

Human papillomavirus and cervical cancer

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Cervical cancer is caused by human papillomavirus infection. Most human papillomavirus infection is harmless and clears spontaneously but persistent infection with high-risk human papillomavirus (especially type 16) can cause cancer of the cervix, vulva, vagina, anus, penis, and oropharynx. The virus exclusively infects epithelium and produces new viral particles only in fully mature epithelial cells. Human papillomavirus disrupts normal cell-cycle control, promoting uncontrolled cell division and the accumulation of genetic damage. Two effective prophylactic vaccines composed of human papillomavirus type 16 and 18, and human papillomavirus type 16, 18, 6, and 11 virus-like particles have been introduced in many developed countries as a primary prevention strategy. Human papillomavirus testing is clinically valuable for secondary prevention in triaging low-grade cytology and as a test of cure after treatment. More sensitive than cytology, primary screening by human papillomavirus testing could enable screening intervals to be extended. If these prevention strategies can be implemented in developing countries, many thousands of lives could be saved.

Introduction

One of the most important scientific discoveries of the past 30 years is the causal link between human papillomavirus infection of the cervix and cervical cancer. This finding resulted from the original seminal findings by Harald zur Hausen and his group, that human papillomavirus 16 can be detected in cervical cancer tissue, and was followed by an enormous worldwide effort involving epidemiologists, molecular biologists, vaccinologists, and clinicians culminating in the development of effective prophylactic vaccines for human papillomavirus, which have the means to prevent 70–80% of cervical cancer. zur Hausen was awarded the Nobel Prize in Physiology or Medicine in 2008, in recognition of his discovery.

Human papillomavirus belongs to the papillomavirus family of viruses, which have a diverse range of hosts in both animals and man. The family has an agreed taxonomy that is based on genome sequence homology, biological function, and pathological effect.¹ More than 100 types of human papillomavirus have been identified, including 13 high-risk types, which are responsible for cervical neoplasias and other anogenital and oropharyngeal cancers.

We review the worldwide epidemiology and natural history of cervical human papillomavirus infection, the virus's lifecycle, and the process of viral oncogenesis. We then discuss how the unique relationship between human papillomavirus and cervical cancer has been exploited for primary (prophylactic vaccines) and secondary (screening) prevention.

Epidemiology

Human papillomavirus infection is the most common sexually transmitted infection worldwide and most sexually active individuals of both sexes will acquire it at some point during their life.² On the basis of a meta-analysis³ of 1 million women with normal cervical cytology, around 291 million women worldwide are estimated to have human papillomavirus infection of the cervix at a given point, corresponding to an average prevalence of 10·4%, though prevalence is higher in women younger than 25 years (16·9%). Human

papillomavirus types 16 and 18 account for roughly 70% of all cervical cancer. Type 16 has been detected in about 24% of women with human papillomavirus infection; type 18 has been detected in about 9%.³

The International Agency for Research on Cancer HPV Prevalence Surveys⁴ included roughly 28 000 women from 26 different regions, mainly in developing countries (figure 1). The Surveys used a standardised protocol for population-based recruitment and detection of human papillomavirus. The prevalence of human papillomavirus was high in countries where the burden of cervical cancer is high—ie, in sub-Saharan Africa, Latin America, and India,⁴ but also in countries, such as Mongolia⁵ and China,⁶ in which the disease burden is uncertain.⁷ Human papillomavirus prevalence in developed countries peaks in young women and decreases after 35 years of age.⁴ In some regions—eg, some Latin American countries⁴—a small second peak in human papillomavirus prevalence occurs in middle-aged women older than 55 years. Human papillomavirus prevalence was high and much the same across all ages in several low-income and middle-income countries (India,⁴ China,⁶ and some African countries^{8,9}). The peak in human papillomavirus prevalence in young women is partly caused by changes in sexual behaviour in some countries.¹⁰ Long-term follow-up studies of human papillomavirus infection should be done to disentangle age-specific and cohort-specific effects and more research is needed to assess the

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Search strategy and selection criteria

We searched the Cochrane Library and PubMed for relevant randomised trials and other high-quality studies (eg, systematic reviews, meta-analyses) between Jan 1, 2000, and July 1, 2012, for the terms "HPV", "human papillomavirus", "HPV vaccination", "cervical cancer", "cervical carcinoma", "cervical neoplasia", and "cervical carcinogenesis". Widely cited older publications that we judged to have remained important references were also included. References from relevant articles identified by our search strategy were also searched.

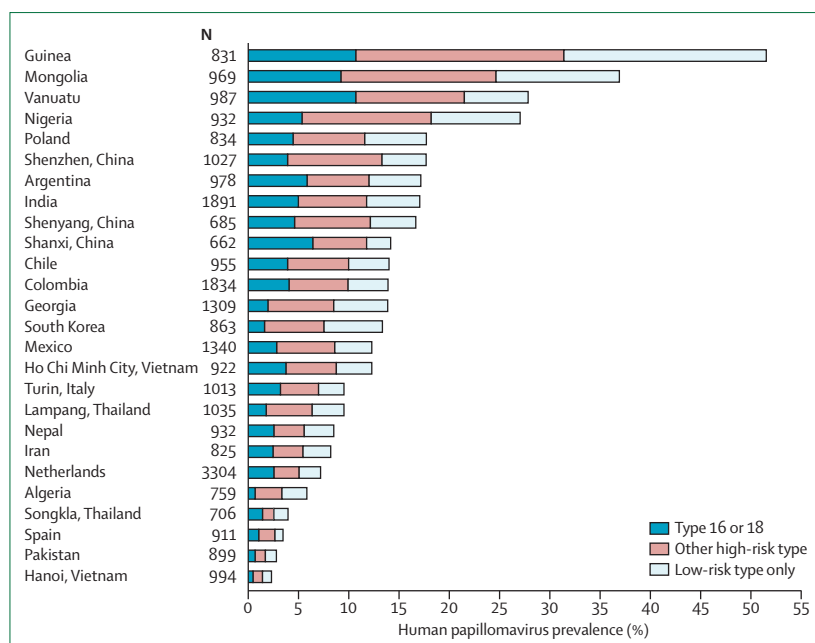


Figure 1: Age-adjusted prevalence of cervical human papillomavirus DNA in sexually active women aged 15–69 years

