Human papillomavirus and cervical cancer

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Summary

Cervical cancer is the second most common cancer in women worldwide, and knowledge regarding its cause and pathogenesis is expanding rapidly. Persistent infection with one of about 15 genotypes of carcinogenic human papillomavirus (HPV) causes almost all cases. There are four major steps in cervical cancer development: infection of metaplastic epithelium at the cervical transformation zone, viral persistence, progression of persistently infected epithelium to cervical precancer, and invasion through the basement membrane of the epithelium. Infection is extremely common in young women in their first decade of sexual activity. Persistent infections and precancer are established, typically within 5–10 years, from less than 10% of new infections. Invasive cancer arises over many years, even decades, in a minority of women with precancer, with a peak or plateau in risk at about 35–55 years of age. Each genotype of HPV acts as an independent infection, with differing carcinogenic risks linked to evolutionary species. Our understanding has led to improved prevention and clinical management strategies, including improved screening tests and vaccines. The new HPV-oriented model of cervical carcinogenesis should gradually replace older morphological models based only on cytology and histology. If applied wisely, HPV-related technology can minimise the incidence of cervical cancer, and the morbidity and mortality it causes, even in low-resource settings.

Burden of cervical cancer

There were about 500 000 incident cases of and 275 000 deaths due to cervical cancer worldwide in 2002, equivalent to about a tenth of all deaths in women due to cancer.[1] The burden of cervical cancer is disproportionately high (>80%) in the developing world.[2] Not only is cervical cancer the most prevalent and important cancer in women in several developing countries, but also the societal importance of the disease is accentuated even further by the young average age at death, often when women are still raising families. Cases are often detected at late stages due to non-existent or inadequate screening, and the standard treatment options are often absent or unaffordable. Promising approaches to cervical cancer prevention have resulted from our new understanding that almost all cases are caused by persistent infection with about 15 genotypes of human papillomavirus (HPV).[3,4] We review recent advances and current issues regarding HPV and cervical cancer.

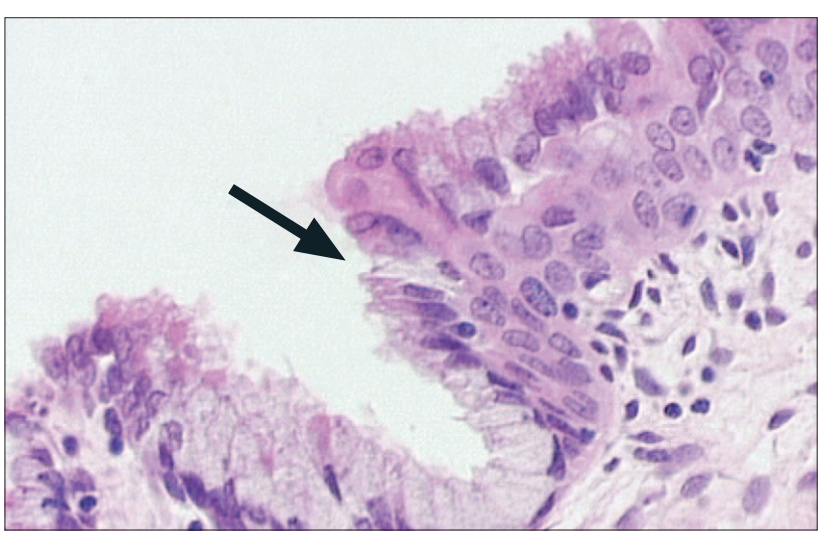

Figure 1: **The cervical transformation zone**
The cervical transformation zone is a ring of active squamous metaplasia where the stratified squamous epithelium of the ectocervix progressively undermines and replaces the glandular epithelium of the endocervix. For unclear reasons, metaplastic tissue is especially susceptible to the carcinogenic potential of persistent HPV infections.

