETERMINATION OF LINEAR B-CELL CONSENSUS EPITOPES

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ABSTRACT

This paper has a dual purpose. First, to determine the stable regions of the capsid human papillomavirus type HPV16 L1 from a molecular dynamics simulation up to 4 ns. This study introduces the concept of standard normalized deviation to confirm that the dynamics converge to a stationary value. We found that the region of greatest mobility is the area of interaction between the monomers to assemble into a pentameric structure. Secondly, it also evaluates a recent methodology for identifying computational linear B-cell consensus epitopes from the available data in the IEDB database that may trigger an immune response. Following this study, 13 potential epitopes were obtained that perfectly match with the capsid of the virus.

Keywords: Molecular dynamics; Capsid; Papillomavirus; HPV16; Epitope B

INTRODUCTION

In the medical literature, it has been shown that the human papillomavirus is the main etiological agent associated with cervical cancer, the second leading cause of death among women in the world. In parallel, it has been estimated that just less than 60 percent of the

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sexually active world population has antibodies, as evidence of contact with the human papilloma virus [1].

It should be noticed that the human papilloma virus was first identified in 1933 by Richard Shope when he characterized the mode of transmission of cutaneous papillomas affecting wild rabbits [2]. It also emerged that such a virus only affected the rabbits; it was therefore called *Cottontail Rabbit Papillomavirus*. This revealed the specificity of the virus with the host, and so far, it is known that papillomaviruses infect more than 20 other species.

Burchell et al. showed in 2006 that the virus causes a disease that is primarily spread through sexual contact [3], but since the last century it was suspected that genital warts could become both squamous cell carcinomas of the vulva and the penis.

HPV was initially classified in the family *Papovaviridae* [4], by the fact of having a genome of double-stranded DNA and presenting a non-enveloped icosahedral capsid. In addition, it was also classified in the same group as *Polyomavirus* and *Simian vacuolating* virus Agent (SV40 - a member of the genus Polyomavirus SV40). However, as reported in the work of Villiers et al. [5], both viruses (*Polyomavirus* and *Papillomavirus*) have different genome sizes and a completely different genomic organization, so they are currently classified into different families according to the International Committee on Taxonomy of Viruses, i.e. *Papillomaviridae* and *Polyomaviridae* [5].

The 8.000 bp genome consists of three regions [5]. The first of them is formed by the early genes that are the first to trigger the viral cycle and are responsible for DNA replication and transmission; they correspond to 45% of the genome (E). The late genes (L) correspond to the second region and to the structural proteins responsible for the virus capsid and constitute 40% of the genome. Finally the LCD region (Long Control Region) does not encode proteins and represents 15% of the genome.

More than 130 different types were accounted for, depending on the variability of the L1 protein sequence. Some have been catalogued as low risk viruses such as type 6 and 11, while high risk viruses include such types as 16, 18 and 35. Of the 130 different types, about 40 are sexually transmitted, the second sexually transmitted disease in the world [6].

According to data from the Pan American Health Organization in 2004 [7], type 16 is the most prevalent and types 18, 45, 58, 33 and 31 present a lesser degree. Types 16 and 18 are the most common worldwide [1,7]. In Venezuela, as indicated by Rivas et al. after a study of 1,545 patients in 2012, the results were positive for the virus in 68 and 78 percent of the samples in women and men respectively [8], the most common types being 52, 51, 18 and 16.

Capsid and Overall L1 Pentamer Structure

The human papilloma virus capsid has icosahedral symmetry T = 7 with a diameter of approximately 55 nm. Such capsid consists of two structural proteins called L1 and L2, where there are 360 copies of L1 organized in 72 pentameric capsomeres and twelve copies of L2. It has been shown that only the recombinant L1 protein self-assembles *in vitro* and *in vivo* in the absence of L2 under certain conditions of low pH or high salt under [9]. Similarly, L1 is involved in the transport of the HPV genome. The L1 protein plays an essential role in viral

DNA binding and subsequent packaging [10], particularly the amino acids from the C-terminus [11].

The 3D structure of the HPV16 L1 capsid virus is pentameric. Each monomer has a β-jelly roll domain [10], which is extended by C- and N-terminals known as "arms". Moreover, it has been determined that an interpentameric disulfide bond exists between the cysteines of the C-terminal arm (i.e. Cys428 Cys175 [10]).

As has been outlined by Chen et al. [10], the elements of the secondary structure of the first 380 amino acids are ten β-sheets, the nomenclature of which is from B to J. The C-terminal region consists of α-helix elements (denoted h1 to h5); out of them, helices h2 to h4 are responsible for maintaining contact with the other monomers. Thus, h2 and h3 acquire an inverted V shape, while the β-sheet called βJ and the last helix (h5) return to the original monomer core.

It is important to note that the capsid is bound by hydrophobic interactions between the h4 pentamer helix and the h2 and h3 helices from other neighboring monomers [12]. In fact, each capsid engages 5 invader arms from each neighboring pentamers similarly to what is described in murine polyoma and in SV40 (13). These contacts involve inter-pentameric cysteines located at positions Cys175 and Cys428, where a disulfide bond stabilizes neighboring pentamer capsids [14].

Finally it must be mentioned that the work of David M. Belnap et al. [15] shows an open and closed conformation of papillomavirus and poliovirus capsids. The open conformation is about 2 nm over the closed one.

Immunity

In the scientific literature there are several studies on the design of therapeutic vaccines from complete capsids formed only by the virus L1 protein HPV16 [16]. In parallel, Alexander et al. 2005 [17] stressed that not all people infected by the virus develop antibodies. Specifically, it was determined that only between 54% and 69% of women with HPV 16, 6 or 18 infections, had serum antibodies.

In parallel, Swarbayel et al. indicated that the best specific characterized antibodies are those which are directed against epitopes which are formed from the L1 protein when it is self-assembled into a capsid virus [18,19].

Stability

Rongcun Yang et al. [19] performed an *in silico* analysis about the contribution of D202, D223, N327 and F446 of HPV16 L1 in the stability of the capsid. It was shown that the D202H mutation affects the EF loop stability and that the position of C175, important for pentamer formation, is consequently shifted. D202H mutation therefore plays a key role in shaping the pentamer and the interaction between the chains that make the virus capsid up.

IEDB Data Base

There are a high number of studies in the scientific literature regarding experimental studies of epitopes in HPV16 L1 capsid. In addition, thanks to the rise of new Information Technologies, most of these studies are available in a database called the Immune Epitope Database (available at www.iedb.org), with the main advantage that the information has been manually verified and reaches over 87,000 peptide epitopes corresponding to parasitic and many other diseases [20].

In that sense, Isea has published a computational approach that leverages all the data published in the IEDB [21,22] to determine linear B consensus epitopes from data in a database, and see if they are best suited for generating an immune response. In this article the mobility and therefore the stability of virus capsid from molecular dynamics calculations up to 4 ns of pentamer of HPV16 L1 capsid was determined. Also, the location of the most suitable linear B cell epitopes were identified in the three dimensional structure of the HPV16 L1 capsid.

MATERIALS AND METHODS

The procedure is divided into two parts. Firstly Molecular Dynamics (MD) were performed with the HPV16 L1 pentamer capsid and, in parallel, the consensus B cell linear epitopes were determined from the data available at IEDB. Finally, these amino acids were located and examined whether they are the best sequences to generate an immune response.

As mentioned above, firstly molecular dynamics calculations were performed on the structure of the L1 capsid from data obtained from the PDB database (http://www.pdb.org), from which the coordinates of the HPV16 L1 pentamer capsid (whose id is 2R5H PDB) were obtained. The calculations did not include the amino acids from 405 to 437 because they were not allocated in the results of the X-ray diffraction studies.

Lately, new simulations were performed with the molecular dynamics software NAMD version 2.5 [23], in the San Diego Supercomputer Center thanks to the access to the IBM Blue Gene/P parallel computer with 256 CPUs; a time step of 2 fs was set. The total time of the molecular dynamic simulations was 4 ns. The force field used was CHARMM version 27 [24]. The terms for non-bond and van der Waals energies were performed with a cut off distance of 12 Å. Solvation was performed with a layer of at least 5 Å around the protein, the dimensions of which are (155.4, 128.7, 86.1), which correspond to the dimensions (-77.2 to 78.2, -69.5 to 59.5 and -42.9 to 43.2). The system was centered in the position (0.5, -4.9, and 0.2). The calculi were performed under periodic boundary conditions. The model of the solvent chosen was 41,569 water molecules and to neutralize the charge of the system, 28 sodium (Na+) counter-ions were inserted (the total number of atoms was 157,896).

The ShakeH algorithm implemented in the VMD software has been used for assigning the protonation states of the ionized protein residues [25]. In addition, the equations of motion have been integrated by means of the explicit velocity- and position-verlet-like algorithms of second order [26]. The firs step was to minimize the energy by using the steepest descent algorithm (preceded by a position restrained stage for protein atoms) and a conjugate gradient until an energy gradient lower than 2.0 kcal/mol/Å was reached. MD simulations were

performed according to the following criteria: 700 ps with the positions of the protein's atoms restrained to allow solvent equilibration; 700 ps with the positions of the backbone's protein atoms restrained to allow gradually liberation of the system; and then, a full MD up to 4 ns with no restrictions (NPT ensemble was completed). The B-factors derived from the trajectory were calculated as $8/3\pi^2 <|\Delta r|^2>$, where $<|\Delta r|^2>$ is the mean square atomic displacement relative to the average position from all trajectories of molecular dynamics.

Finally, the linear B cell consensus epitopes were determined according to the methodology explained by Isea [21,22]. In this procedure, all the derived peptide epitopes of HPV16 L1 capsid that exist in the database IEDB (see http://iedb.org) were used. Next, a local multi-alignment gap scoreless through the Nomad program (ref, http://expasy.org/tools/nomad.html) was conducted. Finally, the obtained blocks were reevaluated using the BepiPred program, which allows the prediction of linear B-cell consensus epitopes based on Markov chain models [27].

RESULTS

The pentamer structure of the major capsid protein L1 of HPV type 16 has been calculated by means of MD simulations. In addition, the root mean square deviation normalized (abbreviate Rn) values of all atoms have been also obtained from the initial X-ray coordinates until a 4 ns simulation was completed (Figure 1). In such a calculation, the highest value obtained in the RMSD entire dynamic is obtained and from it, all data were normalized to see if there are structural changes along the dynamics. This methodology, which has not been used in the literature to the authors' knowledge, is useful to visualize if the dynamic equilibrium is reached throughout the simulation. This state can be observed if the final graph shows that the dynamics converge to an average value of the fluctuation of all atoms, and therefore, never exceed a given maximum RMSD value over time of the dynamics.

Figure 2 shows the average RMDS dynamics throughout the HPV16 L1 up to 4 ns in blue, while red shows the experimental temperature factor obtained by diffraction of the L1 data obtained from the PDB. It is recalled that the temperature factor should be proportional to the displacement of atoms, and therefore, both curves should be comparable in certain regions. The high mobility of the C- and N-terminal regions is also visible, but did not show a similar behavior between the RMSD value obtained (blue color) with respect to the temperature factor (Figure 2). This effect can be observed in the regions between β-B1 and β-B2, β-D and β-E, H1 and β-F and β-H1 and β-H2. On the other hand, regions β-C and β-D and β-G1 and β-G2 show that the RMSD value and the temperature factor present a similar mobility.

In fact, Figure 3 shows the result when averaging separately the mobility of each of the amino acids grouped into five chains identified as A, B, C, D, and E, which constitute the HPV16 L1 capsid. Although it could be thought that the movement between the five strings must be symmetric, results depicted in Figure 3 shows that it is asymmetric. Furthermore, B and C chains have a higher mobility compared to chains E and D, while the chain A is the one with the lowest degree of mobility.

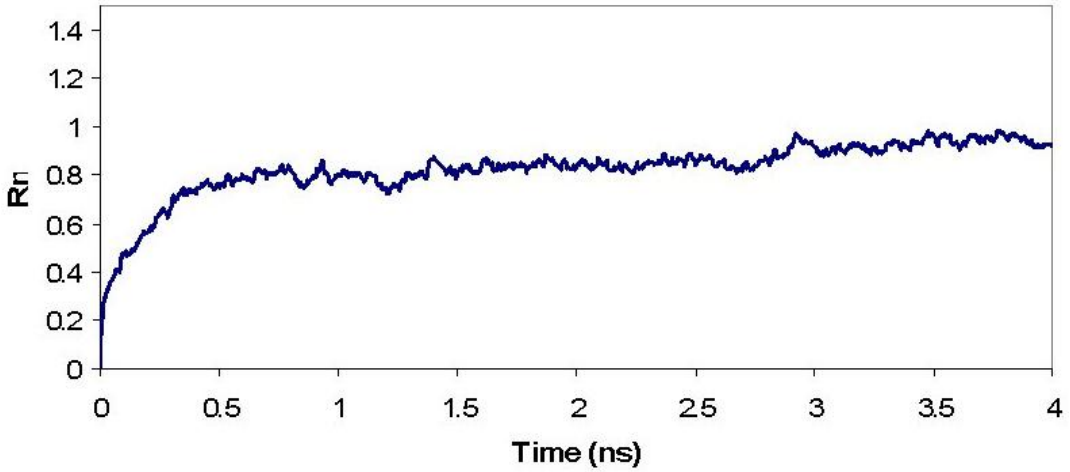

Figure 1. RMSD normalized standard deviation (abbreviated as Rn) during the 4 ns Molecular Dynamics calculation of the virus capsid (more explanation in the text).

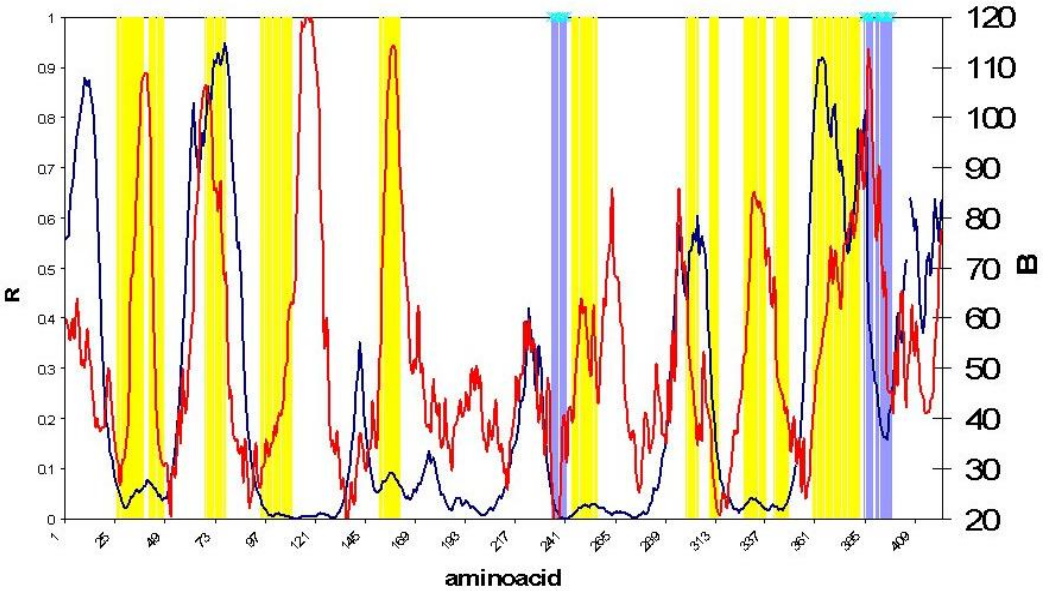

Figure 2. Mobility of the HPV16 L1 capsid. Blue line shows the RMSD value (denoted as R) along the Molecular Dynamics up to 4 ns. Red line shows the experimentally measured temperature factor by X-ray diffraction (B). The yellow stripes identify regions of β-sheets while the blue stripes are those with alpha helix structure.

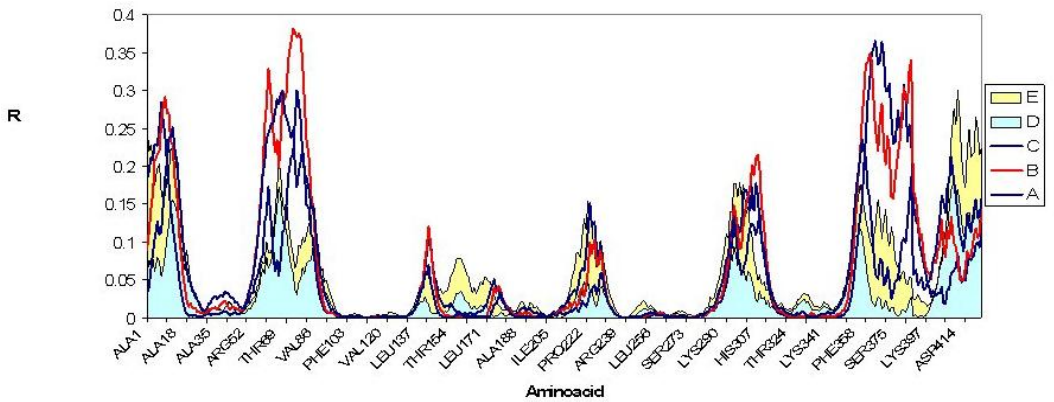

Figure 3. RMSD mean square deviation (abbreviated as R) showing mobility at each of the five chains comprising the HPV16 L1 capsid virus.

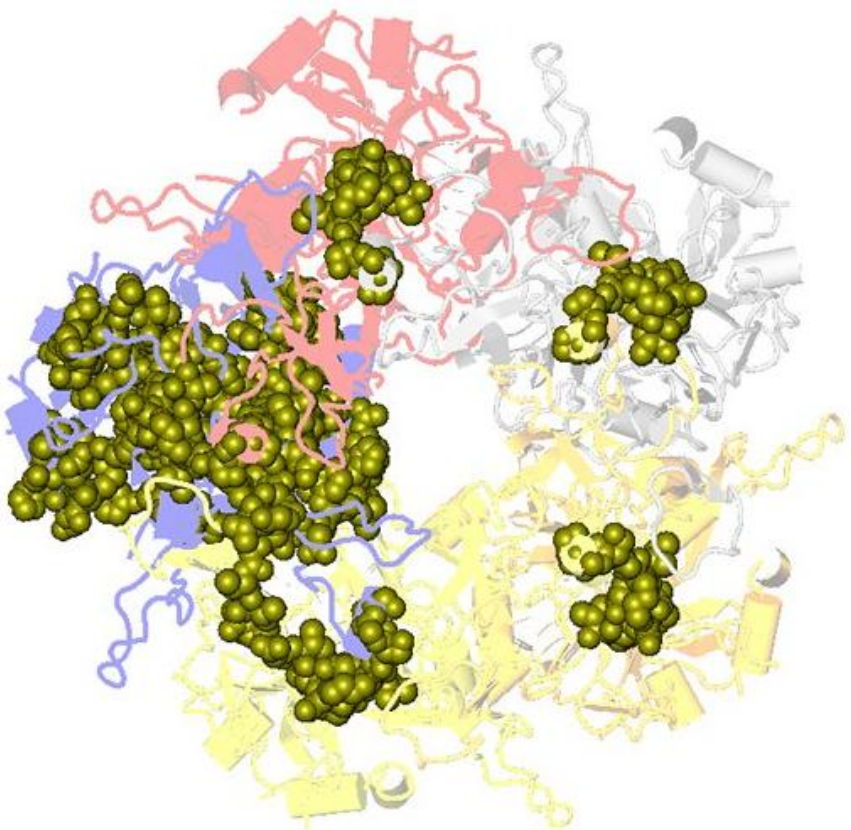

Figure 4. View along the HPV16 capsid channel, where there are five pentamer chains in different colors. The A chain is depicted in blue. The linear consensus epitopes determined in this work are shown in CPK, and only the NYFPTPSGSMVT epitope is shown in each of the rest of the chains for an easy identification.

B Consensus Linear Epitopes of the HPV16 L1 Capsid

All the B and T epitopes already available in the literature concerning the HPV16 L1 capsid were obtained. The end result were 170 epitopes and, in this paper, only those epitopes whose length is less than 12 (see 21, 22 for details) have been considered, reducing the total number to 145 (epitopes such as GFGAMDF, IHSMNSTIL, KYPDYIKM, etc. were discarded). The next step was to generate segments of 12 amino acids by using Nomad (http://web.expasy.org/nomad/), and then, to dismiss all the duplicate ones, so only 73 consensus epitopes were selected. Finally, those fragments that were antigenic were determined using the program BepiPred. From the latter set, the 19 most probable linear consensus epitopes were selected (see Table 1). Such epitopes are shown in Figure 4, where the regions that are antigenic candidates are present in the A chain of the virus (blue), while in the rest of the chains, only the NYFPTPSGSMVT epitope is present (shown in CPK for easy identification).

Figure 5 shows how the thirteen antigenic epitopes according to BepiPred program are aligned with the L1 sequence according to Blasp NCBI server, and how it can be appreciated that these alignments are entirely similar with respect to the primary structure of the virus capsid.

```
L1 AVVSTDEYVARTNIYYHAGTSRLLAVGHPYFPIKKPNNNKILVPKVSGLQYRVFRIHLPD  60
                                                          HLPD
L1 PNKFGFPDTSFYNPDTQRLVWACVGVEVGRGQPLGVGISGHPLLNKLDDTENASAYAANA 120
   PNKFGFPD
          DTSFYNPDTQRL
                                                    DTENASAYAANA
L1 GVDNRECISMDYKQTQLCLIGCKPPIGEHWGKGSPCTQVAVQPGDCPPLELINTVIQDGD 180
                       IGCKPPIGEHWG
                             IGEHWGKGSPCT
                                    PCTNVAVNPGDC
                                      TNVAVNPGDCPP
L1 MVDTGFGAMDFTTLQANKSEVPLDICTSICKYPDYIKMVSEPYGDSLFFYLRREQMFVRH 240
L1 LFNRAGTVGENVPDDLYIKGSGSTANLASSNYFPTPSGSMVTSDAQIFNKPYWLQRAQGH 300
              ENVPDDLYIKGS
                       KGSGSTANLASS
                                    NYFPTPSGSMVT
                            SGSTANLASSNY
L1 NNGICWGNQLFVTVVDTTRSTNMSLCAAISTSETTYKNTNFKEYLRHGEEYDLQFIFQLC 360
                                  ISTSETTYKNTN
L1 KITLTADVMTYIHSMNSTILEDWNGGSGGEDPLKKYTFWEVNLKEKFSADLDQFPLGRKF 420
                                    DPLKKYTF
L1 LLQL                                                          424
```

Figure 5. A multiple alignment where the similarity found in those consensus linear B epitopes that may generate an immune effect and are present in the HPV16 L1 capsid is highlighted (see Table 1).

Table 1. B linear consensus epitopes of the HPV16 L1 capsid virus. Their identification according to IEBD is presented in parentheses. Those epitopes that could present an antigenic behaviour are bold faced according to BepiPred (27)

ACQKHTPPAPKE (110898)	**APKEDPLKKYTF (111113)**	**NYFPTPSGSMVT (112327)**	**PCTNVAVNPGDC (111611)**	**PKEDDPLKKYTF (149873)**
ATPTTSSTSTTA (111733)	DTENASAYAANA (110916)	DTSFYNPDTQRL (111286)	ISTSETTYKNTN (111139)	KGSGSTANLASS (111533)
ENVPDDLYIKGS (108910)	GLQPPPGGTLED (111351)	HLPDPNKFGFPD (111422)	IGCKPPIGEHWG (175613)	**IGEHWGKGSPCT (111418)**
SGSTANLASSNY (110872)	**TNVAVNPGDCPP (111175)**	**TSSTSTTAKRKK (109478)**	**YLPPVPVSKVVS (112625)**	

CONCLUSION

As generally accepted, it is important to estimate the degree of mobility of the macromolecules to determine, for example, how labile they are. In that sense, a molecular dynamics of the HPV16 virus capsid entirely composed of the L1 using the Blue Gene supercomputer at the San Diego Super Computing Centre has been made. This simulation shows the asymmetry of movement of the chains that make the virus capsid up.

When analyzing the result of the mobility of a macromolecule where many atoms are involved, it should be considered if the displacement between the chains is asymmetrical between them, which may result from the lack of considering an icosahedral symmetry in the simulation. While it is true that the modeling performed in this work used periodic boundary conditions, the correlation of movement due to the symmetry T = 7 was not implicitly considered.

In parallel, the normalized standard deviation should be considered in order to identify whether the molecular dynamics achieve a stable behavior and also to monitor the mobility between each of the strings that make the HPV16 L1 capsid. With all this together, it is possible to postulate whether the system is oscillating at equilibrium.

If an optimization of the macromolecule was needed, a molecular mechanics should be performed. The programs that perform this calculation impose the condition that calculations stop when the minimum energy value is reached in a number of iterations. Given the comments above, all conditions should be imposed to the whole system, i.e. to the five separate chains and the normalized RMSD value. Such requirements in such a large number of atoms would probably have as a consequence that convergence would not be reached in the calculation in a finite number of iterations (minimum energy).

It is not surprising that the molecular dynamics show that there is no greater mobility in the C- and N-terminal regions of the protein, and also, that there is a great mobility in the G1-β and H2-H3 regions, which is consistent with the temperature factor data measured experimentally (Figure 2). In this regard, it is important to notice that the β-G1 and β-F regions contain the hydrogen bonds that hold the L1 monomer, which explains the mobility. Furthermore, the H2-H3 region is the area that forms the pentamer, and therefore, it is expected to be displaced. Besides, as evidenced in Figure 3, the mobility in the B and E chains of the HPV16 L1 capsid is larger and hence, more likely to be a target to generate an immune response.

Moreover, regions β-B1 to β-B2, β-E to β-G2, β-D, β-H1, β-H2 and that before H1 present a low RMSD displacement value, which is inconsistent with the regions of the experimental temperature factor. However, it should be noted that these regions have a barrel shape consisting of a range of films, and mobility should not present major changes between them. This assumption can be confirmed by a more advanced molecular dynamics study.

From all the B and T epitopes already available in the literature concerning the HPV16 L1 capsid, we obtained only 19 linear B-cell consensus epitopes which could be considered as antigenic candidates, the effectiveness of which will be further analyzed.

DEDICATION

RI wishes to dedicate this work to his wife Luz Pérez and to their children Jesus Raúl and Luis Miguel for their patience while he spent a lot of time working since they are his inspiration every time.

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Chapter 16

THE ROLE OF HUMAN PAPILLOMAVIRUS IN NON-SMALL CELL LUNG CANCER

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ABSTRACT

Over 120 sub-types of small, non-enveloped, DNA human papillomavirus (HPV) selectively infect keratinocyte stem cells on the surface layer of human skin and mucus. Most of them do not generate any symptom and can be easily cleared up by immune system. However, HPV subtypes with oncogenic potential induce cell proliferation and origin the development of benign warts or malign tumors. HPVs are the most important infectious cause of cancer. Over 5% of worldwide newly diagnosed malignancies are attributable to them. Pelvic and perineal structures are exposed to the virus through direct skin contact during the sexual practices, and can be place of persistent warts known as condylomata acuminata, premalignant lesions, and malignancies. Almost all cervical cancers, the most common female malignancy worldwide, and many vulvar, vaginal, penile, and anal cancers are caused by HPVs. Changes in sexual behavior are increasing the HPV presence in oral and upper respiratory track, and the incidence of HPV-related oropharyngeal cancers. We review current literature about epidemiology, and outcome of HPV-related non-small cell lung cancer.

1. BACKGROUND

Over 120 human papillomavirus (HPV) subtypes are able to infect human skin, mouth, airways, genital mucus, and anus. They belong to the wide Papillomaviridae family, a group

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of host- and tissue-tropic non-enveloped DNA viruses that infect reptiles, birds and mammals [1, 2]. The HPVs take advantage of microtraumas to infect keratinocyte stem cells on the surface layer of epitheliums, and replicate later in the outermost layers of the skin. Some HPV subtypes do not only exhibit tropism for human hosts, they also present a different affinity for specific tissues. For example, the HPV-1 uses to infect the soles of the feet, while the HPV-2 infects the palms of the hands.

Symptoms of HPV infection strongly vary according the virus subtype. Most of them are cleared up by the immune system without treatment or consequences but, if HPV remains and induces the proliferation of epithelium cells, they can origin warts, benign tumors or the development of malignancies. HPV-1, 6 and 11 infections have been reported to induce warts. HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are considered clearly carcinogenic [4].

The genome of HPV [5] is composed of six early (E1, E2, E4, E5, E6, and E7), two late (L1 and L2) genes, and a non-coding long control region (LCR). Early and late genes codify multiple functions: The E1 gene prepares viral genome for replication; The E2 gene regulates viral promoters, and reduces E6 and E7 expression; The E4 gene is expressed in the late infection phase and facilitates the virion release; The E5 gene initiates the epidermal growth factor signal cascade, down-regulates the surface expression of major histocompatibility complex class I, and prevents the HPV elimination by T cells; The E6 gene interacts with MAGUK, MAGI and DLG proteins, reduces response to DNA damage by p53 degradation, reduces protein activity of mammalian cells, and increases cell proliferation; The E7 gene inactivates pRb tumor suppressor protein, prevents apoptosis, and promotes HPV replication [6-8]; L1 protein composes the star-shaped capsomers that made up the capsid of HPV; The L2 protein participates in the virion formation and the infectious process.

