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Overview

Burden and prevention of HPV related diseases: Situation in Croatia

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Abstract

The role of persistent infection of high-risk human papillomaviruses (HR-HPV) in cervical cancer has become convincingly established. Other anogenital cancers in women and men are also caused, to a lesser extent, by HR-HPV. In addition, most of the benign anogenital warts and laryngeal papillomatosis are caused by low-risk (LR) HPV types. All these HPV-associated diseases represent high public health burden worldwide, cervical cancer being the leading one. Nowadays, several opportunities are available to prevent cervical cancer and other HPV-related diseases. The most promising cancer preventing intervention is the HPV vaccination against the most common HPV types (16 and 18). However, secondary prevention like education and early detection of the disease by screening has demonstrated in the past great efficacy and should be continued and improved by novel prognostic methods (HPV testing and other biomarkers) in countries where it is already in place, and implemented in those countries that lack cancer control programmes. Herein, the current knowledge on HPVs, HPV-related diseases and their preventive approaches are discussed. In addition, the situation in Croatia is presented.

INTRODUCTION

In the early 1970s, studies were initiated on the possible role of human papillomaviruses (HPV) in cancers *(1)*. Today it is well established that this very heterogeneous virus family comprehend important human carcinogenic viruses, causing not only the vast majority of cervical, but also a substantial proportion of other anogenital and head and neck cancers. In addition, specific types have been linked to certain cutaneous cancers. HPV infections on a global scale account for more than 50% of infection-linked cancers in females and for approximately 5% in males *(2)*. Vaccines against the high-risk (HR) HPV types 16 and 18 represent the first preventive vaccines directly developed to protect against one of the leading human cancers (cervical carcinoma). This review will cover the essentials about HPVs, the epidemiology and prevention of the major cancer and diseases caused by them, and it will discuss the situation in Croatia.

HUMAN PAPILLOMAVIRUSES

HPV is a strictly epitheliotropic, circular double-stranded DNA virus infecting human mucosal and cutaneous tissues. More than 100 HPV genotypes have been characterized. Approximately 40 types infect the

TABLE 1

Burden of high-risk human papillomavirus (HPV) related diseases.

Disease (cancer site*)	World annual estimates in 2008	Croatian annual estimate in 2008	HPV contribution	Most common HPV types
Cervical cancer (C53):	529,000 cases (WASR 15.2 per 100,000) and 274,000 death (WASR 7.8 per 100,000) *(65)*	360 cases (15.6 per 100,000) *(34)*	100% *(66)*; 85% *(5)*	HPV16 (61%), HPV18 (10%), HPV 31 (4%), HPV33 (4%), HPV35 (2%), HPV45 (6%), HPV52(3%), HPV58 (2%) *(5)*
– SCC	about 90% of all cervical cancer cases		87% (5)	HPV16 (62%), HPV18 (8%), HPV45 (5%) (5)
– adenocarcinoma	about 10% of all cervical cancer cases		62% (5)	HPV16 (50%), HPV18 (32%), HPV45 (12%) (5)
Vulvar cancer (C51)	30,000 cases *(36)*	61 cases (2.7 per 100,000) *(34)*	40% *(37)*	HPV16 (32%), HPV18 (4%) *(37)*
Vaginal cancer (C52)	15,000 cases *(36)*	16 cases (0.7 per 100,000 population) *(34)*	70% *(37)*	HPV16 (54%), HPV18 (8%) *(37)*
Anal cancer (C21)	30,400 (15,900 female and 14,500 male) cases *(36)*	19 (10 female and 9 male) cases (0.4 per 100,000 population) *(34)*	97% *(40)*	HPV16 (75%), HPV18 (3%) *(40)*
Penile cancer (C60)	26,300 cases *(36)*	28 cases (1.3 per 100,000 population) *(34)*	45% *(38)*	HPV16 (60%), HPV18 (13%), HPV6/11 (8%) *(38)*;
Oropharyngeal cancers (C01, C09, C10)	61,500 (12,600 female and 48,900 male) cases *(36)*	147 (20 female and 127 male) cases (1.3 per 100,000 population; 2.1 and 0.6 per 100,000 population in men and women, respectively) *(34)*	47% in oropharyngeal carcinomas (C10) and 11% in oral cavity carcinomas (C01, C09); 64% in female and 42% in male cases *(42)*	HPV16 (90%) in oropharyngeal carcinomas; HPV16 (96%) in oral carcinomas *(42)*

WASR, world age standardized rate; HPV, human papillomavirus; MSM, men having sex with men; SCC, squamous cell carcinoma; *ICD-10 code: C01, *basic linguae*, C09, *tonsilla*, C10, oro-pharynx, C21, *anus et canalis analis*, C51, *vulva*, C52, *vagina*, C53, *cervix uteri*, C60, *penis*.

anogenital tract mucosa, while others infect cutaneous tissues *(3)*. Since the discovery of the HPV genome the presence of viral infection has been evaluated by the detection of viral DNA as viral isolation on cell culture is extremely difficult and natural serology response to viral infection is very weak and often uninformative.

Based on ample scientific evidence, in 1995 a working group of the IARC concluded that some HPV types, also called HR types, are carcinogenic to humans *(4)*. These carcinogenic HPV types can also cause other anogenital cancers in women (vulvar, vaginal and anal cancers) and men (penile and anal cancers), as well as oropharyngeal tumors. Genital HPV types also have a role in squamous cell carcinoma (SCC) of the conjunctiva, but no conclusion could be reached for cancer of the esophagus, lung, colon, ovary, breast, prostate, urinary bladder, or nasal and sinonasal cavities *(4)*.

