Pathogenesis of genital HPV infection

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Abstract

Clinical, subclinical, and latent human papillomavirus (HPV) infections are distinguished from HPV-associated neoplasia. Besides HPV additional cofactors are necessary to transform HPV infected tissue to intraepithelial or invasive neoplasia. Risk factors for the presence of HPV are high number of sexual partners, early cohabitarche, young age at first delivery, suppression and alteration of immune status, young age and hormonal influences. While the fact of a high number of sexual partners exclusively increases the risk of HPV infection, it is not known whether the other factors lead to either an increased risk for HPV infection and/or to HPV-associated neoplasia.

Subclinical and latent genital HPV infections are highly prevalent. The prevalence rate depends on the sensitivity of the HPV detection system used, on age and sexual activity of the population screened, and on the number of subsequent examinations performed for each subject.

Sexual transmission is the main pathway for genital HPV's, however, vertical, peripartal, and oral transmission are also possible.

Seroreactivity against genital HPV 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. Compared with humoral response cellular immune response is probably more important for regression

of genital HPV infection: impaired cellular response is characterized by depletion of T helper/inducer cells and/or Langerhans cells and impaired function of natural killer cells and/or the infected keratinocyte.

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 observed in longitudinal investigations show a constant come-and-go of HPV presence. Subclinical or latent cervical infections with high-risk HPV types (such as HPV 16 and 18) have an increased risk for the development of HPV-associated neoplasia.

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Introduction

There is general consensus that genital human papillomaviruses (HPV) play a major causal role in the development of cervical cancer.¹ Despite increasing knowledge about the biological potential of genital HPV's in connection with anogenital carcinogenesis relatively little is known about the pathogenesis of genital HPV infections. The following review tries to summarise the current knowledge on such aspects as prevalence, transmission, course of infection, immune response and risk factors.

Definition and diagnosis

For the classification of genital HPV-associated lesions a generally accepted nomenclature is not available. However, most clinicians differentiate HPV infections (benign disorders) from HPV associated diseases (pre-malignant or malignant disorders) since in addition to HPV other factors are needed to transform the epithelium to its premalignant or malignant state (fig). Moreover, clinical infections are distinguished from subclinical and latent infections. Clinical HPV infections such as condylomata acuminata cause symptoms and are easily recognised clinically. Subclinical HPV infections such as flat condylomata may be diagnosed by the presence of koilocytes or dyskeratocytes which are

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Fig Risk factor for genital HPV infections and HPV-associated diseases and their assumed points of action (modified after a poster by Bauer and Manos).

Table 1 Spectrum of various HPV types detected by three commonly used HPV detection systems

HPV	6/11	16	18	26	31	33	35	39	40	42	43	44	45	51	52	53	54	55	56	57	59
*	6/11	16	18	26	31	33	35	39	40	42			45	51	52	53	54	55	56	57	59
†	6/11	16	18		31	33	35	39			43	44	45	51	52				56		
‡	6/11	16	18		31	33	35			42	43	44	45	51	52				56		

*Consensus PCR L1: Manos *et al.*, 1989¹⁶⁸
†General/Type-specific PCR L1: Snijders *et al.*, 1990¹⁶⁹
‡Hybrid Capture™: Imprim *et al.*, 1993¹⁷⁰

specific morphological signs for permissive HPV infection.² These signs, however, are only present in the minority of subclinical lesions; other colposcopic or cytomorphologic criteria possibly present in subclinical HPV infection are so subtle and non-specific that their diagnosis is difficult to reproduce.³⁻¹⁵ Therefore, virological assays must be applied for specific diagnosis of subclinical disease, virus detection being the only way for a proper diagnosis of latent HPV infection, since, by definition, morphological changes are absent.

For detection of the various genital HPV types of which 28 have been established so far identification of viral DNAs or RNAs by various hybridisation techniques is used. When analysing results of published material on HPV it is crucial to know the validity of the different techniques, their sensitivity and specificity, and the spectrum of HPV types detectable (Table 1). No perfect hybridisation method exists at present. Southern blot hybridisation is still used as the gold standard for reliable DNA identification of the various HPV types; however, between 5000 and 50 000 HPV DNA copies need to be present in a clinical sample in order to be detected. Recently, the more sensitive technique of HPV DNA amplification using the polymerase chain reaction (PCR) followed by simple methods of hybridisation has been widely applied, especially in large epidemiological studies. This permits between 10 and 100 HPV DNA copies to be discovered. In addition, combined with phylogenetic classification, new HPV types can be established.¹⁶⁻¹⁸ With PCR HPV-type-specific primers allow the detection of one HPV type whereas consensus or general primers amplify a whole panel of different HPV types (table 1). Standardisation of PCR methods will be necessary to allow a valid comparison of results generated by different laboratories.¹⁹