Data are from IARC Prevalence Surveys, 1990–2012.⁴

	High-grade lesions		Cervical cancer	
	Type 16	Type 18	Type 16	Type 18
Europe	54.4 (5.6)	7.7 (1.1)	66.7 (2.0)	16.4 (4.6)
North America	56.8 (3.1)	9.6 (2.7)	61.2 (3.2)	19.6 (4.3)
South and Central America	52.8 (8.1)	9.4 (3.5)	59.5 (2.8)	12.7 (4.5)
West and Central Asia	68.4 (16.4)	6.3 (5.0)	73.0 (4.6)	15.1 (3.7)
East Asia	37.9 (7.1)	7.4 (1.9)	61.7 (5.9)	15.8 (2.6)
Oceania	53.9 (3.5)	9.6 (1.7)	62.6 (5.4)	21.2 (4.2)
Africa	30.3 (5.2)	9.2 (2.8)	53.1 (4.4)	19.8 (4.1)

Data are taken from Guan and colleagues.²² Data are % (±1.96 SE).

Table: Positivity for human papillomavirus types 16 and 18 as a proportion of human papillomavirus-positive samples in high-grade lesions and cervical cancer by region

reasons for the large variations in human papillomavirus prevalence by age across populations.⁴

Prospective studies have shown that the prevalence of human papillomavirus includes a mix of incident and persistent infections that have accumulated over time because of lack of clearance.^{11,12} More than 90% of new human papillomavirus infections at any age regress in 6–18 months¹³ and more persistent infection is a prerequisite for progression to cervical intraepithelial neoplasia (CIN). CIN1 is an insensitive histopathological sign of human papillomavirus infection,¹⁴ CIN2 includes a heterogeneous group of lesions that have different potential to progress to cancer, and CIN3 represents the most clinically relevant lesions and is the best surrogate endpoint for cervical cancer in screening and vaccination trials. The probability of clearance of human

papillomavirus depends on the duration of infection;^{15,16} longer persistence reduces the probability of clearance. Human papillomavirus infections detected in women aged older than 30 years persist for longer than those in younger women because they are more likely to be persistent infections of long duration.^{15,16}

The only clear risk factors for persistence and progression of human papillomavirus are immunodeficiency (eg, HIV-positive women and transplant recipients)¹⁷ and the human papillomavirus type, although sexual and reproductive factors, recent oral contraceptive use,¹⁸ smoking,¹⁹ and *Chlamydia trachomatis* infection²⁰ have also been implicated.¹³ Human papillomavirus types have been classified as either carcinogenic or probably carcinogenic¹⁷ (ie, high-risk human papillomavirus) and type 16 is by far the likeliest to persist and cause CIN3 and cervical cancer.²¹ In a study by Castle and colleagues,¹³ women who twice tested positive for type 16 after a 9–21 month interval had a 3-year cumulative incidence of CIN2 or worse of 40%. The corresponding cumulative incidence was 15% for type 18 and 9% for other high-risk types. Genotyping might therefore improve risk stratification of women with human papillomavirus in cervical screening programmes. A meta-analysis²² has investigated the cross-sectional distribution of high-risk human papillomavirus types across the full spectrum of cytopathological and histopathological cervical diagnoses (table). It included 116 000 women with human papillomavirus (including 36 374 cervical cancers) from 432 studies using PCR-based human papillomavirus DNA testing. Worldwide, the most common human papillomavirus types in cervical cancer were types 16 (57%), 18 (16%), 58 (5%), 33 (5%), 45 (5%), 31 (4%), 52 (3%), and 35 (2%).^{22,23} Types 16, 18, and 45 accounted for a greater or equal proportion of infections in cervical cancer compared with normal cytology (panel 1); the ratio between cervical cancer and normal cytology was 3.1:1 for type 16, 1.9:1 for type 18, and 1.1:1 for type 45. Other high-risk types accounted for substantial proportions of CIN2 and CIN3, but their contribution to cervical cancer was low, with ratios ranging from 0.9:1 for type 33 to 0.2:1 for type 51.

Human papillomavirus is one of the most powerful human carcinogens and has been implicated in cancers at several sites. Roughly 610 000 new cancers per year (5% of all cancers) have been attributed to human papillomavirus infection, of which more than 80% occurred in developing countries.²⁴ Such cancers include effectively all cervical cancer (ie, around 530 000 cases per year) and 88% of anal cancer (around 24 000 cases). Anal cancer is rare in the general population of both sexes (<2 cases per 100 000 people) but it is 20-times more common in men who have sex with men.²⁵ Anal cancer is as common in men who have sex with men who have HIV, as is cervical cancer in women in sub-Saharan Africa.²⁵ Other cancers attributed to human papillomavirus infection include those of the vagina (70%), penis (50%), vulva (43%), and oropharynx (26%).²⁴