In contrast to the HR types, low-risk (LR) HPV types, notably types 6 and 11, cause almost all clinically visible benign lesions, i.e. genital warts (flat and acuminate condylomata) and laryngeal papillomas. In the last classification, there are three main categories of HPV relative to cervical cancer: carcinogenic (high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), probably carcinogenic (type 68) and possibly carcinogenic (types 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97) (Bouvard 2009). In a recent worldwide survey, HPV 16 was the most prevalent type in cervical cancer (61%), followed by HPV 18 (10%), HPV 31 (4%), HPV33 (4%), HPV35 (2%), HPV45 (6%), HPV52 (3%) and HPV58 (2%) *(5)* (**Table 1**).

HPV infection is very common at young age (under 25 years). HPV infections are generally transient, with 60-70% of new infections clearing within one year and 91% clearing within two years *(6)*. Only a small proportion of HPV infections progress to persistent infection, often involving HR HPV types that have been shown to persist longer than LR-HPV types. The estimated average global prevalence of genital HPV infection is 12% *(7)*. The prevalence among women with normal cytology varies between countries from 2 to 42% and depends on the age and population risk *(8)*. High prevalence was observed in Africa and Latin America compared to Europe, North America and Asia.

Besides genital warts, most HR-HPV infections resolve without appearance of any symptoms or in some cases with the occurrence of atypical or low-grade cervical lesion (CIN 1, cervical intraepithelial neoplasia grade 1). Only a small proportion (10-30%) of HR-HPV infection that persists for a long time (several years) poses a risk for the development of high-grade cervical lesions (CIN 2 and 3) that are precursor lesions of invasive cervical cancer (ICC). For instance, CIN 2 lesion will progress to CIN 3 lesion within 2 to 4 years *(9)*. However, for the majority of HPV positive women the precancerous lesion disappears at the same time the HPV infection has cleared. Thus, a small proportion (about 1%) of low grade lesion (CIN 1) and 12% of high grade lesions (CIN3) will progress into invasive cancer if left untreated

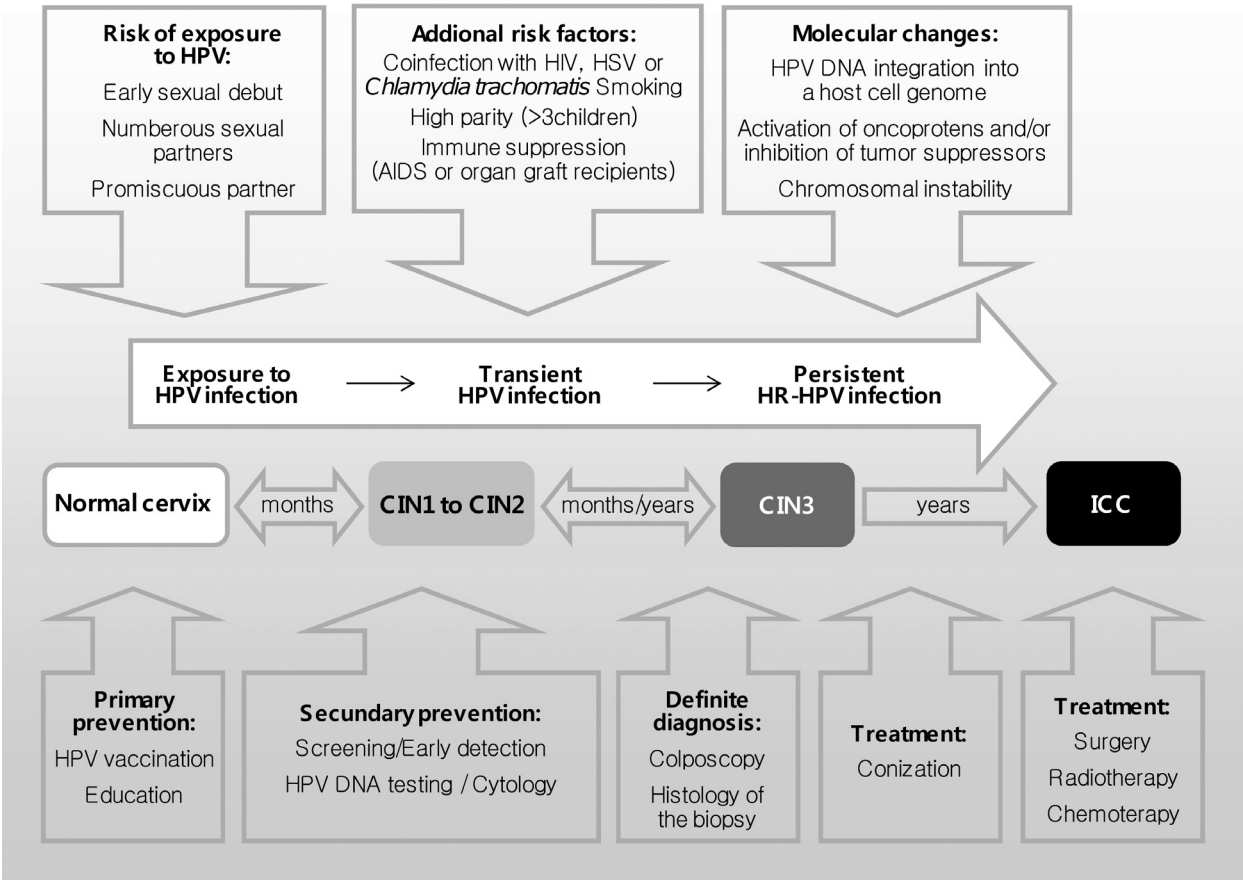

Figure 1. *Risk factors, natural history of human papillomavirus (HPV) infection, cervical cancerogenesis and opportunities for cervical cancer control; pre-invasive cervical lesions or cervical intraepithelial neoplasia (CIN) grade 1 to 3 that may progress to invasive cervical cancer (ICC).*

(10). Progression of precursor lesions to ICC usually takes place over a period of more than 10 years, allowing time for the identification and treatment of precursors before cancer develops (**Figure 1**).