Prevalence and incidence

The prevalence estimates depend on the presence of factors for STDs, on the age of the population screened, the sensitivity of the molecular-biological technique applied, and on the number of examinations performed. In women single-point estimates using dot-blot hybridisation vary between 1% and 20%,²⁰⁻²³ 8% and 11% using filter in situ hybridisation,^{24 25} 3% and 29% using Southern blot,²⁶⁻³⁰ and 5% to 53% using general and HPV type-specific PCR,³¹⁻³⁵ and M. Schiffman and M. Manos (personal

communication). These prevalence estimates comprise subclinical and latent disease since most studies do not include information on whether or not the patients had lesions. The prevalence rates for males may be similar³⁶ but large-scale evaluation with PCR techniques has not been reported so far. Cumulative prevalence estimates are two-four times higher than single-point measurements.^{24 37 38}

With respect to incidence, an eight-fold increase in the age- and sex-adjusted incidence of condylomata acuminata between 1950 and 1978 was reported for Rochester, USA,³⁹ physician visits for condyloma increased 4-5-fold between 1966 and 1984 in the USA⁴⁰ and the incidence of condyloma among males and females in the United Kingdom increased 2.5-fold between 1971 and 1982.⁴¹ For incidence estimates of subclinical and latent HPV infection no reliable data are available as yet.

Transmission

Sexual transmission

For condylomata acuminata the interval between exposure and appearance of clinical disease ranges from 3 weeks to 8 months.^{42 43} Data on incubation periods for subclinical and latent infection are not available. A direct correlation between number of sexual partners and presence of HPV is evident⁴⁴⁻⁴⁶ and was proved to be independent of other risk factors such as age, race or use of oral contraceptives: 21% of women (n = 90) reporting exposure to only one male sexual partner were positive for HPV by consensus PCR compared with 69% of women (n = 102) reporting exposure to 10 or more partners (OR = 11.2, 95% CI 4.9-24.4).⁴⁷ Previous studies which showed no association between HPV detection and sexual activity^{37 48-50} probably misclassified the HPV status of a small fraction of subjects which may distort true association.⁵¹ Subclinical HPV infections and HPV associated disease are prevalent (64% to 70%) in male partners of women with cervical HPV infections and intraepithelial neoplasia.^{52 53}

Non-sexual transmission

Transmission of HPV 6 or 11 to the infant by an infected mother can result in recurrent respiratory papillomatosis (RRP), albeit a rare event.⁵⁴ It is still unclear if transmission is vertical through the intact amnion or during birth. The peripartal transmission is favoured by a study which showed HPV DNA in 33% of 45 nasopharyngeal aspirates from neonates, using Southern blot hybridisation, and HPV DNA in only two samples of amniotic fluid.⁵⁵ Only 2.8% (2 of 72) oropharyngeal swabs of newborns were positive for HPV by dot-blot hybridisation in another study.⁵⁶ Peripartal transmission to the genital area (n = 4) and the oral cavity (n = 2) was shown in newborns from ten HPV-positive mothers by type-specific PCR analysis.⁵⁷ In preschool children (n = 21) oral cavity scrap-

ings were positive for HPV 6 in 24% and for HPV 16 in 19% by type-specific PCR compared with 17% and 23%, respectively, in adult men (n = 35).⁵⁸ Thus, the oral cavity may act as a reservoir for genital HPVs. In addition, presence of “high risk” HPV types such as HPV 16 and HPV 33 may induce malignant lesions as shown for tonsillar carcinomas.⁵⁹

Genital HPV types such as HPV 16 and HPV 35 may also be present in skin lesions of the periungual area and be potentially infective for the genital area.^{60 61}

Fomites may play a role in transmitting the virus since HPV DNA could be demonstrated on a small number of gynaecological instruments after sterilisation.⁶² The infectious potential of such findings has, however, still to be proved.