The first report of a papilloma related cancer was done by Peyton Rous in 1935. Nowadays, we know that 5.2% of malignancies are attributable to HPV. It represents over 561000 new cancers diagnosed worldwide and positions HPV as the most important infectious cause of cancer [4]. Urogenital malignancies as cervix uterine, anal, vulvar, vaginal, and penile tumors have been clasically related to a dozed high-risk oncogenic and sexually transmitted HPV subtypes. Anyway, current literature also suggests an eventual relationship between HPV infection and the development of malignancies distant from the genital area such as oropharyngeal, lung, and esophagus cancers [9, 10]. This chapter tries to review the epidemiology, management, and special features of the main HPV-related human malignancies.

2. Epidemiology and Etiology of the Lung Cancer

Lung cancer is the most common and lethal malignancy in the developed countries. It represents 12-13% of new cancer cases, and 18-28% of cancer deaths in the European Economic Community and the United States of America [18]. However, lung cancer cannot be understood as a unique malignancy. Over 37 histopathological varieties can be distinguished, with a different clinic behavior, molecular expression, response to treatment and, sometimes, a different etiology.

Smoke origins over the 90% of lung cancers. However, they also appear in patients that never smoked, suggesting the existence of any other etiologic agents. Genetic alterations, exposure to chemical substances or to ionic radiations, the presence of previous lung scars, and virus infections such as cytomegalovirus, John Cunningham virus, or the simian vacuolating virus have been considered.

The eventual relationship between the HPV infection and the development of bronchial malignancies was first suggested by Syrjänen in 1979 [19]. Since then, HPV tracks have been detected in non-small cell lung cancer (NSCLC) samples but no definitive association between HPV infection and NSCLC development has been definitively confirmed in Caucasian race. Anyway, the HPV infection rate of NSCLC samples widely varies on literature.

3. HPV INFECTION RATE OF LUNG CANCER SAMPLES

The HPV infection rate of lung cancer samples depends on the geography area [20, 16]. Single studies use to report a low HPV infection rate in NSCLC samples from Mediterranean populations [29, 30]. Galvan et al. [65] does not detect HPV tracks in a series of 100 lung cancer patients from Italy and the United Kingdom. Koshiol et al. [35] reported 0.5% only of weak positivity for HPV DNA in NSCLC samples from 399 Italian patients, our own series detected 2.5% HPV positivity in NSCLC samples from Spanish patients [36]. However, this low HPV infection rate in European populations can be discussed. A recent Finnish series [53] reports 5.2% HPV infection rate in 77 lung cancer histological samples, and three Italian works including over 2500 lung samples reveled 14-21% HPV infection rate in NSCLC tissues [42-44]. And, in the other hand, single studies report a significant higher HPV infection rate in NSCLC samples from Eastern patients [31-34].

Three meta-analyses clearly reflect the wide differences in the HPV infection rate of NSCLC samples between Western and Asian patients. The first of them[20], including 37 publications and 2435 cases, reports 7.1% HPV infection rate in NSCLC samples from Western patients and 20.4% in samples from Asian patients that coincides with a significantly higher intensity of the HPV tracks in the Asian samples (50% vs. 88%). The second meta-analysis [16], including 4508 cases, reported a mean 24.5% HPV infection rate of NSCLC samples worldwide that decreased to 16% in Western patients, and increased to 35.7% in the Asian ones. The third meta-analysis [56], including 100 and 7381 cases, reports 22.4% HPV infection rate in NSCLC and confirms the impact of geography in the incidence of the infection.

The HPV detection rate can be biased by the nature of the analyzed NSCLC sample. The HPV DNA uses to be located mainly in the tumor cells, and not in the healthy adjacent tissues [24]. It could explain why different works failed to detect a significant HPV presence in serum or bronchial aspirates from lung cancer patients [22, 23], but a recent work from the University of Foggia [15] refutes the hypothesis and detects 15.1% HPV positivity on exhaled breath condensates from 89 lung cancer patients that overlaps with paired samples from bronchial brushing and lung tissue.

If the HPV infection rate of NSCLC samples also depends on histology is controversial. Polish and Italian works [45, 52] did not detect significant differences in the HPV infection

rate according to lung cancer histology, while a French work [25] reported a significant higher percentage of HPV infection in lung adenocarcinomas than in any other lung malignancy (11 vs. 25%). A West Chinese analysis [47] reports a significantly higher HPV infection rate in squamous carcinomas than in lung adenocarcinomas (51.4%, 16.2%), a Japanese study [48] did not find statistical differences (10% vs. 9%), and another Japanese work [28] found a significant higher incidence in lung adenocarcinomas than in squamous carcinomas (7% vs. 30%).

The last report coincides with other many Asian works that reveal a higher HPV infection rate in Asian women, non-smokers, and lung adenocarcinomas [49, 51, 24, 26, 15]. Asian origin, female gender, non-smoke habit, and adenocarcinoma histology also increase the percentage of NSCLC harboring sensitizing mutations of the epidermal growth factor receptor (EGFR), a target for the modern tyrosine kinase inhibitors gefitinib and erlotinib. Even the higher incidence of HPV infection in lung cancer samples from elderly patients [53] coincides with a higher presence of EGFR mutation in this population.

Recent investigations preclude that HPV infection rate could depend on EGFR status. Baba et al. [28] reported a higher HPV infection rate in NSCLC samples from patients who responded to gefitinib (75 vs. 0%). Marquez-Medina et al[36] suggested a direct relationship between HPV infection and the presence of EGFR sensitizing mutation in NSCLC, but did find significant differences in the HPV infection rate of EGFR mutation or EGFR wild type NSCLC samples in a series. And, finally, Kato et al. [55] demonstrated a higher incidence of HPV infection among Japanese patients with lung cancer harboring sensitizing mutations of the EGFR (38% vs. 7%, p= 0.021).

4. HPV CONTAGION OF THE RESPIRATORY TRACT

HPV can infect the upper respiratory track by a direct extension during sexual practices. The HPV infection rate of oral cavity ranges from 1% to 60% [57] and increases with the number of oral sex or open-mouthed kissing partners. Later, HPV infection lowers the respiratory track by continuity. HPV infection rate progressively decreases from 59%, to 43%, and 33% in patients with oral, pharyngeal, and laryngeal cancer, respectively, and it is even lower in patient with more distant respiratory malignancies.

Anyway, the presence of oral HPV infections in a significant percentage of children rules out sexual practice as an exclusive infective mechanism, and allows the possibility of a vertical transmissions mother to infant during the birth process although oral infection through salivary or cross transmission is also plausible. Perinatal transmission of HPV-6 and 11 has been related with the onset of the very rare recurrent respiratory papillomatosis in the childhood (0.002%), which incidence rises to 1% among descendants of women with genital warts at the moment of birth. Recurrent respiratory papillomatosis produces warts along the entire respiratory track that interfere with breathing, and recurs instead of repetitive resections [11-13].

A different hypothesis supports that HPV infection could origin in the genital track and to infect the lung tissues through the blood. Hennig [58] and Iwamasa [49] have reported 80-92% incidence of previous cervical diseases, and 74% of cervical HPV active infections, in

patients with HPV-infected NSCLC. However, some other works did not confirm this asosiation [51].

It is not clear if HPV infection modifies the outcome of patients with NSCLC. Patients with HPV-infected oral or tonsillar cancers have a significant better survival that the rest of them [61, 62], and a better prognosis has been also suggested for HPV-infected NSCLC [49, 27, 64]. Anyway, no current study has explored this aspect correctly. The revelation of an eventual relationship between HPV infection and the presence of EGFR mutation in lung cancer patients warrants a deeper investigation of this phenomenon, and of their therapeutic and outcome implications.

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Chapter 17

HPV MODELLING GOES BAYESIAN: INFERENCE VIA ADVANCED MARKOV CHAIN MONTE CARLO METHODS

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Abstract

This chapter details an overview of each of the statistical aspects of HPV modelling in a quantitative manner. We introduce practitioners to development of quantitative models for HPV epidemic dynamics in a generic Bayesian framework. This is done in two settings, when the population dynamics are deterministically described by a dynamical model or are treated as stochastic processes. In each case, under the Bayesian formulation we consider, the model parameters are treated as static random vectors to be estimated from the data along with the dynamics of the epidemic in a population. For these generic classes of Bayesian models we specify how one can statistically formulate key inferential quantities for generic HPV model frameworks related to point estimation, interval estimators, model selection, prediction and forecasting. We then provide explicit details in the HPV epidemics context of popular approaches to constructing the model components underpinning the presented Bayesian framework.

Next we discuss sexual mixing matrices which describe sexual behaviour in a population. We present from first principles the components of such a matrix and then discuss to extend aspects of this model component to incorporate additional flexibility in the populations behavioural assumptions through introducing stochasticity to the mixing matrix.

Having formulated the models we then carefully detail for practitioners the sampling approaches that can be adopted to make inference from the Bayesian models developed, based on Markov chain Monte Carlo sampling algorithms. In particular

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we present for practitioners new to this field, an elementary discussion on Markov chain samplers. Then for the benefit of those familiar with the basic mechanisms involved with such techniques, we present details of state-of-the-art samplers which are adaptive in their Markov chain proposals. We provide algorithms for the Gibbs sampler, the Slice sampler, the Adaptive Metropolis sampler and the Riemann-Manifold Hamiltonian Monte Carlo sampler.

We then conclude the chapter by considering an example based on the actual data, which incorporates calibration as well as an analysis of the impact of vaccination.

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1. Introduction

The human papillomaviruses (HPV) are DNA viruses that usually infect differentiating epithelial cells of the skin and mucosae. Over 100 HPV genotypes have been identified so far. These are classified according to their tissue tropism (mucosal or cutaneous) and oncogenic potential. About 40 HPV types are known to infect the mucosae, including those of the anogenital and oral tracts, and 13-18 of these are considered to be high risk (oncogenic) because of their association with malignancies. Low risk HPV types are associated with benign lesions such as genital warts and low-grade intraepithelial neoplasias of the cervix [102]. HPV is the most common sexually transmitted infection in the world [21]. It is known to contribute to the vast majority of cervical cancer cases and is also involved in a proportion of other anogenital cancers and cancers of the head and neck. Currently, the overall burden of disease attributed to HPV, both cancers (as much as 5.2% of incident cancers worldwide) and benign lesions such as genital warts, remains considerable [89].

Two vaccines have been developed and shown through clinical trials to be highly effective in the prevention of precancerous lesions and persistent infection due to certain HPV types [116, 128]. The quadrivalent vaccine (Gardasil) protects against high risk HPV types 16 and 18 that are associated with 70-75% of cervical cancers, and against low risk HPV types 6 and 11 that cause more than 90% of genital warts. The bivalent vaccine (Cervarix) provides protection against HPV types 16 and 18 only. Both vaccines have been licensed in more than 100 countries and publicly funded national immunisation programmes have commenced in some of these.

National immunisation programmes are costly and decisions regarding their implementation are generally made on the basis of health-economic evaluations. In regard to HPV, these decisions are complicated by the fact that HPV is a sexually transmitted infection, and sexual behaviours in a population are difficult to describe accurately. Also, only a small proportion of infections do not resolve and can lead to cancer many years or decades subsequent to acquisition. Both of these factors have generally been addressed by employing models to estimate the long-term impact of vaccination on the incidence of HPV-related disease so that the costs and benefits can be calculated.

2. Data for HPV Modelling

2.1. Data Required to Describe HPV Transmission

We begin with discussing the data required for specifying the force of infection, which is the rate at which an individual becomes infected per unit time.

Sexual behaviour data are usually presented in HPV models as partner change rates stratified by age and level of sexual activity of individuals, although it can be stratified by other criteria, such as, for example, annual income, ethnicity, place of residence and so on. Extensive sexual behaviour surveys have been conducted only in a few countries. In particular, the National Survey of Sexual Attitudes and Lifestyles (Natsal 2000) done in the UK, interviewed 11,161 respondents (4762 men and 6399 women) aged 16 to 44 [79]. The Australian Study of Health and Relationships [88] covered 19,307 men and women aged 16-59, and the National Health and Social Life Survey [4] questioned 2,500 adults, aged 18 to 44.

HPV transmission rates are used to approximate the probability of HPV transmission from men to women and vice versa per partnership or sexual act. A few studies focused on investigation of these rates. The HITCH Study (HPV Infection and Transmission among Couples through Heterosexual activity) in Canada enrolled 18-24 year old women and their male partners and followed them for about five years [20]. It was established that transmission from men to women (3.5 per 100 person-months, 95%, CI 2.74.5) was very similar to that from women to men (4.0 per 100 person-months, 95% CI, 3.05.5). On the other hand, another study in Hawaii [75] which followed 25 heterosexual couples (mean age for men 28 (range 18-59 years) and for women 26 (range 18-57)) for an average of 7.5 months, found that men-to-women transmission was 4.5 per 100 person-months (95% CI, 1.59.3) and transmission from women to men was 27.8 per 100 person-months (95% CI, 19.0-38.3).

In order to calculate the time dependent force of infection $\lambda(t)$ given the above mentioned data, we usually assume that the true mass action principle holds (also known as frequency dependance, i.e. force of infection does not change with population size, which is sensible for sexually transmitted infections), and then make $\lambda(t)$ proportional to the product of the partner change rate, probability of HPV transmission and HPV prevalence in the population where the partners are chosen from.

Another key data needed to describe HPV transmission is the *recovery rate*, i.e. the rate at which an individual clears HPV infection. It is approximated as the inverse of duration of infection. It is pertinent to mention that it is common to distinguish between transient and persistent HPV infections. HPV types linked to cervical cancer have been of most importance from public health perspective, and it is known that women with persistent HPV infections develop cervical cancer. While it seems natural to define persistence based on the duration of infection, this definition is not uniformly accepted yet (see discussion in [101]). As the reported values for the duration of infection and often divided into those for transient or persistent infections, one has to carefully interpret them prior to incorporating either in a model.

Among the most representative studies reporting mean and/or median time to clearance of HPV infection we would like to mention the HPV in Men (HIM) international study [59] which covered 4,074 men from Brazil, Mexico and the US aged 18-70 years. The

Ludwig-McGill cohort study [132] enrolled 2,462 Brazilian women aged 18-60.

Duration of natural immunity to re-infection with the same HPV type following clearance of infection is crucial in HPV models assuming existence of an immune state. However, there are presently no definitive data on this, due to high costs and organisational difficulties which would inevitably accompany a study requiring a long term following of its participants who would be expected to cope with inconveniences of being investigated for a prolonged period. While it is generally assumed that there is some form of immunity, the lack of solid evidence of its existence should be acknowledged.

2.2. Vaccination Related Data

Currently, there are two HPV vaccines on the market: Gardasil and Cervarix. Both protect against HPV types 16 and 18, and Gardasil also protects against types 6 and 11. Gardasil and Cervarix are preventative (prophylactic) vaccines, that is, they protect vaccinated individuals who are not infected, while infected individuals are protected from subsequent reinfection. This implies that vaccination is most efficient if done before infection with the HPV types covered by the vaccine occurs. In order to model vaccination, it is useful to know the actual *vaccine coverage*, which is a percentage of the targeted population who have received an intended number of vaccine doses. *Vaccine efficacy* is defined as the percentage reduction of cases among vaccinated individuals. Information on efficacy usually comes from clinical trials such as [135] or [125]. Efficacy is measured against persistent or transient infections, or diseases associated with HPV.

Duration of vaccine protection is not known as yet, hence it introduces additional uncertainty into HPV models with vaccination. According to the available studies, for Gardasil protection against HPV 16 and 18 has lasted at least 5 years after vaccination and for Cervarix more than 6 years [66]. It can be reasonable to assume a life-long protection for modelling purposes, which is discussed in Section 7

2.3. Observational Data

Data describing HPV in a population usually come from cross-sectional and longitudinal surveys. Typically, these surveys approximate *HPV DNA prevalence*, that is, the proportion of population with a detected HPV infection. Non-oncogenic HPV types 6 and 11 are known to cause genital warts, so the available genital warts incidence data give an idea about presence of these types in a population. Observational data are used to calibrate mathematical models to: the models should be able to reasonably reproduce these data in order to be granted some plausibility.

3. Statistical Approaches to HPV Modelling

The following subsections outline a set of statistical approaches that summarise how the Bayesian modelling paradigm can be adopted when undertaking statistical calibration in HPV modelling. The presentation style is intentionally general so that interested practitioners can adopt the methods proposed for their specific problems. The aim is to provide for

medical practitioners and modellers in the HPV community a clear understanding of the key features and attributes that such an approach will involve.

The data detailed above are typically costly to obtain in terms of both time and money. It is therefore critical for modellers to maximise their understanding of the dynamics of HPV in light of whatever data are available. Though there may be many challenges associated with performing statistical analysis on these data, the following sections will highlight approaches one may adopt to perform a sound and robust statistical analysis, whilst also allowing a statistically rigorous approach to incorporation of often under-utilised expert opinions on aspects of the model. The incorporation of both observed data and expert opinion is standard under a Bayesian paradigm and this is the motivation for consideration of such approaches in HPV modelling.

In the context of modelling features of HPV it is becoming increasingly important for clinicians, epidemiologists and applied statisticians to validate or invalidate hypotheses postulated about different aspects of HPV transmission and associated diseases. This is especially important to understand in the case of certain HPV types which are involved in genital and throat cancers.

To understand and address these hypotheses from clinicians and public health experts, it is standard practice for epidemiologists and statisticians to develop models for the epidemic dynamics. These will be discussed in the following sections, including the development of dynamic and stochastic compartmental models which are stratified by age, activity group, gender and involve sexual mixing matrices. These models have to be calibrated accurately to observational data, and calibration approaches require several stages as will be discussed.

Calibration, which is the process of determining parameters values such that model output replicates features of empirical data, is a very important component of model development, since a failure to produce outcomes close enough to the actually observed ones indicates possible problems with model structure or modelling assumptions, so credibility of analyses based on this model may appear rather questionable. As discussed in [129], while calibration is performed in many modelling studies, it is rarely explained in an adequate manner. This may be partly due to the lack of systematic approaches to the procedure, which potentially leads to uncertain robustness of the results. In particular, the goodness-of-fit metrics are often not mentioned explicitly or are simply reduced to a visual assessment of fit. In addition, the approaches adopted to perform parameter estimation based typically on search algorithms (i.e. how to organise selection of parameter combinations) are generally not well described. In some cases, the informal "trial-and-error" approaches are used (which is essentially guessing at what values to try) while in other cases some "systematic variation" of model parameter values is mentioned but not explained. More often than not, a single best-fitting parameter set is focused upon to conduct model analyses, which hardly captures parameter uncertainty. We broaden the horizons of practitioners in this regard by explaining how to work with a posterior distribution over the parameters in the model, how to undertake rigorous point estimations as well as interval estimation for parameters and the interpretation of such quantities. Availability of quality calibration methods is especially relevant for HPV modelling. We will detail below how a Bayesian modelling approach can be directly beneficial in the context of HPV model calibration. It allows one to incorporate prior information into the inference on the model parameters either subjectively or objectively. We argue that in the context of HPV modelling where one

has many more parameters in the models to consider, compared to the available observational data, the Bayesian paradigm will be directly beneficial to performing calibration. In particular it provides a rigorous statistical framework to incorporate two additional sources of information into the inference that are often either not utilised or worse still heuristically incorporated in a informal statistical approach. The approach we present is rigorous and incorporates such additional data in a statistically meaningful manner. The first additional data component we consider is prior knowledge about HPV transmission obtained from previous studies on populations which may have similar attributes to the population under study, and the second is clinician's expert opinions into the formulation of plausible prior beliefs about possible model parameters in a HPV dynamic compartmental model and the ranges that such parameters may take. A comprehensive book length survey on such prior elicitation from experts in practical settings including health care settings is provided in [106].

The common feature in addressing all these hypothesis in HPV dynamic modelling can be set up under a few basic statistical inference challenges. We will first present these in a general framework and formally present the inference problems related to calibration and prediction. Then we will detail efficient statistical sampling solutions based on recent advances in Markov chain Monte Carlo.

Notation: In the proceeding sections we denote random variables by upper case and their realisations by lower case. We utilise bold for vectors and matrices and adopt the standard statistical notation of $X_{1:T} = [X_1, \ldots, X_T]$. We will assume all cumulative distribution functions by $F(x)$ and assume that for the context considered in this chapter they will always admit the existence of a probability density that we denote by $f(x)$. Throughout the following sections we will further assume that we consider a Bayesian Paradigm, though of course such ideas presented below are general for any distribution and therefore equally as suitable for application in frequentist modelling frameworks.

3.1. Bayesian Modelling Perspective

The statistical modelling paradigm known as Bayesian methodology has much to offer the HPV modelling community. In this chapter we will highlight several advantageous aspects that such a modelling approach can offer when designing and calibrating HPV models. The idea of Bayesian approach is to represent uncertainty about model parameter $\boldsymbol{\theta}$ with a probability distribution and update this uncertainty taking into account the observational data $\boldsymbol{y}$ which becomes available. The normalised product of such updates is a probability distribution with less uncertainty in the parameters termed the posterior density. Hence, we make conclusions about a parameter $\boldsymbol{\theta}$ in terms of probabilistic statements using the posterior density.

To begin with, we have to specify a probability distribution for the model parameter which should describe our initial knowledge about $\boldsymbol{\theta}$ (this distribution is called "prior" and we denote it as $\pi(\boldsymbol{\theta})$). This step is important, because it has an influence on the resulting inference. There is no unique way of choosing a prior, and usually there are many distributions consistent with the available knowledge about a parameter. In addition, one may be objective of subjective in the specification of the prior distributions hyper-parameters and

structure. Typically the other important feature to consider when multiple parameters are present in the model, so that the prior will be a multivariate distribution, is how to handle the *a priori* beliefs in the likely dependence between parameters. This is typically addressed in practice through the formulation of a hierarchical prior structure, see discussions in [47, 106].

Next, one should specify a "likelihood function" formally defined as a function of the parameters and a distribution for the observations that specifies the model under study, denoted by

$$l(\boldsymbol{\theta}|\boldsymbol{y}) := f(\boldsymbol{y}|\boldsymbol{\theta}).$$

This is effectively the probability density function of the observed data $\boldsymbol{y}$ given parameter $\boldsymbol{\theta}$ which is viewed as a function of the model parameters and constructed based on the statistical assumptions one assumes or makes as a result of the random sampling process - survey or observational study undertaken in collecting the realisations of the observations that comprise the data set under study.

Using these two components, the priors and the likelihood, we then derive a "posterior" distribution, that is, a distribution of $\boldsymbol{\theta}$ given $\boldsymbol{y}$,

$$\pi(\boldsymbol{\theta}|\boldsymbol{y}) \propto l(\boldsymbol{\theta}|\boldsymbol{y})\pi(\boldsymbol{\theta}).$$

Next we apply a sampling technique to sample from the $\pi(\boldsymbol{\theta}|\boldsymbol{y})$ and finally, summarise the posterior using basic descriptive statistical calculations. We note that sampling is not always necessary, but in many cases the posterior distributions do not have a known standard parametric distributional form, i.e. the prior is not conjugate with the likelihood model considered. In addition, typically one would not have a closed from expression for the solution to the normalisation of the posterior distribution which is given in Equation 1. Fortunately, as we will discuss in future sections this does not cause too much difficulty in practice for most models of interest in the HPV setting.

$$\int l(\boldsymbol{\theta}|\boldsymbol{y})\pi(\boldsymbol{\theta})d\boldsymbol{\theta}. \tag{1}$$

The approach typically adopted to overcome this challenge with working with the posterior is to utilise sampling techniques. The basic idea behind all sampling is that one draws a sample (realisation of a random variable or random vector) from the target distribution, in this case the posterior, and then uses discrete formulas from Monte Carlo approximations which are applied to these samples to approximate the integrals of interest when making inference on for example point estimates of the posterior model parameters. There is extensive literature on these concepts in the Bayesian modelling setting in the field of medical statistics and modelling, see examples in [62, 29, 65] and [108].

To relate such a generic modelling framework to the context of HPV models, we consider a HPV model generically parameterised (dependent upon) a d-dimensional vector of parameters denoted $\boldsymbol{\theta} = [\theta_1, \ldots, \theta_d]$. These parameter may be considered as purely static parameters and in general under a Bayesian modelling paradigm they would be assigned prior probability distribution that we will denote generically by $f(\boldsymbol{\theta})$. Given the model parameters the majority of HPV models would also include a set of dynamical latent unobserved states. These dynamical states correspond to the model framework or structure and

relate the spread of the epidemic in the population to the mixing and activity of the population under study in the HPV epidemic. That is they relate mathematically how the epidemic mixes in the population as the population interacts over time and space. If one considers a time interval $[0, T]$ over which the population will be studied, then these dynamical state will generally be either latent and unobserved or they will be partially observed in presence of observation noise.

We denote these dynamics generically by vectors over a discrete time interval as the k-dimensional dynamic (random) vectors for the population under study given by $\boldsymbol{X}_{1:T}$ with $\boldsymbol{X}_t = [X_{1,t}, X_{2,t}, \ldots, X_{k,t}]$. Note, these dynamics of course can also be allowed to evolve on multiple, different time scales either through a system of coupled deterministic dynamic equations such as a system of ordinary differential equations, or perhaps under a system of partial differential equations if there is a spatial component to the model, or through a system of coupled stochastic differential equations (or a mixture of the two). We denote the joint multi-variate distribution of the static model parameters and dynamic state vectors by $f(\boldsymbol{x}_{1:T}, \boldsymbol{\theta})$ which would under most models typically factorise according to $f(\boldsymbol{x}_{1:T}, \boldsymbol{\theta}) = f(\boldsymbol{x}_{1:T}|\boldsymbol{\theta})\, f(\boldsymbol{\theta})$.

Furthermore, consider a generic observational study that produces l-dimensional observation random vectors for a population denoted $\boldsymbol{Y}_{1:T}$ with $\boldsymbol{Y}_t = [Y_{1,t}, Y_{2,t}, \ldots, Y_{l,t}]$. In the following sections we do not require any assumptions on the statistical properties of these observation vectors, nor do we formally specify the relationship such observation vectors have (through the model postulated) with the parameters of the model. We simply assume that the observation process takes a distributional form conditional on the latent dynamics and the static model parameters denoted by the multivariate distribution $f(\boldsymbol{y}_{1:T}|\boldsymbol{X}_t\boldsymbol{\theta})$. In keeping this section generic like this we allow practitioners to utilise the following concepts for inference in their own models.

To complete this section, we will consider the general case in which the modeller may be faced with making inference on hypotheses that are consistent with different model choices. We denote the i-th possible model under consideration by a model index $\mathcal{M}_i$ and prior to assessing the models plausibility given observation data, we assume a prior belief is given by $f(\mathcal{M}_i)$.

Having defined these basic model components generically the inference involved in HPV modelling can be summarised under the following contexts either for point estimation, interval estimation, forecasting and prediction and model selection. All such inference requires one to work with the distributional model formulation under a Bayesian paradigm given in Equation 2 for the i-th model.

$$f(\boldsymbol{x}_{1:T}, \boldsymbol{\theta}|\boldsymbol{y}_{1:T}, \mathcal{M}_i) = \frac{f(\boldsymbol{y}_{1:T}|\boldsymbol{x}_{1:T}, \boldsymbol{\theta}, \mathcal{M}_i)\, f(\boldsymbol{x}_{1:T}|\boldsymbol{\theta}, \mathcal{M}_i)\, f(\boldsymbol{\theta}|\mathcal{M}_i)}{\int f(\boldsymbol{y}_{1:T}|\boldsymbol{x}_{1:T}, \boldsymbol{\theta}, \mathcal{M}_i)\, f(\boldsymbol{x}_{1:T}|\boldsymbol{\theta}, \mathcal{M}_i)\, f(\boldsymbol{\theta}|\mathcal{M}_i)\, d\boldsymbol{x}_{1:T} d\boldsymbol{\theta}}. \quad (2)$$

From this posterior distribution in the following sections we describe the statistical quantities of direct relevance to inference in HPV models that may be considered.