The most prevalent HR-HPV types in Croatian women with precancerous lesions (high-grade squamous cell intraepithelial lesion [HSIL]) were, as expected, HPV 16 (23%) followed by HPV 31 (12%), HPV 33 (6.1%), HPV 18 (5%), HPV 52 (2.3%), HPV 58 (1.1%) and HPV 45 (0.9%) *(11)* (**Figure 2**). It is important to note that LR-HPV 6/11 was present in 7% of HSIL cases (data not shown). The HSIL lesions positive for LR-HPV types will probably clear and the lesion will regress, while those lesions that are associated with HR-HPV types have a higher risk of progression to invasive cervical cancer.

Cervical cancer precursor lesions persist longer and progress more quickly in women with HPV 16 and/or 18 infections than in women with other HR-HPV types and LR-HPV types *(12)*. HPV 16 and 18 positive women have a 200-fold increased risk of cervical cancer *(13)*. Factors that may influence progression (**Figure 1**) include co-infection with other sexually transmitted infections such as *Chlamydia trachomatis*, *herpes simplex* virus (HSV) or human immunodeficiency virus (HIV), tobacco smoking, high parity (>3 children) and immune suppression (organ graft recipients or acquired immunodeficiency syndrome [AIDS] patients) *(14)*. The role of nutrition and long-term use of oral contraceptives in cervical cancerogenesis is still unclear.

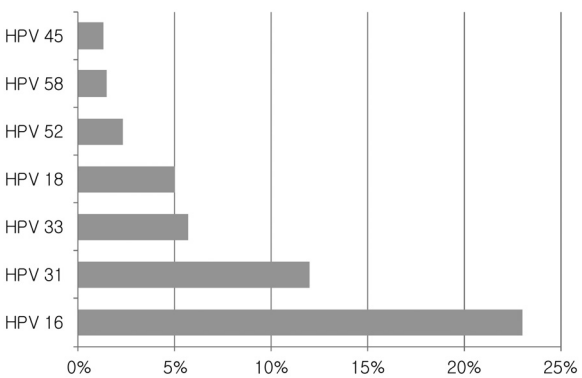

Figure 2. *Prevalence of specific human papillomavirus (HPV) types including single and multiple infections in high-grade squamous cell intraepithelial cervical lesion (HSIL) in Croatia (11).*

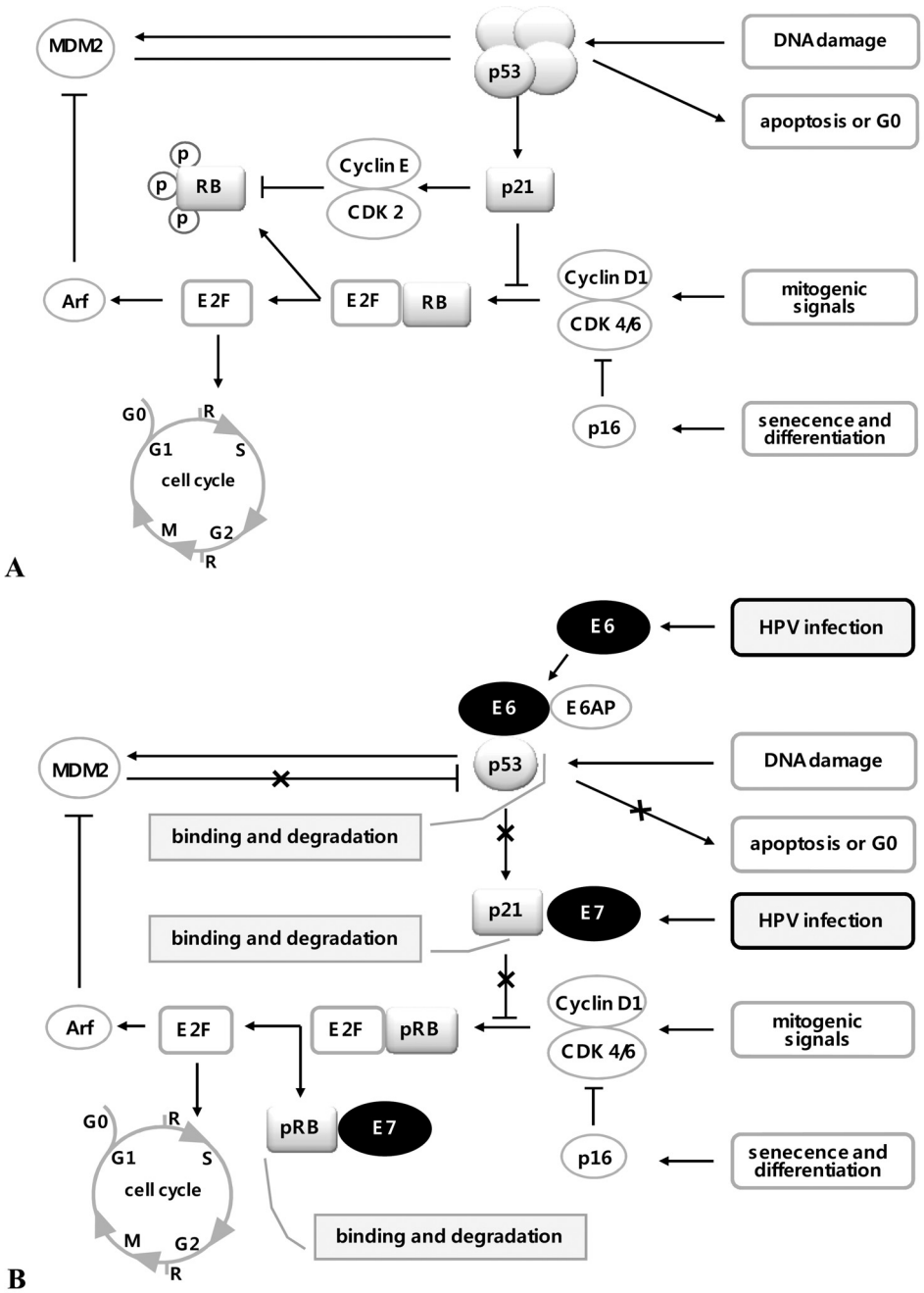

Figure 3. *Schematic presentation of essential cell cycle signaling pathways (A) and the interference of the HPV E6 and E7 oncoproteins (B).*