Viral replication, malignant transformation, and immunology

The viral lifecycle

Human papillomavirus infects only epithelial cells and depends on the differentiation pathway of epithelial cells to complete its lifecycle.²⁶ Human papillomavirus infects cells in the basal layer of the epithelium, probably via microabrasions in the epithelial surface. It capitalises on the lateral extension of basal cells that accompanies wound healing to gain entry to the cell. Infectious internalisation takes several hours, after which viral DNA is released from the capsid and transported into the nucleus as free genetic material or extrachromosomal episomes. Early gene expression is tightly controlled in the basal epithelial cells with substantial amplification of viral DNA. Replication (figure 2) occurs only in suprabasal, differentiating cells²⁷ that are destined for maturity and senescence, and as thus do not naturally express the replicative machinery that the virus depends on for survival. To circumvent this problem, human papillomavirus encodes two proteins—E6 and E7—which together promote cellular proliferation, prolong cell-cycle progression, and prevent apoptosis.²⁸ The cell becomes permissive for viral replication and hundreds or even thousands of human papillomavirus genomes are generated within a single cell. The capsid proteins L1 and L2 are expressed in the most superficial layers of the epithelium, where viral assembly takes place, and finally, new infectious viral particles (virions) are shed from the epithelial surface (figure 2). The papillomavirus lifecycle takes 2–3 weeks, the time necessary for a cervical cell to migrate from the basal to most superficial layers of the epithelium, mature, undergo senescence, and die.²⁹

Malignant transformation

To complete the infectious lifecycle of the virus, the cell must undergo terminal differentiation, an essential prerequisite for virion assembly and release. However, for some high-risk papillomavirus infections, E6 and E7 are so effective at blocking negative regulators of the cell cycle that the infected cells never mature. The cells remain actively involved in cell-cycle progression and cease to apoptose. The resulting genomic instability enables genetic alterations to accumulate, ultimately driving malignant transformation of a cell infected with human papillomavirus into an invasive cancer cell.

E6 and E7 start oncogenesis through well-characterised interactions with products of tumour suppressor genes—TP53 for E6 and retinoblastoma proteins for E7. TP53 has a crucial role in protecting genomic integrity by forcing apoptosis or inducing cell-cycle arrest until errors in DNA replication can be repaired. E6 targets TP53 for degradation via the ubiquitin pathway, preventing apoptosis and enabling potentially transformed cells to replicate.³⁰

E7 contributes to oncogenesis through its interaction with the retinoblastoma family members RB1, RBL1, and RBL2, the so-called pocket proteins. E7 binds these proteins and targets them for degradation.^{31,32} This action

Panel 1: Epidemiology of human papillomavirus infection and prophylactic vaccination strategy

The most common human papillomavirus types in cervical cancer are types 16 (57%) and 18 (16%)²³ with small regional variations.²² Type 16 makes up the largest proportion in western-central Asia (73%) and the smallest in Africa (53%). Types 16 (48%) and 18 (10%) are also the most common types in high-grade precancerous lesions.²² Current vaccines—which target types 16 and 18—should therefore prevent roughly 70% of cervical cancers worldwide and half of high-grade precancerous lesions.²² Thus, human papillomavirus vaccination has the potential to prevent most deaths caused by cervical cancer in unscreened populations and to substantially reduce the anxiety and costs associated with detection and treatment of cervical intraepithelial neoplasias.

results in the release and activation of E2F transcription factors that drive the expression of S-phase genes, including those that encode cyclins A and E, which in turn precipitates cell-cycle entry and promotes DNA synthesis. High-risk E5 works with E6 and E7 to drive cellular proliferation and might be a weak cofactor in development of malignancy.³³ Both episomal and integrated copies of the human papillomavirus genome frequently co-occur, often within the same cell. In this case, E6 and E7 expression might not be significantly increased.

Immune evasion

The development of cancer depends not only on efficient negative regulation of cell-cycle control supporting the accumulation of genetic damage, but also on sophisticated techniques of immune evasion that enable the virus to be undetected for long periods.³⁴ No cell death, necrosis, or viraemic phase exists that would trigger an inflammatory response. Viral antigens are detectable only in superficial epithelial cells destined for desquamation and remote from immunological surveillance.³⁵ High-risk papillomaviruses have evolved several mechanisms that minimise their risk of detection by the immune system. High-risk E6 reduces the surface expression of CDH1 by epithelial cells, reducing their ability to present human papillomavirus antigens.³⁶ Toll-like receptors activate antigen-presenting cells as part of the innate immune response to viral infection, but transcription of toll-like receptor 9 is inhibited by expression of high-risk E6 and E7.³⁷ E7 reduces expression of TAP1—a key component of the peptide processing and presentation pathway—preventing activation of specific cytotoxic T lymphocytes.³⁸ High-risk E6 and E7 inhibit interferon synthesis through specific interactions with IRF-1 and IRF-3.^{39,40} Changes from proinflammatory to anti-inflammatory signals—ie, the cytokine milieu—can affect whether or not an infection is cleared. High-risk human papillomavirus downregulates the expression of proinflammatory cytokines including