In the following subsections we outline how this generic Bayesian modelling paradigm and posterior construction can be understood in terms of inference goals and calibration. This will allow HPV modellers to understand how working with a Bayesian posterior model can be performed and how one can interpret calibration in such settings. Following this, we

detail important models that are consistent with such a modelling paradigm and which are standard frameworks for HPV modelling and analysis in Section 4

3.2. Bayesian Point Estimators for HPV Modelling

It is often a common practice in reporting results in HPV model calibrations to consider the reporting of a single numerical summary of the model parameters obtained from the calibration of the model given data. In the Bayesian paradigm, it is possible to produce such a summary and to indeed interpret directly what this summary means statistically. The most common examples include the posterior maximum a-posteriori (MAP) corresponding to the posterior mode and the minimum mean square error (MMSE) relating to the posterior mean. These two estimators can be computed either on the full posterior space of parameters and dynamics given by

$$\begin{aligned}\left[\widehat{\boldsymbol{\Theta}}^{MAP}, \widehat{\boldsymbol{X}}_{1:T}^{MAP}\right] &= \arg\max f\left(\boldsymbol{x}_{1:T}, \boldsymbol{\theta} | \boldsymbol{y}_{1:T}, \mathcal{M}_i\right) \\ &= \arg\max f\left(\boldsymbol{y}_{1:T} | \boldsymbol{x}_{1:T}, \boldsymbol{\theta}, \mathcal{M}_i\right) f\left(\boldsymbol{x}_{1:T} | \boldsymbol{\theta}, \mathcal{M}_i\right) f\left(\boldsymbol{\theta} | \mathcal{M}_i\right)\end{aligned} \tag{3}$$

and for the posterior mean

$$\left[\widehat{\boldsymbol{\Theta}}^{MMSE}, \widehat{\boldsymbol{X}}_{1:T}^{MMSE}\right] = \int \boldsymbol{\theta} \int \boldsymbol{x}_{1:T} f\left(\boldsymbol{x}_{1:T}, \boldsymbol{\theta} | \boldsymbol{y}_{1:T}, \mathcal{M}_i\right) d\boldsymbol{x}_{1:T} d\boldsymbol{\theta}, \tag{4}$$

or marginally. It is typically the marginal results that are directly interpretable for medical health professionals and clinicians and correspond to

$$\left[\widehat{\boldsymbol{\Theta}}^{MAP}\right] = \arg\max \int f\left(\boldsymbol{x}_{1:T}, \boldsymbol{\theta} | \boldsymbol{y}_{1:T}, \mathcal{M}_i\right) d\boldsymbol{x}_{1:T} \tag{5}$$

and

$$\left[\widehat{\boldsymbol{\Theta}}^{MMSE}\right] = \int \boldsymbol{\theta} \int f\left(\boldsymbol{x}_{1:T}, \boldsymbol{\theta} | \boldsymbol{y}_{1:T}, \mathcal{M}_i\right) d\boldsymbol{x}_{1:T} d\boldsymbol{\theta}. \tag{6}$$

Of course, being point estimators these should preferably be reported with some measure of confidence. This is addressed in the following section.

3.3. Bayesian Interval Estimators for HPV Modelling

To report a marginal posterior confidence interval or a measure of precision for the posterior point estimators previously defined for either the i-th static parameter $\boldsymbol{\Theta}_i \sim F(\boldsymbol{\Theta}_i)$ or at time t the j-th component of the dynamic state vector $\boldsymbol{X}_{j,t} \sim F(\boldsymbol{X}_{j,t})$, one would typically utilise the following posterior interval $[L(\alpha), U(\alpha)]$. The lower and upper limits are functions of the level of confidence that the interval is defined by with $\alpha \in [0, 1]$ and given for the i-th static parameter $\boldsymbol{\Theta}_i$ by

$$L\left(\alpha\right) = \inf\left\{\boldsymbol{\Theta}_i \in R : 1 - \alpha \leq F(\boldsymbol{\Theta}_i)\right\}, \quad U\left(\alpha\right) = \inf\left\{\boldsymbol{\Theta}_i \in R : \alpha \leq F(\boldsymbol{\Theta}_i)\right\}, \tag{7}$$

and at time t the j-th component of the dynamic state vector $\boldsymbol{X}_{j,t}$ given by

$$L\left(\alpha\right) = \inf\left\{\boldsymbol{X}_{j,t} \in R : 1 - \alpha \leq F(\boldsymbol{X}_{j,t})\right\}, \quad U\left(\alpha\right) = \inf\left\{\boldsymbol{X}_{j,t} \in R : \alpha \leq F(\boldsymbol{X}_{j,t})\right\}. \tag{8}$$

3.4. Bayesian Model Selection

It is often overlooked in the literature on HPV modelling that instead of just postulating one model after another for individual hypotheses, there is the possibility to consider a set of plausible models and to rigorously decide between such models from a statistical perspective using Bayesian methods of model selection. Here we outline, for those unacquainted with the rich literature on model selection methods in the statistics literature, one such method that is widely used in practice and based on the notion of Bayes Factors, see in the Bayesian context discussions in [76], [81] and [22].

We define for each potential model a posterior odds (denoted $\text{PO}_{k|l}$) for the model $\mathcal{M}_k$ compared to model $\mathcal{M}_l$ as a function of the Bayes factor (denoted $\text{BF}_{k|l}$) and the prior model probabilities defined by

$$\text{PO}_{k|l} = \text{BF}_{k|l} \cdot \frac{p(k)}{p(l)}. \tag{9}$$

Using the posterior odds between pairs of plausible models that may explain the variation in the observed data one can then construct the marginal posterior distribution for the probability of each of the considered HPV models in explaining the data. This is particular advantageous for practitioners as it provides a quantity that has a direct interpretation, is on an interpretable scale corresponding to a probability in $[0, 1]$ and is directly applicable for use in model selection, model averaging and model comparison. The posterior model probabilities can be obtained based on the posterior odds by,

$$p(k|Y) = \frac{\text{PO}_{k|l}}{\sum_{m=0}^{M} \text{PO}_{k|m}}. \tag{10}$$

One of the main challenges faced by statisticians and practitioners in utilising this model selection approach is the ability to accurately evaluate for each pair of models the Bayes factors $\text{BF}_{k|l}$. The reason for this difficulty arises from the fact that the Bayes factor is defined by the evaluation of the ratio of the evidence under each model, where the log BF between a model indexed by k and model indexed by l involves

$$\text{BF}_{k|l} = \log p\left(y|M_k\right) - \log p\left(y|M_l\right). \tag{11}$$

The model evidence involves evaluation of the high dimensional integrals, for model $\mathcal{M}_k$, given by

$$p\left(y|M_k\right) = \int \ldots \int f\left(\boldsymbol{y}_{1:T}|\boldsymbol{x}_{1:T}, \boldsymbol{\theta}, \mathcal{M}_k\right) f\left(\boldsymbol{x}_{1:T}|\boldsymbol{\theta}, \mathcal{M}_k\right) f\left(\boldsymbol{\theta}|\mathcal{M}_k\right) d\boldsymbol{x}_{1:T} d\boldsymbol{\theta}, \tag{12}$$

which are almost always intractable to evaluate in closed from and due to the very high dimensions typically basic quadrature methods will fail. It is therefore common practice for the evaluation of the model evidence to be performed using samples from a Markov chain Monte Carlo sampler, which are discussed below in detail.

There are a number of approaches one could adopt to estimate the model evidence and therefore the Bayes factors, see references and discussion on this matter in for example a selection of the many papers on this topic such as [22], [25], [126] and [82]. In this section

we advocate the use of evaluating the ratio of the evidence under each model given via the Chibb estimator, see [22].

The Chibb estimator that we propose for practitioners to utilise for the estimation of the Bayes Factors is still based on sample output from the posterior model, typically from Markov chain samplers that will be discussed in detail in the following sections. An important statistical property of this estimator from Chibb is that it satisfies a standard Gaussian Central Limit Theorem and has finite variance. Therefore we can estimate not only the model evidence but also for a given set of simulations we can report as a measure of uncertainty in our model selections through an assessment of the accuracy of our evidence estimation. This is unlike other commonly used evidence estimators such as the harmonic mean, see [82], which will produce an estimator with infinite variance as discussed in [140].

In the following we provide a brief description of the simplest form of the Chibb estimator, the single block estimator. Under this approach one proceeds to evaluate the evidence for the i-th model, denoted by $\log \widehat{p\left(y|M_i\right)}$ according to the log decomposition as a function of the posterior with generic vector of parameters $\boldsymbol{\theta}$ and data $\boldsymbol{y}$ as follows

$$\log \widehat{p\left(\boldsymbol{y}|M_i\right)} = \log p\left(\boldsymbol{y}|\boldsymbol{\theta}^*, M_i\right) + \log p\left(\boldsymbol{\theta}^*|M_i\right) - \log \widehat{p\left(\boldsymbol{\theta}^*|\boldsymbol{y}, M_i\right)}, \tag{13}$$

where $\boldsymbol{\theta}^*$ represents a point estimator for the parameters obtained from the MCMC output, such as the posterior mean (MMSE) or the posterior mode (MAP) estimators. Here the estimator of $\widehat{p\left(\boldsymbol{\theta}^*|\boldsymbol{y}, M_i\right)}$ obtained via Chibb's approach is given for J samples from the proposal $\left\{\boldsymbol{\theta}^{(j)} : \boldsymbol{\theta}^{(j)} \sim q\left(\boldsymbol{\theta}, \boldsymbol{\theta}^*|\boldsymbol{y}\right)\right\}_{j=1:J}$ and M samples from the MCMC output (i.e. correlated draws from the posterior) by

$$\widehat{p\left(\boldsymbol{\theta}^*|\boldsymbol{y}, M_i\right)} = \frac{\frac{1}{M}\sum_{m=1}^{M} \alpha\left(\boldsymbol{\theta}^{(m)}, \boldsymbol{\theta}^*|\boldsymbol{y}\right) q\left(\boldsymbol{\theta}^{(m)}, \boldsymbol{\theta}^*|\boldsymbol{y}\right)}{\frac{1}{J}\sum_{j=1}^{J} \alpha\left(\boldsymbol{\theta}^*, \boldsymbol{\theta}^{(j)}|\boldsymbol{y}\right)}. \tag{14}$$

Here, the function $\alpha\left(\boldsymbol{\theta}, \boldsymbol{\theta}'|\boldsymbol{y}\right)$ represents the standard Metropolis-Hastings acceptance probability given by

$$\alpha\left(\boldsymbol{\theta}^*, \boldsymbol{\theta}^{(j)}|\boldsymbol{y}\right) = \min\left\{1, \frac{p\left(\boldsymbol{y}|\boldsymbol{\theta}'\right) p\left(\boldsymbol{\theta}'\right) q\left(\boldsymbol{\theta}', \boldsymbol{\theta}\right)}{p\left(\boldsymbol{y}|\boldsymbol{\theta}\right) p\left(\boldsymbol{\theta}\right) q\left(\boldsymbol{\theta}, \boldsymbol{\theta}'\right)}\right\}. \tag{15}$$

The proposal often considered is a multivariate student-t distribution for $q\left(\boldsymbol{\theta}, \boldsymbol{\theta}'\right)$ given for location parameter vector $\boldsymbol{\theta} \in \mathbb{R}^p$ and covariance matrix $\Sigma \in SP^+(p)$ for parameter $\boldsymbol{\theta}' \in \mathbb{R}^p$ by a probability density of

$$q\left(\boldsymbol{\theta}, \boldsymbol{\theta}'\right) = \frac{\Gamma\left(\frac{(n+p)}{2}\right)}{\Gamma\left(\frac{n}{2}\right) n^{p/2}\pi^{p/2}|\Sigma|^{1/2}\left[1 + \frac{1}{n}\left(\boldsymbol{\theta}' - \boldsymbol{\theta}\right)^T \Sigma^{-1}\left(\boldsymbol{\theta}' - \boldsymbol{\theta}\right)\right]^{(n+p)/2}} \tag{16}$$

where one could estimate the covariance of the proposal, Σ, from the empirical sample covariance obtained from the samples out of the MCMC output for the M_i-th model according to

$$\widehat{\Sigma} = \frac{1}{M-1}\sum_{m=1}^{M}\left(\boldsymbol{\theta}^{(m)} - \bar{\boldsymbol{\theta}}\right)\left(\boldsymbol{\theta}^{(m)} - \bar{\boldsymbol{\theta}}\right)^T. \tag{17}$$

3.5. Bayesian Prediction and Forecasting for Modelling HPV Transmission and the Impact of Vaccination

Often one constructs models for HPV and calibrates them using observed data with the aim to utilise such models for forecasting and prediction. This can involve making predictions about future possible population characteristics, equilibrium behaviour and dynamics as well as possible scenario analysis. For example the study of vaccine efficacy or policy interventions that may be considered in a public health campaign and should have things like a cost-benefit analysis undertaken and an assessment of their potential influence on the dynamics of the HPV epidemic in the longer term. To study such questions statistically one may adopt a Bayesian approach and consider a core quantity that may be adopted for use in such situations, known as the posterior predictive distribution comprised of the study of the conditional distribution of the observable characteristics of the population, and possibly attributes and features of the unobservable characteristics given the observations $\boldsymbol{Y}_{1:T}$, an addition K periods into the future (for example months, years etc.). This is statistically specified through the conditional distribution for a given model $\mathcal{M}_i$ given by

$$\begin{aligned} &f\left(\boldsymbol{y}_{T+1:T+K}|\boldsymbol{y}_{1:T},\mathcal{M}_i\right) = \\ &\int f\left(\boldsymbol{y}_{T+1:T+K}|\boldsymbol{x}_{T+1:T+k},\boldsymbol{\theta},\mathcal{M}_k\right) f\left(\boldsymbol{x}_{T+1:T+K}|\boldsymbol{x}_{1:T},\boldsymbol{\theta},\mathcal{M}_k\right) f\left(\boldsymbol{x}_{1:T},\boldsymbol{\theta}|\boldsymbol{y}_{1:T},\mathcal{M}_k\right)\, d\boldsymbol{x}_{1:T+k}\, d\boldsymbol{\theta} \\ &= \mathbb{E}_{f(\boldsymbol{x}_{1:T},\boldsymbol{\theta}|\boldsymbol{y}_{1:T},\mathcal{M}_k)}\left[f\left(\boldsymbol{y}_{T+1:T+K}|\boldsymbol{x}_{T+1:T+k},\boldsymbol{\theta},\mathcal{M}_k\right) f\left(\boldsymbol{x}_{T+1:T+K}|\boldsymbol{x}_{1:T},\boldsymbol{\theta},\mathcal{M}_k\right)\right] \end{aligned} \tag{18}$$

The estimation of these predictive intervals will naturally be required as in the majority of models that involve non-linear state dynamics and/or non-Gaussian observational errors, these integrals are typically intractable. As is the case with the posterior distribution, the assessment for a given model of a predictive distribution can also be summarised by analogous point estimators for the predictive observations as those previously presented for the posterior.

Now that the basic quantities that one wishes to estimate are defined for the HPV model context, it is now important to detail the different Monte Carlo strategies that may be considered to work with such distributions. In this regard, it is beneficial to start with a basic intro to Monte Carlo in general, followed by examples of an auxiliary variable method that is practically useful in many settings and works efficiently, known as the slice sampler. This will present a particular variant of the class of Markov chain Monte Carlo algorithms available in many statistical software packages that are utilised to address such inference problems as those proposed above. Following this, the presentation of two novel classes of Adaptive Markov chain Monte Carlo and Adaptive Particle Markov chain Monte Carlo are presented. These represent state of the art methods that may be utilised to efficiently solve the inference problems above for challenging HPV models. The adaptive MCMC method combined with forward projection was utilised successfully in calibration and inference of HPV models and for predictive analysis and scenario analysis of vaccine efficacies in [85]. The Particle MCMC approach is utilised if one believes the dynamic process may be stochastic.

4. Statistical Epidemic Models

There are several interesting references available surveying different stochastic epidemic models, see for example [17, 92, 6, 131]. We note that there are many different aspects one could consider when developing HPV models, this section presents a very high level discussion of the basic types of models that are available and provides some further links to in-depth discussions on the properties of such models. As such the aim of this section is to establish the types of statistical inference problems that will be encountered in the HPV epidemics modelling context, and then the majority of this chapter will present generic methods to address these inferential problems that are widely applicable to wide classes of such models.

Any HPV transmission model incorporates a number of states that the modelled population can be in with respect to HPV infection. These states would normally include the following:

- *Susceptible*: the part of the population who are at a risk of becoming infected;
- *Infected*: those who are infected;
- *Removed* or *Immune*: those who have been infected before, but cleared HPV and are immune to re-infection.

Currently, there is no satisfactory understanding of the natural immunity to re-infection, so HPV models can be divided into several basic types depending on what is assumed about natural immunity (see Figure 1). Models of SIS type allow for no immunity at all, and so the infected individuals in such models become susceptible immediately after they clear HPV. In contrast, models of SIR type assume a life-long natural immunity following clearance - individuals become ”removed”, meaning that they can never be susceptible or infected again. Finally, SIRS type models are like SIR models, but they allow individuals to be removed only temporarily. The possibility that someone can be born infected with or immune to any HPV type is usually ignored in HPV models, so individuals entering a model are placed into the susceptible compartment.

4.1. Dynamic versus Static Models

All HPV models are either static (also known as cohort or Markov models) or dynamic models.

- **Cohort or Markov epidemic models:** Cohort models aim to mimic the progression of HPV for a single cohort over its lifetime. Transitions from one state to another are occurring at some fixed time intervals and are defined by a set of transition probabilities. These probabilities only depend on the current health state. They may be stratified by various population characteristics and vary in time. Models of this type are discussed in, for example, [32, 61, 16, 136].
- **Dynamic epidemic models:** The structure of dynamic models is typically deterministic and nonlinear. Unlike cohort models, dynamic models describe changes in a population over time, not a single cohort. The execution of a dynamic model does

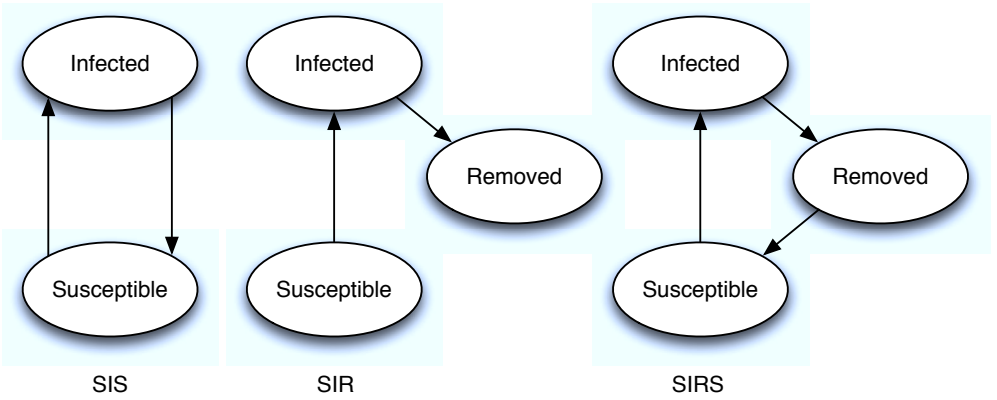

Figure 1. Common HPV model types specified based on the assumptions regarding natural immunity.

not have a natural stopping point, as such models include ongoing and death processes. Instead, one approaches the analysis by focusing on the long-run behaviour of the system which is usually expected to reach an equilibrium state t some time. A key point to note in the consideration of dynamic models is that the force of infection depends on the number of infectious individuals in the population (which is changing), whereas in static models it is fixed. Consequently, the force of infection will be reduced as a result of a vaccination in dynamic models but not in static models. Thus, dynamic models capture herd immunity effects, whereas static models omit them. This is an important feature that clinicians may wish to consider when trying to decide between different approaches.

4.2. Deterministic Dynamic Compartmental Models

Deterministic dynamic compartmental models of HPV transmission are arguably the simplest HPV models currently used. They model a population as a set of large non-overlapping groups (compartments) which are defined based on the characteristics of interest to the modellers. These characteristics are averaged over large portions of the population. Models based on this approach, sometimes referred to as the mean-field approach, are successfully applied in many fields such as statistics and physics.

The basic premise of such an approach is to recognise that instead of having a large number of individuals in a model, one alternatively chooses to assume that since there are so many individuals of a particular kind in the population, it is reasonable to view them for example on average as a single group and therefore any stochastic effects, present due to the fact that all individuals are actually different in some respects, can be safely omitted for modelling purposes. For example, the entire population can be seen as two compartments, susceptible and infected. Then the process of becoming infected and clearing infection are seen as movements between the two compartments. The rates at which this happens are mean rates per unit time (a year, for instance), which are usually approximated as the inverse of mean times required to change compartments. The models in question are described by nonlinear ordinary differential equations (ODE's) supplemented with initial

conditions.

Model of SIS type: In this model, susceptible individuals, after contracting infection from infected individuals, become infected but never develop immunity to re-infection with HPV. Therefore, they eventually return to the susceptible state. One can describe such a model by a system of ODE's governing the changes in system states at time t denoted as S_t and I_t,

$$\begin{aligned} \dot{S} &= -\frac{\beta}{N} S\, I + (b+\gamma) I, \\ \dot{I} &= \frac{\beta}{N} S\, I - (b+\gamma) I, \end{aligned} \tag{19}$$

where derivatives are with respect to time, $\beta > 0$ is a contact rate, γ is a recovery rate and the birth rate is given by b.

Remark. *In the context of HPV models we consider in this chapter, we would not typically admit births into the infected state as there is little clinical evidence to suggest that this may be plausible. Therefore, we consider the sub-class of ODE models given by* $b = 0$.

In this model the total population size at each time instant is then given by $N = S_t + I_t$ and the initial conditions are given by $S_0 > 0$, $I_0 > 0$ and $S_0 + I_0 = N$. As discussed in [5] and in [100], the long term solutions of these dynamic epidemic equations for the SIS model are characterized by the basic reproduction number, defined as the number of secondary infections caused by one infected individual in an entirely susceptible population given by the so called $\mathcal{R}_0$ with the SIS model producing

$$\mathcal{R}_0 = \frac{\beta}{b+\gamma}.$$

which in the context of the simplified HPV models discussed here would collapse to $\frac{\beta}{\gamma}$. One can then show that based on values of the $\mathcal{R}_0$ the system of ODE.'s for this SIS deterministic dynamic model will produce one of two steady state behaviours:

$$\lim_{t\to\infty}(S_t, I_t) = \begin{cases} (N, 0), & \text{if } \mathcal{R}_0 \leq 1 \text{ disease free equilibrium;} \\ \left(\frac{N}{\mathcal{R}_0}, N\left(1 - 1/\mathcal{R}_0\right)\right), & \text{if } \mathcal{R}_0 > 1 \text{ endemic equilibrium.} \end{cases} \tag{20}$$

Model of SIR type: In this model susceptible individuals become infected and subsequently on clearing the infection become immune. Such a model has three states at time t, given by S_t, I_t and R_t, and can be described by a system of ODE's

$$\begin{aligned} \dot{S} &= -\frac{\beta}{N} S\, I + b(I+R) \\ \dot{I} &= \frac{\beta}{N} S\, I - (b+\gamma) I \\ \dot{R} &= \gamma I - bR \end{aligned} \tag{21}$$

where $\beta > 0$, $\gamma > 0$, $b \geq 0$.

Remark. *As in the SIS models, we also have that in the context of the simple SIR HPV models we consider in this chapter, we would not typically admit a birth into the infected category as there is little clinical evidence to suggest that such transmissions occur. Therefore, we again consider the sub-class of ODE models given by* $b = 0$.

Therefore, the total population size given by $N = S_t + I_t + R_t$ at time t and initial condition $S_0 > 0$, $I_0 > 0$ and $R_0 \geq 0$ with initial population size $S_0 + I_0 + R_0 = N$. In this case one can also characterize the steady state long run dynamic behaviour as follows:

$$\lim_{t\to\infty}(S_t, I_t, R_t) = \left\{\left(\frac{N}{\mathcal{R}_0}, \frac{bN}{b+\gamma}\left(1-\frac{1}{\mathcal{R}_0}\right), \frac{\gamma N}{b+\gamma}\left(1-\frac{1}{\mathcal{R}_0}\right)\right), \text{if } \mathcal{R}_0 > 1 \text{ endemic equilibrium.}\right. \quad (22)$$

and $\lim_{t\to\infty} I_t = 0$ when $\mathcal{R}_0 \leq 1$.

4.3. Stochastic Dynamic Epidemic Models

Stochastic dynamical models can be a viable alternative to the deviously discussed deterministic models. Typically one may consider one of three broad classes of modelling process given by either discrete time Markov chain (DTMC) models; continuous time Markov chain (CTMC) models or stochastic differential equation (SDE.) models. The difference between each stochastic model arises from their underlying assumptions regarding the time and state variables. There are however alternative classes of non-Markovian models based on survival analysis that are also proposed for epidemics such as the works of [131].

DTMC In the DTMC models the random variables for the susceptible S_t, Infected I_t and Immune R_t are from distributions with a discrete support for each time instant t and furthermore the time units in the model are discrete $t \in \{0, \triangle, 2\triangle, \ldots\}$.

CTMC In the CTMC models the random variables for the susceptible S_t, Infected I_t and Immune R_t are from distributions with a discrete support for each time instant t, however now the time unit is considered a continuous variable say $t \in [0, \infty)$.

SDE In the SDE models the random variables for the susceptible S_t, Infected I_t and Immune R_t are from a distribution with a continuous support for each time instant t, and in addition the time unit is considered a continuous variable say $t \in [0, \infty)$.

One can now consider different mechanisms to construct the relevant models, for example one could construct the SDE models by taking the ODE models and assuming that the rates of transition between the compartments are not only changing in time, but that they change according to some stochastic processes, which on average may behave like the system of ODE's in the non-stochastic setting. An example of such processes is presented generically below in this context, see Section 4.5. For the above specified ODE models of SIS and SIR types, one can derive the equivalent SDE models as detailed explicitly in [5]. This results in the discrete time analog models for the SIR and SIS models under the stochastic dynamical models given by the following SDE structures:

- Considers the formulation of an SIS stochastic SDE model over an interval of time $t \in [0, \infty)$ with continuous state processes $\{S(t)\}$ and $\{I(t)\}$. As detailed extensively in [5] the behaviour of the stochastic SIS model will be strongly dependent on the process for the number of infective members of the population as characterised by the finite dimensional probability distributions such as $p(x,t)$ defined by

$$\Pr\left(l \leq I(t) \leq u\right) = \int_l^u p(x,t)dx \tag{23}$$

with also the assumption that the process will satisfy the Markov property that

$$\Pr\left\{I(t_n) \leq y | I(t_0), I(t_1), \ldots, I(t_{n-1})\right\} = \Pr\left\{I(t_n) \leq y | I(t_{n-1})\right\} \tag{24}$$

no matter what sequence of ordered times. In addition it will be often important to consider a diffusion process structure in which one assumes that the Markov process has a well defined infinitesimal mean and variance process that exist. To proceed to the SDE formulation of the SIS model, one then assumes that the transition distribution of the process given by $p(y, t_n; x, t_{n-1})$ for any $0 \leq t_{n-1} < t_n \leq T$ is well defined in the sense that one can write down the forward Kolmogorov differential equation and impose some boundary conditions to ensure the numerical solutions can be obtained. As discussed in [5] this is non-trivial in this example to solve numerically and so they propose instead to develop an SDE model for the SIS given by the following form, whose solution is a sample path of the stochastic process of interest. The interesting feature of this SDE structure representation is that it can be trivially discretised and worked with as we discuss below since the drift and volatility functions are simple well behaved functions of the state variable $I(t)$ as given by:

$$dI = \mu(I)dt + \sigma(I)dW \tag{25}$$

with dW an increment of a Wiener process and the drift function is given by

$$\begin{aligned} \mu(I) &= \frac{\beta}{N} I(N-I) - (b+\gamma)I \\ \sigma(I) &= \sqrt{b(I) + d(i)} = \sqrt{\frac{\beta}{N} I(N-I) + (b+\gamma)I}. \end{aligned} \tag{26}$$

Note again we would consider for the HPV models the setting of $b = 0$ in the above structures. In addition, having defined the SDE structure for the infected, one can obtain the process for the state variable $S(t)$ due to the symmetry property of the system of ODE's from which this model was based in equilibrium. In general one should note there will be an absorbing state of this system corresponding to the intuitive case of the disease free state.

- In the SIR case there are three state variables $S(t)$, $I(t)$ and $R(t)$ and one can follow a similar approach, also spelled out specifically in [5] to obtain the following system of two SDE's, each driven by two independent Wiener process:

$$\begin{aligned} dS &= -\frac{\beta}{N} SIdt + \sigma_{11}dW_1 + \sigma_{12}dW_2 \\ dI &= \frac{\beta}{N} SIdt - \gamma Idt + \sigma_{21}dW_1 + \sigma_{22}dW_2 \end{aligned} \tag{27}$$

with the state $R(t)$ having a process of the form of $I(t)$ and the matrix $\Sigma(\triangle t)$ giving the components $\sigma_{i,j}$ given by

$$\Sigma(\triangle t) = \begin{bmatrix} \frac{\beta}{N}SI & -\frac{\beta}{N}SI \\ -\frac{\beta}{N}SI & \frac{\beta}{N}SI + \gamma I \end{bmatrix}. \tag{28}$$

Remark. *As noted in [78] in general we can consider that under a well defined stochastic model with the standard regularity conditions on the drift function and volatility function, any stochastic epidemic model will have an analogue deterministic system obtained by simply setting the driving noise processes to zero. In addition it can be shows that if one considers the true SIR HPV model SDE dynamics constructed from the Kolmogorov forward equations (if it was approximated numerical in the case with $b = 0$) for the states $S(t)$, $I(t)$ and $R(t)$ then looking at the average behaviour one has for the infectious state*

$$\mathbb{E}\left[I(t+dt) - I(t) | S(t) = s_t, I(t) = i_t, R(t) = r_t\right] = \frac{\beta}{N}S(t)I(t)dt - \gamma I(t)dt + o(dt) \tag{29}$$

this matches the deterministic process for the dynamic we defined in the SIR ODE model. However, it can be shown that if one considers the equation

$$\frac{d}{dt}\mathbb{E}\left[I(t)\right] = \frac{\beta}{N}\mathbb{E}[S(t)]\mathbb{E}[I(t)] - \gamma\mathbb{E}[I(t)] + \frac{\beta}{N}\mathbb{C}\text{ov}\left(S(t), I(t)\right), \tag{30}$$

it does not make the deterministic solution from the ODE dynamics, simply the mean of the SDE dynamics!