The cervical transformation zone

Cervical cancer usually arises from a ring of mucosa called the cervical transformation zone (figure 1). For reasons that we do not understand, persistent HPV infections cause cancers mainly at the transformation zones between different kinds of epithelium (eg, cervix, anus, and oropharynx).[2] Illustrating the importance of the transformation zone, cancer-associated (carcinogenic) HPV infections are equally common in cervical and vaginal specimens;[5] however, cervical cancer is the second most common tumour in women worldwide, whereas vaginal cancer is exceedingly rare.[2] The position of the cancer-susceptible transformation zone is dynamic, gradually shifting over years towards, and into, the endocervical canal[6] as stratified squamous epithelium replaces the mucus-producing glandular epithelium.[7]

Prevention of cervical cancer after abnormal screening results depends on the destruction or excision of the

Search strategy and selection criteria

We searched the Cochrane Library (2000–07) and Medline (1980–2007) with the terms "human papillomavirus", "HPV", "CIN", "cervix cancer", "cervical carcinoma", "cervical neoplasia", "cervix cancer", "cervix carcinoma", and "cervix neoplasia". We largely selected publications from the past 5 years, but chose some commonly referenced, important older publications. Review articles and book chapters are cited to provide readers with additional details and references. Our reference list was modified on the basis of comments from peer reviewers. We searched for papers in English, Spanish, and French.

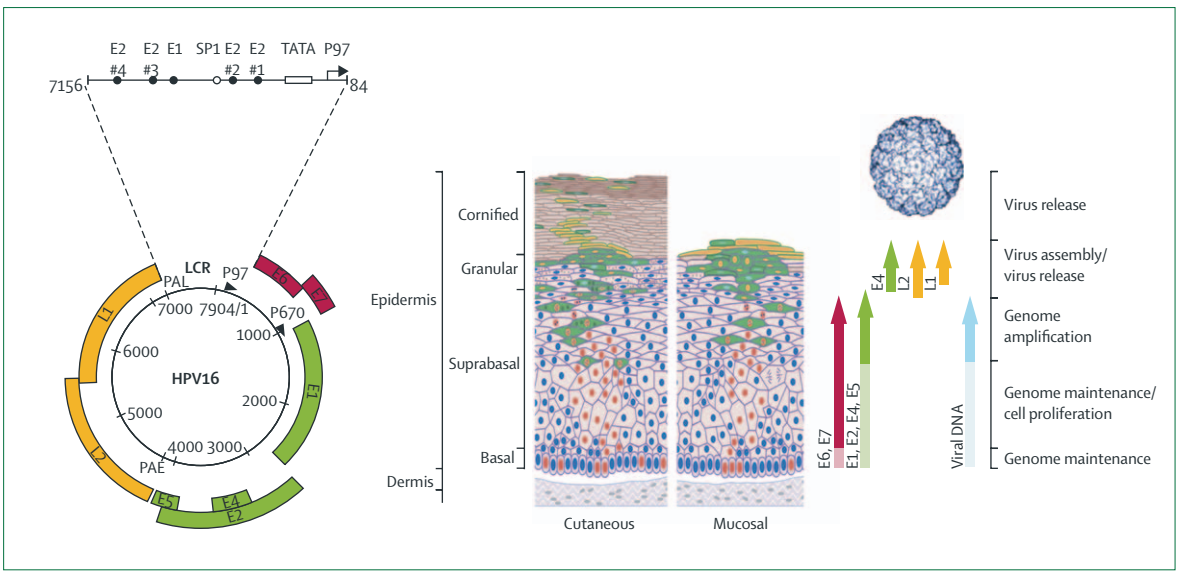

Figure 2: **The HPV genome and its expression within the epithelium**
The HPV genome consists of about 8000 bp of single-stranded, circular DNA. There are eight open reading frames and an upstream regulatory region. HPV genes are designated as E or L according to their expression in early or late differentiation stage of the epithelium: E1, E2, E5, E6, and E7 are expressed early in the differentiation, E4 is expressed throughout, and L1 and L2 are expressed during the final stages of differentiation. The viral genome is maintained at the basal layer of the epithelium, where HPV infection is established. Early proteins are expressed at low levels for genome maintenance (raising the possibility of a latent state) and cell proliferation. As the basal epithelial cells differentiate, the viral life cycle goes through success stages of genome amplification, virus assembly, and virus release, with a concomitant shift in expression patterns from early genes to late genes, including L1 and L2, which assemble into viral capsid. L1 is the major capsid protein while L2 serves as the link to the plasmid DNA. Adapted from Doorbar.[21]

entire transformation zone epithelium, not specific precancerous lesions; this method is effective in about 80–95% of cases.[8,9] The site of a biopsy showing cervical precancer is not necessarily the exact site of subsequent cancer development but rather is evidence of a field of increased risk. Therefore, exfoliative cytological and virological measurements of the transformation zone can sometimes predict cancer risk even when histopathology from a colposcopically derived biopsy does not confirm the presence of a precancer.[10]