In addition to carcinogenic alpha-HPV types (mucosotropic), several beta-HPV types, notably HPV types 5 and 8, are associated with SCC of the skin and thus considered possibly carcinogenic to humans *(4)*. Data for this association is strong in patients with *Epidermodysplasia verruciformis* (EV), but less so in general population. EV is a rare genetically heterogeneous disease, either autosomal recessive or X-linked but also associated with a high-risk of non-melanoma skin cancer *(15)*. EV is a unique model where genetic susceptibility to HPVs is demonstrated. In 75% of EV-patients, one of two related genes, EVER1 or EVER2, has a non-sense mutation. The complex of zinc-transporting proteins EVER1, EVER2, and ZnT-1 that maintain cellular zinc homeostasis plays a central role in the HPV-specific barrier which protects cells against infection with beta-HPVs *(16)*.

HPV induced cancerogenesis

The HPV genome contains several early and late open reading frames coding for replication and transcription regulating proteins, E1, E2, E5, E6, E7 and viral capsid proteins, L1 and L2, respectively. Two early proteins, E6 and E7, act as major viral oncoproteins

which, besides being over-expressed in HPV-associated cancers, have potent transforming activity in tissue culture and tumorigenic action in transgenic mice *(12)*.

The key activity of E7 protein is to overcome tumor suppressor block controlled by the retinoblastoma pocket protein (pRB), while that of E6 protein is to overcome the p53 protective control pathways which are important in preventing the genetic damage leading to cellular transformation. Thus, these oncoproteins promote genetic instability through induction of cellular proliferation, disruption of cell cycle checkpoints, inhibition of apoptosis, induction of telomerase activity, and finally lead to cancer (Figure 3).

Briefly, in quiescent cells, pRB is present in a hypophosphorylated form and associates with E2F transcription factor, thereby inhibiting their transcriptional activity (Figure 3A). Under exposure to a mitogenic signal, complexes of cyclin and cyclin-dependent kinase (CDK) are activated, notably cyclin D1-CDK4 and cyclin D1--CDK6 complexes, which induce pRB phosphorylation leading to the disruption of pRB/E2F complexes. Such activated E2F transcription factors then induce cyclin E and subsequent additional phosphorylation of RB by cyclin E-CDK2 complex that initiates entry into S phase. The protein $p16^{INK4A}$ (p16) mediates senescence and differentiation by inhibiting cyclin D1-CDK4 and cyclin D1-CDK6 complexes. The interplay between the cyclins, CDKs and their inhibitors determines whether the restriction point (R on Figure 3) can be passed. A second important control mechanism of the cell cycle occurs during the G2 phase, when p53 plays a crucial role during DNA replication. Usually, p53 is maintained at low concentrations by MDM2-mediated degradation but when replication errors or other DNA damages occurs the checkpoint kinases CHK1 and CHK2 induce increased p53 activity by phosphorylation of various downstream molecules, including p53 itself. The p53 tetramer acts as a stress-induced transcription factor and induces the expression of $p21^{CIP}$ (p21) which inhibits several cyclin-CDK complexes and stops the cell cycle allowing correction of DNA errors or induction of apoptosis if the damage is too extensive. Besides its crucial role in cell cycle control, p53 is also a master regulator of many other stress-associated cellular functions, and is therefore often inactivated or mutated in many different cancer types *(17)*.

Upon HPV infection, the E7, when binding to pRB and its related members (p107 and p130), mimics the effects of pRB phosphorylation resulting in the release of active E2F transcription factors which in turn activate the transcription of a group of genes encoding proteins essential for cell cycle progression (Figure 3B). Thus, E7 expressing cells can enter the S phase in the absence of mitogenic signals *(17)*. E7 also binds and activates complexes of cyclins and CDKs which control progression through the cell cycle. On the other hand, E6 associates with the ubiquitin-protein ligase E6AP which then binds to p53 and targets the p53 protein for multi-ubiquitination and consequent proteasomal degradation *(18)* (Figure 3B). HPV oncoproteins E7 and E6, both have multiple other functions by binding numerous target proteins and degrading some *via* proteasomal degradation, i.e. family proteins of pRB (p107, p130) *(19)*, and p53 (p63, p73) *(18)*, and p21(CIP1) protein *(20)*.

In conclusion, expression of E6 and E7 viral oncoproteins allows infected cells to re-enter or remain in the cell cycle and furthermore inhibits p53-mediated apoptosis, which consequently allows the virus to replicate as it is otherwise dependent on the host cell DNA replication machinery. Both HR and LR-HPV oncoproteins bind their target proteins but HR do this with higher affinity and also degrade them unlike LR-HPV oncoproteins *(21)*.

Immunology of HPV infection

Natural HPV infection of the genital tract gives rise to a slow and modest serum antibody response in most but not all infected individuals. Humoral response to HPV infection is limited to only 50-80% of women with persistent infection seroconvert, while incident infections very rarely lead to seroconversion, which usually occurs from 6-18 months from infection *(22)*.

Presentation of viral antigens, major capsid protein (L1) and the minor capsid protein (L2) *(23)* to the host immune system is limited as the HPV infection is restricted to epithelial cells. Virus-neutralizing anti-L1 antibodies are essentially type-specific. As the L2 protein is situated more internally into the capsid, the anti-L2 antibodies are less potent than anti-L1 antibodies, but they appear to show some cross-reactivity to heterologous HPV types *(24)*. Antibody responses against E6 and E7 proteins from HPV 16 are most prevalent in patients with advanced cervical cancer and as such do not have prognostic value *(25)*.