Since the most common form of dynamical model in the HPV context arises in the deterministic case, and is specified by a system of nonlinear ODE's, it is therefore prudent to briefly mention the role played by numerical solvers for such systems.

4.4. The Role of ODE Solvers in Deterministic Compartmental HPV Models

Typically, deterministic compartmental HPV transmission models are described by the first order nonlinear differential equations, that is, equations of the form

$$\dot{y} = f(y, t),$$

with nonlinearity in the right-hand side introduced by the force of infection traditionally denoted λ, which depends on the number of infected individuals in a model.

Solving such equations analytically is rarely possible, so one has to seek an approximate solution using a numerical method. There are many numerical methods to consider (see, for example, [71] for discussion), and even the simplest of them, such as Euler's method where one would progress the solution y_n from a time point t_n to the next point $t_{n+1} = t_n + h$ as

$$y_{n+1} = y_n + hf(y_n, t_n),$$

may be completely suitable for modelling purposes.

In fact, the simplest methods (and consequently, the least expensive in terms of computer time) may be given the highest priority. This will often be assessed based on the

quality of the observational or survey data one has access to when performing the model calibration. For instance it is important to realise that there would be little gained in applying a highly accurate ODE solver if one eventually found that such accuracy was wasted due to large uncertainty in the estimation arising form poor data quality. Indeed, data utilised for calibration of HPV models are often not particularly accurate. Hence, one would be interested in a numerically robust and fast solver. To ensure an improved robustness, one might look at solvers which will adaptively select the 'step' size as a function of the model parameters, under a pre-specified accuracy criterion.

An important practical issue is that a system of ODE's may be "stiff". There is no universally agreed upon definition of stiffness, but a reliable empirical indication of the phenomenon is that explicit methods cease to work. One may observe that they do find a solution, but only if the selected time step is extremely small, so the computational time is unacceptably long. Then so-called implicit numerical methods should be used, among which implicit Runge-Kutta methods are arguably most popular. An extensive overview of these and many other methods is available in [72], so we do not discuss them here. However, we would like to mention that there are four ODE solvers aimed at stiff problems in Matlab (ode15s, ode23s, ode23t and ode23tb). Also, we can recommend a free Intel ODE Solver Library, which implements explicit, implicit and hybrid numerical methods.

4.5. Generic Examples of Dynamical HPV Models

In the following two examples we present illustrations of dynamic deterministic and stochastic models that can be considered in the context of HPV modelling.

Example 1. Deterministic dynamic ODE model with unknown static parameters
The first illustrates a common case in which the component of the model specified by the dynamics turns out to be deterministically specified by a system of ODE's. In other words in we do not require the distribution $f(\boldsymbol{x}_{1:T}|\boldsymbol{\theta}, \mathcal{M}_i)$ in such a case. Instead one may utilise the method termed "forward projection" in [85], which treats the static dynamic parameters, which are deterministically specified given a sample of $\boldsymbol{\Theta}$, through a forward projection ODE solver to obtain the dynamic trajectories and evaluate the likelihood $f(\boldsymbol{y}_{1:T}|\boldsymbol{x}_{1:T}, \boldsymbol{\theta}, \mathcal{M}_i)$. Then ultimately the target posterior for such examples is given by $f(\boldsymbol{\theta}|\boldsymbol{y}_{1:T}, \mathcal{M}_i)$, however to evaluate the likelihood, one must perform the forward project dynamic solutions for deterministic trajectories $\boldsymbol{x}_{1:T}$ given samples of $\boldsymbol{\Theta}$, see detailed discussion in [85].

Modellers may therefore consider a system of coupled (referring to the fact that the systems shares parameters and states) ODE.'s which could for example be generically specified for the i-th component of a general n-dimensional ODE., according to,

$$dx_t^i = a^i(t, \boldsymbol{x}_t)\, dt$$

where each non-linear function for the i-th state, $a^i(t, \boldsymbol{x}_t)$, is a non-linear mapping of the state vector $\boldsymbol{x}_t$ to the new value of the i-th state element instantaneously at time t. Such a dynamic system of ODE.'s would then in practice be discretised to a discrete time grid $t \in \{1, 2, \ldots, T\}$ via a particular discretisation scheme, such as an Euler method describe previously.

A very simple example to illustrate this class of dynamical models in a HPV context we consider a model of SIRS type (see Figure 1) and assume that there is no demography, that is, the modelled population is closed (no births, no deaths, nobody leaves or enters the population in any other way). Let a mean rate at which susceptibles (denote them S) become infected (denoted by I) be λ. This rate depends on the number of infected I, hence we will write $\lambda(I)$ to emphasise this fact. The infected recover at a mean rate γ and the removed (R) lose natural immunity to reinfection at a mean rate w. Then the dynamics of this model are described by the following equations:

$$\dot{S} = -\lambda(I)S + wR, \tag{31}$$

$$\dot{I} = \lambda(I)S - \gamma I, \tag{32}$$

$$\dot{R} = \gamma I - wR, \tag{33}$$

where dot means a derivative with respect to time.

Example 2. Dynamic coupled SDE epidemic model with unknown static model parameters and latent states

The second possible case involves formulation of a stochastic model, in which case the dynamics evolve either in discrete time via a state space model structure or in continuous time via instead of a system of coupled ODE's one may consider a system of coupled SDE's. In this case it is required to incorporate the distribution $\pi\left(\boldsymbol{x}_{1:T}|\boldsymbol{\theta}, \mathcal{M}_i\right)$. There are several modelling approaches for which such a choice may arise. Consider the compartmental model described in the example above, in this case the simplest approach adopted to obtain a stochastic model would be in practice to consider that the rate parameters describing the flow of members of the population between different disease states become time varying and stochastic. This makes the system of ODE's into a system of SDE's which could for example be generically specified for the i-th component of a general n-dimensional SDE, with m-dimensional Wiener process according to,

$$dX_t^i = a^i\left(t, X_t\right) dt + \sum_{j=1}^{m} b^{i,j}\left(t, X_t\right) dW_t^j.$$

Hence, we have seen particular examples above of SDE models for both the SIR and SIS formulations, as long as the drift and volatility functions are known for these s.d.e models, then they can all be considered under the general formulation for discretisation and inference as detailed below.

Such a dynamic system of SDE's would then in practice be discretised to a discrete time grid $t \in \{1, 2, \ldots, T\}$ via a particular stochastic discretisation scheme, see extensive discussion in [84]. For example, two examples of popular schemes for this would be either an Euler scheme or a Milstein second order scheme described below. A Milstein discretisation

scheme of the i-th component is given by,

$$X_t^i = X_{t-1}^i + a^i\left(t-1, X_{t-1}\right)\triangle t + \sum_{j=1}^{m} b^{i,j}\left(t-1, X_{t-1}\right)\triangle W_{t-1}^j + \sum_{j_1,j_2}^{m} L^{j_1} b^{i,j_2}\left(t-1, X_{t-1}\right) I_{(j_1,j_2)\triangle t}$$

In this expansion we define the operator $L^j = \sum_{i=1}^{n} b^{i,j}\frac{\partial}{\partial x_i}$ and the Ito multiple integral $I_{(j_1,j_2)\triangle t}$ is given by,

$$I_{(j_1,j_2)\triangle t} = \int_{t_n}^{t_{n+1}} \int_{t_n}^{s_1} dW_{s_1}^{j_1} dW_{s_1}^{j_2}.$$

These integrals have the useful properties that,

$$I_{(j_1,j_1)\triangle t} = \frac{1}{2}\{(\triangle W_t)^2 - \triangle_t\},$$

and $I_{(j_1,j_1)\triangle t}$ with $j_1 \neq j_2$ not easily expressed.

As pointed out in [84], when $j_1 \neq j_2$ the Ito and Stratonovich integrals are equal,

$$I_{(j_1,j_2)} = J_{(j_1,j_2)} = \int_{\tau_n}^{\tau_{n+1}} \int_{\tau_n}^{s_1} dW_{s_2}^{j_1} dW_{s_1}^{j_2}.$$

This leads to an infinite series representation that they provide, which up to p-th order is given by

$$J_{(j_1,j_2)\triangle t}^{p} = \triangle t\left(\frac{1}{2}\zeta_{j_1}\zeta_{j_2} + \sqrt{\rho_p}\left(\mu_{j_1,p}\zeta_{j_2} - \mu_{j_2,p}\zeta_{j_1}\right)\right) + \frac{\triangle t}{2\pi}\sum_{r=1}^{p}\frac{1}{r}\left(\psi_{j_1,r}\left(\sqrt{2}\zeta_{j_2} + \nu_{j_2,r}\right) - \psi_{j_2,r}\left(\sqrt{2}\zeta_{j_1} + \nu_{j_1,r}\right)\right),$$

where $\zeta_j, \mu_{j,p}, \nu_{j,r}$ and $\psi_{j,r}$ are all independent $\mathcal{N}(0;1)$ Gaussian random variables with,

$$\rho_p = \frac{1}{12} - \frac{1}{2\pi^2}\sum_{r=1}^{p}\frac{1}{r^2}, \quad \zeta_j = \frac{1}{\sqrt{\triangle t}}\triangle W^j.$$

In these cases clearly the stochastic nature of the dynamics ensures that one must jointly make inference on the full posterior $f\left(\boldsymbol{\theta}, \boldsymbol{x}_{1:T}|\boldsymbol{y}_{1:T}, \mathcal{M}_i\right)$ for the latent states and the static parameters of the HPV model.

5. Sexual Behaviour and Sexual Mixing Matrices

HPV is sexually transmitted, so modelling of sexual behaviour within a population is a necessary part of any HPV model. Unfortunately, availability and reliability of sexual behaviour data are both limited. Sexual behaviour is a very private activity dependent on

social, cultural and other norms, varying from one country, state or even a town to another. As discussed in [45], sexual behaviour studies generally fall into four main groups: general population surveys, studies on population subgroups, partner and network studies, and also ethnographic and qualitative studies. None of these can provide truly trustworthy results. Even comparatively large cross sectional population surveys, such as the ASHR in Australia [88], struggle to capture all essential details. The ASHR was a computer-assisted telephone survey of a random sample of about 20, 000 aged from 16 to 59. No matter that the number of participants was substantial, people under 30 were clearly under-represented, while well-educated people in full-time employment were over-represented. Besides, the questions asked were often vague and focusing on the behaviour of individuals with little information about their partners. Additionally, the study only provided a snapshot on the sexual behaviour in time, which implies that multiple studies of this sort would be required to monitor temporal changes in sexual behaviour, which are not unlikely. Extensive studies like the ASHR may fails to cover enough of representatives of small subgroups whose sexual behaviour may be notably different from that in the rest of the population (men who have sex with men, drug users, sex workers and so on). Hence, small scale studies are needed to address these groups. All sexual behaviour surveys are prone to participation and reporting biases. Participation bias arises due to systematic differences in the characteristics of study participants compared with those who do chose to not participate. Those with more conservative lifestyles are less likely to participate or answer certain more intrusive questions [30]. Reporting bias is important because in the vast majority of cases the surveys under discussion rely on self-reported recall of behaviours. Respondents may tend to forget the number of their sexual partners or frequency of intercourse. It is also commonly known that women are more likely to under-report the number of their sexual partners, while men are usually over-reporting, which may be explained by a so-called social desirability bias [24].

Usually, sexual behaviour is described in terms of mixing between population groups, defined based on one or more population characteristics, such as age. It is convenient to represent this mixing via matrices containing probabilities of partnership formation between any two groups. Matrices of this sort are known as sexual mixing matrices.

Following the notations used in [137], let g denote a gender, and g' an opposite gender; a and a' are some age groups, and s and s' are some sexual activity groups; $P_{g'as}$ is the number of partnerships generated by people of gender g' in age group a and sexual activity group s. Then, according to the formulation introduced in [49], the probability that someone of gender g in age group a and sexual activity group s will form a partnership with someone of gender g' in age group a' and sexual activity group s' is defined as

$$\rho_{gasa's'} \equiv \left(\varepsilon^{(A)} \delta_{aa'} + (1 - \varepsilon^{(A)}) \frac{\sum_{\beta=1}^{n_s} P_{g'a'\beta}}{\sum_{\alpha=1}^{n_A} \sum_{\beta=1}^{n_s} P_{g'\alpha\beta}} \right) \times \left(\varepsilon^{(S)} \delta_{ss'} + (1 - \varepsilon^{(S)}) \frac{\sum_{\beta=1}^{n_s} P_{g'a's'}}{\sum_{\beta=1}^{n_s} P_{g'a'\beta}} \right) \quad (34)$$

where $\varepsilon^{(A)}$ and $\varepsilon^{(S)}$ are the degrees of assortativity by age and sexual activity group, and

$$\delta_{aa'} = \begin{cases} 1, & a = a' \\ 0, & a \neq a' \end{cases}$$

Note that if $\varepsilon^{(A)} = 0$, the first factor in the product is simply the proportion of all partnership generated by people of gender g' attributed to the age group j, and sexual mixing is called proportional by age. If $\varepsilon^{(A)} = 1$, then the first factor is non-zero only if $a = a'$, which means that a partnership can be established strictly between people from the same age group. Then sexual mixing is called fully assortative by age. Similar considerations are valid for the second factor, representing mixing by sexual activity.

As recently discussed in [137], formulation (34) is widely used but not entirely correct, and it is more appropriate to define ρ_{kihjm} as below.

$$\rho_{gasa's'} \equiv \varepsilon^{(A)}\varepsilon^{(S)}\delta_{ij}\delta_{ss'} + \varepsilon^{(A)}(1-\varepsilon^{(S)})\frac{P_{g'a's'}}{\sum_{\beta=1}^{n_s} P_{g'j\beta}}\delta_{aa'} + \\ (1-\varepsilon^{(A)})\varepsilon^{(S)}\frac{P_{g'a's'}}{\sum_{\alpha=1}^{n_A} P_{g'\alpha s'}}\delta_{ss'} + (1-\varepsilon^{(A)})(1-\varepsilon^{(S)})\frac{P_{g'a's'}}{\sum_{\alpha=1}^{n_A}\sum_{\beta=1}^{n_s} P_{g'\alpha\beta}}. \quad (35)$$

The degrees of assortativity are very hard to determine based on the results of sexual behaviour surveys, no matter that some reasonable assumptions regarding them may be made. For example, one might assume that $\varepsilon^{(A)}$ is higher for those attending school than for other people, because their social contacts are largely limited to their own age group; or, $\varepsilon^{(S)}$ might be low for the most sexually active group because this group may mainly include sex workers servicing less active clients. However, such assumptions are rather speculative and rarely backed by sufficient data. Therefore, it is beneficial to consider $\varepsilon^{(A)}$ and $\varepsilon^{(S)}$ as model parameters with their own prior distributions assigned.

6. Performing Model Calibration and Inference for HPV Models via Monte Carlo Methods

The aim of this section is to explain in detail the approaches and properties of several classes of statistical sampling techniques that practitioners can utilise to perform estimation, calibration and make inference in HPV models that are developed. In almost all of the inference, calibration and model selection goals presented previously in this chapter, one is required to work with the posterior model for the parameters and states given data. In performing calibration this often requires one to make inference on either point estimates or integral identities. To achieve this it is often impossible to do so in closed form and in addition, owing to the often high dimension of the posterior parameter vector and dynamic states, such analytic expressions for integrals with respect to the HPV model posterior can not be performed via numerical quadrature integration techniques. We therefore typically approach inference for such models using more sophisticated techniques based on obtaining samples from the posterior distribution which can then be used to estimate these integrals with known accuracy properties.

In this section we detail the class of Monte Carlo approaches to such problems. There is a vast literature on Monte Carlo integration and sampling methods, in particular we focus on the class known as Markov chain Monte Carlo methods extensively reviewed in [55, 56, 47, 103, 14] and recently applied in the context of HPV modelling [85]. There are also many accessible tutorial papers on this class of algorithm such as [26, 8, 108, 80, 18] and the extensive reviews found in [50].

All approaches that will be described below and in these other references share the same common goal: to accurately and efficiently sample from the resulting posterior distribution, in this case for the HPV model parameters given the observed data so that calibration and inference can be performed. The biggest difference between each approach to be discussed relates to the accuracy that one can perform such inference given a computational budget such as total samples, total simulation time etc. When we consider accuracy of such approaches from a statistical perspective, all methods to be presented will provide unbiased estimators for the inference quantities of interest, however the finite sample variance obtained from each method could differ significantly.

Typically there will be a trade-off for practitioners related to the complexity of the sampling algorithm they wish to consider versus the reduction in variance in estimated quantities of interest. Therefore we provide a range of methods one may consider suiting those interested in very simple approaches with non-restrictive simulation budgets through to those looking for state of the art sampling methods that will provide sample estimates accurately for constrained computational budgets. Often in HPV models, this can be of direct importance due to the excessive cost associated with running the HPV model for too many iterations of the sampler, which may involve for example finding discretised long run solutions to a system of ODE's for each parameter change.

Consider a general problem of drawing samples from a posterior distribution $\pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)$ and we drop the model index for notational convenience in the below discussion, with the understanding that such a density will correspond to the posterior of a particular parametric HPV model. Let $\boldsymbol{\Phi}$ be a d-dimensional random variable which will be used throughout the following sections to denote all the random quantities in the HPV model that are to be estimated in the posterior inference. For example, all the parameters in a compartmental HPV model which would correspond in a deterministic dynamic model to the unknown static model parameters (i.e. $\boldsymbol{\Phi} = \boldsymbol{\Theta}$, and in the case of the stochastic dynamic models this random vector $\boldsymbol{\Phi}$ would denote $\boldsymbol{\Phi} = [\boldsymbol{\Theta}, \boldsymbol{X}_{1:T}]$. Hence, the proceeding sections will subsume all these different models into a general framework of posterior inference characterized by the density of interest $\pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)$. In the special case that one can draw independent and identically distributed samples from this posterior distribution, then a standard Monte Carlo estimation can proceed as detailed below.

First consider the generic question of quantification of the probability of a particular event or set of events that are measurable with respect to outcomes of the model, denoted by $A \subset \mathbb{R}^d$ some measurable subset of the support of the posterior for the HPV model. Now consider computation of quantities such as $\mathbb{P}(\boldsymbol{\Phi} \in A) := \int_A \pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right) d\boldsymbol{\phi}$.

Given a sequence of samples $(\boldsymbol{\phi}^i)_{1\leq i\leq N}$ of independent copies of the random variable $\boldsymbol{\Phi}$. In this situation, the traditional Monte Carlo approximation of quantities such as $\mathbb{P}(\boldsymbol{\Phi} \in A)$ (which is the most simple special case of the inference problems previously defined) is

given by the empirical measures with N samples by

$$\widehat{\pi}\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right) = \frac{1}{N}\sum_{1\leq i\leq N}\delta_{\boldsymbol{\Phi}^i} \longrightarrow_{N\uparrow\infty} \pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)$$

Under this most basic of Monte Carlo estimators, the convergence of the empirical measure is understood as a weak convergence of empirical measures in the following sense for any bounded and measurable test function ψ on $\mathbb{R}^d$,

$$\begin{aligned}\widehat{\pi}(\psi) &:= \int \psi(\boldsymbol{\phi})\,\widehat{\pi}\left(d\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right) = \frac{1}{N}\sum_{1\leq i\leq N}\psi(\boldsymbol{\phi}^i)\\ \longrightarrow_{N\uparrow\infty}\pi(\psi) &:= \int \psi(\boldsymbol{\phi})\,\pi\left(d\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right) = \mathbb{E}_{\pi(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})}(\psi(\boldsymbol{\Phi}))\text{ a.s.}.\end{aligned} \tag{36}$$

It is often highly informative for practitioners to consider the marginal behaviour of sub-blocks of the parameter vector $\boldsymbol{\Phi}$. From the samples obtained under a basic Monte Carlo strategy, we observe that through the use of indicator functions for cells in $\mathbb{R}^d$ one can study visualisations for the shape of the posterior distribution marginally simply by plotting the histograms of the samples $\boldsymbol{\Phi}^i$ in every dimensions.

In the specific choice of test functions given by the indicator function on a set of events A denoted $\psi = \mathbb{I}_A$, with the notational convention that the empirical measure of the posterior $\widehat{\pi}\left(\mathbb{I}_A|\boldsymbol{y}_{1:T}\right)$ and the true posterior measure $\pi\left(\mathbb{I}_A|\boldsymbol{y}_{1:T}\right)$ is denoted by $\widehat{\pi}\left(A|\boldsymbol{y}_{1:T}\right)$ and $\pi\left(A|\boldsymbol{y}_{1:T}\right)$. Hence, for indicator function $\psi = \mathbb{I}_A$ one has by the a.s. convergence of the empirical measure of the test function ψ above results in an unbiased estimator given by

$$\mathbb{E}\left[\widehat{\pi}\left(A|\boldsymbol{y}_{1:T}\right)\right] = \pi\left(A|\boldsymbol{y}_{1:T}\right) \tag{37}$$

and a variance in this estimator given by

$$\mathbb{V}\mathrm{ar}\left(\widehat{\pi}\left(A|\boldsymbol{y}_{1:T}\right)\right) = \frac{1}{N}\,\pi\left(A|\boldsymbol{y}_{1:T}\right)\;\left(1-\pi\left(A|\boldsymbol{y}_{1:T}\right)\right). \tag{38}$$

6.1. Standard Markov Chain Monte Carlo for HPV Model Inference

Unfortunately in practice one can typically not easily generate i.i.d. samples from the target posterior distribution corresponding to a particular HPV model given by $\pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)$, due to the intractability of the inverse of the distribution function (i.e. the quantile function is not known in closed form). In such cases, which incidentally correspond to the majority of situations in practice, one must resort to alternative statistical approaches to provide samples. There are numerous such examples which include importance sampling (IS), Markov chain Monte Carlo (MCMC), Sequential Monte Carlo (SMC), Sequential Monte Carlo Samplers (SMCSamplers), Particle Markov chain Monte Carlo (PMCMC) and their adaptive versions. Each of these classes of algorithms are significantly different in their attributes and in the problems for which it is appropriate to utilise them when making inference in HPV modelling. It is the intention of the following sections to introduce practitioners to a subset of the many possible choices that are selected as their performance is efficient and widely applicable for the types of problems discussed above for the context of HPV modelling in

a Bayesian setting. We begin with MCMC general properties, then present a special case of what is known as an auxiliary variable sampler illustrated by the Slice sampler of [104]. This is widely applicable to many Bayesian inference problems and is now a standard package in statistical software such as R and Matlab.

It is first worth noting that all of these approaches are generally going to produce samples from the target posterior distribution which are correlated, as opposed to independent in the standard Monte Carlo described above. The direct consequence of this is an increase in variance of estimates formed with such samples, relative to an i.i.d. sample. The amount by which this variance increases is directly related to what is known as the mixing of the Markov chain Monte Carlo sampler, see detailed discussions in [97].

Nevertheless, the samples obtained are still instrumental in Bayesian inference for HPV models to estimate the quantities discussed previously. The following basic introduction is based on a recent tutorial work of [36] which is summarised below for the HPV practitioners to understand the basic concepts of MCMC methods, before providing particular algorithmic examples that can be utilised directly in practice.

The MCMC approach constructs an ergodic Markov chain $\{\boldsymbol{\Phi}_1, \ldots, \boldsymbol{\Phi}_N\}$, taking values in $\mathbb{R}^d$. This Markov chain is constructed to have the property that it has a limiting, invariant distribution, which is the target distribution of interest $\pi\left(d\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)$. This invariant distribution is the target distribution, which in the cases considered in this chapter, will correspond to the posterior for the HPV model. For the Markov Chain samples to be used as samples from the target distribution, it is necessary that there exist a unique invariant distribution of the Markov chain corresponding to the posterior of interest for the HPV model. A detailed review of the properties of more general state space Markov chain theory can be found in for example [97] and [33].

To achieve this construction of a Markov chain with desired stationary distribution, the majority of methods developed in the statistics literature have focussed on the case in which the Markov chain created satisfies the condition of reversibility, whereby the following holds

$$\pi\left(d\boldsymbol{\phi}_i|\boldsymbol{y}_{1:T}\right) Q(\boldsymbol{\phi}_i, d\boldsymbol{\phi}_j) = \pi\left(d\boldsymbol{\phi}_j|\boldsymbol{y}_{1:T}\right) Q(\boldsymbol{\phi}_j, d\boldsymbol{\phi}_i), \tag{39}$$

where $\boldsymbol{\phi}_i$ and $\boldsymbol{\phi}_j$ represent states of the Markov chain and $Q(\boldsymbol{\phi}_j, d\boldsymbol{\phi}_i)$ denotes the Markov transition representing the probability of starting in state $\boldsymbol{\phi}_j$ and transition to a neighbourhood of the state $\boldsymbol{\phi}_i$. Under this condition there is a wide range of methods that one may utilise to construct the desired Markov chain, which in a large number of instances involve the careful design of the transition kernel $Q(\boldsymbol{\phi}_i, d\boldsymbol{\phi}_j)$. These algorithms are generally considered as special cases of the general framework established by [96] and extended in [74]. It is instructive to first present the basic Metropolis-Hastings MCMC sampler and the univariate Gibbs sampler prior to explaining how more recent advances in these methods can be utilised effectively for HPV model inference.

6.2. Metropolis-Hastings Samplers for HPV Model Inference

The Metropolis-Hastings algorithm has a proposal distribution, denoted by $q(\boldsymbol{\phi}_i, \boldsymbol{\phi}_j)$, which conditioned on the current state $\boldsymbol{\phi}_i$ is used to sample the new state of the Markov chain given by random variable $\boldsymbol{\Phi}_j$. Then the proposed new state along with the current state are used to calculate an acceptance probability. This corresponds to the probability

that the Markov chain makes the proposed state transition or remains where it currently is located in the parameter space. This acceptance probability is crucial in ensuring that the Markov chain constructed in this manner will produce the appropriate invariant distribution $\pi(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})$. The basic algorithm is then defined by the following sequence of stages used to produce J-samples as detailed in the proceeding Algorithm 1. We also note that typically the initial 10-20% of the J samples may be discarded as what is known as "burn-in", see discussion in [56].

The Markov chain that is created by such a rejection mechanism in Algorithm 1 is reversible and has the required stationary distribution given by the target HPV model posterior $\pi(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})$. The transition kernel for this Markov chain is given by

$$\begin{aligned} Q(\boldsymbol{\phi}_j, d\boldsymbol{\phi}_{j+1}) &= q(\boldsymbol{\phi}_j, d\boldsymbol{\phi}_{j+1})\,\alpha(\boldsymbol{\phi}_j, d\boldsymbol{\phi}_{j+1}) \\ &+ \left[1 - \int q(\boldsymbol{\phi}_j, \boldsymbol{z})\,\alpha(\boldsymbol{\phi}_j, \boldsymbol{z})\,d\boldsymbol{z}\right] \mathbb{I}[\boldsymbol{\phi}_{j+1} = \boldsymbol{\phi}_j]\,. \end{aligned} \tag{40}$$

where $\left[1 - \int q(\boldsymbol{\phi}_j, \boldsymbol{z})\,\alpha(\boldsymbol{\phi}_j, \boldsymbol{z})\,d\boldsymbol{z}\right]$ is the probability that the chain remains in the current state value $\boldsymbol{\phi}_j$. Now, to understand how the correct stationary distribution is preserved under such a transition kernel, the reader is referred to the simple derivation in [26, Equations 3 and 4].

Algorithm 1: Metropolis-Hastings

1. Initialization: $j = 0$, $\boldsymbol{\Phi}_0 = \boldsymbol{\phi}_0$

   (a) **NOTE:***If working with ODE. dynamical system - solve the coupled ODE to obtain $\boldsymbol{X}_{1:T}$ in time interval of interest $[0, T]$. Otherwise, if working with discretised SDE dynamical system the latent state trajectories for the stochastic dynamics of the system $\boldsymbol{X}_{1:T}$ in time interval of interest $[0, T]$ are considered contained in the vector $\boldsymbol{\Phi}_0$ and so no additional Forward Projection stage is required under the standard Metropolis-Hastings sampler, as these would have been sampled along with the static model parameters.*

2. For $j = 1$ to J

   (a) Draw the $j+1$-th proposal vector $\boldsymbol{\Phi}_{j+1}$ from proposal distribution $q(\boldsymbol{\phi}_j, \boldsymbol{\phi}_{j+1})$

   (b) Evaluate the acceptance probability:

$$\alpha(\boldsymbol{\phi}_j, \boldsymbol{\phi}_{j+1}) = \min\left(1, \frac{\pi(\boldsymbol{\phi}_{j+1}|\boldsymbol{y}_{1:T})\,q(\boldsymbol{\phi}_j, \boldsymbol{\phi}_{j+1})}{\pi(\boldsymbol{\phi}_j|\boldsymbol{y}_{1:T})\,q(\boldsymbol{\phi}_j, \boldsymbol{\phi}_{j+1})}\right) \tag{41}$$

   (c) Sample a random uniform variable $U \sim U[0, 1]$

   (d) If $U \leq \alpha(\boldsymbol{\phi}_j, \boldsymbol{\phi}_{j+1})$ then set $\boldsymbol{\Phi}_{j+1} = \boldsymbol{\phi}_{j+1}$ else set $\boldsymbol{\Phi}_{j+1} = \boldsymbol{\phi}_j$.