Histopathology

In poorly screened populations, squamous cell carcinomas constitute most cases of cervical cancer. In regions with good cervical cancer screening programmes, the proportion of adenocarcinomas is increased (15–20%) compared with unscreened populations, presumably because they arise from the poorly sampled glands of the canal or from poorly recognised precursor lesions.[11] Beyond the relative increase, absolute rates of cervical adenocarcinomas are thought to have increased in various countries over the past two to three decades,[12,13] for uncertain reasons. Infection with a carcinogenic HPV is a necessary cause of both squamous cell carcinoma and adenocarcinoma. However, the distribution of carcinogenic HPV types and variants detected in these two histopathological types (eg, adenocarcinoma is more strongly linked with HPV18) and the roles of non-viral cofactors (eg, smoking and parity) differ.[14,15]

Basics of HPV virology

Papilloma (wart) viruses have co-evolved with animal hosts over millions of years and the life cycle of each genotype of HPV is tied closely to the differentiation of its specific epithelial target (eg, sole of foot, non-genital skin, anogenital skin, anogenital/oropharyngeal mucosa).[16] The relations between HPV genotypes can be expressed in the form of phylogenetic trees based on DNA sequence and protein homologies, which serve as unifying tools in understanding HPV classification and behaviour.[17] HPV16 and HPV18 are the two most carcinogenic HPV types, and are responsible for 70% of cervical cancer and about 50% of cervical intraepithelial neoplasia (CIN) grade 3 (CIN3);[18] by contrast, HPV6 and HPV11 are responsible for about 90% of genital warts.

When we refer to HPV infection in this Seminar, we are referring to the genetically related group of genotypes that are linked to cancer risk—ie, the carcinogenic types—unless specified. For cytopathology, we refer to the 2001 Bethesda System[19] and for histopathology, we use the WHO classification.[20]

The human papillomavirus genome codes for only eight genes (figure 2).[21] E6 and E7 are the primary HPV oncoproteins. Each has numerous cellular targets,[21–23] with p53 and retinoblastoma tumour suppression protein (pRB) being the most important. E6 inhibition of p53 blocks apoptosis, whereas E7 inhibition of pRB abrogates cell-cycle arrest. E7 is the primary transforming protein. Both proteins are expressed at low levels during the infectious process. At some still undefined point in progression to

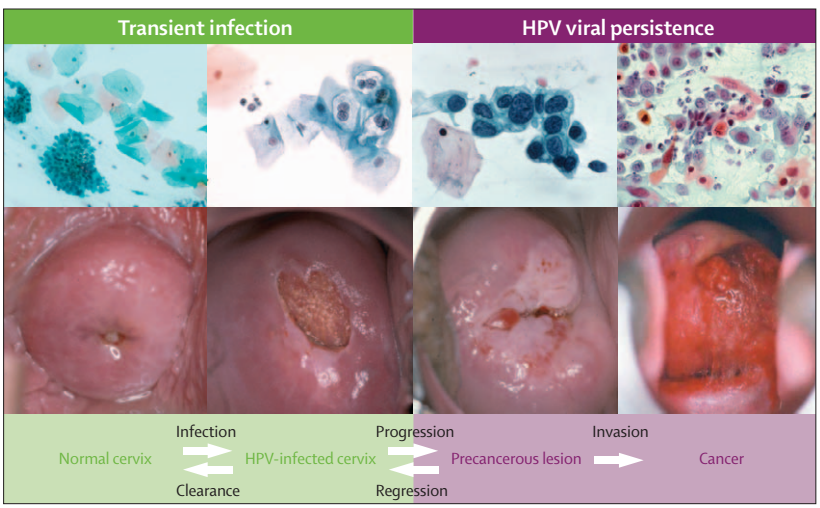

Figure 3: **Major steps in the development of cervical cancer**
Top row shows cytology, bottom row colposcopy. The major steps in cervical cancer development can be understood best in relation to age at first sexual intercourse as a proxy for age at first infection. The typical age of cervical HPV infection is similar to other sexually transmitted infections, with a large peak rapidly following average age of sexual initiation. This average age of HPV infection varies by culture, affecting average ages of subsequent stages. Incident HPV infection is best measured by molecular tests. Cross-sectionally, most HPV infections show no concurrent cytological abnormality. About 30% of infections produce concurrent cytopathology, usually non-classical (equivocal) changes. Most HPV infections clear within 2 years; the 10% that persist for 2 years are highly linked to precancer. Detection of precancers is delayed by their initially small size and the typically low sensitivity of screening methods. Precancers are usually detected around age 25–30 years (about 10 years after sexual debut) in regions with cytological screening. Adapted from Schiffman and Castle.[24]