Innate immunity, also known as non-specific immune response is as important as the adaptive immunity in case of HPV infection. The first line of defense is the skin or mucosa, which are either a layer of keratinized cells that provide a physical barrier against HPV entry or mucin that the cells release on the mucosal surface. This is the reason why HPV are thought to infect cells only after micro-lesions of the skin or mucosa *(26)*. Innate immunity additionally activates interferon response, macrophages and natural killer (NK) cells to help clear the infection. Finally, innate immunity has a significant role in activating and directing later activated adaptive immunity for the HPV clearance. However, as HPV life cycle is exclusively linked with differentiating keratinocytes *(27)* there is limited opportunity for immune system activation as in the lower layers of the epithelium only few viral proteins are expressed at low levels. Furthermore, the virus does not kill or destroy infected cells but is shed when those cells undergo programmed apoptosis as a part of their terminal differentiation programme. Thus, during HPV active infection there is no inflammation that would otherwise activate the immune response. And finally, viral particles are released out of the

epithelium where they cannot efficiently activate host immunity *(22)*. In addition to the specific life cycle that efficiently hides the virus from the immune system, HPV proteins directly interfere with several aspects of immune system *(28)*.

Despite all the mechanisms of immune evasion that HPV employs, adaptive immunity does play a role in the clearance of infection. Thus, in specific cell-mediated immune response dendritic cells (or Langerhans cells), present in the cervical epithelium, play an important role in recognizing HPV-infected cells and stimulate T helper 1 (Th1) cells, which elicits the production of cytotoxic T-lymphocytes *(29)*. It was shown that regressing genital warts contain infiltrating cytotoxic T-lymphocytes and macrophages *(30)*. Furthermore, it was shown that in immunocompromised patients (i.e. HIV positive patients) HPV-induced lesions recur more often after treatment *(31)*.

HR HPV-RELATED DISEASES

Cervical cancer

Cervical cancer is the third most common cancer, after breast and colorectal cancer, among women worldwide, with 529,500 estimated new cases and 275,000 deaths in the year 2008 *(32)* (**Table 1**). It accounts for 8.8% of all female cancer cases with the estimated incidence and mortality rates (world age standardised rate [WASR]) of 15.2 and 7.8 per 100,000, respectively. Cervical cancer is the second most common cancer in developing regions (452,000 cases representing 85% of all cervical cancer cases or 15% of female cancers) and only the 10th most common cancer in developed regions (76,000 cases or 3.6% of female cancers). In Croatia, cervical cancer is the 8th most common female cancer, with approximately 350 new cases and about 100 deaths per year *(33)*. In 2008, there were 360 cases of cervical cancer (C53) with the incidence of 15.6 per 100,000 population and 556 precancer cases (carcinoma *in situ* [CIS]) with the incidence of 24.2 per 100,000 population *(34)* (Figure 4).

About 90% of cervical cancer cases are SCC, while 10% are adenocarcinoma *(5)*. Both types of cancer are mainly caused by HPV type 16 (62% and 50%, respectively), but adenocarcinoma is significantly more associated with HPV types 18 (32%) and 45 (12%) (**Table 1**) which are phylogenetically closer to each other than to HPV 16. In a Croatian study on HPV prevalence in cervical cancer, HPV 16 and HPV 18 were identified in 52% and 28% of SCCs, while in adenocarcinomas there were found in 27% and 68% cases, respectively *(35)*. The lower prevalence found in the Croatian study may be attributed to the lower sensitivity of the genotyping methods used.

Other HR-HPV related cancers

In addition to cervical cancer, HPV infection is associated with the cancer of the vulva and vagina in women, cancer of the penis in men, and anal cancer and oropharyngeal cancer in both sexes (**Table 1**).

Worldwide, about 30,000 cases of vulvar cancer were identified in 2008 *(36)*. In Croatia, in the same year, 61 cases were identified with the incidence of 2.7 per 100,000 population *(34)*. Most of these vulvar cancers were SCCs and HPV positive in 40% cases, mostly for HPV 16 (32%) followed by HPV 18 (4%) *(37)*.

Worldwide, there are about 30,000 and 15,000 new vulva and vagina cancer cases per year, respectively *(36)*. In Croatia, in 2008, 61 and 16 cases of vulvar and vaginal cancer occurred, with the incidence rate of 2.7 and 0.7 per 100,000 population, respectively *(34)* (Figure 4). Most of these cancers are SCC affecting older women. The HPV contribution in vulvar cancer is 40%, mostly HPV 16 (32%) and then HPV 18 (4%) *(37)*. In vaginal cancer the HPV contribution is higher being 70%, mostly HPV 16 (54%) followed by HPV 18 (8%) *(37)*.

The penile cancer affect about 26,300 men aged over 50 years *(36)*. Similarly to the vulvar cancer, the HPV contribution is 45%, mostly attributed to the two most common HR-HPV types, HPV 16 (60) and 18 (13%) but also to the two most common LR-HPV types, 6 and 11 (together 8%) *(38)*. In Croatia, in 2008, 28 cases of penile cancer occurred (incidence of 1.3 per 100,000 population) *(34)* (Figure 4).

About 30,400 cases of anal cancer occur annually worldwide *(36)*. There are slightly more cases in women (15,900) than men (14,500). In Croatia, in 2008, 19 cases of anal cancer occurred, 10 and 9 cases in women and men, respectively, with the incidence of 0.4 per 100,000 population in both sexes *(34)*. The risk of anal carcinoma is increased among men having sex with men (MSM) and in immunosuppressed population including those with the HIV and organ graft recipients *(39)*. Similarly as the cervix, the anus has a transformation zone that is highly susceptible to HPV infection. Thus, 97% of anal cancer cases are HPV positive, mostly with HPV 16 (75%) followed by HPV 18 (3%) *(40)*.