6.3. Gibbs Samplers for HPV Model Inference

Gibbs sampling is one of the most widely used forms of single component Metropolis-Hastings algorithm. It involves sampling from what are known as the full conditional distributions, given for model parameter vector $\boldsymbol{\phi} = \left[\phi^{(1)}, \ldots, \phi^{(d)}\right]$, for the i-th component by the posterior density conditional,

$$\pi\left(\phi^{(i)}|\boldsymbol{\phi}^{(-i)}, \boldsymbol{y}_{1:T}\right) = \frac{\pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)}{\int \pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right) d, \phi^{(i)}} \tag{42}$$

where $\boldsymbol{\phi}^{(-i)} = \left[\phi^{(1)}, \ldots, \phi^{(i-1)}, \phi^{(i+1)}, \ldots, \phi^{(d)}\right]$. A an explanation of the validity of such a Markov chain sampling algorithm is provided in the accessible review for practitioners in [23].

The Gibbs sampler is applicable in cases in which one can sample exactly from these full conditional distributions via inversion or rejection sampling or in Block-Gibbs the equivalent distributions corresponding to the full conditional distributions for each sub-vector in each block. It turns out that in several models this is possible when conjugacy properties are exploited in the posterior distribution. In cases in which no conditional conjugacy can be exploited for efficient and exact sampling, one may resort to other alternatives to remain using the block-Gibbs structures. Examples of general Gibbs sampler algorithms then include the use of conditionally conjugate models as well as rejection sampling approaches such as the adaptive rejection sampler approach of [57]. If this is not possible to utilise these structures then it is also valid to run a Metropolis-Hastings within Gibbs sampler on any sub-blocks with a random or deterministic scan of each sub-block of parameters comprising $\boldsymbol{\phi}$, see discussions on adaptive versions of such algorithms in [91].

Due to this construction of the Gibbs importance distribution, the acceptance probability of a proposed new state for the Markov chain in the exact Gibbs sampler is always identically unity, hence every move proposed is automatically accepted, this is simply proven by considering the solution to the fixed point equations.

In the next section we detail a special case of an auxiliary variable Gibbs sampler which has efficient sampling properties, is widely applicable to many HPV Bayesian models and is known as the Slice sampler.

6.4. Generic Univariate Auxiliary Variable Gibbs Sampler: Slice Sampler

The full conditional distributions obtained for the models in a HPV context are typically only known up to normalising constants and can not be sampled from directly via inversion sampling since they will not typically admit known parametric density representations. Therefore this will exclude straightforward inversion or basic rejection sampling being used to sample from these distributions. As an alternative, one may adopt a Metropolis Hastings (MH) within Gibbs sampler to obtain samples, see for example [54] and [118] for detailed expositions of such approaches, that were discussed briefly above.

To utilise such algorithms it is important to select a suitable proposal distribution. Quite often in high dimensional problems such as those encountered in HPV modelling, this requires tuning of the proposal for a given target distribution. Hence, one incurs a significant additional computational expense in tuning the proposal distribution parameters off-line so

that mixing of the resulting Markov chain is sufficient. To avoid this consider utilising the full conditional distributions within the sampler known as a univariate Slice sampler, see [104].

Slice sampling was developed with the intention of providing a "black box" approach for sampling from a target distribution which may not have a simple form. The Slice sampling methodology we develop will be automatically tailored to the desired target posterior. As such it does not require pre-tuning and in many cases will be more efficient than a MH within Gibbs sampler. The reason for this, pointed out by [104], is that a MH within Gibbs has two potential problems. The first arises when a MH approach attempts moves which are not well adapted to local properties of the density, resulting in slow mixing of the Markov chain. Secondly, the small moves arising from the slow mixing typically lead to traversal of a region of posterior support in the form of a Random Walk. Therefore, L^2 steps are required to traverse a distance that could be traversed in only L steps if moving consistently in the same direction. A univariate Slice sampler can adaptively change the scale of the moves proposed avoiding problems that can arise with the MH sampler when the appropriate scale of proposed moves varies over the support of the distribution.

The intuition behind Slice sampling arises from the fact that sampling from a univariate distribution, in this case given by say the i-th component full conditional of the posterior for the HPV Bayesian model, given by $\pi\left(\phi^{(i)}|\boldsymbol{\phi}^{(-i)}, \boldsymbol{y}_{1:T}\right)$, can always be achieved by sampling uniformly from the region under the distribution $\pi\left(\phi^{(i)}|\boldsymbol{\phi}^{(-i)}, \boldsymbol{y}_{1:T}\right)$.

The procedure in Algorithm 2 is repeated and by discarding the auxiliary variable sample u_{j+1} one obtains correlated samples $\boldsymbol{\phi}_{j+1}$ from $\pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)$. [104], demonstrates that a Markov chain $(U, \boldsymbol{\Phi})$ constructed in this way will have stationary distribution defined by a uniform distribution under $\pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)$ and the marginal of $\boldsymbol{\Phi}$ has desired stationary distribution $\pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)$. Additionally, [99, 98] proved that the Slice sampler algorithm, assuming a bounded target distribution $\pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)$ with bounded support, is uniformly ergodic.

Similar to a deterministic scan Gibbs sampler, the simplest way to extend the Slice sampler to a multivariate distribution is by considering each full conditional distributions in turn or randomly selected as detailed above. Note, discussion relating to the benefits provided by Random Walk behaviour suppression, as achieved by the Slice sampler, are presented in the context of non-reversible Markov chains, see [37].

Algorithm 2: Obtaining a univariate Slice sample at iteration $j+1$.

1. Random select an index i uniformly from the set $i \sim U\left\{1, 2, 3, \ldots, d\right\}$.
2. Sample a value $U_{j+1} \sim U\left[0, \pi\left(\phi_{j+1}^{(i)}|\boldsymbol{\phi}_j^{(-i)}, \boldsymbol{y}_{1:T}\right)\right]$
3. Estimate the level set(s) of the univariate distribution at point U_{j+1} corresponding to the possibly disjoint intervals given by,
$$\mathcal{A}_{j+1} = \left\{\phi^{(i)} : \pi\left(\phi_{j+1}^{(i)}|\boldsymbol{\phi}_j^{(-i)}, \boldsymbol{y}_{1:T}\right) = U_{j+1}\right\}. \tag{43}$$
4. Sample a value uniformly from the corresponding level set $\mathcal{A}_{j+1}$ a new value of $\phi_{j+1}^{(i)} \sim U\left[\mathcal{A}_{j+1}\right]$.

Additionally, we only need to know the target full conditional posterior up to normalisation, see [104, p. 710]. This is important in this example since solving the normalising constant in this model is not possible analytically. To make more precise the intuitive description of the Slice sampler presented above, we briefly detail the argument made by Neal on this point. Suppose we wish to sample from a distribution for a random vector $\boldsymbol{\Phi} \in \mathbb{R}^d$ whose density $\pi(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})$ is proportional to some function $g(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})$. This can be achieved by sampling uniformly from the $(d+1)$-dimensional region that lies under the plot of $g(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})$. This is formalised by introducing the auxiliary random variable U and defining a joint distribution over $\boldsymbol{\Phi}$ and U which is uniform over the region $\{(\boldsymbol{\Phi}, U) : 0 < u < g(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})\}$ below the surface defined by $g(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})$, given by

$$p(\boldsymbol{\phi}, u) = \begin{cases} 1/Z, & \text{if } 0 < u < g(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}), \\ 0, & \text{otherwise,} \end{cases}$$

where $Z = \int f(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})\, d\boldsymbol{\phi}$. Then the target marginal density for $\boldsymbol{\Phi}$ is given by

$$p(\boldsymbol{\phi}) = \int_0^{g(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})} \frac{1}{Z} du = \frac{g(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})}{Z},$$

as required. There are many possible procedures to obtain samples of $(\boldsymbol{\Phi}, U)$.

6.5. MCMC Convergence Diagnostics

In all cases of the use of MCMC algorithms in practice one has to decide on how many samples to draw from the Markov chain mechanism, ie. how long a Markov chain to run. In principle this will dependent on a few different factors such as the precision in the estimated inferential quantities that one wishes to achieve, since we have seen that the accuracy of Monte Carlo estimates increases with the number of samples.

In addition, the additional feature to consider when using MCMC samplers is that if one initialises the sampler from a arbitrary point in the parameters space, it will take a certain number of iterations before the Markov chain reaches what can be considered the stationary regime, i.e. begins to sample from the true posterior target distribution. Therefore it is common in practice to do two things, the first is discard the initial samples which may not have come from the stationary distribution (know as discarding 'burn-in'), and the second thing often done is to monitor the mixing (exploration of the Markov chain) around the support of the posterior distribution. This helps to ensure that we are not using samples which are too autocorrelated. Both these tasks require some version of monitoring, and generally there have been statistical approaches developed to monitor these aspects as the Markov chain progresses, these are known as convergence diagnostics, see the review in [95].

Hence, we stress that when using an MCMC algorithm, it is crucial to carefully monitor the convergence diagnostics of the Markov chain. This is more important in general MCMC contexts due to the possibility of extended rejections where the Markov chain can stick in a given state for long periods.

If the total chain has length T, the initial burn-in stage will correspond to the first T_b samples and we define $\widetilde{T} = T - T_b$. In this particular section we will denote by $\{\boldsymbol{\phi}^i_{(t)}\}_{t=1:\widetilde{T}}$

the Markov chain of the i-th parameter after burn-in. The diagnostics we consider are given as follows, where the upper script index i used throughout this subsection refers to the i-th element of the state vector and the lower subscripts will refer to the iteration index of the Markov chain samples.

- *Autocorrelation.* This convergence diagnostic will monitor serial correlation in the Markov chain. For given Markov chain samples for the i-th parameter $\{\boldsymbol{\phi}^i_{(t)}\}_{t=1:\widetilde{T}}$, we define the biased autocorrelation estimate at lag τ by

$$\widehat{ACF}(\boldsymbol{\phi}_i, \tau) = \frac{1}{(\widetilde{T}-\tau)\hat{\sigma}\left(\boldsymbol{\phi}^i\right)} \sum_{t=1}^{\widetilde{T}-\tau} [\boldsymbol{\phi}^i_{(t)} - \widehat{\mu}\left(\boldsymbol{\phi}^i\right)][\boldsymbol{\phi}^i_{(t+\tau)} - \widehat{\mu}\left(\boldsymbol{\phi}^i\right)], \tag{44}$$

  where $\widehat{\mu}\left(\boldsymbol{\phi}^i\right)$ and $\hat{\sigma}\left(\boldsymbol{\phi}^i\right)$ are the estimated mean and standard deviation of the i-th component of the state vector $\boldsymbol{\phi}^i$.

- *Geweke [53, 31] time series diagnostic.* For the i-th component of the parameter $\boldsymbol{\phi}^i$ it is calculated as follows:

  1. Split the Markov chain samples into two sequences, $\{\boldsymbol{\phi}^i_{(t)}\}_{t=1:T_1}$ and $\{\boldsymbol{\phi}^i_{(t)}\}_{t=T^*:\widetilde{T}}$, such that $T^* = \widetilde{T} - T_2 + 1$, and with ratios $T_1/\widetilde{T}$ and $T_2/\widetilde{T}$ fixed such that $(T_1+T_2)/\widetilde{T} < 1$ for all $\widetilde{T}$.
  2. Evaluate $\widehat{\mu}\left(\boldsymbol{\phi}^i_{T_1}\right)$ and $\widehat{\mu}\left(\boldsymbol{\phi}^i_{T_2}\right)$ corresponding to the sample means on each sub sequence.
  3. Evaluate consistent spectral density estimates for each sub sequence, at frequency 0, denoted $\widehat{SD}(0; T_1, \boldsymbol{\phi}^i)$ and $\widehat{SD}(0; T_2, \boldsymbol{\phi}^i)$. The spectral density estimator considered in this paper is the classical non-parametric periodogram or power spectral density estimator. We use Welch's method with a Hanning window; for details of the power spectral density in [109].
  4. Evaluate convergence diagnostic given by
     $Z_{\widetilde{T}} = \frac{\widehat{\mu}\left(\boldsymbol{\phi}^i_{T_1}\right) - \widehat{\mu}\left(\boldsymbol{\phi}^i_{T_2}\right)}{T_1^{-1}\widehat{SD}(0;T_1,\boldsymbol{\phi}^i) + T_2^{-1}\widehat{SD}(0;T_2,\boldsymbol{\phi}^i)}$.
     According to the central limit theorem, as $\widetilde{T} \to \infty$ one has that $Z_{\widetilde{T}} \to \mathcal{N}(0,1)$ if the sequence $\{\boldsymbol{\phi}^i_{(t)}\}_{t=1:\widetilde{T}}$ is stationary.

- *Gelman-Rubin [51, 19] R-statistic diagnostic.* This approach to convergence analysis requires that one runs multiple parallel independent Markov chains each starting at randomly selected initial starting points (we run five chains). For comparison purposes we split the total computational budget of $\widetilde{T}$ into $T_1 = T_2 = \ldots = T_5 = \frac{\widetilde{T}}{5}$. The convergence diagnostic for parameter $\boldsymbol{\phi}_i$ is calculated using the following steps:

  1. Generate five independent Markov chain sequences, producing the chains for parameter $\boldsymbol{\phi}_i$ denoted $\{\boldsymbol{\phi}^{(t)}_{i,k}\}_{t=1:T_k}$ for $k \in \{1, \ldots, 5\}$.
  2. Calculate the sample means $\widehat{\mu}\left(\boldsymbol{\phi}^i_{T_k}\right)$ for each sequence and the overall mean $\widehat{\mu}\left(\boldsymbol{\phi}^i_{\widetilde{T}}\right)$.

3. Calculate the variance of the sequence means $\frac{1}{4}\sum_{k=1}^{5}\left(\widehat{\mu}\left(\boldsymbol{\phi}^i_{T_k}\right)-\widehat{\mu}\left(\boldsymbol{\phi}^i_{\widetilde{T}}\right)\right)^2 \stackrel{def.}{=} B^i/T_k$.

4. Calculate the within-sequence variances $\widehat{s}^2\left(\boldsymbol{\phi}^i_{T_k}\right)$ for each sequence.

5. Calculate the average within-sequence variance, $\frac{1}{5}\sum_{k=1}^{5}\widehat{s}^2\left(\boldsymbol{\phi}^i_{T_k}\right) \stackrel{def.}{=} W^i$.

6. Estimate the target posterior variance for parameter $\boldsymbol{\phi}^i$ by the weighted linear combination $\widehat{\sigma}^2\left(\boldsymbol{\phi}^i_{\widetilde{T}}\right)=\frac{T_k-1}{T_k}W^i+\frac{1}{T_k}B^i$. This estimate is unbiased for samples which are from the stationary distribution. In the case in which not all sub chains have reached stationarity, this overestimates the posterior variance for a finite $\widetilde{T}$ but asymptotically, $\widetilde{T}\rightarrow\infty$, it converges to the posterior variance.

7. Improve on the Gaussian estimate of the target posterior given by $\mathcal{N}(\widehat{\mu}\left(\boldsymbol{\phi}^i_{\widetilde{T}}\right),\widehat{\sigma}^2\left(\boldsymbol{\phi}^i_{\widetilde{T}}\right))$ by accounting for sampling variability in the estimates of the posterior mean and variance. This can be achieved by making a Student-t approximation with location $\widehat{\mu}\left(\boldsymbol{\phi}^i_{\widetilde{T}}\right)$, scale $\sqrt{\widehat{V}^i}$ and degrees of freedom df^i, each given respectively by:
$\widehat{V}^i=\widehat{\sigma}^2\left(\boldsymbol{\phi}^i_{\widetilde{T}}\right)+\frac{B^i}{\widetilde{T}}$ and $df^i=\frac{2(\widehat{V}^i)^2}{\widehat{\text{Var}}(\widehat{V}^i)}$, where the variance is estimated as

$$\begin{aligned}\widehat{\text{Var}}\left(\widehat{V}^i\right)=&\frac{1}{5}\left(\frac{T_1-1}{T_1}\right)^2\widehat{\text{Var}}\left(\widehat{s}^2\left(\boldsymbol{\phi}^i_{T_k}\right)\right)+\left(\frac{6}{\sqrt{2\widetilde{T}}}\right)^2(B^i)^2\\&+\frac{12(T_1-1)}{25T_1}\widehat{\text{Cov}}\left(\widehat{s}^2\left(\boldsymbol{\phi}^i_{T_k}\right),\widehat{\mu}\left(\boldsymbol{\phi}^i_{\widetilde{T}}\right)\right)\\&-\frac{24(T_1-1)}{25T_1}\widehat{\mu}\left(\boldsymbol{\phi}^i_{\widetilde{T}}\right)\widehat{\text{Cov}}\left(\widehat{s}^2\left(\boldsymbol{\phi}^i_{T_k}\right),\widehat{\mu}\left(\boldsymbol{\phi}^i_{\widetilde{T}}\right)\right).\end{aligned}\tag{45}$$

Note, the covariance terms are estimated empirically using the within sequence estimates of the mean and variance obtained for each sequence.

8. Calculate the convergence diagnostic $\sqrt{\widehat{R}}=\sqrt{\frac{\widehat{V}^i df^i}{W^i(df^i-2)}}$, where as $\widetilde{T}\rightarrow\infty$ one can prove that $\widehat{R}\rightarrow 1$. This convergence diagnostic monitors the scale factor by which the current distribution for $\boldsymbol{\phi}^i$ may be reduced if simulations are continued for $\widetilde{T}\rightarrow\infty$.

Having specified a few popular approaches to monitoring convergence of an MCMC sampler, we next turn to the question of how one can improve the design of the Markov chain proposal mechanism. This will involve modifying the proposal to help to make it adaptively learn the appropriate structure so as to improve the exploration and mixing of the resulting Markov chain.

6.6. Introduction to Adaptive Markov Chain Monte Carlo

As has now been demonstrated in the previous few sections, Markov chain Monte Carlo (MCMC) sampling has gained a wide recognition in all areas of modelling and statistical estimation as an essential tool for performing inference in Bayesian models (see reviews

and discussions in [54] and [18]). In this section we discuss two recently developed class of algorithms known as forms of adaptive MCMC (see a review in [8]), and demonstrate how they may be utilised to solve statistically challenging estimation and prediction problems of direct relevance to the interpretation and analysis of the calibration and impact of vaccine dynamics for HPV epidemic models. We illustrate this on a model we develop for HPV-6 and -11.

As discussed in the section on Metropolis-Hastings algorithms, standard MCMC algorithms that do not incorporate adaptation often require a degree of "tuning" of the parameters controlling the algorithms' performance. This is typically performed by off-line simulations to assess performance of the mixing of the resulting Markov chain followed by numerical investigation of the convergence rates to stationarity of the chain for different algorithmic settings of the proposal distribution. For example, the variant of the Metropolis-Hastings algorithm, the random walk Metropolis algorithm with the widely used multivariate Gaussian proposal has mixing performance that is controlled through specification of the Markov chain proposal distributions covariance matrix. Tuning this matrix for optimal performance can be computationally expensive and inefficient (see detailed discussions in [54, 18, 26]). Optimal performance of an MCMC algorithm is typically either specified by the convergence rate of the Markov chain to stationarity or through the related quantity, the acceptance probability of the rejection step in the MCMC algorithm. In this regard, theoretically optimal results have been derived for several classes of statistical models, which now act as guides for more complicated sampling problems (see discussions in [119]).

In general if one considers an ODE HPV epidemic model, it will typically be constructed on a high dimensional space both in the parameters of the model and also in the latent ODE state trajectories solved for at each discrete time point in the "forward projection" ODE solver, see discussions in [85]. This high dimensionality in the posterior parameter space provides a significant challenge for standard MCMC algorithms with respect to the design of an efficient proposal mechanism for the Markov chain. In particular, in the model considered in this paper the fact that we incorporate a forward projection stage for the ODE solver adds additional complications in the design of the proposal. Therefore, it is desirable to automate this proposal construction for the MCMC sampler, avoiding computationally expensive tuning processes. Hence, we develop an adaptive version of the random walk Metropolis algorithm, coupled with forward projection. The incorporation of an adaptive proposal mechanism in a Markov chain Monte Carlo algorithm has been demonstrated to improve the performance of the sampling algorithm relative to standard MCMC approaches (see reviews of several examples of this improvement in [8]). The improvement is achieved by learning the structure of the Markov chain proposal distribution on-line in an automated fashion, avoiding off-line tuning of the MCMC proposal mechanism.

There are several classes of adaptive MCMC algorithms and each class has several adaptation strategies [122, 12, 8]. These approaches can be classified as either internal adaptation mechanisms, including controlled MCMC methods, or external adaptation strategies (see discussion in [12]). The distinguishing feature of adaptive MCMC algorithms, when compared to standard MCMC, is that the Markov chain is generated via a sequence of transition kernels. Adaptive algorithms get their name from the fact that they utilise a combination of time or state inhomogeneous proposal kernels. Each proposal in the sequence is allowed to depend on the past history of the Markov chain generated, resulting in many

possible variants. When using inhomogeneous Markov kernels it is particularly important to ensure the generated Markov chain is ergodic, with the appropriate stationary distribution. Several recent papers proposing theoretical conditions that must be satisfied to ensure ergodicity of adaptive algorithms include [12] and [69, 68]. The papers [121, 90, 13] consider properties such as the ergodicity of the adaptive MCMC under conditions such as *Diminishing Adaptation* and *Bounded Convergence*.

Designing an adaptation strategy that satisfies these conditions guarantees asymptotic convergence of the law of the Markov chain samples to the target posterior and ensures the Weak Law of Large Numbers holds for bounded test functions of the parameter space (an interested reader is referred to [123] for details).

The practitioner reading this section may wish to skip the following technical notes relating to the validity of the Adaptive MCMC algorithm. Though they are provided for those interested in the design of such MCMC methods and their associated conditions for validity. In stating these conditions more precisely we consider the distance between two probability distributions generically denoted by ν and μ, which are formed from measures which we assume admit a density with respect to say Lebesgue measure. We can define this distance generically according to

$$d(\mu,\nu) = \sup\left\{\left|\int \varphi d\mu - \int \varphi d\nu\right| : \varphi \in \mathcal{D}\right\} \tag{46}$$

for some test function φ in a class of functions denoted $\mathcal{D}$, for example the class of all bounded and k-th order differentiable functions, etc. In the case of considering the Total Variation distance $||\mu-\nu||_{TV}$ we consider the space of Borel sets $\mathcal{B}$ and define the distance

$$d(\mu,\nu) = ||\mu-\nu||_{TV} := \sup_{A\in\mathcal{B}} |\nu(A)-\mu(A)| \tag{47}$$

with the class of functions given by the indicators on the Borel sets $\mathcal{D} = \{\mathbb{I}_A : A \in \mathcal{B}\}$. This distance in the case of two probability measures ν and μ will clearly be between $[0,1]$ and will provide a comparison of convergence between two probability measures which will imply weak convergence (convergence in distribution).

One can formally consider this distance and utilise it to develop a condition that will succinctly state one of the required conditions for an adaptive Markov chain to satisfy ergodicity. This condition is known as

Diminishing Adaptation:

$$\lim_{n\to\infty} \sup_{\boldsymbol{\phi}\in\mathbb{R}^d} \| Q_{\Gamma_{n+1}}(\boldsymbol{\phi},\boldsymbol{\phi}') - Q_{\Gamma_n}(\boldsymbol{\phi},\boldsymbol{\phi}') \|_{TV} = 0 \text{ in prob.}$$

where $\boldsymbol{\phi}$ is the vector of parameters in the HPV Bayesian model and the measures ν and μ are selected to be the Markov transition kernel $Q_{\Gamma_{n+1}}$ at a random time denoted by index Γ_{n+1} when the $n+1$-th update of the kernel (in the learning phase) was applied.

The second condition required for a transition kernel to satisfy ergodicity is given by

Bounded Convergence:

$$\{M_\epsilon(\Theta^n,\Gamma_j)\}_{j=0}^{\infty} \text{ is bounded in prob., } \epsilon > 0$$

with convergence time defined as $M_\epsilon(\theta,\gamma) = \inf\left\{n \geq 1 :\| Q_\gamma^n(\theta,.) - P(\cdot) \| \leq \epsilon\right\}$ Creating an AdMCMC sampler with a proposal distribution that satisfies these technical conditions ensures:

- Asymptotic convergence:

$$\lim_{n\to\infty} \| \mathcal{L}(\Theta^n) - \pi(.) \| = 0 \text{ in prob.}$$

  where $\pi(\cdot)$ is the target posterior distribution - intended stationary distribution of the Markov chain.

- Weak Law of Large Numbers (for all bounded functions g: E $\rightarrow$ R)

$$\lim_{n\to\infty} \frac{1}{n}\sum_{i=1}^{n} g(\Theta^i) = \pi(g) = \int g(\theta)\pi(\theta)\, d\theta$$

In the following we discuss one particular illustrative choice of transition kernel that satisfies these conditions and has been used successfully in several applications (see [8], [113, 85] and [122]).

6.7. Adaptive MCMC Strategies Combined with Forward Simulation for Dynamic HPV Model Calibration

In this section we present details for the adaptive Markov chain Monte Carlo (AdMCMC) algorithm that can be combined with say a Forward simulation (ODE. solver) algorithm in order to sample from the posterior distribution for the static model parameters of the HPV model, whilst also solving for the required state trajectories of the system (required for evaluation of the likelihood in the posterior density). In particular, we first introduce the background of an AdMCMC algorithm, before detailing the adaptation strategy we will explore in this paper based on the adaptation rules developed in [12, 123].

In essence, we first construct a MCMC proposal distribution to sample the static posterior parameters, denoted by vector $\boldsymbol{\Theta}$. Then, conditional on these model parameters proposed, we obtain the state trajectories for the ODE HPV epidemic model, given by $\boldsymbol{X}_{1:T} = [\boldsymbol{X}_1, \ldots, \boldsymbol{X}_T]$, which are generated using forward simulation, see a detailed discussion on this algorithm recently proposed in [85].

The MCMC proposal distribution is parameterised by parameter vector or matrix $\boldsymbol{\Psi}$. The idea of adaptive MCMC methods is to learn appropriate values for $\boldsymbol{\Psi}$ recursively utilising the previous samples of the Markov chain that have been accepted under the MCMC accept-reject mechanism. This is achieved on-line, adapting according to the support of the posterior distribution, thereby allowing the Markov chain to discover and explore the regions of the posterior distribution that have the most mass. Through this on-line adaptive learning mechanism the Markov chain proposal distribution can significantly improve the acceptance rate of the Markov chain, enabling efficient mixing and improving the samples obtained from the posterior.

We consider a popular adaptation scheme proposed in the literature and analyse its ability to explore the very high dimension of the posterior distribution developed in the

nonlinear HPV epidemic model. Before presenting the details of the adaptation scheme for the Markov proposal, we first present the generic algorithm developed for sampling from the posterior. In the following sequence of steps for the j-th iteration of the AdMCMC Forward Simulation algorithm, we will update the state of the Markov chain from $\boldsymbol{\Theta}_{j-1}$, with corresponding states $\boldsymbol{X}_{1:T}[j-1]$, to parameter vector $\boldsymbol{\Theta}_j$ with associated state trajectories $\boldsymbol{X}_{1:T}[j]$. This algorithm is summarised below for one step of the AdMCMC Forward project algorithm:

Algorithm 3: Adaptive Markov chain Monte Carlo Algorithm

1. Sample $\boldsymbol{\theta}_* \sim q\left(\boldsymbol{\theta}_{j-1}, \cdot\right)$ from an adaptive MCMC proposal constructed using previous Markov chain samples $\{\boldsymbol{\Theta}_1, \ldots, \boldsymbol{\Theta}_{j-1}\}$.

2. Solve the nonlinear ODE system for the particular dynamic HPV model of interest, by running a forward simulation of the nonlinear ODE solver, conditional on proposed parameter vector $\boldsymbol{\theta}_*$, to obtain the state of the ODE system at times $t \in \{1, 2 \ldots, T\}$, given by $\boldsymbol{X}_1, \ldots, \boldsymbol{X}_T$, which correspond to the observations $\boldsymbol{Y}_1, \ldots, \boldsymbol{Y}_T$.

3. Accept the proposed new Markov chain state comprised of $\boldsymbol{\theta}_*$ with acceptance probability given by

$$\begin{aligned} &\alpha\left(\boldsymbol{\theta}_{j-1}, \boldsymbol{\theta}_*\right) \\ &= \min\left(1, \frac{\mathcal{L}\left(\boldsymbol{\theta}_*; \boldsymbol{Y}_1, \ldots, \boldsymbol{Y}_T\right) p\left(\boldsymbol{\theta}_*\right) q\left(\boldsymbol{\theta}_{j-1}, \boldsymbol{\theta}_*\right)}{\mathcal{L}\left(\boldsymbol{\theta}_{j-1}; \boldsymbol{Y}_1, \ldots, \boldsymbol{Y}_T\right) p\left(\boldsymbol{\theta}_{j-1}\right) q\left(\boldsymbol{\theta}_*, \boldsymbol{\theta}_{j-1}\right)}\right) \end{aligned} \tag{48}$$

   where we evaluate this acceptance probability utilising the expressions detailed previously. These steps are repeated for $j \in \{1, \ldots, J\}$.