Course of infection

In order to establish infection, HPV needs access to the basal cells of the differentiating epithelium which occurs most commonly through microlacerations. The time course of infection has been studied on human xenografts in nude mice infected with HPV 11 using *in situ* hybridisation.⁶³ The first signs of transcription from the early (E) open reading frames (ORF) of the viral genome can be seen 4 weeks after infection.⁶³ Between weeks 6 and 8 viral replication, transcription of early and late (L) ORFs and cellular proliferation reach a plateau and by weeks 10 to 12 condylomata are morphologically fully established. The amount of viral DNA increases towards the epithelial surface with the highest amount of viral replication and antigen production in the most differentiated, superficial cells which was also shown on human tissues.⁶⁴ The viral DNA is encoated by viral capsid proteins and infectious viral particles are released by interference with the viral E4 protein which destabilises the intracellular cytokeratin network.⁶⁵ Whereas in low grade lesions viral transcription is weak in the basal and parabasal layers, strong transcriptional activity is seen throughout the whole epithelium in high grade lesions.⁶⁶ Little is known about the time course of events in subclinical and latent infected tissues since conventional *in situ* hybridisation techniques fail to detect viral DNA in the majority of tissues^{11 12 14 15} shown to be HPV-infected by hybridisation techniques which destroy the morphology of the lesions.^{67 68} This discrepancy may be solved in the future by the combination of PCR and *in situ* hybridisation.^{69 70} A collagen raft culture system containing cells derived from a cervical intraepithelial neoplasia (CIN) lesion

recently allowed the production of HPV 31b virions.⁷¹ More data on the cellular and viral factors responsible for maintaining or terminating latency, and on the synthesis and assembly of HPV virions can be expected from studies using this system.

No systematic evaluation has so far been carried out to study the spontaneous regression rate for condylomata; however, in placebo-controlled studies this rate varies between 0%, 17%, 18%, and 69%.⁷²⁻⁷⁵ After regression or treatment of clinical disease 45% of patients retain latent infection with a recurrence rate of clinical disease in 67% of latently infected patients.⁶⁸

Longitudinal evaluation of subclinical or latent HPV infection is difficult since reactivation or regression may be masked by reinfections through the same or a new sexual partner. In a follow-up study of 51 adolescents using Southern blot analysis HPV was detected in 58% (n = 30/52) on at least one occasion. Only 9.6% (5/52) of patients were HPV positive at two visits, but only one patient had an identical HPV type at both visits.⁷⁶ When HPV 16 type-specific PCR was used in 21 women over a one-year period 14 patients (66.7%) were HPV 16 positive at least once.³⁸

The oncogenic potential of specific genital HPV types such as HPV 16 and 18 was demonstrated by *in vitro* transformation assays involving human keratinocytes and viral DNA.⁷⁷ The viral proteins derived from the E6 and E7 ORFs interact with cellular proteins such as p53 and p105-RB which control cell cycle and DNA repair.⁷⁸⁻⁸⁰ *In vivo* the biological potential of the various genital HPV types has been assessed in large cross-sectional series and could be confirmed in case-control studies^{81 82} (table 2). The meaning of HPV negativity for cervical carcinogenesis especially in the elderly is not yet clear,⁸³⁻⁸⁶ whereas in vulvar cancer HPV negativity represents a specific entity more common in older patients.⁸⁷⁻⁹⁰ The extent of risk of subclinical and latent HPV infection for the induction of anogenital neoplasia has been determined prospectively: early prospective studies on patients with cervical condyloma or CIN I showed an increased risk for progression in the presence of HPV 16 or 18.⁹¹⁻⁹³ Subclinical and latent HPV infection was the starting point for prospective follow-up of cytologically negative women: Consensus primer PCR was used for HPV detection in 18000 women and after a median of 360 person-days 155 women had developed squamous intraepithelial lesions (SIL) of varying severity (M Schiffman, M Manos, personal communication). When analysed in a nested case-control evaluation with three controls per case the presence of high risk HPV types 16 or 18 in the enrolment smear was associated with a relative risk (RR) of 7.9 (95% CI 4.4, 14.4) for the development of cervical neoplasia. Positivity of HPV at enrolment and diagnosis had a RR of 16.1 for SIL. A second cohort study of 241 STD clinic attendants with a history of nega-

Table 2 Biological potential of minor genital HPV types

● “low risk”:	HPV 6,11,42,43,44	mainly in “low grade SIL”
● “intermediate risk”:	HPV 31,33,35,51,52,58	mainly in “SIL”
● “high risk, HPV 16”:	HPV 16	“high grade SIL” & cancer
● “high risk, HPV 18”	HPV 18,45,56	more frequent in cancer than in “SIL”