Globally, about 61,500 oropharyngeal cancers that occurred in 2008 were associated to HPV infection, including lingual cancer (C01), tonsil cancer (C09) and

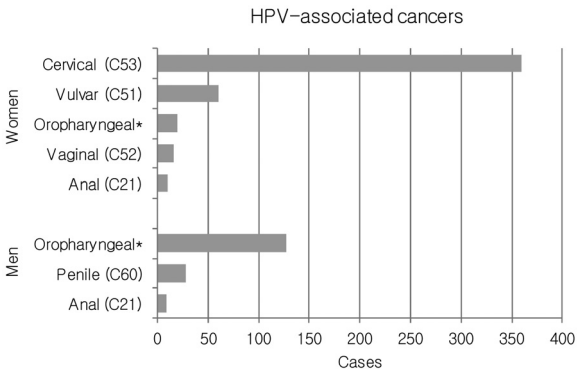

Figure 4. *Human papillomavirus (HPV) associated cancers among men and women in Croatia in the year 2008 (34); *oropharyngeal cancers represents a sum of C01* (basic linguae), *C09* (tonsilla) *and C10 (oropharynx) cancers.*

oropharyngeal cancer (C10) *(36)* (Table 1). They are much more common in men (48,900 cases) than women (12,600 cases). In Croatia, in 2008, there were 147 oropharyngeal cancers, 127 in men and 20 in women, with the incidence of 1.3, 2.1 and 0.6 per 100,000 population, respectively *(34)* (Figure 4). HPV-positive oropharyngeal tumors are associated with oral sex, age under 60 years, infrequent p53 gene mutation and a more favorable clinical outcome, while other HPV-negative cancers are associated with smoking, excessive alcohol use, age above 60 years, frequent p53 gene mutation and poor prognosis *(41)*. HPV contributes to 47% oropharyngeal carcinomas and 11% of oral cavity (C01 and C09) carcinomas, of which 64% and 42% cases in women and men, respectively *(42)*. HPV16 was the most commonly found in oropharyngeal carcinomas (90%) and oral carcinomas (96%) *(42)*.

LR-HPV RELATED DISEASES

Genital warts are highly infectious, with an estimated transmission rate of >60% and the average incubation period of 2-8 months. If left untreated, genital warts may show minimal change, grow larger and more numerous, or regress spontaneously. Genital warts are largely attributable to LR-HPV types 6 (89%) and 11 (11%) *(43)*. They are very common in young people (up to 25 years), spontaneous remissions occur frequently (up to 40%) *(44)*. However, the psychological burden of the patient as well as the cost of treatment are very high as incidence rate is estimated to be 1-2 per 1000 person annually *(36)*.

Laryngeal papillomatosis (also known as recurrent respiratory papillomatosis) is a rare medical condition affecting 1.1 per 100,000 children *(45)*. Laryngeal papillomatosis is caused by HPV types 6 and 11, in which benign tumors form on the larynx or other areas of the respiratory tract. Since the disease is most commonly found in children, the infection may be acquired by vaginal childbirth from HPV infected mother. Without treatment, it is potentially fatal as uncontrolled growths obstruct the airway. These tumors can reoccur frequently, may require repetitive surgery, and may cause problem with breathing *(46)*.

PREVENTIVE STRATEGIES

As mentioned before, there are three levels of public health strategies to prevent cervical cancer: 1) education, 2) prophylactic cervical cancer vaccination, and 3) screening for early detection of the disease. The knowledge about the cause of cervical cancer and the way how to prevent this cancer has been quite recently so, even though we have advanced HPV-related technologies (HPV testing and HPV vaccination), the delivery and acceptance of these tools are quite difficult in some countries.

Education of young people about sexually transmitted diseases, which is poor in Croatia, could largely help preventing HPV infection on the individual basis. Although some HPV subtypes cause genital warts, most infections with HPV cause no symptoms; therefore preventive measures should be conducted in any case. Consistent use of condoms among partners may reduce but not eliminate the risk of male-to-female genital HPV transmission since HPV can be present in skin throughout the anogenital area as well as in genital secretions *(47)*. There is also evidence that male circumcision reduces the risk of acquisition of HPV infection among men, which in turn lowers the risk of subsequent transmission and infection of their partners *(48)*.

Prophylactic HPV vaccination

Prophylactic HPV vaccines were primarily designed and produced to prevent infection with the most common cancer causing HPV types, HPV 16 and 18, which cause about 70% of cervical cancer cases. Nowadays, two vaccines against HPV 16 and 18 are commercially available *(49)* One vaccine is also designed to protect against HPV 6 and 11 which together cause 90% of genital warts. Since March 2008, both vaccines have been approved for use in many countries.

Both vaccines are safe, generally well tolerated and very immunogenic (100%) against vaccine-included HPV types. Vaccine efficacy in HPV-negative women in preventing persistent infection with vaccine-included HPV types and related diseases is very high (>90%) for both vaccines *(50, 51)*. The primary expectation of vaccine efficacy was a reduction in precursor lesions by approximately 50% and in cervical cancer by approximately 70%. This expectation was achieved; in addition, both vaccines procure cross-protection against non-vaccine--HR-HPV types (HPV 31, 33, 45, 52 and 58) and associated cervical disease. In HPV-negative women, cross--protection by the quadrivalent vaccine was most notable against HPV 31 *(50)*. In case of bivalent vaccine, the best individual cross-protection was observed against HPV 31, HPV 33 and HPV 45 *(51)*. Both HPV 31 and HPV 45 are very prevalent in cervical cancer, following HPV types 16 and 18 in SCC or HPV 18 and 16 in adenocarcinoma and adenosquamous carcinoma *(5)*. This is particularly important for protection against adenocarcinoma and adenosquamous carcinoma which are more difficult to identify by cervical screening. Together, HPV types 16, 18, 31 and 45 cause 81% of cervical cancer cases *(5)*, which indicates that cross-protection against those non--vaccine-HR-HPV types increases vaccine efficacy. A limitation to HPV vaccination on an individual or population basis is its currently very high price (Table 2).