6.8. An Example of an Adaptive MCMC Algorithm: Adaptive Metropolis

To provide practitioners with perhaps the simplest version of an Adaptive MCMC algorithm proposal that could be considered, we present the internal adaptation strategy based on the adaptive Metropolis algorithm detailed in [123] and recently utilised in HPV modelling in [85]. This is a variant of the approach proposed in [70] which develops a Random Walk Metropolis-Hastings (RWMH) that estimates the global covariance structure from the past samples.

Under an adaptive Metropolis algorithm, the proposal distribution is based on a Gaussian mixture kernel detailed in [123]. The proposal, $q\left(\boldsymbol{\phi}_{j-1}, \boldsymbol{\phi}_*\right)$, involves an adaptive Gaussian-mixture Metropolis proposal, one component of which has a covariance structure that is adaptively learnt on-line as the algorithm explores the posterior distribution. For iteration j of the Markov chain the proposal is

$$q_j\left(\boldsymbol{\phi}_{j-1}, \cdot\right) = \gamma \mathcal{N}\left(\boldsymbol{\phi}_*; \boldsymbol{\phi}_{j-1}, \frac{(2.38)^2}{d}\Sigma_j\right) + (1-\gamma)\mathcal{N}\left(\boldsymbol{\phi}_*; \boldsymbol{\phi}_{j-1}, \frac{(0.1)^2}{d} I_{d,d}\right). \tag{49}$$

Here, Σ_j is the current empirical estimate of the covariance between the parameters of $\boldsymbol{\phi}$, estimated using samples from the Markov chain up to time $j-1$. Small positive constant γ is usually taken equal to 0.05 [123]. The theoretical motivation for the choices of scale factors 2.38, 0.1 and dimension d are all provided in [123] and are based on optimality conditions presented in [120].

We note that the update of the covariance matrix, can be done recursively on-line via the following recursion (as detailed in [11]), see the recursion below:

$$\begin{aligned}\mu_{j+1} &= \mu_j + \frac{1}{j+1}\left(\boldsymbol{\phi}_{j-1} - \mu_j\right), \\ \Sigma_{j+1} &= \Sigma_j + \frac{1}{j+1}\left(\left(\boldsymbol{\phi}_{j-1} - \mu_j\right)\left(\boldsymbol{\phi}_{j-1} - \mu_j\right)' - \Sigma_j\right).\end{aligned}$$

6.9. Riemann-Manifold Hamiltonian Monte Carlo Sampler (Automated Local Adaption)

By this stage we have established clearly the fact that design of the proposal distribution for the Markov chain that is created for a MCMC method with target HPV model posterior $\pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)$ can directly effect the ability to make accurate inference. In addition we have discovered that the transition kernel for the class of MCMC methods of interest is typically given by

$$\begin{aligned}Q\left(\boldsymbol{\phi}_j, d\boldsymbol{\phi}_{j+1}\right) &= q\left(\boldsymbol{\phi}_j, d\boldsymbol{\phi}_{j+1}\right)\alpha\left(\boldsymbol{\phi}_j, d\boldsymbol{\phi}_{j+1}\right) \\ &+ \left[1 - \int q\left(\boldsymbol{\phi}_j, \boldsymbol{z}\right)\alpha\left(\boldsymbol{\phi}_j, \boldsymbol{z}\right)d\boldsymbol{z}\right]\mathbb{I}\left[\boldsymbol{\phi}_{j+1} = \boldsymbol{\phi}_j\right],\end{aligned} \tag{50}$$

where the (adaptive) design of $q\left(\boldsymbol{\phi}_j, d\boldsymbol{\phi}_{j+1}\right)$ is of direct interest for reducing variance in Monte Carlo estimates. Next we present another more advanced class of algorithms known as HMC methods. These are a recently introduced class of Markov chain Monte Carlo samplers in [58] was developed to help automate the design of the proposal distributions within the Markov kernel, in this case this will be achieved through the use of what is known as Riemann-Manifold Hamiltonian Monte Carlo, that we will briefly describe next, see a tutorial overview in [105].

We first define the basic principles from which Riemann-Manifold Hamiltonian Monte Carlo (hereafter RM-HMC) as discussed in detail in [43, 58, 105]. The context of the RM-HMC algorithm derives from the design of a Markov chain proposal obtained from a discretised Langevin diffusion with two components, a stochastic discretised diffusion component and a second component based on a discretised deterministic component constructed from gradient information of the target density (HPV model posterior density). This first class of algorithm was known as the Metropolis Adjusted Langevin Algorithm (MALA) method of [130] and adaptive versions in [94]. Alternative approaches of a similar nature were also developed and are generally known as Hybrid Monte Carlo (hereafter HMC) proposals as they also involve a combination of deterministic and stochastic components obtained from discretisation of a physical stochastic process. Such HMC algorithms typically produce an ergodic Markov chain in which large traversals of the posterior support are accepted with high probability.

6.9.1. Sampling the HPV Posterior Density via Establishing Related Hamiltonian Mechanics

This section provides basic details to aid the understanding of how one develops such a HMC proposal mechanism to efficiently explore the support of the target posterior distribution for a given HPV model. it is instructive to consider the following non-standard formulation of a posterior distribution, which we repose as the equations of motion under Hamiltonian mechanics. This involves specification of a system of partial differential equations that can be solved to provide the building blocks of the HMC algorithms. Consider the random vector of posterior parameters $\boldsymbol{\Phi} \in \mathbb{R}^d$ with $\boldsymbol{\Phi} \sim f(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})$ and an independent auxiliary random vector denoted by $\boldsymbol{Z} \in \mathbb{R}^d$ with $\boldsymbol{Z} \sim N(0, \Sigma)$. Now consider construction the negative log joint density given by the equivalent interpretation as a Hamiltonian $H(\boldsymbol{\phi}, \boldsymbol{z})$ of a energy conserving physical dynamic system described by

$$H(\boldsymbol{\phi}, \boldsymbol{z}) = -\ln \pi(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}) + \frac{1}{2}\log(2\pi)^d|\Sigma| + \frac{1}{2}\boldsymbol{z}^T\Sigma^{-1}\boldsymbol{z}, \tag{51}$$

with $-\ln \pi(\boldsymbol{\phi}|\boldsymbol{y}_{1:T})$ the accumulated potential energy at location $\boldsymbol{\Phi}$, the term $\frac{1}{2}\boldsymbol{z}^T\Sigma^{-1}\boldsymbol{z}$ representing the kinetic energy and $\boldsymbol{z}$ the momentum and mass matrix Σ, see discussion in [58, 105]. To understand why this interpretation may be of relevance to the design of an MCMC sampler one needs to consider the score function of the joint distribution of random vectors $\boldsymbol{\Phi}$ and $\boldsymbol{Z}$ given by

$$\frac{\partial H}{\partial \boldsymbol{z}} = \Sigma^{-1}\boldsymbol{z}, \quad -\frac{\partial H}{\partial \boldsymbol{\phi}} = \nabla_{\boldsymbol{\phi}} \ln \pi(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}). \tag{52}$$

This deterministic system of partial differential equations can be used to re-interpret, with respect to the joint distribution of the two random vectors, a dynamical system with artificial 'time' unit τ given by $\frac{d\boldsymbol{\phi}}{d\tau} = \frac{\partial H}{\partial \boldsymbol{z}}$ and $\frac{d\boldsymbol{z}}{d\tau} = -\frac{\partial H}{\partial \boldsymbol{\phi}}$.

By linking the joint distribution of random vectors $\boldsymbol{\Phi}$ and $\boldsymbol{Z}$ to a physical system evolution one may now construct from these artificial dynamics a dynamic proposal mechanism for a Markov chain sampler, see algorithm 4.

It should be noted that the numerical integrator should provide a dynamic solution which is interpreted as a transformation mapping from the parameter vector $(\boldsymbol{\phi}, \boldsymbol{z})$ to new 'proposed' parameter vector $(\boldsymbol{\phi}', \boldsymbol{z}')$. If this mapping is time-reversible (in the artificial time τ) and volume preserving then it can be utilised to design a Metropolis-Hastings Markov chain reject-accept sampler.

Fortunately, by constructing such a Hamiltonian dynamical system for utilisation in the proposal as an efficient and perhaps adaptive MCMC sampler one automatically satisfies the following important properties, see proofs in [105]:

1. **Reversible Proposals:** Hamiltonian mechanics preserve the reversibility of a Markov chain constructed with a proposal that utilises such a dynamic system to explore the support of the posterior. In other words, one may define a mapping from the state of the system at time τ_1 given by $(\boldsymbol{\theta}(\tau_1), \boldsymbol{z}(\tau_1))$ to a new state at time $\tau_2 > \tau_1$, denoted $(\boldsymbol{\theta}(\tau_2), \boldsymbol{z}(\tau_2))$, which is one-to-one and therefore invertible.

2. **Invariance of the Hamiltonian System:** Designing a proposal from a Hamiltonian system of equations will create dynamics which are invariant within the Hamiltonian system. That is the dynamics preserve the structure of the Hamiltonian system.

3. **Volume Preservation (Liouvilles Theorem):** It is well known that Hamiltonian system is volume preserving. The consequence of this is that using this dynamic system to construct a proposal in MCMC will result in an acceptance probability that does not require a Jacobian mass transform. This is a significant advantage of such a transformational proposal.

6.9.2. Sampling the HPV Posterior via Discretization of the Hamiltonian Mechanics

Utilising this Hamiltonian dynamic system for the MCMC proposal therefore requires a numerical solver for the two PDEs in order to generate the proposal at each iteration of the HMC. Therefore, the challenge lies in finding numerical integrators that are both time reversible and volume preserving. Fortunately, one such explicit class of such integrators are known as the symplectic class, a particular example from this class is the leapfrog integrator, see [43]. This was utilised to define a Hamiltonian Monte Carlo (HMC) solution, where one iteration of the HMC algorithm therefore involves drawing randomly a realised vector $\boldsymbol{z}$ and then iterating the Leap Frog integrator defined by deterministic recursions for step size ϵ in Algorithm 4.

Hence the generation of an MCMC proposal under the HMC system would proceed as follows:

Algorithm 4: Hybrid Monte Carlo Algorithm Proposal

1. Sample a realisation of auxiliary random vector $\boldsymbol{Z} \sim N(0, \Sigma)$.

2. Perform numerical integration to solve $\frac{d\boldsymbol{\phi}}{d\tau} = \frac{\partial H}{\partial \boldsymbol{z}}$ and $\frac{d\boldsymbol{z}}{d\tau} = -\frac{\partial H}{\partial \boldsymbol{\phi}}$, using the sampled value of the auxiliary variable, thus providing an evolution equation in the joint distribution space for random vectors $\boldsymbol{\Phi}$ and $\boldsymbol{Z}$.

   (a) $\boldsymbol{z}(\tau + \epsilon/2) = \boldsymbol{z}(\tau) + \epsilon \nabla_{\boldsymbol{\phi}} \ln \pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)\big|_{\boldsymbol{\phi}=\boldsymbol{\phi}(\tau)} /2$

   (b) $\boldsymbol{\phi}(\tau + \epsilon) = \boldsymbol{\phi}(\tau) + \epsilon \Sigma^{-1} \boldsymbol{z}(\tau + \epsilon/2)$

   (c) $\boldsymbol{z}(\tau + \epsilon) = \boldsymbol{z}(\tau + \epsilon/2) + \frac{\epsilon}{2} \nabla_{\boldsymbol{\phi}} \ln \pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)\big|_{\boldsymbol{\phi}=\boldsymbol{\phi}(\tau+\epsilon)}$.

Iteration of this algorithm generates a sequence of random proposals of initial value for $\boldsymbol{Z}$ followed by a deterministic trajectory solution for n-steps of size ϵ via a leap-frog integration iteration for the proposal. Taking the last point $\boldsymbol{\phi}_T = \boldsymbol{\phi}_*$ as the proposal one then accepts this proposed point under the MCMC accept reject mechanism with the following probability which involves the Hamiltonian energy functions,

$$\alpha(\boldsymbol{z}, \boldsymbol{\phi}; \boldsymbol{z}_*, \boldsymbol{\phi}_*) = \min\left(1, -H(\boldsymbol{z}_*, \boldsymbol{\phi}_*) + H(\boldsymbol{z}, \boldsymbol{\phi})\right). \tag{53}$$

It was recently realised that one could further adapt this Hamiltonian proposal through observing that the behaviour of the simulated trajectory was directly affected by "tuning"

the matrix Σ, therefore one could try to find a way to learn efficient choices for Σ to improve the acceptance probability of a move by adapting Σ to local structure of the target distribution posterior. Therefore the MCMC proposal constructed in this fashion is then tuned via the selection of the mass matrix Σ, the number of iteration steps n and the step size ϵ. In general one may summarise this HMC algorithm according to the Langevin discretised diffusion recursion,

$$\boldsymbol{\phi}(\tau+\epsilon) = \underbrace{\boldsymbol{\phi}(\tau) + \frac{\epsilon^2}{2}\Sigma^{-1}\,\frac{\epsilon}{2}\nabla_{\boldsymbol{\phi}} \ln \pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right)\Big|_{\boldsymbol{\phi}=\boldsymbol{\phi}(\tau)}}_{\text{Preconditioned deterministic innovation}} + \underbrace{\epsilon\Sigma^{-2}\boldsymbol{z}(\tau)}_{\text{Stochastic innovation}} . \tag{54}$$

The adaptive MCMC development of this algorithm is discussed extensively in [58] and involves primarily the adaption of the mass matrix Σ. The other algorithmic parameters to consider involve the number of steps n and the step size ϵ, these may typically be effectively estimated from acceptance probabilities of the MCMC chain. The following two key points were noted in [58] to consider in order to improve the performance of the HMC algorithm:

> **Remark 1.** Stochastic transitions that account for local geometric structure of the target distribution when making proposals to different regions of the distributional support can improve the Markov chain exploration and mixing. One way to achieve this is to replace the HMC global covariance matrix proposal Σ (mixing matrix) with a position specific version.

> **Remark 2.** Under the HMC algorithm described, the deterministic component of the Langevin proposal involves the gradient of the target distribution which is preconditioned by the inverse global mass matrix. It was noted that adapting this mass matrix Σ to local structure of the target distribution would improve mixing performance. This can be achieved by exploiting a Riemannian structure of the target distribution parameter space using a localised metric tensor.

To address these remarks the approach of [58] was to develop a Riemann Manifold Hamiltonian Monte Carlo (RM-HMC) algorithm. Here we briefly discuss this adaptive HMC algorithm and present the details so that it may be utilised to make inference in HPV model estimation.

In the RM-HMC setting one considers locally adapting the generic Hamiltonian given by $-\log p(\boldsymbol{\phi},\boldsymbol{z}) = -\log p(\boldsymbol{\phi}) - \log p(\boldsymbol{z})$ in the HMC setting. This is achieved by interpreting the family of parameterised probability densities for d-dimensional random vector $\boldsymbol{\Phi}$ given by $\log p(\boldsymbol{\phi})$ as defining a Riemannian manifold which has an associated metric tensor, which may for example be selected to be the Fisher information matrix for the target distribution model given by $\mathcal{I}(\boldsymbol{\phi}) = \mathbb{E}\left[\nabla_{\boldsymbol{\phi}} \ln p(\boldsymbol{\phi}) \nabla_{\boldsymbol{\phi}} \ln p(\boldsymbol{\phi})^T\right]$.

Under this modified specification using the alternate metric tensor, say the Fisher Information matrix $\mathcal{I}(\boldsymbol{\phi})$ one obtains a Hamiltonian equation given by

$$H(\boldsymbol{\phi},\boldsymbol{z}) = -\ln f\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right) + \frac{1}{2}\log(2\pi)^d|\mathcal{I}(\boldsymbol{\phi})| + \frac{1}{2}\boldsymbol{z}^T\mathcal{I}(\boldsymbol{\phi})^{-1}\boldsymbol{z}. \tag{55}$$

Under this formulation one can sample the auxiliary variable vectors in the RM-HMC scheme for $\boldsymbol{z}$ given by a conditionally Gaussian distribution $\boldsymbol{Z} \sim N(\boldsymbol{z}; \boldsymbol{0}, \mathcal{I}(\boldsymbol{\Phi}))$. It is now clear that such a modification through the Riemannian structure of the target distribution allows one to utilise a locally adapted proposal, however the consequence of this structure is that the Hamiltonian is no longer separable. The consequence of this loss of separability is that the symplectic integration procedure previously proposed for the standard HMC algorithm will be required to be modified as detailed in [58] and detailed in Algorithm 5.

Consider designing a RM-HMC algorithm to move from state $(\boldsymbol{z}_0, \boldsymbol{\phi}_0)$, i.e. some previous RM-HMC state, to proposed state $(\boldsymbol{z}_*, \boldsymbol{\phi}_*)$. If one defines the metric tensor for the local adaption of the covariance Σ in which $\Sigma\left(\boldsymbol{\phi}_{j-1}\right) = \mathcal{I}(\boldsymbol{\phi})$ and the integration step size is given by ϵ and total number of iterations by T, then the full symplectic integrator for the RM-HMC algorithm is now adjusted to the following 5 steps:

$$
\begin{aligned}
\boldsymbol{z}_1 &= \boldsymbol{z}_0 - \frac{\epsilon}{2}\nabla_{\boldsymbol{\phi}} \left(-\ln \pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right) + \frac{1}{2}\log(2\pi)^d |\mathcal{I}(\boldsymbol{\phi})| \right)\Bigg|_{\boldsymbol{\phi_0}} \\
\boldsymbol{z}_2 &= g\left(\boldsymbol{\phi}_0, \boldsymbol{z}_1, \frac{\epsilon}{2}\right) \\
\boldsymbol{\phi}_* &= \boldsymbol{\phi}_0 + \epsilon \mathcal{I}(\boldsymbol{\phi})^{-1} \\
\boldsymbol{z}_3 &= g\left(\boldsymbol{\phi}_*, \boldsymbol{z}_2, \frac{\epsilon}{2}\right) \\
\boldsymbol{z}_* &= \boldsymbol{z}_3 - \frac{\epsilon}{2}\nabla_{\boldsymbol{\phi}} \left(-\ln \pi\left(\boldsymbol{\phi}|\boldsymbol{y}_{1:T}\right) + \frac{1}{2}\log(2\pi)^d |\mathcal{I}(\boldsymbol{\phi})| \right)\Bigg|_{\boldsymbol{\phi_*}}
\end{aligned}
$$

with the vector valued function k defined as presented in the technical appendix in [58] for both the scalar and multivariate parameter $\boldsymbol{\phi}$ cases.

This is then iteratively applied in Algorithm 4 above with the new symplectic integration scheme, then the final proposed state after T iterations of the proposal is accepted with the MH-rejection scheme with acceptance probability again presented with the appropriate exponential of the difference between the non-separable Hamiltonian (see equation 55) at the old proposed state minus the Hamiltonian at the newly proposed Markov chain state obtained from the symplectic integrator proposal.

6.10. Particle Markov Chain Monte Carlo and Adaption for Stochastic Dynamic HPV Models

So far we have discussed MCMC for general classes of HPV epidemic models, either with deterministic or stochastic dynamics. Now in this section we make a particular focus on stochastic dynamic models. In particular we will be interested in the continuous time SDE epidemic processes, discretised as discussed previously so that a state space structure is obtained. Then given a batch of HPV observational survey data, the task is to obtain samples from the joint posterior distribution both for the static HPV model parameters as well as the latent state trajectories. This can be achieved via a recent class of MCMC algorithms known as Particle Markov chain Monte Carlo. Throughout the following section we will denote the state of the system at time t by vector $\boldsymbol{X}_t$ and the static parameters by $\boldsymbol{\Theta}$, then the j-th

sample from the PMCMC algorithm will be now denoted for the i-th particle in the SMC proposal by $[\boldsymbol{X}_{1:T}, \boldsymbol{\Theta}](j,i)$.

6.10.1. Particle MCMC Methods

In this section we detail carefully the attributes of direct relevance for clinical practitioners on the implementation and stages of understanding for the particular family of MCMC algorithms known as the PMCMC class of algorithms. We feel this is particularly important to provide practitioners with confidence and understanding in order that they can develop such algorithms in practice correctly. We begin with the specific details of the marginal PMCMC class of algorithm. Then we detail carefully different mechanisms one may consider to incorporate adaptive extensions which improve the efficiency of the PMCMC sampler by reducing the auto-correlation in the Markov chain samples ie. improve exploration of the posterior support.

The algorithm developed to sample from the posterior distribution is based on a version of the PMCMC family proposed in [7, Section 2.4.2], recently developed for use in state-space models and known as the marginal PMCMC or Particle Metropolis-Hastings. This is perhaps the simplest of the algorithms in the PMCMC class of samplers as its validity can be shown to only rely on the unbiasedness of the marginal likelihood estimator utilised.

The key advantage of this PMCMC algorithm is that it allows one to jointly update the entire set of posterior parameters $(\boldsymbol{\Theta}, \boldsymbol{X}_{1:T})$ efficiently via an approximation to the marginal acceptance probability in the Metropolis-Hastings algorithm, as detailed below. This allows the Markov chain to mix efficiently in the high-dimensional posterior parameter space, even in the presence of strong posterior parameter dependence between static model parameters and latent states.

The marginal PCMCM is often referred to as the "exact approximation" of an ideal Metropolis sampler that targets the marginal distribution of the static model parameters given the observed data, $p(\boldsymbol{\theta}|y_{1:T}) = \int p(\boldsymbol{y}_{1:T}|\boldsymbol{\theta}, \boldsymbol{x}_{1:T})\, p(\boldsymbol{x}_{1:T}|\boldsymbol{\theta})\, d\boldsymbol{x}_{1:T}$. It is generally impossible however to analytically integrate over the probability of the latent states in order to obtain the marginal likelihood, $p(\boldsymbol{y}_{1:T}|\boldsymbol{\theta})$. Marginal PMCMC replaces this intractable integral quantity by an unbiased Monte Carlo estimate denoted by $\widehat{p}(\boldsymbol{y}_{1:T}|\boldsymbol{\theta})$. This Monte Carlo estimate is specifically obtained from a Sequential Monte Carlo algorithm (ie. particle filter), see detailed reviews in [40]. It is therefore termed an "exact approximation" of the ideal algorithm, this is understood in the sense that a PMCMC kernel leaves invariant the "exact" posterior distribution of interest, (see detailed theoretical discussion in [7]).

To understand this it is useful for practitioners to consider the Marginal PMCMC as creating samples from the target distribution $\pi(\boldsymbol{\theta}, \boldsymbol{X}_{1:T}|\boldsymbol{y}_{1:T})$ by approximating the ideal marginal (over the latent states) Metropolis-Hastings acceptance probability

$$\alpha\left([\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j-1), [\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j)'\right) = \min\left(1, \frac{\overbrace{p\left([\boldsymbol{\theta}](j)'|\boldsymbol{y}_{1:T}\right)}^{\text{Marginal Posterior}} \overbrace{q\left([\boldsymbol{\theta}](j)', [\boldsymbol{\theta}](j-1)\right)}^{\text{MCMC Static Parameter Proposal}}}{p\left([\boldsymbol{\theta}](j-1)|\boldsymbol{y}_{1:T}\right) q\left([\boldsymbol{\theta}](j-1), [\boldsymbol{\theta}](j)'\right)}\right). \tag{56}$$

The PMCMC algorithm we present here does this by factoring the standard Metropolis-

Hastings proposal distribution into two components:

$$q\left([\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j-1), [\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j)'\right) = \overbrace{q\left([\boldsymbol{\theta}](j-1), [\boldsymbol{\theta}](j)'\right)}^{\text{First Component}} \overbrace{p\left([\boldsymbol{x}_{1:T}](j)' | \boldsymbol{y}_{1:T}, [\boldsymbol{\theta}](j)'\right)}^{\text{Second Component}}, \tag{57}$$

Substituting Equation (57) into the standard Metropolis Hastings acceptance probability we achieve the marginalization and recover Equation (56),

$$\begin{aligned}
&\alpha\left([\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j-1), [\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j)'\right) \\
&= \min\left(1, \frac{p\left([\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j)' | \boldsymbol{y}_{1:T}\right) q\left([\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j-1), [\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j)'\right)}{p\left([\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j-1) | \boldsymbol{y}_{1:T}\right) q\left([\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j)', [\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j-1)\right)}\right) \\
&= \min\left(1, \frac{p\left(\boldsymbol{y}_{1:T} | [\boldsymbol{\theta}](j)'\right) p\left([\boldsymbol{\theta}](j)'\right) q\left([\boldsymbol{\theta}](j)', [\boldsymbol{\theta}](j-1)\right)}{p\left(\boldsymbol{y}_{1:T} | [\boldsymbol{\theta}](j-1)\right) p\left([\boldsymbol{\theta}](j-1)\right) q\left([\boldsymbol{\theta}](j-1), [\boldsymbol{\theta}](j)'\right)}\right).
\end{aligned}$$

In the ideal special case of a linear Gaussian SSMs the marginalization of the likelihood can be performed by the Kalman Filter, see details in [73]. In non-linear, non-Gaussian SSMs the evaluation of $p\left(\boldsymbol{y}_{1:T} | [\boldsymbol{\theta}](j)'\right)$ exactly can not be performed. Therefore, the key result in the marginal PMCMC sampler is to replace the ideal PMCMC above with an SMC based approximation as discussed in Remark 1 and detailed in the following sections.

Remark 1. *The use of an SMC proposal within an MCMC proposal distribution allows one to construct as a by-product an unbiased estimator of the marginal likelihood* $p(\boldsymbol{y}_{1:T}|\boldsymbol{\theta})$ *given by*

$$\widehat{p}(\boldsymbol{y}_{1:T}|\boldsymbol{\theta}) := \prod_{t=2}^{T} \widehat{p}(\boldsymbol{y}_t|\boldsymbol{y}_{1:t-1}, \boldsymbol{\theta}), \tag{58}$$

where an SMC approximation with L*-particles produces for all* t *at the* j*-th iteration of the MCMC*

$$\widehat{p}(\boldsymbol{y}_t|\boldsymbol{y}_{1:t-1}, \boldsymbol{\theta}) = \frac{1}{L} \sum_{i=1}^{L} w_t(\boldsymbol{X}_{1:t}[j, i]) \tag{59}$$

where $w_t(\boldsymbol{X}_{1:t}[j, i])$ *denotes a generic incremental importance sampling weight, which will dependent on the type of SMC algorithm used, see examples in [40]. Then one may form an unbiased particle estimate for all time* t *of*

$$p(\boldsymbol{y}_t|\boldsymbol{y}_{1:t-1}, \boldsymbol{\theta}) = \int w_t(\boldsymbol{x}_{1:t}) q(x_t|\boldsymbol{y}_t, x_{t-1}) p(\boldsymbol{x}_{1:t-1}|\boldsymbol{y}_{1:t}, \boldsymbol{\theta}) d\boldsymbol{x}_{1:t}. \tag{60}$$

This non-trivial unbiasedness was first presented in [33, Proposition 7.4.1] and has since been utilised to great advantage as explained in [28, Section 3.2 and 3.3]. In addition the variance of this estimator typically only grows linearly with T*.*

To conclude this section we provide an example of the simplest PMCMC algorithm Markov chain proposal that one may construct, which corresponds to an Sequential Importance Resampling particle filter proposal with an adaptive Metropolis proposal for the static parameters as detailed below.

Algorithm 1 Construction of optimal SIR based path space proposal given by $\widehat{p}\left(\boldsymbol{x}_{1:T}|\boldsymbol{y}_{1:T},[\boldsymbol{\theta}]\left(j\right)'\right)$

Initialisation of SIR filter at iteration (j) *of the Markov chain*

SIR particle filter for $\boldsymbol{X}_{1:T}$: initialise L particles $\{[\boldsymbol{x}_1]\left(j,i\right)'\}_{i=1:L}$ via sampling from the priors

for $t=2,\ldots,T$ **do**

Perform mutation of the L *particles at time* $t-1$ *to obtain new particles at* t *via state evolution.*

Sample the i-th particle $[\boldsymbol{x}_t]\left(j,i\right)'$ from particle filter proposal according to state equation given by a discretisation of the stochastic dynamic SDE model such as via a Milstein scheme as detailed previously in Example 2, i.e. simulate one iteration of the discretised SDE for the model states.

Incremental SIR importance sampling weight correction.

Evaluate the un-normalised importance sampling weights, $\left[\tilde{W}_t\right]\left(j,i\right)$, for the L particles, with the i-th weight given by

$$\begin{aligned}\left[\tilde{W}_t\right]\left(j,i\right) &\propto \left[W_{t-1}\right]\left(j,i\right)\left[w_t\right]\left(j,i\right)\\ &\propto \left[W_{t-1}\right]\left(j,i\right)p(\boldsymbol{y}_t|\left[\boldsymbol{x}_t,\boldsymbol{\theta}\right]\left(j,i\right)),\end{aligned} \tag{61}$$

5: Normalise the importance sampling weights $\left[W_t\right]\left(j,i\right)=\frac{\left[\tilde{W}_t\right](j,i)}{\sum_{i=1}^{L}\left[\tilde{W}_t\right](j,i)}$

Evaluate the importance estimate and resample adaptively.