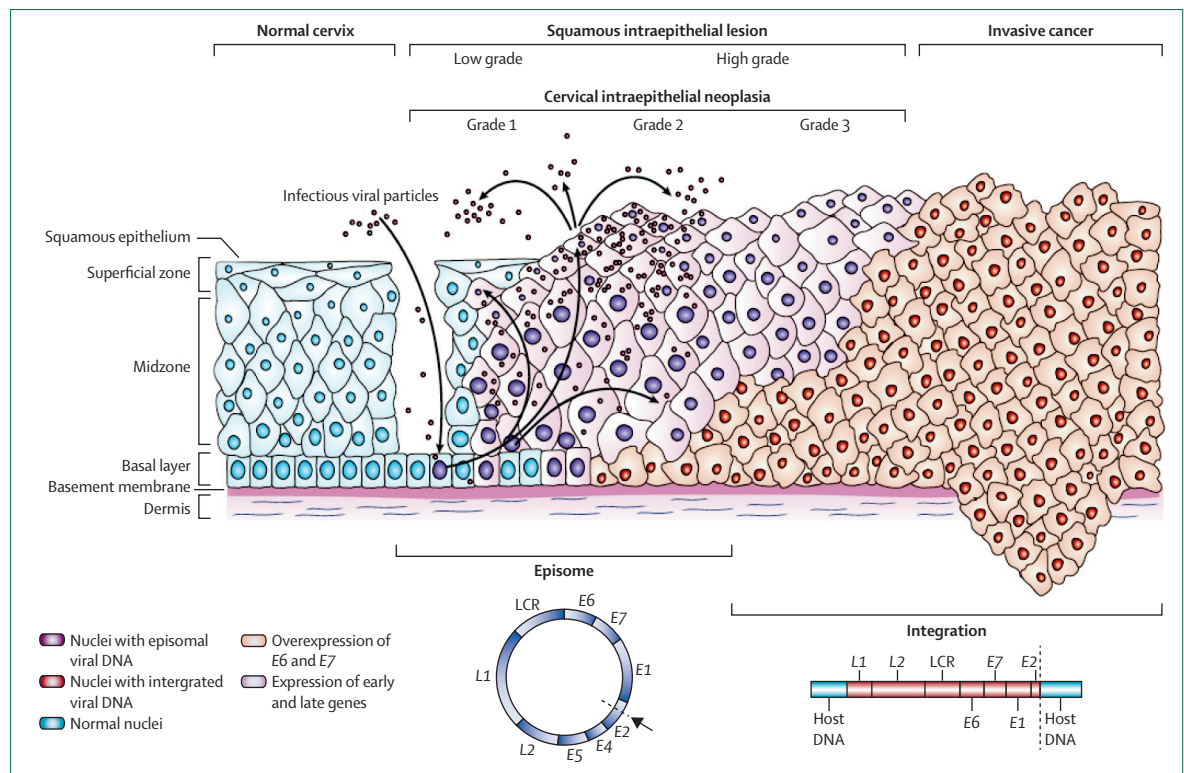


Figure 2: Human papillomavirus lifecycle and organisation of its genome

Basal cells in the cervical epithelium rest on the basement membrane, which is supported by the dermis. Human papillomavirus is thought to access the basal cells through microabrasions in the cervical epithelium. After infection, the early human papillomavirus genes E1, E2, E4, E5, E6, and E7 are expressed and the viral DNA replicates from episomal DNA. In the upper layers of epithelium (the midzone and superficial zone) the viral genome is replicated further, and the late genes L1 and L2, and E4 are expressed. L1 and L2 encapsidate the viral genomes to form progeny virions in the nucleus. The shed virus can then initiate a new infection. Low grade intraepithelial lesions support productive viral replication. An unknown number of high-risk human papillomavirus infections progress to high-grade cervical intraepithelial neoplasias. The progression of untreated lesions to microinvasive and invasive cancer is associated with the integration of the human papillomavirus genome into the host chromosomes (red nuclei), with associated loss or disruption of E2, and subsequent upregulation of E6 and E7 oncogene expression. Reproduced from Woodman and colleagues.²⁷ LCR=long control region.

tumour necrosis factor α , while anti-inflammatory cytokines that prevent migration of immune cells to the site of infection (eg, IL-10), are upregulated.⁴¹ The concentrations of antimicrobial peptides—eg, SLPI and human β defensins 2 and 3—are low in the cervical-vaginal tract of women with CIN.⁴²

Preinvasive disease that progresses to cancer accumulates genetic alterations that further assist with immune evasion. This process is a result of the continuous pressure exerted on the developing tumour by the immune system and is known as cancer immunoeediting.⁴³ The tumour might have MHC class I down-regulation and impaired antigen-processing ability, insensitivity to and avoidance of T-cell mediated killing, increased immunosuppressive T regulatory cell infiltration, and produce immunosuppressive cytokines.³⁴

Natural immune responses

Despite this impressive array of immune evasion mechanisms, most papillomavirus infections are cleared within 12 months. Cell-mediated immunity is implicated in viral clearance through several lines of evidence:

(1) naturally regressing warts are associated with an influx of T lymphocytes;⁴⁴ (2) cell-mediated immune deficiency such as HIV infection can lead to extensive human-papillomavirus-induced lesions; and (3) an increased risk of progressive disease^{45,46} and human papillomavirus-specific immune responses have been detected in the lesions and peripheral blood of people with active and resolving human papillomavirus-associated disease.^{47–49}

Antibody responses to the major viral capsid protein, L1, can be detected from about 6 months after infection⁵⁰ and can still be measured up to 5 years later in individuals who have cleared human papillomavirus infection.⁵¹ Type-specific L1 antibody responses have also been detected in people with persistent disease and cancer, although roughly 50% of individuals never seroconvert.⁵² The presence of L1 antibody might therefore represent previous or persistent infection³³ and it is unclear whether naturally induced L1-specific antibody responses protect against new infection.

Several different variables affect the measurement of these immune responses. Each research-based assay that has been developed to detect anti-L1 human

papillomavirus responses measures different things. Some measure specific immunodominant epitopes (eg, Luminex immunoassays), whereas others measure total IgG (eg, standard ELISA).⁵⁴

Understanding the immunological mechanisms that underpin natural viral clearance and disease regression is needed to inform design of a therapeutic vaccine. T-cell responses specific to human papillomavirus have been detected by measuring proliferation, cytokine release, or cytotoxicity after extensive in-vitro stimulation with peptides or viral constructs expressing the human papillomavirus antigen of interest. T cells are likely to be important effectors for clearance of established disease, but the prevalence of T cells specific to human papillomavirus in the peripheral blood of women with intra-epithelial lesions is extremely low⁵⁵ as a result of the low antigen load and strict epithelial compartmentalisation of premalignant disease.