Lörincz *et al.*, 1992⁸¹
SIL: squamous intraepithelial lesion.

tive Pap smears over a two-year period using dot-blot and Southern blot hybridisation showed biopsy-confirmed CIN II-III in 3% of those without detectable HPV DNA, compared with 39% of women positive for HPV 16 or 18 infection (RR = 11.03, 95% CI 4.64, 26.23).⁹⁴ Besides the presence of high risk HPV types, persistent detection and, possibly, the amount of viruses are important prognostic factors.³⁸ In addition, mutations in the transforming HPV genes as shown for E7 may explain variations of biological behaviour in different geographic regions.⁹⁵

Immune response of the host

Antibody response

Serological assays have the potential of being more accurate, sensitive and relevant for the detection of disease progression, compared with direct HPV DNA detection in gynaecologic smears. After several genital HPV-types had been sequenced, structural and regulatory HPV proteins could be expressed in various vectors and synthetic peptides designed. HPV 11 virions became available through the nude-mouse xenograft system. The raft culture system will provide an additional source for viral particles of other genital HPV types. Bacterial fusion proteins have been generated from some early and all late ORFs of HPV 6, HPV 11, HPV 16, and HPV 18 and were used in Western blot assays and ELISA to detect HPV typespecific antibodies.^{58 96-102} Immunoreactive synthetic peptides were selected by different methods designated for HPV 6 L2, HPV 11 L2, HPV 16 E2, L1, L2, and HPV 18 E7, L2.¹⁰³⁻¹¹⁴ Recently, HPV 16 E7 was generated for radioimmuno-precipitation analysis (RIPA) of human sera using an *in vitro* transcription-translation system.¹¹⁵

The results of these studies can be summarised as follows: (i) prevalence rates of antibody responses to late proteins are more common than to early proteins ranging between 25% and 65%,^{58 96 97 99 100 116} (ii) the antibodies detected seem HPV type-specific and do not cross-react,^{58 100 108 112 116 117} (iii) antibodies to HPV 16 E7 are strongly associated with the presence of cervical cancer,^{99 101 107 113 115 118} (iv) antibodies to HPV 16 E4 may also be markers of cervical cancer risk^{99 118} and of current or recent HPV infection,¹⁰¹ (v) prevalence estimates of IgG antibodies for adults and children do not differ and vary between 10 and 75% for the different HPV types,^{58 101 119} (vi) in only 50% to 70% of patients with proven HPV 16 or HPV 18 positive tumors antibodies can be detected.^{101 102 108 113 115} Compared with bacterial fusion proteins or synthetic peptides, intact virions present conformational epitopes which may be more specific.¹²⁰ HPV 11 virions generated in the nude-mouse xenograft system were used to examine sera from patients with condylomata acuminata and RRP; significantly higher absorbance values were found in cases compared with controls.^{121 122} Besides the possibility of creating

whole virions with the raft system, *in vitro* production is also feasible by way of recombinant vaccinia virus vectors¹²³ which may be used for immunological studies in the near future.

Cell-mediated immune response

Competent cell-mediated surveillance is required for the control of HPV infection as immunosuppression or immunocompromising diseases increase the incidence of genital HPV infections and HPV associated disease. Mononuclear-cell inflammatory infiltrates are the major morphological feature in regressing skin warts.¹²⁴ Invasion of T-suppressor cell subsets and macrophages is probably stimulated by the virus. The role of T lymphocytes, Langerhans cells, natural killer cells and keratinocytes for local immune control in genital HPV-infected lesions has been investigated in a number of studies: condylomata are depleted of T-helper cells with a reversed T4/T8 ratio to less than 1 (95% CI 0.29, 0.98).¹²⁵ A significantly higher percentage of suppressor/cytotoxic T-cells (T8), a lower proportion of helper/inducer T-cells (T4) and a lower helper/suppressor T-cell ratio (1.72 against 3.21, $p < 0.05$) is found in the peripheral blood of patients with condylomata.^{126 127} *In vitro* translated viral fusion proteins, tested in a skin test, evoked reactions against proteins of the L1 ORF more frequently in patients with CIN (six out of seven patients) when compared with controls (0 out of 10).¹²⁸