Calculate the Effective Sample size, $ESS=\frac{1}{\sum_{i=1}^{L}[W_t](j,i)^2}$

If the Effective Sample size is less than 80% then resample the particles at time t using stratified resampling based on the empirical distribution constructed from the importance weights to obtain new particles with equal weight.

end for

Evaluate marginal likelihood $\widehat{p}\left(\boldsymbol{y}_{1:T}|\left[\boldsymbol{\theta}\right]\left(j\right)\right)=\prod_{t=1}^{T}\left(\frac{1}{L}\sum_{i=1}^{L}\left[w_t\right]\left(j,i\right)\right)$

6.10.2. Examples of Adaptive Strategies for PMCMC

Generally, experts in PMCMC will be aware of numerous approaches one could adopt to develop adaptive strategies to automate and remove the need to tune certain parameter settings in PMCMC algorithms. These settings are often critical factors of the relative efficiency and properties of the obtained inference. It is the intention of this section to discuss and explain for practitioners a few possibilities to consider when developing PMCMC algorithms for stochastic dynamic models in a HPV context.

Generally adaptive strategies can be applied to several key parameters in the PMCMC algorithm, some choices include: the number of iterations J of the MCMC; the number of particles L in the SMC component; the rate at which resampling is applied in the SMC proposal construction (adaptive resampling via e.g. Effective Sample Size criteria see discussion in [40]); the learning of an optimal mutation kernel within the SMC proposal; the adaption of the Markov chain Monte Carlo proposal for the static parameters. These dif-

ferent adaptive ideas can be developed based on the observations $\boldsymbol{y}_{1:T}$ and elements of all previously sampled PMCMC output such as $\{[\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j)\}_{1\leq j\leq J}$ and possibly even genealogical path-space ancestry information for each iteration of the SMC in the PMCMC algorithm. We detail three examples of these adaptions split into two parts below, those for the static parameter MCMC proposal $\boldsymbol{\theta}$ and then those for the SMC proposal.

6.10.3. Second Component of PMCMC Proposal: Adaptive Sequential Monte Carlo

In this section we discuss the SMC proposal approximation to what we termed the Second Component in the optimal PMCMC proposal (57). SMC methods emerged out of the fields of engineering and statistics with variants of the methods appearing under the names of particle filtering or interacting particle systems. The theoretical analyses of particle filtering algorithms is a mature topic with consistency and fluctuation properties of particle algorithms in terms of the number of particles L studied in [35, 27, 87, 39, 34, 112]. Other theoretical properties for different modes of convergence and the stability of these algorithms is discussed in [138] and the references therein.

To construct a sampler for the optimal proposal (second component) $p\left([\boldsymbol{x}_{1:T}](j)'|\boldsymbol{y}_{1:T}, [\boldsymbol{\theta}](j)'\right)$ we consider a particle filter approximation. This is constructed recursively based on the sequence of distributions $\{p\left([\boldsymbol{x}_1](j)'|\boldsymbol{y}_1, [\boldsymbol{\theta}](j)'\right), p\left([\boldsymbol{x}_{1:2}](j)'|\boldsymbol{y}_{1:2}, [\boldsymbol{\theta}](j)'\right), \ldots, p\left([\boldsymbol{x}_{1:T}](j)|\boldsymbol{y}_{1:T}, [\boldsymbol{\theta}](j)'\right)\}$ which satisfy the following two stage ideal filter recursion construction, given static parameters obtained from the first component proposal.

The prediction stage calculates an *a priori* estimate of the posterior distribution $p\left([x_t](j)'|\boldsymbol{y}_{1:t}, [\boldsymbol{\theta}](j)'\right)$ at time *t*. The *a priori* estimate is calculated by using the *a posteriori* estimate at the previous time step and the identity from the Chapman-Kolmogorov equation,

$$p([\boldsymbol{x}_t](j)'|\boldsymbol{y}_{1:t-1}, [\boldsymbol{\theta}](j)') = \int p([\boldsymbol{x}_t](j)'|\boldsymbol{x}_{t-1}, [\boldsymbol{\theta}](j)')p(\boldsymbol{x}_{t-1}|\boldsymbol{y}_{1:t-1}, [\boldsymbol{\theta}](j)')dx_{t-1}. \tag{62}$$

The update stage produces an *a posteriori* estimate of the posterior distribution at time *t*,

$$p([\boldsymbol{x}_t](j)'|\boldsymbol{y}_{1:t}, [\boldsymbol{\theta}](j)') = \frac{p(\boldsymbol{y}_t|[\boldsymbol{x}_t, \boldsymbol{\theta}](j)')p([\boldsymbol{x}_t](j)'|\boldsymbol{y}_{1:t-1}, [\boldsymbol{\theta}](j)')}{p(\boldsymbol{y}_t|\boldsymbol{y}_{1:t-1}, [\boldsymbol{\theta}](j)')}, \tag{63}$$

where the normalizing constant and marginal likelihood are given by,

$$\begin{aligned} p(\boldsymbol{y}_t|\boldsymbol{y}_{1:t-1}, [\boldsymbol{\theta}](j)') &= \int p\left(\boldsymbol{y}_t|\boldsymbol{x}_t, [\boldsymbol{\theta}](j)'\right) p\left(\boldsymbol{x}_t|\boldsymbol{y}_{1:t-1}, [\boldsymbol{\theta}](j)'\right) d\boldsymbol{x}_t \\ p(\boldsymbol{y}_{1:T}|[\boldsymbol{\theta}](j)') &= \prod_{t=1}^{T} p(\boldsymbol{y}_t|\boldsymbol{y}_{1:t-1}[\boldsymbol{\theta}](j)'). \end{aligned} \tag{64}$$

This optimal solution is conceptual due to the fact that in general the integrals in the prediction and update stages cannot be determined analytically. Therefore, as recognised in [7], constructing a recursive particle based approximation to the sequence of distributions $\{p([\boldsymbol{x}_{1:t}](j)'|\boldsymbol{y}_{1:t}, [\boldsymbol{\theta}](j)')\}_{t=1:T}$ is achieved by the PMCMC algorithm through the class of

Sequential Monte Carlo (SMC) estimators to approximate the optimal proposal distribution in the PMCMC algorithm. We briefly detail the SMC proposal algorithm and then we discuss examples of adaptive SMC proposals that can be considered.

All SMC proposals will for each t construct an empirical particle estimate of the path space distribution, with L particles for the jth Markov chain iteration, given by

$$\widehat{p}([\boldsymbol{x}_{1:t}](j)'|\boldsymbol{y}_{1:t},[\boldsymbol{\theta}](j)') = \sum_{i=1}^{L} W_{1:t}^{(i)} \delta_{[\boldsymbol{X}_{1:t}](j,i)'}(\boldsymbol{x}_{1:t}),$$

where $W_{1:t}^{(i)}$ represents the normalised importance weight for the ith particle on the path space (to time t), and $\delta_{[\boldsymbol{X}_{1:t}](j,i)'}(\boldsymbol{x}_{1:t})$ is the Dirac measure, representing the particle's path locations over times 1 to t.

Under the simplest version of an SMC algorithm, given by the SIR filter [63], the mutation kernel at time t, given parameters $[\boldsymbol{\theta}](j)'$ is selected as the latent state process $p([\boldsymbol{x}_t](j)'|[x_{t-1}](j)', [\boldsymbol{\theta}](j)')$ and the importance weights are calculated recursively according to

$$\begin{aligned} W_t([\boldsymbol{x}_{1:t}](j)) &= \frac{p([\boldsymbol{x}_{1:t}](j)|\boldsymbol{y}_{1:t},[\boldsymbol{\theta}](j))}{q([\boldsymbol{x}_{1:t}]|\boldsymbol{y}_{1:t})} \\ &\propto \frac{p(\boldsymbol{y}_t|[\boldsymbol{x}_t](j),[\boldsymbol{\theta}](j))p([\boldsymbol{x}_t](j)|[x_{t-1}](j),[\boldsymbol{\theta}](j))p([\boldsymbol{x}_{1:t-1}](j)|\boldsymbol{y}_{1:t-1},[\boldsymbol{\theta}](j))}{q([x_t](j)|[\boldsymbol{x}_{1:t-1}](j),\boldsymbol{y}_{1:t-1})q([\boldsymbol{x}_{1:t-1}](j)|\boldsymbol{y}_{1:t-1})} \\ &= W_{t-1}([\boldsymbol{x}_{1:t-1}](j))p(\boldsymbol{y}_t|[\boldsymbol{x}_t,\boldsymbol{\theta}](j)). \end{aligned}$$

Remark 2. *We note that the simple SIR filter is suitable for PMCMC applications because it is only used as a proposal distribution and not as an empirical estimate of the posterior (see discussion of the standard application in [40]). In addition, it may be the only feasible choice in settings in which the latent path-space is intractable to evaluate but can be simulated efficiently see examples in [111].*

Procedurally, particles obtained from an initial distribution $p(\boldsymbol{x}_1|\boldsymbol{y}_1,\boldsymbol{\theta})$, with a set of corresponding initial weights, are sequentially propagated through each distribution in the sequence via three processes: *mutation* (or move), *correction* (or importance weighting) and *selection* (or re-sampling). There are many choices for each stage that can be considered in the design of these algorithms, see detailed discussion in [40] and the references therein.

Remark 3. *In the PMCMC context [7] have proven that even in the extreme case of $L = 1$ particle the resulting PMCMC chain will still have the appropriate stationary distribution, although the mixing rate of the resulting Markov chain will be affected by L. One of the adaptive strategies discussed below will explore this feature.*

Below, we detail three adaptive SMC proposal strategies that can improve the performance but are relatively straightforward for practitioners to implement in practice. They are all based on the observation that besides the choice of proposal utilised for all MCMC schemes, the main practical choice to be made by practitioners that affects computational cost and performance of the sampler is the selection of the appropriate number of particles L.

Basic SMC Adaptive Resampling Proposal Choice 1. Consider the simplest version of an SMC algorithm, based on a simple SIR filter [64], then introduce adaptive stratified resampling based on for example an effective sample size criterion for when resampling occurs, see discussions in [33, 40] and a basic derivation of this criterion in [110, Chapter 2] and references therein. This then represents the most basic adaptive SMC proposal one could develop, upon which all other adaptive strategies can be constructed.

Advanced SMC Adaptive Particle Number (L) Choice 2. A recent more sophisticated adaptive strategy [41] involves increasing adaptively at each iteration of the PMCMC algorithm, the number of particles L such that the resulting log-likelihood estimator discussed in Remark 1 has a standard deviation in the estimator of the marginal likelihood which is optimal. It can be proven that the optimal value for accuracy in this estimator is around unity, regardless of the mutation proposal distribution in the SMC, see technical justifications in [41, Theorem 1]. This can be considered a global proposal as L is a function of the properties of the entire path space trajectory and future work can also consider local adaptivity.

The global path space based approach developed in [41] is based upon the fact that when forming an average utilising samples from a PMCMC algorithm at equilibrium, there will be an associated computational time (efficiency) that is proportional to the integrated autocorrelation time (IACT) multiplied by the number of particles used in the proposal construction, L. If one sets out to minimise this criterion (efficiency loss due to a particle approximation) as measured by the IACT then it produces an problem which is intractable to minimise explicitly. However, [41] show how an upper bound may be achieved under a Normality assumption for the estimated marginal likelihood as $L \to \infty$. The result is that the standard deviation of the estimated marginal likelihood at each iteration should be selected to be around unity, and this can be achieved in a number of ways. The simplest of these would be to devise a strategy that works with an adaptive number of particles L as a function of $\boldsymbol{\theta}$ for each iteration, in which the number of particles is systematically increased until a variance of around one in the estimator is achieved.

Advanced SMC Adaptive Fitness Partial Rejection Control Proposal Choice 3. A mechanism to control the fitness of the particles selected to perform the estimation of the marginal likelihood through extending ideas in [93] has been recently proposed, see [112]. Under this adaptive strategy one can consider the true number of particles L_T as a random variable with a effective number of particles fixed at L. Effectively, each stage of mutation of a particle in the SIR filter is attempted under a rejection mechanism until it achieves a desired "fitness", which may correspond to for example a lower bound threshold on the incremental importance sampling weight in each stage of the filter, see derivation of this mechanism and a study of its theoretical properties in [112] and the references therein.

6.10.4. Summary for Adaptive Particle MCMC

Combining all these features of the AdMCMC and adaptive SMC proposals into the PMCM algorithm is then summarised by the following generic version of the AdPMCMC Algorithm. The jth iteration of the AdPMCMC algorithm proceeds as follows:

1. Sample $[\boldsymbol{\theta}](j)' \sim q\left([\boldsymbol{\theta}](j-1), \cdot\right)$ from an Adaptive MCMC proposal.(Algorithm 1. and Algorithm 2).

2. Run an SMC algorithm with L particles to obtain:

$$\begin{aligned}\widehat{p}\left(\boldsymbol{x}_{1:T}|\boldsymbol{y}_{1:T}, [\boldsymbol{\theta}](j)'\right) &= \sum_{i=1}^{L} W_T^{(i)} \delta_{[\boldsymbol{x}_{1:T}](j,i)'}\left(\boldsymbol{x}_{1:T}\right) \\ \widehat{p}\left(\boldsymbol{y}_{1:T}|[\boldsymbol{\theta}](j)'\right) &= \prod_{t=1}^{T}\left(\frac{1}{L}\sum_{i=1}^{L} w_t\left([\boldsymbol{x}_t](j,i)'\right)\right)\end{aligned} \tag{65}$$

   where $w_t\left([x_t](j,i)'\right)$ is the incremental particle weight and under the SIR filter given by $p(\boldsymbol{y}_t|[x_t, \boldsymbol{\theta}](j))$. Then sample a candidate path from the SMC algorithm, to obtain $[\boldsymbol{X}_{1:T}](j)' \sim \widehat{p}\left(\boldsymbol{x}_{1:T}|\boldsymbol{y}_{1:T}, [\boldsymbol{\theta}](j)'\right)$, for evaluation of $W_{1:T}^{(i)}\delta_{[\boldsymbol{x}_{1:T}](j,i)'}(\boldsymbol{x}_{1:T})$ and $w_t\left([x_t](j,i)'\right)$

3. Accept the proposed new Markov chain state comprised of $[\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j)'$ with unbiased Monte Carlo approximation of the optimal marginal Metropolis-Hastings acceptance probability given by

$$\begin{aligned}&\alpha\left([\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j-1), [\boldsymbol{\theta}, \boldsymbol{x}_{1:T}](j)'\right) \\ &= \min\left(1, \frac{\widehat{p}\left(\boldsymbol{y}_{1:T}|[\boldsymbol{\theta}](j)'\right) p\left([\boldsymbol{\theta}](j)'\right) q\left([\boldsymbol{\theta}](j)', [\boldsymbol{\theta}](j-1)\right)}{\widehat{p}\left(\boldsymbol{y}_{1:T}|[\boldsymbol{\theta}](j-1)\right) p\left([\boldsymbol{\theta}](j-1)\right) q\left([\boldsymbol{\theta}](j-1), [\boldsymbol{\theta}](j)'\right)}\right)\end{aligned}$$

   where $\widehat{p}\left(\boldsymbol{y}_{1:T}|[\boldsymbol{\theta}](j-1)\right)$ is obtained from the previous iteration of the PMCMC algorithm.

In general to study theoretically the behaviour of embedding of the Adaptive Metropolis proposal within the PMCMC family of algorithms could proceed in a block-Gibbs type PMCMC structure based on results developed in [90]. However, for the case of the Particle Metropolis-Hastings algorithm we conjecture that the extended space auxiliary variable representation of the PMCMC algorithm in [28] will suffice.

Conjecture 1. *Consider the Particle Marginal Metropolis-Hastings class of samplers with a proposal distribution constructed from a particle filter and the static parameter proposal is an adaptive Markov kernel satisfying diminishing adaptation and bounded convergence. If one consider the target distribution not as $p(\boldsymbol{\theta}, \boldsymbol{X}_{1:T}|\boldsymbol{y}_{1:T})$ but instead, think of this as the marginal distribution of a target distribution defined on an extended auxiliary variable augmented space. Where the additional auxiliary variables correspond to all additional random variables used in the sampling and construction of the PMCMC algorithm, as utilised in the proofs deriving the validity of the basic PMCMC and SMC^2 algorithms in [7] and [28], then on this space utilisation of a joint MCMC proposal with a homogeneous proposal component as well as a component which is adaptive and satisfying diminishing adaptation and bounded convergence, will produce a joint proposal which is also satisfying diminishing adaptation and bounded convergence.Hence, ergodic averages from marginal samples of this algorithm will be preserved.*

Table 1. Stratification of the modelled Australian heterosexual population. Sexual activity groups are defined as in [117]

attribute	index	value	label	interpretation
gender	g	1	M	males
		2	F	females
sexual activity	r	1		the least active (60% of population)
(risk) group		2		moderately active (21% of population)
		3		highly active (11% of population)
		4		the most active (2% of population)
age group	a	1	12	12 year old
		2-53	13-64	13-63 year old
infection state	i	1	S	susceptible
		2	I	infected asymptomatic
		3	W	infected with untreated genital warts
		4	T	infected treated for genital warts
		5	R	removed, or immune
sexual activity	s	1	act	active
status		2	inact	inactive
vaccination status	v	1	V	vaccinated
		2	U	unvaccinated

7. Detailed Bayesian Application of HPV Modelling and Calibration

In this section we present a detailed application of several ideas presented previously to specifically perform Bayesian model analysis and calibration for inference in HPV 6 and 11 for the epidemic in Australia.

7.1. Model Structure

We consider a model of HPV-6/11 transmission in Australian heterosexual population stratified as shown in Table 1.

The model is schematically shown in Figure 2. Changes in the number of individuals in each of the model compartments in time are described by a system of ODE's

$$\begin{aligned} \dot{S} &= -\lambda S + r_{li} R, \\ \dot{I} &= -(r_{cl} + r_{gw}) I + \lambda S, \\ \dot{W} &= -(1/T_{med}) W + r_{gw} I, \\ \dot{T} &= -(1/T_{clt}) T + (1/T_{med}) W, \\ \dot{R} &= -r_{li} R + r_{cl} I + (1/T_{clt}) T, \end{aligned} \tag{66}$$

where dot denotes a derivative with respect to time. This is only a simplified representation of the movements between compartments. In reality, we compose differential equations for a multi-dimensional vector $\bar{x}$ containing the number of individuals in subpopulations

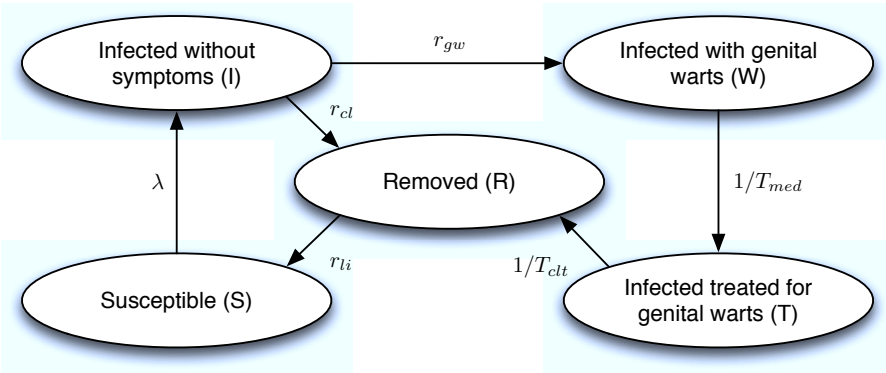

Figure 2. HPV-6/11 transmission model with genital warts. Here λ is the force of infection, r_{gw} is the rate of development of genital warts, r_{cl} is the rate of clearance of asymptomatic HPV infection, T_{med} is the time between detection of genital warts and beginning of their treatment, T_{clt} is the time to clear HPV under treatment for genital warts, r_{li} is the rate of loss of natural immunity. Model parameters are stratified as shown in Table 2.

identified by the attributes shown in Table 1. Each of the model parameters can in principle be dependent on any of these attributes. So, for example, the equation describing the changes in the number of females with genital warts is implemented as a set of the following equations:

for all r, a, v

$$\dot{x}(F, r, a, W, act, v) = -(1/T_{med,F})x(F, r, a, W, act, v) + r_{gw,F}x(F, r, a, I, act, v).$$

It is pertinent to note that we stratified the majority of model parameters by gender only (see Table 2, so in this equation, for example, r_{gw} is the same for both unvaccinated and vaccinated sexually active females, which implies that we assume vaccination with a strictly preventative vaccine (i.e. the vaccine has no effect on development of genital warts in infected females).

7.2. Model Parameters and Their Prior Distributions

According to Bayesian approach schematically described in Figure 3, we should specify the prior distributions for model parameters. These distributions should reflect our knowledge about the range of values the parameters are likely to take. We selected the priors based on the findings available in literature following considerations outlined below.

Table 2. Model parameters and their prior distributions. Note that all distributions are uniform, and all parameters denote quantities averaged over the modelled population; rates are annual

model parameter	notation	prior distribution	reference(s)
probability of transmission per partnership	β	$U(0.1, 1.0)$	[20]
rate of clearance of asymptomatic HPV in females	$r_{cl,F}$	$U(0.3, 1.0)$	[132]
rate of clearance of asymptomatic HPV in males	$r_{cl,M}$	$U(0.4, 0.8)$	[60]
proportion of infected females who develop genital warts	$p_{gw,F}$	$U(0.45, 0.8)$	[139]
proportion of infected males who develop genital warts	$p_{gw,M}$	$U(0.3, 0.8)$	[9, 10]
rate of genital warts development in females	$r_{gw,F}$	$U(0.5, 1.1)$	[48, 139]
rate of genital warts development in males	$r_{gw,M}$	$U(0.5, 1.6)$	[9, 10]
delay before seeking medical help after detection of genital warts for females	$T_{med,F}$	$U(0.02, 0.25)$	[42, 141]
delay before seeking medical help after detection of genital warts for males	$T_{med,M}$	$U(0.08, 0.55)$	[42, 141]
time to clear HPV with treatment for genital warts in females	$T_{clt,F}$	$U(0.15, 0.4)$	[139, 77]
time to clear HPV with treatment for genital warts in males	$T_{clt,M}$	$U(0.2, 0.4)$	[77]
rate of loss of natural immunity in females	$r_{li,F}$		N/A
rate of loss of natural immunity in males	$r_{li,M}$		N/A
degree of assortativity by age group	$\varepsilon^{(A)}$	$U(0.1, 1.0)$	[117]
degree of assortativity by sexual activity group	$\varepsilon^{(S)}$	$U(0.1, 1.0)$	[117]

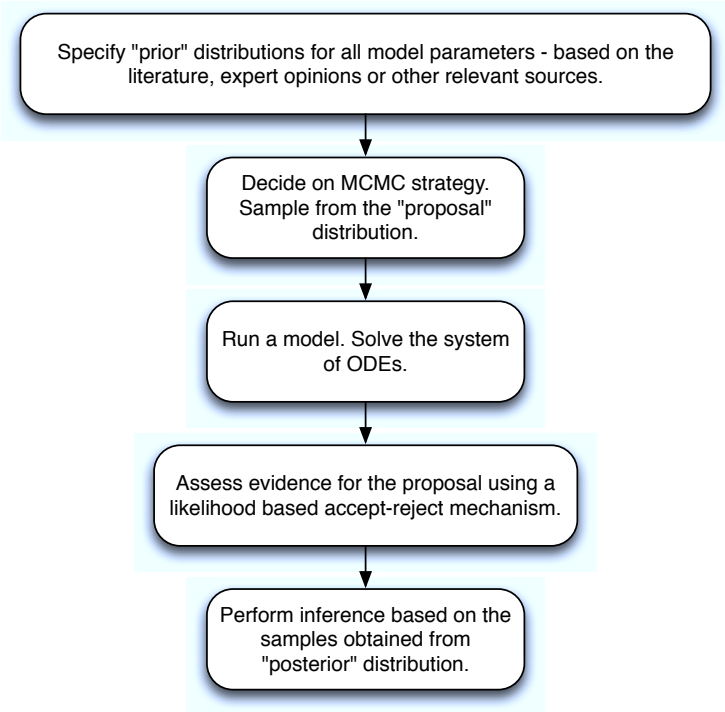

Figure 3. Model calibration based on Bayesian approach.

Probability of HPV-6/11 transmission per partnership

An estimation of this probability at 0.20 (95% CI, 0.16-0.24) was reported by the HITCH Study (HPV Infection and Transmission among Couples through Heterosexual activity) [37]. The study enrolled 18- 24 y.o. women and their partners, 179 discordant couples in total. No notable differences were detected between the probabilities female-to-male and male-to-female transmissions. However, taking into account a considerable uncertainty surrounding this probability, especially, in the context of compartmental models where all partnerships are treated as instantaneous, we decided to use the reported estimation to define only the lower boundary of the prior, that is, chose a uniform distribution U(0.1,1.0);

Rate of clearance of asymptomatic HPV in female and males

This rate is assumed to be approximately equal to the inverse of the mean duration of HPV-6/11 infection. As reported in [132], an extensive Ludwig-McGill cohort study involved 2,462 women aged 18-60 from São Paulo, Brazil who attending a maternal and child health program in a low-income neighborhood. The women were recruited between 1993 and 1997 and followed for up to 10 years. It was found that the mean duration of the first incident HPV-6/11 infection among these women was 9.5 months (95% CI: 6.9-12.1), or in years, 0.79 (95% CI: 0.57-1.0). Then the inverse of the mean duration, that is, $r_{cl,F}$ is1.26 (95% CI: 1.0-1.75). Hence we can assume that is uniformly distributed in this confidence interval, i.e. $r_{cl,F} \sim U(1.0, 1.75)$.

A prospective study of the natural history of HPV infections among 290 men aged 18-

44 years conducted in Tucson, Arizona. Cohort participants were recruited between 2003 and 2005 and followed until December 2005. The maximum duration of follow-up was 24.7 months. The median times to clearance of HPV-6/11 infection were 5.4 months (95% CI: 5.1-5.7). In years these are 0.45 (95% CI: 0.425-0.475), which corresponds to the rate of clearance $r_{cl,M}$ of 2.22 (95% CI: 2.10-2.35). Note that in our model mean durations of infections are used, not median ones. We assume that the mean durations are longer than median and allow them to be up to 1 year. Then we conclude that it is reasonable to let $r_{cl,M} \sim U(1.0, 2.35)$.

Proportion of infected females and males who develop genital warts

A study [139] followed 603 young females, who were the University of Washington students aged 18-20, for a mean duration of 38.8 months. The students were recruited between July 1990 and September 1997. Cumulative incidence of developing genital warts among women with incident HPV-6/11 infection was around 0.6 at 1 year. In view of this, we decided that it would be sufficient to assume that $p_{gw,F} \sim U(0.5, 0.8)$.

An international study the HPV in Men (HIM) reported that among 2,487 men aged 18-70 years, who resided in Florida, the US, São Paulo, Brazil, or the state of Morelos, Mexico, at 1 year after an incident HPV infection, 8.9% (95% CI: 0.0-18.1) of men with HPV 6/11 only and 5.2% (95% CI: 1.8-8.5) of men with HPV 6/11 and other types developed genital warts.

On the other hand, another study [10] which covered 473 18-21-year-old male students attending the University of Washington, reported the cumulative incidence of developing genital warts among men infected with HPV- 6/11 at about 0.35 at 1 year, and close to 0.6 at 2 years. Therefore, we chose to assume that $p_{gw,M} \sim U(0.02, 0.8)$.

Rate of genital warts development

This rate is assumed to be an inverse of the mean time between detection of HPV-6/11 infection and detection of genital warts (i.e. genital warts incubation period).

According to [48], among 17,622 women 15-26 years old, enrolled in an HPV vaccine efficacy trial between December 2001 and May 2003, the median incubation period was 6 months (0.5 years). Another study we have previously mentioned, [139], found that the median period was 2.9 months (IQR: 0-5.7), or in years, 0.24 (IQR: 0-0.475). Assuming that the mean duration of genital warts incubation period has be longer than the median, we let it be uniformly distributed in (0.2,0.6). Then the rate is distributed as $r_{gw,F} \sim U(1.66, 5.0)$.

As reported in [10], among the 18 men with incident HPV-6/11 infection who developed warts, the median time between incident detection of infection and genital warts was 11.0 months (IQR: 0-16.1), which in years is 0.91 (IQR: 0-1.34).

Interestingly, according to [9], the median incubation period in men infected with HPV 6/11 only was 6.2 months (95% CI: 5.6-24.2), or in years, 0.51 (95% CI: 0.46-2.01), while in men infected with HPV-6/11 and other types it was 13.3 months (95% CI: 6.3-19.6), in years 1.10 (95% CI: 0.525-1.63). Assuming that the mean duration of the incubation period is longer than the median duration, we decide that it can be uniformly distributed in (0.4,1.7). Then $r_{gw,M} \sim U(0.58, 2.5)$.