A longitudinal, non-intervention study⁴⁷ of patients with cytological evidence of low grade CIN reported that CD4+ T-helper 1 responses to E2 coincided with viral clearance. CD4+ T-helper 1 responses against E2 and E6 have also been detected in healthy volunteers, possibly as a result of previous viral clearance.⁵⁶ Crucially, such responses are either absent or dysfunctional in patients with low grade CIN, high grade CIN, or cancer.^{55,57,58} Regression of disease is associated with lesion-infiltrating CD8+ cytotoxic T-cell responses (strongly positive for granzymes),^{46,47} whereas regulatory T cell infiltrates, which maintain an immunologically tolerant environment, occur in persistent and progressive disease.⁵⁹

Human papillomavirus vaccination

Prophylactic vaccines

Understanding of the ubiquitous role of human papillomavirus infection in all CIN and cervical cancer combined with an understanding of human papillomavirus natural history has led to the development of the first prophylactic cancer vaccines. These vaccines contain human papillomavirus L1 self-assembling virus-like particles (figure 3), which induce strong neutralising antibody responses against human papillomavirus infection.⁶⁰ These antibodies are thought to block the human papillomavirus virions before they gain access to the proliferating basal cell layer of the epithelial surface through microabrasions.⁶¹ Two vaccines are available—the quadrivalent vaccine (Merck, Whitehouse Station, NJ USA), which contains virus-like particles to human papillomavirus types 6, 11, 16, and 18, and the bivalent vaccine (GlaxoSmithKline, Rixensart, Belgium), which contains virus-like particles to human papillomavirus types 16 and 18.

Both vaccines protect against precancerous lesions associated with human papillomavirus types 16 and 18, as shown in global randomised clinical trials.^{62–64} In the pivotal randomised, placebo-controlled trial of the quadrivalent vaccine,⁶⁴ vaccine efficacy in patients who

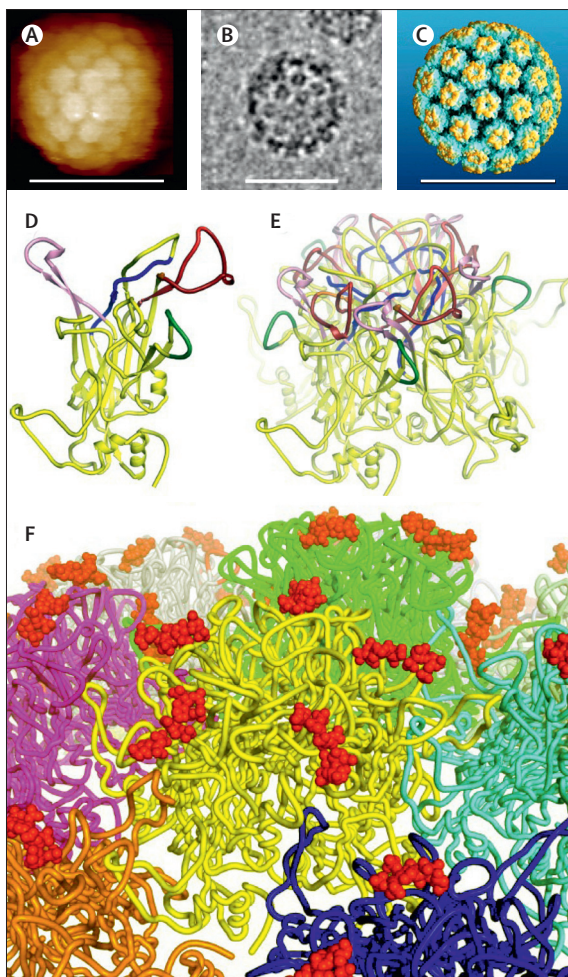


Figure 3: Human papillomavirus type 16 virus-like particles

(A) Atomic force microscopy image of a human papillomavirus type 16 virus-like particle after disassembly and reassembly treatment; adapted from Rodriguez and colleagues.⁴⁶ The scale bar is 50 nm. (B) Cryo-electron microscopy image of a human papillomavirus type 16 virus-like particle after disassembly and reassembly treatment. The scale bar is 50 nm. (C) Atomic model of the T=7 structure human papillomavirus type 16 virus-like particle. The scale bar is 50 nm. (D) A single subunit of L1 in the standard orientation. Residues 261–297 are in red and form part of the H16.V5, H16.E70, H263.A2, and H16.J4 epitopes. Residues 339–365 are in pink and form part of the H263.A2, H16.V5, and H16.E70 epitopes. Residues 174–185 are in dark green and form the H16.H5 epitope. Residues 111–130 are in blue and form the H16.I23 epitope. (E) A pentameric L1 capsomere with the same colouring as in panel D and with the subunit in the foreground in roughly the same orientation. (F) The human papillomavirus type 16 virus-like particle surface with the capsomere in yellow, in the same orientation as in panel E. Heparin oligosaccharides as they bind to L1 capsomeres shown in red. Reproduced from Zhao and colleagues.⁶⁰

had not previously had human papillomavirus was 98% (95% CI 86–100). As expected, efficacy was lower (44%, 95% CI 26–58), for women who had had an active human papillomavirus 16 or 18 infection at baseline or had previous exposure to human papillomavirus based on the presence of human papillomavirus antibodies to vaccine-related human papillomavirus types at baseline. In the pivotal phase 3 bivalent vaccine clinical

trial,⁶⁵ vaccine efficacy was 93% (95% CI 80–98) in human papillomavirus naive patients and less in those with active or previous human papillomavirus infection. Given that the optimum efficacy is in human papillomavirus-naive women, the focus of vaccine programmes worldwide has been on female adolescents.