A significant decrease of Langerhans cells is seen in cervical condylomata and CIN lesions.^{125 129-131} This applies to all Langerhans cell subpopulations examined¹²⁵ and is more significant in HPV 18-infected lesions compared with HPV 16-positive lesions.¹³⁰ Expression of the MHC class II antigen (HLA-DR and HLA-DQ) is increased in Langerhans cells of condylomata and CIN ($p < 0.05$),¹²⁹ but Langerhans cell activity does not seem to be predictive for recurrence of CIN.¹³² Production of interferon gamma and interleukin 2 by natural killer cells is decreased in condylomata.¹²⁷ Natural killer cells from patients with Bowenoid papulosis and anogenital cancer show decreased lytic activity against HPV 16-bearing keratinocytes which appears to be due to defective recognition of the disease-specific target cells.¹³³

In keratinocytes of cervical condylomata major histocompatibility class II antigens (HLA-DR) were not expressed.¹³⁴ This malfunction of antigen presentation leads to defective immunological surveillance.¹³¹

HLA type

In a recent cross-sectional study presence of the HLA class II DQw3 antigen was associated with a seven-fold increased risk for cervical cancer, with a less strong association for DR5 antigen and a protective effect of DR6 antigen.¹³⁵ However, another investigation could not confirm the hypothesis that certain HLA haplotypes carry an increased risk for possible acquisition of HPV and, thus, for the

development of squamous carcinoma of the cervix.¹³⁶

Risk factors for detection of genital HPV infection

A number of risk factors have been associated with the development of cervical cancer.¹³⁷ Only part of these factors have been examined in connection with genital HPV infection so far. The majority of studies are not designed to clearly pinpoint the scope of action of the risk factor examined: does it increase the risk for HPV infection, for HPV associated neoplasia or for both (fig)?

Sexual behaviour

The number of sexual partners has been recognised as an independent risk factor for the acquisition of genital HPV infection (see under *Sexual transmission*) and is probably not associated with events which transform HPV infection to HPV associated neoplasia. This may be different for early cohabitation which was shown to be an independent risk factor for cervical cancer with an odds ratio (OR) of 4.3 (95% CI 2.1, 9.0) for age <16 years vs. 24+ years in a case-control study in Colombia and Spain where HPV status was measured by consensus-PCR.⁸⁵

Immunosuppression

Renal allograft recipients have an increased risk for genital warts.^{138, 139} This was confirmed for latent and subclinical HPV infections when 27% of women (n = 49) with renal allografts were positive for HPV-16 or 18, compared with only 6% in the controls (n = 69).¹⁴⁰

HIV and other infectious agents

Individuals positive for HIV have a high rate of HPV infections and HPV associated neoplasia. This phenomenon is currently explained by the alteration of the immune status in the HIV-infected individual which increases the risk for acquisition of a new HPV infection or reactivation of a latent HPV infection. Transactivation of viral replication may be another pathway of interaction between these viruses but seems unlikely since there is no overlap in the human cell types targeted by HPV and HIV.

Cytological signs of HPV infection were found in women positive for HIV at a significantly higher rate compared with women at risk for HIV infection¹⁴¹ or with women negative for HIV.¹⁴² Symptomatic HIV-infected women (n = 33) showed a 70% HPV prevalence in cervico-vaginal lavages by Southern blot which was significantly higher than in HIV-positive asymptomatic women (4 of 18, 22%) or in HIV-negative high-risk women (10 of 45, 22%).¹⁴³ Symptomatic HIV-infected women showed a strong association between HPV infection and the presence of CIN with an OR of 12 (95% CI 1.3, 108). In an East-African population detection of HPV 16 and 18 by Southern blot and PCR was 2.2-times more frequent in HIV-positive

women; this was, however, not associated with a higher rate of abnormal PAP smears.¹⁴⁴

In men analysis of anal smears (n = 105) by dot blot hybridisation and PCR revealed a significantly higher rate of HPV positivity in 53% of HIV positives, compared with 29% in HIV negatives.¹⁴⁵ A low T-helper cell count was an independent risk factor for the presence of HPV. In another study of homosexual men (n = 120) anal smears were scrutinised for HPV by dot blot hybridisation and a T-helper/T-suppressor cell ratio of <0.4 was associated with a significantly higher HPV positivity of 35.3% of men positive for HPV DNA compared with only 7.3% of men with a ratio of >1.0.¹⁴⁶ Cytological abnormalities in anal smears were strongly correlated with the presence of HPV in these two studies of homosexual men.^{145, 146}

Besides HPV, HSV2 may play an independent or supporting aetiological role for the development of cervical neoplasia. A significant interactive effect between HPV 16 and 18 measured by filter in situ hybridisation and detection of seropositivity for HSV2 for invasive cervical cancer was seen in a Latin American case-control study.¹⁴⁷ These results have still to be confirmed by studies with more reliable virological assays.