Rate at which medical help is sought after detection of genital warts

Between September 2006 and February 2008, as a part of a Canadian study [42], 42 physicians across Canada recruited men and women aged over 18 (mean age for women 28.1, and for men 33.3 years) with a first or recurrent episode of anogenital warts. It was established that the median delay between the onset of genital warts and the first visit to a doctor was 30 days (IQR: 761), or in years, 0.08 (IQR: 0.02-0.16) for women, and 76 days (IQR: 29-198), in years 0.2 (IQR: 0.08-0.54) for men.

Another study carried out in a convenience sample of seven sexual health clinics in England and one in Northern Ireland [141] recruited 895 participants aged over 16. It turned out that for men, the mean delay was 144 days (95% CI: 112-174), or in years, 0.39 (95% CI: 0.3-0.47) while for women it was only 69 days (95% CI: 48-90), in years 0.19 (95% CI: 0.13-0.24).

This information suggests that it is reasonable to think that the mean delay is somewhere in (0.01, 0.25) for women, and (0.1,0.5) for men.

Rate of clearance of HPV under treatment

This rate is an inverse of the mean time to clearance under treatment. An extensive US study [77] analysed the Medstat Marketscan database, which contains information on inpatient, outpatient, and pharmacy claims for 3,664,686 privately insured individuals in the United States. The mean time to clearance for women was found to be 84.8 days (95% CI: 67.5-102.1), or in years 0.23 (95% CI: 0.18-0.28), while for men it was 102.6 days (95% CI: 77.8-127.4), in years 0.28 (95% CI: 0.21-0.35). According to [139], the median time to clearance with treatment for females was 5.9 months (IQR: 3.9-8.0), which is in years 0.49 (IQR: 0.325-0.66). Thus we assume that the mean time to clearance under treatment for females is uniformly distributed in (0.15,0.7), while for males it is also uniformly distributed in (0.2,0.4).

Rate of loss of natural immunity

There is virtually no information on this parameter, since even the very existence of natural immunity is not yet a commonly accepted notion at this time. That is why we assume that the mean duration of natural immunity may be anywhere between 0.5 and 100.0 years for both females and males, and then the rates in question will be uniformly distributed as follows: $r_{li,F} \sim U(0.01.2.0)$ and $r_{li,M} \sim U(0.01.2.0)$. While such assumptions may seem debatable in terms of their physical meaning (how to understand a 100 year long immunity if individuals tend to die before 100 years since they clear HPV infection), they are common in HPV modelling literature (see, for example, [15]).

7.3. Sexual Mixing and Force of Infection

In our model, the pattern of sexual partnership formation between males and females depending on their age and level of sexual activity is described by a sexual mixing matrix. The entries of this matrix are specified as in (35) for the sexually active population comprising a part of the entire population according to Table 3. To fill the matrix we used the data presented in Table 4 below, which originally was extracted from the findings of Australian

Table 3. Assumed percentage of sexually active individuals in Australian population [44, 67]

year old	12	13	14	15	16	17	18	19	20	21	22	23	24	25-64
%	0	20	30	40	50	60	70	80	82	85	90	92	95	100

Study of Health and Relationships (ASHR) [88] in [117]. These data are relative sexual partner acquisition rates for each age group a (we denote them r_a) and each sexual activity (risk) group s (r_s). The overall sexual partner change rate $\bar{c}$ averaged over the entire population was fixed at 0.437. We should emphasise the assumption that there is no difference in sexual activity between females and males from the same group.

Table 4. Relative sexual partner change rates in Australian population

age groups (years)							risk groups (% of population)			
15-19	20-24	25-29	30-34	35-39	40-44	45-59	1 (60)	2 (21)	3 (17)	4 (2)
5.28	6.06	4.37	2.57	1.61	1.43	1.00	1.00	4.76	24.83	105.67

In order to calculate the force of infection, we follow the scheme outlined in [49] and shown in Figure 4.

Step 1. We have the relative rates r_a and r_s, but not the actual ones. Denote a rate at which an individuals of gender g in age group a and sexual activity group s acquire new sexual partners as c_{gas}. Importantly, this rate, just like r_a and r_s, does not describe sexual activity between particular groups, but rather shows the turnover of partners in general, between the individuals in question and the rest of the population of the opposite gender. Let for all g, a and s

$$r_a r_s c_{min} = c_{gas},$$

i.e. c_{gas} can be expressed via some minimal rate c_{min}. It is assumed that we know the number of individuals in any subdivision (gas) of the population demoted by N_{gas} (usually such information is available from a national bureaus of statistics or a similar organisation). Then we can calculate

$$c_{min} = \frac{\bar{c} N_g}{\sum_{a,s} r_a r_s N_{gas}},$$

where N_g is the number of all individuals of gender g. Then

$$c_{gas} = r_a r_s c_{min} = r_a r_s \frac{\bar{c} N_g}{\sum_{as} r_a r_s N_{gas}}.$$

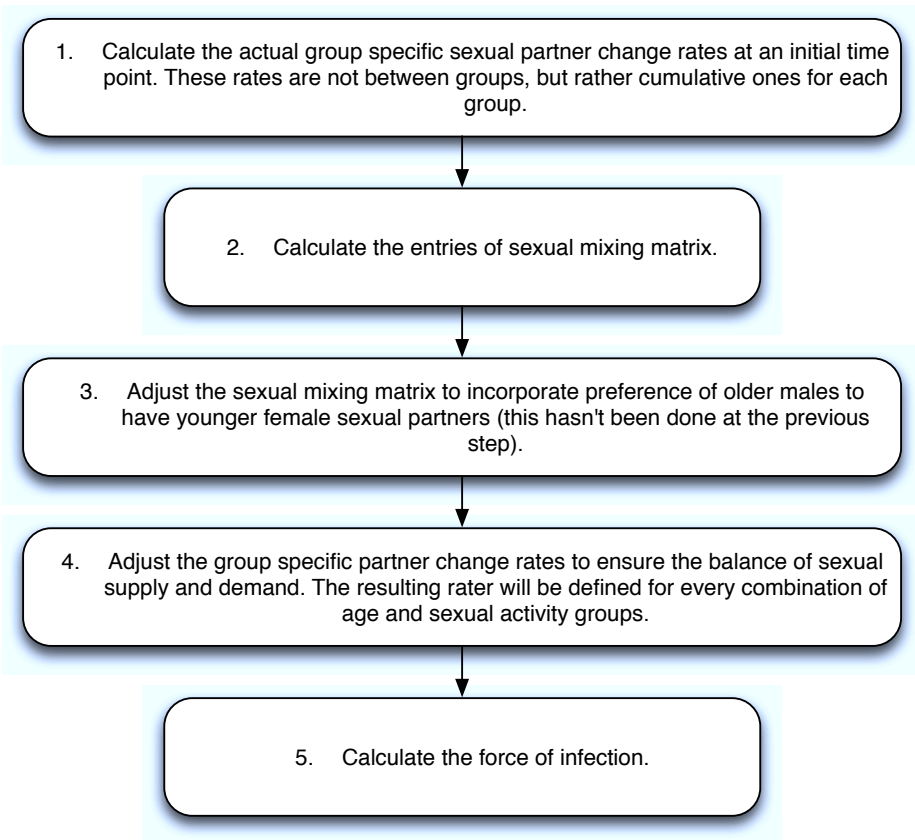

Figure 4. Steps required to calculate the force of infection λ.

Step 2. Given the rates c_{gas}, we can immediately fill the entries of the sexual mixing matrix ($\rho_{gasa's'}$ specified in (35)). Note that the numbers of generated partnerships $P_{ga's'}$ needed to calculate $\rho_{gasa's'}$ are obtained by multiplying rates c_{gas} by the number of individuals in (gas).

Step 3. Next we modify the matrix to take into account the preference of older males to form partnerships with younger females. In particular, we reduce each probability of the form (35) for males to have a female partner of from the same age group to its proportion $1-\Gamma$, and add its remaining proportion Γ to the probability that these males have a female partner from the adjacent younger age group. The entries of (35) corresponding to females are adjusted accordingly.

Step 4. Now we balance supply and demand of sexual partnerships: this simply means that we adjust the actual partner change rates so that for any two subpopulations of female and males, the number of male sexual partners acquired by females from the male subpopulation is equal to the number of female sexual partners acquired by males. This makes the rates c_{gsa} group-specific in terms of the groups the sexual partners are selected from. The details as below.

We want the following to hold for all (gas) and $(g'a's')$:

$$c_{gas}\rho_{gasa's'}N_{gas} = c_{g'a's'}\rho_{g'a's'as}N_{g'a's'}. \tag{67}$$

Here $c_{gas}\rho_{gasa's'}$ is the rate of acquisition of new sexual partners by individuals who belong to (gas) from $(g'a's')$, and $c_{gas}\rho_{gss'aa'}N_{gas}$ is the total number of new partners acquired by (gas) from $(g'a's')$. Let

$$c_{gas}\rho_{gasa's'}N_{g,s,a} \neq c_{g'a's'}\rho_{g'a's'as}N_{g'a's'}.$$

We want to find such a multiplier B that

$$B^{\theta_1}c_{gas}\rho_{gasa's'}N_{gas} = B^{\theta_2}c_{g'a's'}\rho_{g'a'sas}N_{g'a's'}.$$

Then

$$B^{\theta_1-\theta_2} = \frac{c_{g'a's'}\rho_{g'a's'as}N_{g'a's'}}{c_{gas}\rho_{gasa's'}N_{gas}}.$$

To keep things simple, let $\theta_1 - \theta_2 = 1$. Note that B serves as a degree of imbalance: the balance is established if $B = 1$. So, to ensure that (67) is true it is enough to introduce the group-specific rates $c_{gasa's'}$ to be used instead of c_{gas}:

$$c_{gasa's'} = c_{gas}B^{\theta_1}, \quad c_{g'a'sas} = c_{g'a's'}B^{\theta_1-1} \tag{68}$$

We limit the range of value of parameter θ_1 to $[0, 1]$. Suppose the supply and demand are not balanced and $\theta_1 = 0$. Then as follows from (68), $c_{gasa's'} = c_{gsa}$ (the rates for gender g do not get adjusted), but $c_{g'a'sas} = c_{g'a's'}B^{-1}$ (the rates for gender g' are adjusted). If $\theta_1 = 1$ it is the other way around. Consequently, θ_1 indicates to what extent individuals of each gender adjusts their sexual partner acquisition rates (i.e. we may say, sexual behaviour) in case the available supply of sexual partners does not meet demand.

Step 5. Hence, we have the mean per capita annual rates at which an individual of gender g from a sexual activity group s and age group a acquires new sexual partners of the opposite gender g' from a sexual activity group s' and age-group a' denoted as $c^*_{gasa's'}$. Then the force of infection on an average individual from (gas) is defined as follows

$$\lambda_{gas} = \sum_{s'a'} \left(\beta c^*_{gasa's'}I_{g'a's'}\right), \tag{69}$$

where β is the transmission probability per partnership for gender g as given in Table 2, and $I_{g'a's'}$ is the proportion of infected (with or without treated or untreated genital warts) individuals of gender g' of age a' from sexual activity group s'. Importantly, the force of infection is time dependent, because so is $I_{g'a's'}$.

7.4. Underlying Modelling Assumptions

Here we briefly comment on the key modelling assumptions we make.

The model reaches a steady state regardless the initial conditions

This is a standard assumption (see textbooks on infectious disease modelling, for example, [83]) which is rarely mentioned explicitly in modelling studies. This is probably because the existence of a steady state independent of the initial conditions may be challenging to prove, so it is especially convenient to only verify it empirically.

Only heterosexual population is modelled

We modelled the heterosexual Australian population using data from the Australian Study of Health and Relationships (ASHR) [88]. Excluding non-heterosexual population from our models may be an important omission. According to ASHR, there were about 300,000 homosexuals or individuals who identified themselves as heterosexual but had some experience of homosexual contacts in Australia [115] in 2003. We, however, had very little of representative data on their sexual mixing patterns. Especially this is true for men who have sex with men (MSMs) who can also be proactive in establishing sexual partnerships with women. Therefore, to avoid unnecessary hypothesising supported by minimal evidence, we consider only heterosexual HPV transmission to be able to rely on the results of ASHR.

Impact of immigrants and visitors ignored

As a means of simplifying the model, we ignore the impact of immigrants, temporary visitors, and Australian travellers overseas. This is potentially important given that the Australian population has been steadily growing due to immigration, and the country is a popular destination for young travellers who may engage in a high level of sexual activity (this issue has been constantly acknowledged, most recently in [38], but not properly investigated yet).

Sexual activity starts at 13 and at 25 all individuals are sexually active

Based on a number of studies focused on sexual practices among young people [44, 67] we concluded that it would be reasonable to allow for some sexual activity at age 13. According to the Australian Study of Health and Relationships (ASHR), the median age at first intercourse in Australia is 16 for both males and females, and by the age of 25 more than 95% of individuals are sexually active [88]. Similarly to [127], we used the results of these studies to estimate the proportions of sexually active individuals of age 13, 14, and up to 25 (it was assumed that all people over 25 are sexually active).

Modelled population is 12-64 year old

We do not include in our models individuals of all ages. Instead, we include only those who are old enough to engage in sexual activity and not too old to maintain this activity at a level non-negligible in the context of HPV transmission. It may seem that the modelled population should not contain individuals under 12. However, we need to include the 12 y.o. since they are vaccinated within the Australian HPV immunisation program (which is motivated by the assumption that they are predominantly virgins). The oldest individuals in the model are 64 y.o. The reason for this is the absence of reliable data on sexual habits of the 65+ cohort and the fact that ASHR data (covering people up to 60 y.o.) revealed roughly uniform rates of new sexual partner acquisition, which were also quite low, in the 50+ group. There is currently

no convincing evidence that the 65+ cohort may be more sexually active than the 50+ cohort. So we assumed that the 60-64 year old are as active as the 50+ and viewed the exclusion of 65+ cohort from the model as an acceptable simplification.

HPV-6 and -11 are treated as a combined HPV-6/11 type

For the sake of simplicity, we consider HPV types 6 and 11 as one "combined" type referred to as HPV-6/11. This simplification is justified by the following factors: (a) parameter values for the two types which can be found in the literature are usually close together, while there is no evident reason to assume that the uncertain parameter values related to HPV 6 and 11 are likely to differ significantly; (b) from the public health perspective, it is important to estimate reductions in genital warts incidence, and therefore, as a prerequisite, the reductions in prevalence of a group of HPV types contributing to genital warts; in view of this and (a), simulated effect of vaccination on prevalence of HPV-6/11 type will be more relevant than that on each of these types separately.

Instantaneous partnerships

It has to be noted that all compartmental models treat the partnerships formed between individuals as instantaneous. This immediately has several important implications: they do not allow for reinfection within stable partnerships, partnership overlap is not captured, and the gap between successive partnerships is overestimated. Therefore any such model, in order to achieve a better calibration, will have to artificially increase sexual activity, which can be expressed as higher probabilities of transmission and/or longer durations of infection (see [133, 46] for discussion).

Simplified population dynamics

Based on the data provided by Australian Bureau of Statistics, we found it reasonable to assume that the birth rates and age-specific death rates used in the models are constant (since the observed variations in these rates between 2005 and 2010 appear insignificant [2, 3]). Also, we fixed the number of males in the whole population and its every subdivision is to be the same as the number of females [1]. We found that it would be acceptable to simplify the models by assuming that the size of modelled populations does not change, and the number of individuals exiting the population every year is immediately compensated by the number of 12 y.o. individuals entering the population.

Instantaneous ageing

We use a discrete age structure which gained considerable popularity in infectious disease modelling since its appearance in [124]. Namely, at the end of each year the whole population gets older at once by 1 year. The aged population serves forms an initial condition for advancing the model on the next 1-year time interval, at the end of which the population get older by 1 year once again. This process continues until the pre-specified stopping point is reached.

7.5. Bayesian Model Specification

Recall that according to Bayesian approach, we have to define the parameter vector θ and specify what is the observed data y for our model. We begin with the latter.

Data Our data y are the genital warts incidence [114] in the Australian population (Table 5), estimated from the Bettering the Evaluation of Care and Health (BEACH) cross sectional database. Genital warts incidence is effectively the number of individuals who have been treated for genital warts for the first time during a year. In our model, it is calculated at a time point when the model have already reached a steady state, which means that the number of individuals in each compartment does not change anymore. Then, for an age group i,

$$y_a^{(F)} = [\text{infected females with genital warts in age group } a] \times r_{med,F} \times 1000 \qquad (70)$$

Note that 1,000 appears here because we calculate the incidence not per capita, but per 1,000. We assumed that HPV-6/11 is responsible for 90% of the genital warts and adjusted the reported data accordingly. We assume that we know what kind of probability distribu-

Table 5. Genital warts incidence data for Australia measured as the mean number of new cases per 1,000 persons per year (as reported in [114])

age group		males		females	
number	ages	%	95% CI	%	95% CI
1	15-19	1.66	0.46 - 2.86	7.28	4.18 - 10.38
2	20-24	6.27	3.77 - 8.77	8.61	5.61 - 11.61
3	25-29	7.4	4.4 - 10.4	6.37	3.77 - 8.97
4	30-34	4.64	2.44 - 6.84	4.33	2.13 - 6.53
5	35-44	2.34	1.34 - 3.34	2.19	1.19 - 3.19
6	45-59	0.83	0.33 - 1.33	0.48	0.08 - 0.88

tion function describes the data y, but parameters of this distribution are unknown. Let us write the reported genital warts incidence by age group for females as a vector

$$\mathbf{y}^{(F)} \equiv \left[y_1^{(F)}, \ldots, y_6^{(F)}\right], \qquad (71)$$

where subscripts denote an age group from Table 5. A similar factor for males would be

$$\mathbf{y}^{(M)} \equiv \left[y_1^{(M)}, \ldots, y_6^{(M)}\right]. \qquad (72)$$

Now we assume that both $\mathbf{y}^{(F)}$ and $\mathbf{y}^{(M)}$ are drawn from a multivariate normal distribution denoted $\mathcal{N}(\mu, \Sigma)$, with an unknown mean μ and covariance matrix Σ. Let the covariance matrix has the form

$$\Sigma \equiv \sigma I, \qquad (73)$$

where σ is an unknown parameter and I is a unit matrix.
Parameter vector. The model parameter vector θ is

$$\theta \equiv (\beta, r_{cl,F}, r_{cl,M}, p_{gw,F}, p_{gw,M}, r_{gw,F}, r_{gw,M}, T_{med,F}, T_{med,M}, T_{clt,F}, T_{clt,M}, r_{li,F}, r_{li,M}, \varepsilon^{(A)}, \varepsilon^{(S)}, \sigma), \quad (74)$$

which is simply a vector containing all model parameters listed in Table 2 and σ which we also view as a parameter with a prior distribution $Be(2, 2)$.
Prior structure. The basic prior structure we introduce is simply the product of priors for all entries of the parameter vector θ:

$$\pi(\theta) \equiv \pi(\beta)\pi(r_{cl,F})\pi(r_{cl,M})\pi(p_{gw,F})\pi(p_{gw,M})\pi(r_{gw,F})\pi(r_{gw,M}) \pi(T_{med,F})\pi(T_{med,M})\pi(T_{clt,F})\pi(T_{clt,M})\pi(r_{li,F})\pi(r_{li,M})\pi(\varepsilon^{(A)})\pi(\varepsilon^{(S)})\pi(\sigma). \quad (75)$$

Likelihood function. Let the probability density function of $\mathcal{N}(\mu, \Sigma)$ (where we think the data are drawn from) evaluated at a vector x be mvnpdf(x, μ, Σ). Then the likelihood function can be chosen as below,

$$l(\theta|y) = \text{mvnpdf}(\mathbf{y}^{(F)}, \mu^{(F)}, \Sigma) \times \text{mvnpdf}(\mathbf{y}^{(M)}, \mu^{(M)}, \Sigma), \quad (76)$$

where $\mu^{(F)}$ and $\mu^{(M)}$ are simulated vectors of genital warts incidence by age group for females and males. Note that this choice is not unique and merely a suggestion.

7.6. Modelling a Vaccination Program

Here we demonstrate a simple way to introduce a vaccination in our model. We assume that the vaccination is carried out with Gardasil (recall that it protects from HPV-6/11, as well as against HPV-16 and -18) as under the Australian National HPV Vaccination Program. This means that we assume that the vaccine is strictly prophylactic, i.e. the only action of vaccine is to prevent infection amongst susceptible individuals. Hence, an infected individual who has been vaccinated does not benefit from vaccination at all until he or she naturally clears HPV infection and becomes susceptible. Additionally, we assume that the duration of vaccine protection is life-long. This is justified by an argument that in view of the reported duration of protection amounting to at least five years [134] and possible existence of a post-vaccination immune memory [107], it is reasonable to assume the life-long protection because even if it was not life-long as such, it could be maintained at an appropriate level by boosters (see [86, 127] for details). School-based vaccination covering 12-13 year old girls began in Australia in April 2007 and is now ongoing. The available data [52] suggests that two dose coverage of 80% has been achieved by the program. Therefore, we fixed the vaccine coverage in our model at 80%. Vaccine efficacy (VE) was 100%, which is reasonable according to [134]. Efficacy as such means a degree of protection against infection per partnership. Currently, there is no solid understanding of what is the correct implementation of efficacy. We interpret it as follows: for a vaccinated person the transmission probability per partnership from this person to her sexual partner is only VE% of the transmission probability calculated for this person if she were not vaccinated. For

simplicity, vaccination in our model is carried out at a constant yearly rate r_v. Then the time dependent number of vaccinated individuals $V(t)$ changes as below

$$\frac{dV(t)}{dt} = r_v U(t),$$

while the number of unvaccinated individuals $U(t)$ changes accordingly:

$$\frac{dU(t)}{dt} = -r_v U(t).$$

We also need to impose some boundary conditions which reflect vaccination targets for a given year. More specifically, suppose we want to vaccinate P% of a group of N unvaccinated individuals during this year. Then we state that

$$U(t=0) = N,\ V(t=0) = 0$$

(at the beginning of the year the whole group was unvaccinated) and

$$U(t=1) = (1 - P/100)N,\ V(t=1) = (P/100)N$$

(at the end of the year P% of individuals will be vaccinated, while the rest will not). Finally, it is pertinent to note that we activate vaccination when the model have already reached its steady state, and it is expected that as a result of vaccination, the model will reach another steady state. This state is referred to as post-vaccination endemic equilibrium and the ultimate impact of vaccination is measured using model outcomes corresponding to this state.

7.7. Results

Following the scheme shown in Figure 5, we successfully calibrated our model to Australian genital warts incidence data (see Table 5). For 120,000 iterations the model was run from time 0 to $T_{eq} = 70$. At that time it had reached an equilibrium state already. As it can be seen from Figure 6 below, the simulated genital warts incidence was close to the reported one in for all age groups except for the 15-19 year old males. However, we are inclined to think that incidence at only 1.66 per 1,000 in these males is not to be treated as reliable, since it is anomalously low as compared with that for females of the same age (7.28 per 1,000), and it is not clear what might explain such striking difference.

We ran the model from T_{eq} to $T_{eq2} = 140$ with the female vaccination activated from T_{eq} and on. The time point T_{eq2} was chosen empirically so that the model is at a post-vaccination equilibrium at that point. Figure 7 illustrates how the HPV-6/11 population prevalence was affected by vaccination.

We can see that indeed, about 70 years from the beginning of vaccination, the prevalence has dropped to a steady low level. To evaluate the impact of vaccination, we compared HPV-6/11 prevalence at that level to the one observed before vaccination began (which corresponds to point 0 in Figure 7). The mean relative reduction in prevalence was 93.3% (95% confidence interval: 85.2% - 99.8%). Hence, we conclude that vaccination will drastically reduce HPV in the modelled population.

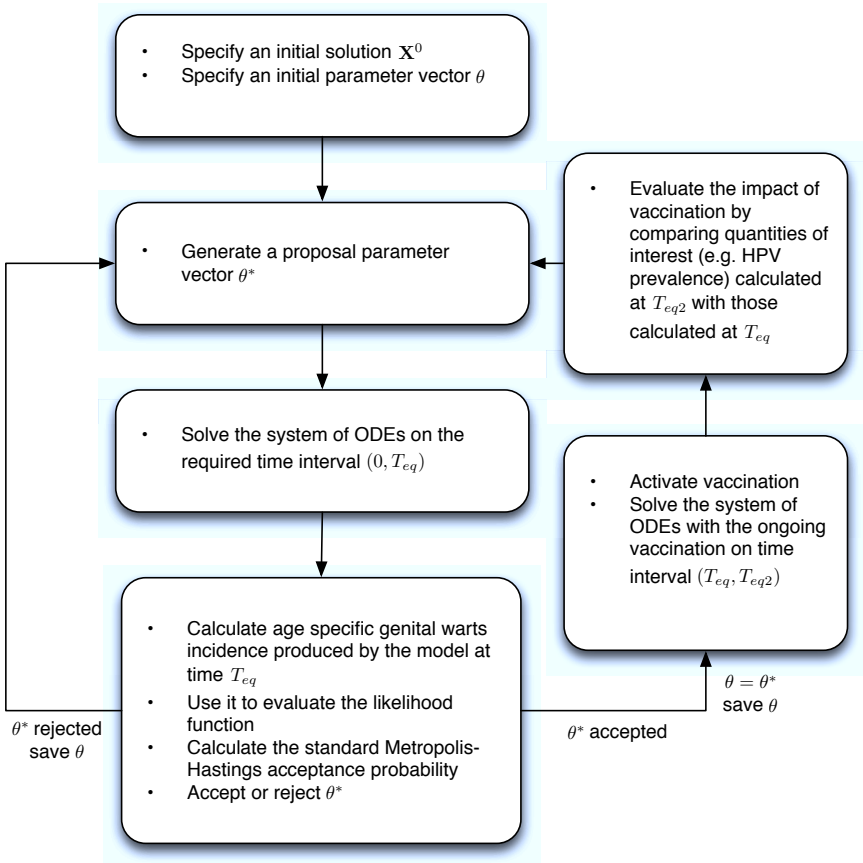

Figure 5. Schematic description of the procedure. The proposal parameter vector θ^* is generated as detailed in Algorithm 3, Section 6.7.

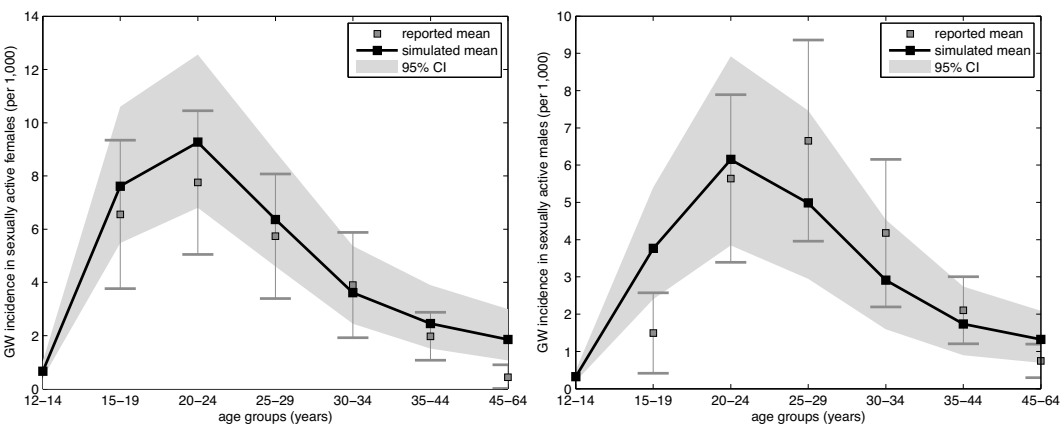

Figure 6. Calibration plots. Genital warts incidence is yearly.

Conclusion

In this chapter we reviewed a number of model formulations suitable for modelling HPV transmission, and discussed both novel and well established sampling algorithms which can

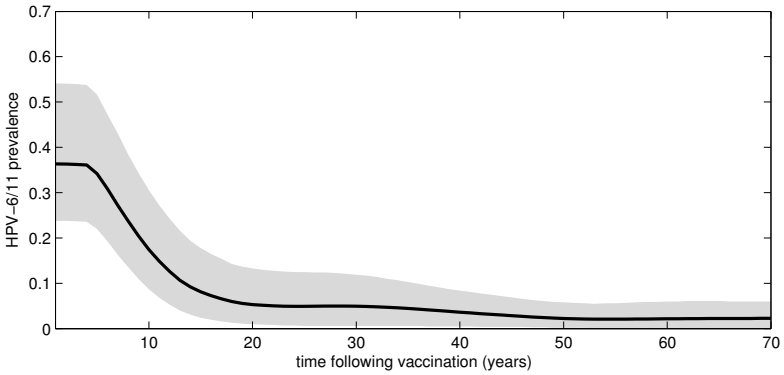

Figure 7. HPV-6/11 prevalence in the modelled population is decreasing after commencement of vaccination.

potentially be efficiently incorporated in both deterministic and stochastic HPV models developed according to Bayesian framework. We then developed a detailed example utilizing several features of the developed models and statistical inference methodologies presented to calibrate and study the HPV epidemic in Australia.

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