These vaccines seem to confer cross-protection to non-vaccine human papillomavirus types. In a subset analysis⁶² of the cohort of women who tested negative for human papillomavirus DNA for each of the four types used in the quadrivalent vaccine, protection against human papillomavirus 31 was 46% (95% CI 15–66) for persistent infection and 57% (29–75) for any CIN or adenocarcinoma in situ. An end-of-study analysis⁶⁶ of the PATRICIA trial reported that the bivalent vaccine showed cross-protection against CIN2 or worse associated with human papillomavirus 31 and 33 in lesions with no co-infection with the vaccine types, and to a lesser extent against human papillomavirus 45 and 51. This cross-protective efficacy is associated with cross-protective immune responses in human papillomavirus types that are phylogenetically related to human papillomavirus 16 and 18, respectively.⁶⁷

Although the primary focus of human papillomavirus vaccination programmes has been on women and girls—who bear the greatest burden of human-papillomavirus-associated cancers⁶⁸—recent clinical trial data showing efficacy of vaccination in men⁶⁹ and the potential for herd immunity, has led to vaccination of adolescent boys to be recommended in some developed regions. Australia provides an example of a successful publicly funded mass-vaccination programme. Since its widespread vaccination programme using the quadrivalent vaccine, which began in April, 2007, uptake and completion of the full three-dose regimen recommended for human papillomavirus vaccination in Australia has been one of the highest in the world. Monitoring of vaccination and registries for tracking incident sexually transmitted infections in Australian clinics is comprehensive and centralised. According to these registries, since the introduction of vaccination, new cases of genital warts have not only fallen by 73% in vaccine-aged young women, but also by 44% in young men, who were not part of the free vaccination programme. These findings strongly suggest that mass vaccination of girls provides substantial herd immunity.⁷⁰ In the UK, the uptake of vaccination in a school-based programme for girls aged 12–13 years was 83%,⁷¹ whereas in the catch-up campaign for older teenagers, which relied largely on general practices, only 41% of eligible individuals had three doses.⁷²

These vaccines were not designed as therapeutic vaccines and have little, if any, prophylactic effectiveness in people that have been previously exposed to the virus types contained in the vaccine.^{64,73} Vaccination policy in some countries, such as the USA, includes a routine recommendation, but vaccination is given on request. To accommodate a wider range of on-request vaccinations, the USA

and other countries have included a catch-up age range for vaccination, which overlaps with the typical age of onset of sexual activity. Because the effectiveness of the vaccine as prophylaxis is higher for a fully vaccinated woman who has not had any sexual activity compared with a woman vaccinated after the onset of sexual activity, the catch-up vaccination policy has implications for health economics. The policy also has implications—which might include extended screening intervals for vaccinated individuals—for future cervical cancer screening recommendations.

Prophylactic human papillomavirus vaccination has been estimated to be cost effective, with the up-front expenditure for vaccination offset by costs averted through disease prevention.⁷⁴ This assumption depends on age at vaccination, screening intervals, female only or male and female programmes, and the cost of the vaccine. Models suggest that costs of cervical cancer screening could be substantially reduced by ensuring high coverage human papillomavirus vaccination and the consequent drop in high-grade cytology and colposcopy referrals. The association between human papillomavirus infection and several other anogenital diseases—including anal, vaginal, and vulval cancers—as well as oropharyngeal cancers,⁷⁵ suggests that prophylactic human papillomavirus vaccination might protect against some of these cancers as well. Despite proven efficacy against human papillomavirus-associated anal disease,⁶⁹ cost effectiveness models in regions of the world with vaccination for both girls and boys have shown that vaccination of both sexes is considerably less cost effective than is vaccination of girls only, unless vaccine costs substantially decrease or high coverage in adolescent girls cannot be achieved.⁷⁶ Arguments for male vaccination do not relate solely to cost, but also to the additional health benefits of moving from a sex-specific strategy to a vaccination policy seeking to prevent disease in both sexes, with the potential for herd immunity.

Ongoing studies are assessing the next generation L1 virus-like particle human papillomavirus vaccines that include additional oncogenic human papillomavirus types. Prophylactic vaccines against the human papillomavirus minor capsid protein L2 are also in clinical development.^{77,78} Vaccination of rabbits with peptides from the human papillomavirus minor capsid protein L2 induces broadly cross-neutralising antibodies, sufficient for broad protection against several phylogenetically related papillomavirus types.^{79,80} Unlike L1 virus-like particles, the number of types covered could be enhanced by generating a multimer of the protective epitope from diverse papillomavirus types.⁸¹

Therapeutic vaccines

Human papillomavirus is an ideal target for a therapeutic vaccine. Therapeutic vaccines targeting human papillomavirus E6 and E7^{82–84} along with broadly targeting immunotherapies or peptides⁸⁵ are in clinical development. The rationale of these vaccines is to avoid the

need for surgical procedures by developing immune responses specific to human papillomavirus. The vaccines in development have several drawbacks. Limitations of peptide vaccines include HLA restriction for either vaccination or for the ability to measure cell-mediated immune responses after vaccination. Some of the broad immunotherapies have non-specific toxic effects that might not be attractive to the generally young healthy women who have CIN, for which very effective outpatient surgical treatments exist. Although many vaccines seem to boost immune responses specific to human papillomavirus, it is unclear whether such systemic responses are appropriate surrogate markers for what is happening at the site of the infection.⁸⁴ To date, no effective therapeutic vaccines have completed clinical development. Several likely reasons exist for this lack of success, including the ability of human papillomavirus to evade immune recognition,⁸⁶ a paucity of effective strong immune responses generated by current vaccine technologies,⁸⁷ and inadequate time without treatment to see a response within the time scales of randomised trials in women with CIN.

Human papillomavirus testing in cervical screening

Because of its crucial causal role and the need for continued expression to maintain the disease phenotype, human papillomavirus can be used as a biomarker of cervical cancer and precancer. Randomised trials^{88–90} have shown that human papillomavirus DNA testing provides greater sensitivity than does cytology for detection of CIN. Human papillomavirus testing is more reproducible with less subjective analytical characteristics,⁹¹ and users need less training and expertise. The major drawback of human papillomavirus testing is that infection is far more common than is underlying disease—particularly in women younger than 30 years—and therefore needs reflex cytology to achieve the specificity needed to detect an underlying abnormality. The ARTISTIC trial included 24 000 women aged 20–64 years screened in Manchester, UK. It showed a large fall in high-risk human papillomavirus infection with age: from 40% in those aged 20–24 years to 7% in those aged 50–54 years.⁹² However, human papillomavirus is not only capable of detecting prevalent disease, but it is also a biomarker of increased future risk.