No conclusive data are available for other infectious agents such as chlamydia, syphilis, gonorrhoea, cytomegalovirus, Epstein-Barr virus and bacterial vaginosis.¹⁴⁸

Age

The peak prevalence rate of HPV in gynaecologic smears spans the age range of 20 to 24 years, with a steady decline as age progresses^{24, 47} and M Manos, personal communication. Consensus PCR applied to smears obtained from patients under 25 years (n = 872) showed that 43% were HPV-positive, compared with 32% in the 26- and 35-year age group (n = 617) and 21% in women older than 35 years (n = 21%) (M Manos, personal communication). Since the various HPV types showed a similar distribution pattern throughout all age groups a cohort effect cannot explain this phenomenon. Acquisition of immunity with increasing life span is a more likely explanation.

Pregnancy

The influence of pregnancy on HPV detection is controversial although the majority of studies find a higher HPV detection rate during pregnancy: 8% to 20% in non-pregnant and 9% to 35% in pregnant women.^{27, 149-154} Four out of six studies found an increasing prevalence during pregnancy and three out of four studies describe a decrease of HPV detection post partum. Early age at first birth was an independent risk factor for cervical cancer with an OR of 5.0 (95% CI 1.8, 14.2) for age < 16 years vs. 24 years in a consensus PCR-based case-control study.⁸⁵ It may be speculated, that an active transformation zone, exposed to a high HPV load at the end of pregnancy and to trauma during birth at

young age when immunity against HPV is still immature, may be the scenario required for the development of cervical neoplasia.

Oral contraceptives, hormones

Patients on oral contraceptives (OC) have a RR of 1.5 for condylomata acuminata which increases to 9.8 after long-term use.¹⁵⁵⁻¹⁵⁶ Using Southern blot analysis, current OC use was associated with HPV positivity in patients with normal Pap smears ($p < 0.001$) and patients with reactive atypia ($p = 0.03$) but not in patients with CIN.¹⁵⁷ An independent and partly linear association between OC use and HPV detected by consensus primer PCR was seen in female students with an OR of 2.8 after one year OC use and OR of 4.6 after 4 to 5 years of OC use.⁴⁷ OC use may have a synergistic effect with HPV since increased risk for cervical cancer was only seen in HPV positive women.⁸⁵ Hormonal factors influence transcription and/or translation of the HPV genome also *in vitro*. An eight-fold overexpression of transcripts from the HPV 16 E6 and E7 open reading frames is achieved by treatment of Siha cells, a cervical cancer cell line, with β -oestradiol,¹⁵⁸ and oncogenic transformation of primary baby rat kidney cells in the presence of HPV 16 DNA and the *ras* oncogene is achieved by treatment with progesterone or progestins used in OCs.¹⁵⁹ The expression of progesterone receptors is significantly associated with high-grade CIN and HPV 16 and HPV 18 positive cervical lesions.¹⁶⁰

Smoking

History of smoking is associated with an increased risk for condylomata acuminata (RR = 3.7; 95% CI 1.8-7.6).¹⁵⁶ Elevated concentrations of nicotine and cotinine were found in the cervical mucus of smokers and may transform HPV infected tissue.¹⁶¹ Smoking acts immunosuppressively on the cervix by decreasing the Langerhans cell population¹⁶² and may, alternatively or synergistically, promote the acquisition of HPV infection. A synergistic effect between smoking and HPV has been suggested by a case-control study which found a limited effect on cervical cancer risk only in HPV positive women.¹⁶³

Nutritional factors

For HPV-associated disease an increased risk is reported in patients with deficiencies of vitamin A, β carotene, vitamin C, and folic acid.¹⁶⁴ Using Southern blot for HPV 16 detection, low folate level was an independent risk factor for HPV with a RR of 1.1 among women with folate above 660 nmol/l, compared with a RR of 5.1 (95% CI 2.3, 11) among women with lower levels.¹⁶⁵ No data on the influence of antioxidants or folic acid on genital HPV infections are available. *In vitro* bovine papilloma virus (BPV)-containing mouse cells can be depleted of virus with an all-*trans*-retinoic acid treatment, and cell transformation is reversed.¹⁶⁶ In rabbits infected with cottontail rabbit

papilloma virus (CRPV) vitamin A treatment leads to regression of papillomas.¹⁶⁷

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