Prevention of other cancers and HPV-related diseases in men and women

Pap test can also detect precancerous lesion of the vulva (VIN, vulvar intraepithelial neoplasia) and the vagina (VaIN, vaginal intraepithelial neoplasia). Preventing VIN and VaIN by HPV vaccination is far more efficacious (Table 2). However, as vulvar and vaginal cancer generally occur in older women, who usually do not benefit from HPV vaccination, regular gynecological examination should be performed even after the age of

TABLE 2

Essential facts about prophylactic cervical cancer vaccines.

Commercially available vaccines	The quadrivalent vaccine (Gardasil®, Merck & Co., Inc., Whitehouse Station, NJ USA) and the bivalent vaccine (Cervarix®, GlaxoSmithKline Biologicals, Rixensart, Belgium) have been available since 2006 and 2007, respectively.
Nature of the vaccines	Prophylactic vaccines are made by recombinant technology and self-assembled major HPV capsid protein L1 into VLPs that exhibit morphological and antigenic properties identical to native virions; VLPs do not contain viral genetic material or live biological product, and are therefore not infectious.
Targeted HPV types	The bivalent vaccine is composed of HPV 16 and 18 VLPs with AS04 adjuvant containing aluminum hydroxide and 3-deacylated monophosphoryl lipid, while the quadrivalent vaccine is composed of HPV 6, 11, 16 and 18 VLPs with aluminum hydroxyphosphate sulphate adjuvant.
Safety	Both vaccines are safe, without major adverse effects and generally well tolerated.
Immunogenicity	Both vaccines show high immunogenicity (100%) against vaccine-related HPV types. Higher antibody response against HPV 16 and 18 has been observed for the bivalent vaccine with AS04 adjuvant compared with the same antigens with only aluminum hydroxide adjuvant.
Efficiency	HPV vaccines are most efficient if administered to preadolescent or adolescent and young women before sexual debut. Boys may be vaccinated as well.
Efficacy	High efficacy (>90%) of both vaccines in preventing high-grade cervical disease (CIN2+ and AIS) caused by HPV 16 and 18 among women who were naive to these HPV types before vaccination, and received all three doses. High efficacy in preventing vulvar (VIN) and vaginal (VaIN) intraepithelial lesions caused by vaccine-related HPV types was observed with the quadrivalent vaccine (not assessed for the bivalent vaccine) in women who were naive to these HPV types before vaccination, and received all three doses.
Time of protection	Both vaccines provide high efficacy for several years after the first dose.
Protection against non-vaccine HR-HPV types	Vaccination with the bivalent vaccine has resulted in high seropositivity for non-vaccine types, HPV 31 (>70%) and 45 (>98%) types (phylogenetically close to HPV 16 and 18, respectively). Both vaccines show some group cross-protection against non-vaccine-HR-HPV types (HPV 31, 33, 45, 52 and 58) and associated cervical disease, as well as individual cross-protection against persistent infection with the non-vaccine HPV types and related CIN2+.
Protection against LR-HPV types	Vaccination with the quadrivalent vaccine shows high efficacy in preventing vaccine HPV types 6 and 11 and associated disease.
Therapeutic effect	Neither vaccine has shown a therapeutic effect against disease due to HPV types with which subjects were infected at baseline.
Delivery	National immunization HPV vaccination programmes have been introduced faster than any other new vaccine in the past in more developed countries but not in less developed countries. School-based programmes seems to be most efficient in coverage.
Limitation	High price of the vaccine, complex three dose vaccination schedule, and in some countries low compliance because of the insufficient advocacy campaigns before delivery. Vaccinated women have to be regularly screened because HPV vaccines will reduce, but not eliminate, the risk of cervical cancer.

HPV, human papillomavirus; HR, high-risk; LR, low-risk; VLP, virus-like particles; CIN, cervical intraepithelial neoplasia; AIS, adenocarcinoma *in situ*; VIN, vulvar intraepithelial neoplasia; VaIN, vaginal intraepithelial neoplasia.

60 years when cervical screening is no longer recommended for those women.

For oropharyngeal cancers, primary prevention by prophylactic HPV vaccination seems to be very promising. Besides HPV vaccination, especially against HPV 16, there is no secondary prevention by screening of early stages of the disease as there are no precancerous lesions that can be easily identified to be prognostic. However, prognosis of a treated patient can be determined by either HPV DNA assay, E6/E7 expression assay or $p16^{INK4A}$ immunostaining (a surrogate marker for HPV infection) of archival formalin fixed paraffin embedded specimens. Different treatment options for HPV-positive head and neck SCC are under consideration as HPV-positive head and neck SCC usually have better prognosis

Cervical screening

Cervical cancer has a long pre-clinical phase with precursor lesions that can be identified by cervical cytology and easily treated using simple outpatient procedures if they are detected at an early stage (**Figure 1**). Incidence and mortality rates for cervical cancer in deve-

loped countries have decreased dramatically in the past 25 years largely due to cervical cancer screening using Pap tests which allows for detection and subsequently treatment of precancerous lesions. Indeed, organized screening programmes have been proven to reduce cervical cancer mortality by more than 80%, notably when the compliance of the target population is high, which is the case in British Columbia (Canada) and Nordic countries (Finland, Denmark, Iceland, Norway and Sweden) *(52)*.