The first test to achieve the status of a standard commercial kit relied on DNA–RNA hybridisation and chemiluminescence (Hybrid Capture-2; Qiagen, Gaithersburg, MD, USA).⁹³ During the past few years other tests have been developed, including RNA-based tests, which have been claimed⁹⁴ to be more specific, since RNA transcription is likely to be a clearer indicator of oncogenesis. Some newly developed tests can provide a readout specific for human papillomavirus type 16 or 18 as well as non-specific high-risk human papillomavirus. These tests might prove to be clinically useful,

Panel 2: Human papillomavirus as a test of cure after treatment of cervical intraepithelial neoplasia

Human papillomavirus testing—either in combination with cytology or as a single test—can be used as a test of cure after treatment of high-grade cervical intraepithelial neoplasia. The rationale behind this approach is that human papillomavirus will be a more sensitive detector of residual untreated cervical intraepithelial neoplasia than cytology, and has the advantage of enabling women who test negative to be deemed low risk, and so return to routine recall rather than need to attend yearly follow-up for up to 10 years. Women who test positive for human papillomavirus, even in the presence of normal cytology, would be referred for colposcopic examination. This approach has been shown to be a very safe and successful way of stratifying treated women. In a UK study of 744 treated women who were cytology negative and human papillomavirus negative at 6 months, only five were known to have developed high grade cervical intraepithelial neoplasia during 2 years.⁹⁹ Both human papillomavirus triage and test of cure are being implemented at present in the English NHS Cervical Screening Programme, as well as in several other countries. In the English programme, women who have been referred for colposcopy and who have a negative and adequate colposcopic examination are referred back to routine recall. In this way human papillomavirus testing is being used to enhance the detection of disease and at the same time streamline management, speeding up diagnosis and return to routine recall. By capitalising on the negative predictive value of a human papillomavirus test, many women will not need to undergo unnecessary colposcopy.

since infection with human papillomavirus type 16 or 18 has a relative risk for CIN3 of 7–10 compared with any high-risk human papillomavirus.^{95,96}

Selection of the most appropriate human papillomavirus tests for cervical cancer screening depends on several considerations: validation in large trials with regulatory approval, the need for high-throughput tests or tests that can be cost-effectively done in smaller numbers, the necessary balance of sensitivity and specificity, and whether platforms used for human papillomavirus testing are already used in a given clinical laboratory. Low cost is also a prerequisite, especially for medium-resource and low-resource countries.

The role of human papillomavirus testing

Atypical cytology of unknown significance or borderline cytology are associated with underlying CIN2 or worse in roughly 10% of cases. In atypical cytology of unknown significance or borderline cytology, about 60% test positive for high-risk human papillomavirus, which enables human papillomavirus testing to identify those at very low risk (human papillomavirus negative), who can be routinely recalled at standard screening intervals, whereas a human papillomavirus positive result warrants referral to colposcopy. The US ALTS trial⁹⁷ showed the effectiveness of human papillomavirus triage and a large implementation study⁹⁸ confirmed the clinical use of restricting colposcopy referral. In this study, the positive predictive value for underlying CIN2 or worse varied between referring laboratories, but the mean was 16% (range 9–22). Human papillomavirus triage of equivocal cytology enables immediate referral for those at risk and routine recall for those who test negative. This approach

avoids the need for repeat testing, which is not only inefficient—risking reduced adherence to recall—but also causes distress by prolonging uncertainty and necessitating repeated examination. Human papillomavirus testing might also be useful as a test of cure after treatment of high grade CIN (panel 2).

The additional sensitivity of human papillomavirus testing and the potential for high-throughput automated testing make it an attractive alternative to cytology for primary screening. Large randomised trials have been done in Europe and Canada,^{88–90,100} in which human papillomavirus DNA testing combined with cytology was compared with cytology alone. Apart from ARTISTIC, each trial showed significantly greater detection of disease in the first screening round by cotesting compared with cytology alone. All of the trials showed a reduction in the detection of high grade CIN in the second screening round, presumably because of

improved disease detection in the initial round. In a large, four-group, controlled trial in India, screening based on human papillomavirus testing was associated with a reduction in the incidence of advanced cervical cancer and death compared with cytology or visual inspection with acetic acid.¹⁰¹

A major benefit of human papillomavirus primary screening is the potential for human papillomavirus testing to lengthen screening intervals—as shown in a European cohort study¹⁰²—and follow-up data from ARTISTIC⁹⁶ confirm that human papillomavirus primary screening provides a longer duration of negative prediction than does cytology, which could enable screening intervals to be safely extended to 6 years.

The main difficulty of human papillomavirus screening is the management of women who are positive for human papillomavirus testing and negative for cytology. The risk of underlying disease in this group is low, but over 6 years it is twice that of the screened population as a whole.⁹⁶ Data from ARTISTIC also showed that both the prevalence and cumulative 6 year rate of disease is three times higher in women who test positive for human papillomavirus type 16 compared with women with all high-risk human papillomavirus types. Furthermore, in a study from the USA,⁹⁵ women who were positive for human papillomavirus type 16 but who had negative cytology had a disease prevalence of 11%, similar to that for atypical cytology of unknown significance triaged by Hybrid Capture-2. These data also suggest a possible role for human papillomavirus type 16, or indeed other biomarkers, in identification of the highest risk group who warrant referral for colposcopy and using early recall at less frequent intervals for the remaining women, given that most will become negative for human papillomavirus, with only 30–40% expected to have type-specific persistence. Biomarkers such as p16 and Ki67 might offer alternative means of increasing the specificity of screening strategies based on human papillomavirus.¹⁰³

Primary screening based on human papillomavirus testing could use cytology triage for women who are positive for human papillomavirus and colposcopic referral for those with abnormal cytology (figure 4). Women who were positive for human papillomavirus but negative for cytology need not immediately have colposcopy, but could be offered early recall at 12–24 months, in the expectation that most would revert to being negative for human papillomavirus. If they are still human papillomavirus positive, cytology negative, human papillomavirus genotyping could be used to triage those who were positive for human papillomavirus type 16 or 18, and therefore at highest risk, to colposcopy. The overall benefits of this approach are (1) greater sensitivity for detection of CIN2 or higher, though colposcopic referrals would probably increase, and (2) extended screening intervals for human papillomavirus-negative women. The reliance on early recall for women who are human papillomavirus positive, cytology negative