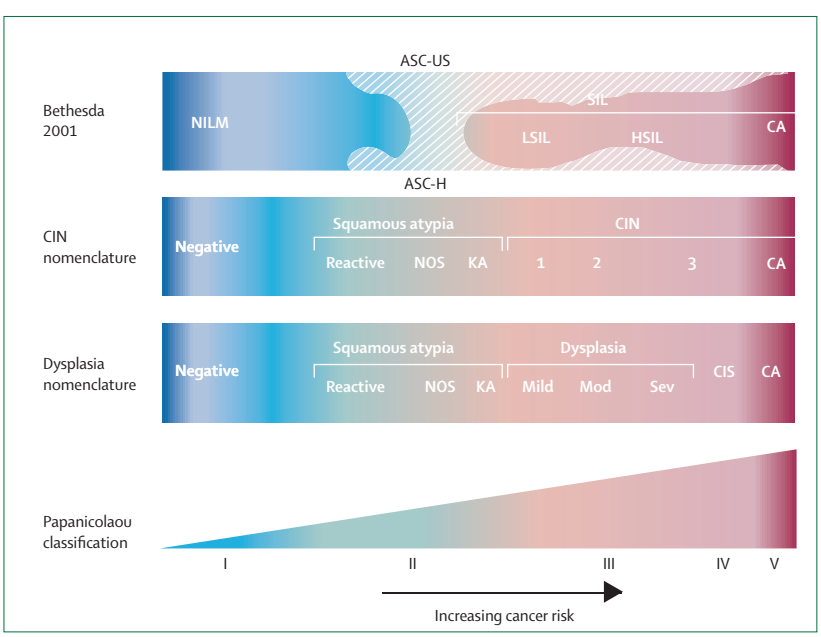

Figure 4: **Comparative classifications of HPV-related microscopic abnormalities**
To discuss HPV infection and cervical cancer with colleagues from other settings requires understanding the many different terms used. Equivocal interpretations of ASC-US (atypical squamous cells of undetermined significance) and ASC-H (atypical squamous cells, cannot rule out high-grade squamous intraeptithelial lesions) are noted with stippling, the amount and colour of which suggests the expected frequencies within the differential diagnosis. Adapted from Sherman.[36]

precancer, E6 and E7 expression is deregulated by either genetic or epigenetic changes, leading to their over-expression in the full-thickness epithelial lesion.

Development of cervical cancer

Cervical cancer arises via a series of four steps—HPV transmission, viral persistence, progression of a clone of persistently infected cells to precancer, and invasion—that can be reproducibly distinguished and which provide a rational starting point for any discussion of optimum prevention efforts (figure 3). Backward steps occur also, namely clearance of HPV infection and the less frequent regression of precancer to normality. The molecular virology underlying HPV persistence, progression, and invasion is not well understood, but this causal model is supported by epidemiological and laboratory data and does not require unreliable morphological distinctions like histological CIN grade 1 (CIN1) or cytological atypical squamous cells of undetermined significance (ASC-US) analogous to borderline dyskaryosis.[25,26]

HPV transmission

Anogenital HPV infections are transmitted mainly by skin-to-skin or mucosa-to-mucosa contact.[27,28] The probability of infection per sexual act is not known but is clearly high,[27] with no known difference between HPV types. Because of their common transmission route, HPV types tend to be transmitted together,[29,30] resulting in a high proportion (20–30%) of concurrent infections with several different types when women in the general population are sampled.[31] Men are also often infected with several HPV types concurrently, implying that a sexual act could transmit several types at once.

Independent of type, infecting viral particles reach the germinal cells in the basal layer presumably via tiny tears to the mucosa.[4] Male circumcision might decrease male HPV infection and carriage, possibly due to the toughness of keratinised epithelium, thereby reducing transmission.[32] Penetrative sexual intercourse is not strictly necessary for transmission and HPV types can apparently be transferred to the cervix from original infection at the introitus.[33]

Most women in the world are probably infected with at least one if not several types of HPV during their sexual life.[34] Total exposure is difficult to measure because DNA detection is usually transient and serology is not accurate.[35] Thus, a substantial proportion of HPV DNA negative, seronegative women have nonetheless been exposed.