Although opportunistic screening can also substantially reduce cervical cancer rates, it will never attain the levels of reductions seen with an organized programme. In addition, opportunistic screening has been demonstrated to over-screen the wealthy and well-educated and under-screen lower socioeconomic groups and minorities, perpetuating health inequalities and wasting scarce healthcare resources *(53)*. This has been recognized by many international, European and national institutions, including: 1) the World Health Organisation's recommendation from 2006 *(54)* stating that cervical screening should only be offered in organized programmes, rather than opportunistically, 2) the European Code Against Cancer from 2003 *(55)* stating that women from 25 to 60 years of age should be screened at 3 to 5 year intervals within programmes with proper quality control procedures, and 3) the new European Guidelines for Quality Control in Cervical Cancer Screening that specifically state that cervical cancer screening should not be offered opportunistically *(56)*.

The limitation of cytology-based screening is the need to perform it regularly in intervals of generally 2-3 years, thus increasing the overall cost of the programme but also the low sensitivity of the Pap test. On the other hand, a negative HPV test virtually excludes any risk of having significant prevalent cervical disease and provides the same degree of protection over 5 years that the accepted standard of a negative Pap smear provides over 2 years *(57)*. These results indicate that HPV testing could also provide substantial cost savings for most countries by reducing the screening frequency with no increase in risk for the women being screened. Further savings could be realized because one laboratory technician could process more samples for HPV testing in a single working shift compared to the work done by several cytotechnicians that is required to process the same number of cytology samples *(56)*. In addition, training technicians to process HPV tests is far simpler and quicker than training cytologists to screen Pap smears and, with the dichotomous result produced by HPV testing, quality assurance procedures are also far simpler.

The use of HPV testing for primary cervical cancer screening, either alone or in combination with the Pap test, has been evaluated in large-scale population-based randomized clinical trials (RCT) in Canada, Finland, the Netherlands, Italy, Sweden, and the United Kingdom. From the combined findings of all these RCTs, HPV testing for primary screening consistently showed to be substantially more sensitive in detecting severe cervical lesions, but still less specific than cytology *(53, 58)*. The sensitivity of HPV testing was uniformly high at all ages, whereas the sensitivity of cytology was substantially better in women over the age of 50 than in younger women, while the specificity of both tests increased with age. The loss of specificity could be minimized by avoiding HPV screening in young women (age <30 year), using more specific HPV tests (RNA test for HPV oncogenes), and by appropriate triage algorithms.

The cytology could be recommended for triage of HPV-positive women. Other candidate markers for triage of HPV-positive women such as immunostaining of p16 or p16/Ki67 are being evaluated *(59)*. In addition, restricted genotyping of HPV types 16, 18 and 45 should also be considered for triage of HPV-positive women, especially because cervical cancer cases associated to those types appear at the average age of 50.0, 48.2 and 46.8 years, respectively, earlier than any other single HPV infection (55.5 years) *(5)*. The triage of HPV-positive screened women is justified especially for HPV types 18 and 45 which are mostly associated with adenocarcinoma that is more difficult to diagnose by cytology than SCC.

The overall results from the RCTs support the use of HPV testing as the sole primary screening test in women older than 30 years, with cytology reserved for women who test HPV positive. This approach does not cause substantial increases in diagnostic work-up and overtreatment and can be safely implemented within organized and quality-controlled population-based cervical screening programmes. Thus, the Netherlands is the first country with an official recommendation to introduce HPV-based primary screening *(60)*. However, further research is needed to establish the optimal age to start screening (30 or 35), the screening interval in HPV--negative women (5 or more years) and the optimal management of HPV-positive women (cytology, HPV genotyping or other biomarkers that can identify women at risk for progressive disease) (Figure 1).

As the size of the vaccinated population increases, HPV vaccination will produce progressive reductions in the prevalence of HPV infection and, with this, progressive reductions in the prevalence of cytological abnormalities *(49)*. The mathematical consequence of this is that the PPV of cervical cytology will steadily diminish eventually to the point when virtually all positives are false positives and the use of cytology will be neither cost-effective nor ethically justifiable. At this time, it is inevitable that cytology will have to be applied to those who are at highest risk, i.e. those who are HPV-positive.

An important issue for the implementation of HPV testing as primary screening is its cost-effectiveness in large-scale population-based screening programmes. The cost analysis of the NTCC trial indicates that if HPV testing is applied with cytology triage, a single HPV test may cost 20-30% more than a conventional Pap test and still result in the same overall cost per CIN 2+ detected *(61)*. However, if the price of HPV testing decreases in the future, better estimates can be expected.

The situation of cervical cancer prevention in Croatia is hardly well established, in spite of the fact that opportunistic screening has been applied for long time. A matter of concern is the steady state of the mortality rate. Thus, appropriate cervical cancer prevention programme including organized screening with HPV testing as primary screening and cytology triage, and organized HPV vaccination should be set up without delay in Croatia *(33*, *62*, *63*).

CONCLUSION

The presented data suggest that cervical cancer is the most important HPV-related disease although other related cancers and benign proliferation (genital warts and laryngeal papillomatosis) represent a high public health burden. Nationwide population-based organized cervical cancer prevention programmes that include both, primary prevention (HPV vaccination) and secondary prevention (screening) with preliminary comprehensive organized education can effectively reduce cervical cancer rates. In addition, offering free services within a programme of cervical cancer prevention (invitation to HPV vaccination and screening) could be a solution to the problem of cervical cancer control but also of other HPV-related cancer and diseases. However, the price of HPV vaccination is a limiting factor for its implementation, especially in low-income countries. Therefore, cervical cancer policies (vaccination and/or screening) will probably have to be adapted to the specific needs and the availability of resources of each country. The choice of a type of the primary screening test (cytology or HPV testing), the length of screening intervals, and the nature of the follow-up strategy will largely influence the cost--effectiveness of a cervical cancer screening programme. However, future cervical cancer control primarily has to rely on cervical screening, and both vaccinated and non--vaccinated women have to be regularly screened because HPV vaccines will reduce, but not eliminate, the risk of cervical cancer. A more distant goal is the development of therapeutic vaccines as adjuvant treatment for infections or cancers associated with HPV *(64)*.

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