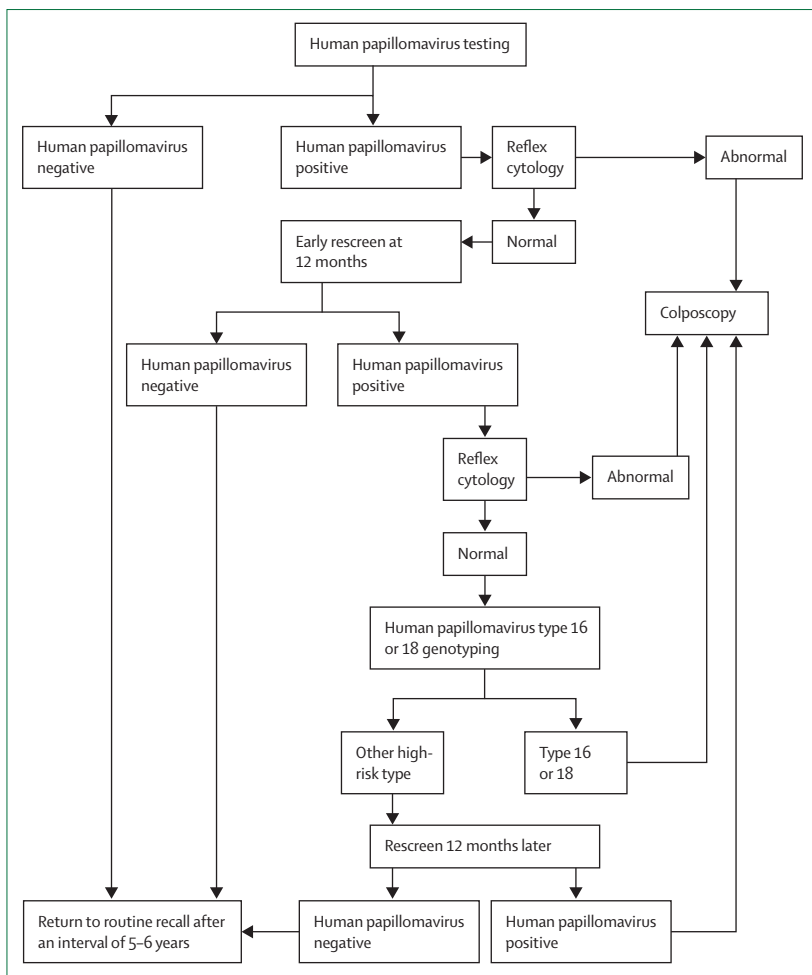


Figure 4: Algorithm for primary human papillomavirus-based cervical screening

Four general principles in the algorithm are: the need for reflex cytology after a positive human papillomavirus test; the need for early recall of women who are human papillomavirus positive and cytology negative; the use of type 16 or 18 genotyping at early recall to identify those at greatest risk; and the use of extended screening intervals for women who are human papillomavirus negative.

will inevitably lead to some loss to follow-up, which could undermine the potential for added sensitivity from human papillomavirus testing. For countries without effective cervical screening, human papillomavirus testing could offer a simpler strategy for population screening than does cervical cytology and if implemented could start to save lives within a few years, as shown by screening trials, for example, in rural India.¹⁰¹ Human papillomavirus testing could be done with a point-of-care human papillomavirus test and where colposcopy is not available, women positive for human papillomavirus could be offered visual inspection with acetic acid,¹⁰⁴ and suspected CIN treated with cryotherapy.

Conclusion

Human papillomavirus is a well-established cancer-causing infectious agent that is almost exclusively sexually transmitted and has increased in prevalence in many parts of the world during the past few decades.^{10,105} Only comprehensive cervical screening programmes have avoided a corresponding epidemic of cervical cancer.¹⁰⁵ Thus, economic improvements in developing countries will not be sufficient to overcome the present international disparities in cervical cancer burden unless human papillomavirus vaccination and cervical screening are implemented. Prophylactic vaccination will likely take more than 20 years to have an effect on cervical cancer.

The progress made has been so successful that a means exists to prevent most cervical cancers worldwide. Unfortunately these benefits will only be felt in the developing world if major and far-reaching political initiatives such as the Global Alliance for Vaccines and Immunisation succeed in gathering and providing the resources to implement these advances in under-resourced regions. These regions bear the ever increasing burden of disease, and thus suffering and morbidity, from cervical cancer. The Millennium Development Goals identified maternal death as a priority and as a consequence the number of maternal deaths has fallen worldwide since 2010. Almost as many women now die from cervical cancer and this issue should be addressed by national governments and the non-governmental agencies that have the means to target resources and build capacity.

Scientific discovery has delivered the means to prevent millions of deaths. It is imperative that those who carry the responsibility for the health and wellbeing of women around the world act to ensure that this benefit is realised.

Contributors

All authors wrote and revised the review.

Conflicts of interest

EJC received an honorarium from GlaxoSmithKline for serving on a human papillomavirus advisory board in November 2010, and was sponsored by GlaxoSmithKline to attend EUROGIN and the International Papillomavirus meetings in 2011. MHE has advised or participated in educational speaking activities, with travel costs paid for, from Merck, GlaxoSmithKline, Roche, Hologic, Advaxis, Inovio, and Photocure. His institution received payment for these activities, but he has not received honoraria. His institution has also received grants for

clinical trials that MHE was the principal investigator for from Merck, GlaxoSmithKline, Roche, Advaxis, Photocure, Inovio, and Hologic. HCK and SF declare that they have no conflicts of interest.

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