While looking for uncommon, significant cervical lesions, pathologists and clinicians encounter a huge assortment of abnormalities that are minor or, even more commonly, equivocal (figure 4). Many millions of women are diagnosed every year with such abnormalities.[37] An aggressive management approach cannot be justified because almost all abnormalities clear without treatment.[38] However, these abnormalities cannot be ignored because most precancers and cancers are diagnosed in women with equivocal or mildly abnormal cytological findings.[39]

Only about a third of women with HPV infections detectable by DNA testing have recognised cytopathology.

Cytological abnormalities are less sensitive for detection of HPV than molecular testing. HPV16 and related types are most likely to produce high-grade squamous intraepithelial lesions; by contrast HPV18 (the second most common type in cancers) causes a disproportionately low fraction of such lesions.[40,41] A lack of HPV18-induced high-grade squamous intraepithelial lesions could explain, at least in part, the poor performance of screening for endocervical or glandular lesions and the increased proportion of adenocarcinoma, which are associated with HPV18,[11,42] in well-screened populations.

In longitudinal studies of cytologically normal adult women who are HPV DNA positive at enrolment, the cumulative risk of incident equivocal and minor cytological abnormalities rises to a high level (about 25–50% of smears) 1–2 years after enrolment and declines thereafter, returning to baseline (<5% of smears) at about 4 years.[43,44] The smaller cumulative risk of precancer and cancer continues to rise for as long as we have been able to observe prospectively (≥15 years), suggesting that a few women remain persistently infected.[45,46] How often precancer arises from an evident mild lesion versus an equivocal lesion or cytologically normal, HPV-infected tissue is not known.

HPV clearance versus persistence

Most cervical HPV infections (with cytological abnormality or not) are cleared or suppressed by cell-mediated immunity within 1–2 years of exposure[47] (figure 5). The most persistent HPV types tend to be the most common.[17] This correspondence is to be expected because prevalence equals incidence multiplied by duration (ie, persistence). The prevalence of different types of HPV is modified by differential censoring due to detection and treatment, which are more common for lesions caused by HPV16 than other types.

With longer HPV persistence of a given type, the probability of subsequent clearance over a fixed interval decreases and the risk of precancer diagnosis increases.[30] However, the average persistence of some non-carcinogenic types (eg, HPV61) can also be long.[17] Prevalent infections detected in cross-sectional screening persist longer in older women than in younger women, probably because they are more likely to represent infections already of long duration.[48] The median time to clearance of HPV infections detected during screening studies is 6–18 months.[30] There is no accepted definition of clinically important persistence, but follow-up strategies targeting abnormalities lasting more than about 1 year (and especially 2 years) seem to distinguish infections and associated lesions posing greater risk to the patient from transient infections.[49] The small proportion (about 10%) of carcinogenic infections persisting for several years is strongly linked to a high absolute risk of diagnosis of precancer.[17]

Ongoing cohort studies with up to 10 years of follow-up data have shown that, after clearance, the same HPV type can occasionally re-appear.[50] Whether infections resolve by complete viral clearance or by maintenance of a latent state in the basal-cell epithelium, in which the virus replicates at extremely low levels without full viral expression, is unclear. The appearance of many HPV infections among immunosuppressed HIV-positive individuals suggests that latency is a possibility.[51] In populations with secondary peaks of HPV infection—eg, post-menopausal women—re-emergence from latency due to senescence of cell-mediated immune control could have a role, as well as new sexual partners (of the women or their partners) or cohort effects. However, older women with a long period without cytological signs of HPV infection show very small risk of subsequent cervical cancer, suggesting that re-activation from latency typically does not cause harm.[52]

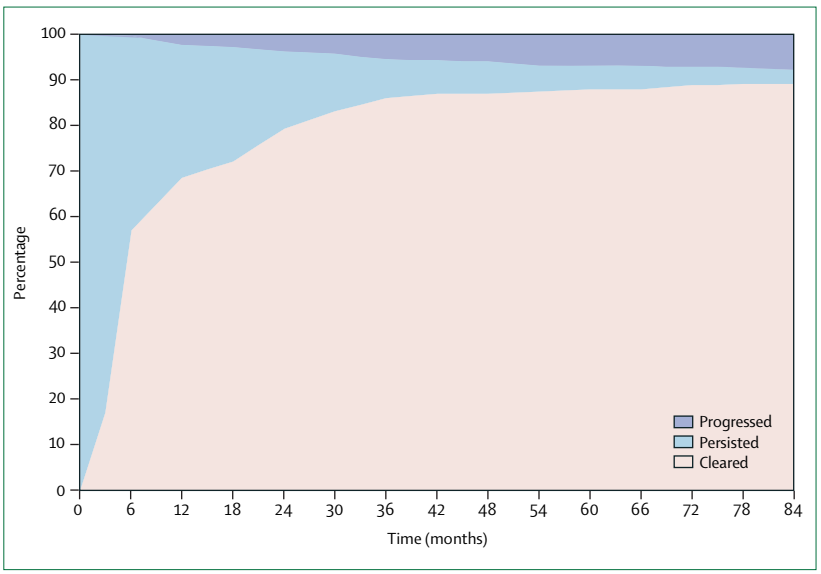

Figure 5: **Average clearance, persistence, and progression of carcinogenic HPV infections**
Carcinogenic HPV infections detected by DNA testing tend to resolve quickly within a year of detection. Details vary by population, cytological status, and age, but this diagram of 777 infections found at enrolment visits of a large population-based cohort study (Guanacaste, Costa Rica; unpublished data) illustrates a typical pattern. Over time, the risk of a precancer diagnosis rises while the probability of eventual clearance among the still-persistent infections falls. Among women with no fertility concerns, treatment for carcinogenic infections, especially with HPV16 (the type most linked to risk of precancer and cancer), that persist beyond an appropriate period of watchful waiting (eg, 12–24 months), might be justified.

Progression to cervical precancer

In terms of histopathology, precancer includes the fairly reliable morphological diagnoses of CIN3, severe dysplasia or dyskaryosis, or carcinoma in situ (figure 6). In precancer, undifferentiated cells with fixed genetic abnormalities have replaced almost the full thickness of the cervical epithelium.[53] To discuss what lesions do not represent precancer is also important for diagnostic specificity. CIN grade 2 (CIN2) is heterogeneous: it is sometimes produced by non-carcinogenic types of HPV and, therefore, is equivocal in cancer potential.[54] CIN1 is an insensitive histopathological sign of HPV infection, and is not precancer. Careful study of cases of

Figure 6: **What defines precancer?**
Heterogeneity in biology (and definition) still exists in precancer, even as it remains the prime target of screening programmes and preventive treatment as well as the scientific surrogate for cancer risk. As the functional definition of precancer has expanded to include smaller and less serious lesions due to difficulties distinguishing the true cancer precursors, the risk (predictive value) of the diagnosis of precancer as a surrogate for predicting invasive cancer has declined. These changes can alter the effect and assessment of prevention programmes. The most certain surrogate for invasive cancer is full-thickness carcinomas in situ; however, many CIN3 lesions detected by screening are very small and less certain to pose an eventual risk of invasion. Nonetheless, CIN3 shares virtually the same HPV type spectrum and causal cofactors as cancer. By contrast, CIN2 can be caused by HPV types rarely found in cancer, and has a sizeable regression potential. Thus, CIN2 represents equivocal precancer, but it is, nonetheless, treated in some regions to provide a safety margin against cancer risk. Molecular markers to define precancer would be helpful, but prospective validation showing risk of invasion would not be ethical.

histologically confirmed CIN1 have revealed that such lesions actually represent a lower risk of progression to cervical cancer than does interpretation of cytological low-grade squamous intraepithelial lesions.[10] Diagnosis of CIN1 incorporates the errors of placement, processing, and interpretation of colposcopically guided biopsy, although such a diagnosis is poorly reproduced even when made on the basis of large tissue specimens.[26] For any given type of carcinogenic HPV, diagnosing CIN1 does not predict meaningfully higher risk of CIN3 than does negative biopsy.[10]

The lag time between infection and appearance of the first microscopic evidence of precancer can be surprisingly short, often within 5 years.[55] In fact, histological precancer has been diagnosed within 2 years of sexual debut.[33,56,57] The biological meaning and clinical importance (ie, risk of invasion) of these early precancers are unknown. The average age of diagnosis of precancer varies from 25 to 35 years and depends both on the average societal age at first intercourse, which can serve—with notable exceptions such as early sexual abuse—as a crude proxy for first exposure to HPV, and on the intensity of screening. Screening by HPV testing can promptly detect precancers that would otherwise grow slowly to the point of detection by less sensitive methods like cytology and colposcopic impression.[58] However, more sensitive screening will also classify as abnormal more lesions and infections that would clear without treatment.

The risk factors for persistence and precancer have not been disentangled. HPV type is the strongest factor that affects the absolute risks of viral persistence and of progression to precancer given viral persistence.[17,45] HPV16 is remarkably carcinogenic, with an absolute risk of a precancer diagnosis approaching 40% after 3–5 years of persistent infection.[17,46,59] The total risk of precancer for a woman carrying several HPV types is increased compared with women infected with any one of the HPV types she carries, but it is not clear whether her risk is greater than the sum of the risks posed by individual HPV types.[31]

Viral load measurements are not clinically useful. Levels detectable only by PCR (eg, below the threshold of detection of the commercially available Hybrid Capture 2 [Digene Corporation, Gaithersburg, MD, USA]) are associated with microscopic normality and with low risk of subsequent precancer or cancer, but increasingly high viral loads do not imply increasing prospective risk,[60] except for HPV16.[61,62] The amount of HPV DNA measured in scrapes of the cervical epithelium is a complex sum of the number, size, and grade of the HPV-associated lesions,[63] and therefore the meaning of viral load is ambiguous. Some of the highest viral loads can be attributed to recently acquired minor lesions producing large amounts of virus, analogous to benign warts.

Risk of cervical cancer is mainly a function of HPV infection and lack of effective screening. External factors (apart from screening) are minor compared with the extremely high primary risks of the most carcinogenic HPV types. Smoking,[64] multiparity,[65] and long-term use of oral contraceptives[66] can double or triple the risk of precancer and cancer among women infected with carcinogenic types of HPV. The role of chronic inflammation, especially due to coinfection with *Chlamydia trachomatis*, is less certain.[67] Further, there has been no confirmation of a role for any one micronutrient in observational studies and supplementation trials,[68] although there is some evidence of a possible protective association between higher folate and the risk of precancer.[68] Among HPV-infected women, low socioeconomic status might remain a risk factor for precancer even when recent medical care is taken into account.[69] Interestingly, a preliminary association between condom use and decreased persistence or progression has been seen in a few studies.[70,71] The mechanisms of action for HPV cofactors (whether immune, genotoxic, or hormonal) are not well understood.

Poorly understood cellular immune responses strongly affect whether an infection is ultimately cleared or persists to pose a risk of precancer. Efforts to identify specific subsets of T cells responsible for clearance remain inconclusive.[47] Epidemiological studies in diverse populations have shown the human leucocyte antigen DRB1*1301 to be protective.[72] In addition to acquired immune responses, innate immune responses—the first line of mucosal defence—could also have an important role.[73]

HPV infection in people living with HIV/AIDS has been addressed in detail elsewhere.[51,74,75] In brief, HIV

status, defined by CD4+ T-cell counts and HIV viral load, strongly affects the early natural history of HPV. HIV-positive individuals experience increased HPV prevalence and persistence, and decreased viral clearance compared with HIV-negative individuals for types other than the uniquely persistent and carcinogenic type HPV16. HIV-infected individuals are often infected with more HPV types than are non-infected individuals, suggesting type differences in the success of immune suppression.[76] The probability of invasion is not strongly affected by HIV. Highly active antiretroviral therapy (HAART) does not seem to affect HPV natural history or to reduce the risk of cervical precancer and cancer; however, the relation between such therapy and risk is perhaps confounded because HIV-infected women on HAART are living longer and allowing the cervix to have prolonged exposure to carcinogenic HPV in the context of relative immunosuppression.

Invasive cervical cancer

In unscreened populations, the peak risk of invasive cervical cancer occurs earlier than for most adult cancers, peaking or reaching a plateau from about 35 to 55 years of age.[77] This distribution is due to the fact that cervical cancers originate mainly from HPV infections transmitted sexually in late adolescence and early adulthood. The average time between HPV infection and establishment of a (small) precancer seems to be much shorter than the average duration of precancer growth leading to invasion. There are many more precancers than cancers, suggesting that only a minority invade. The precise magnitude and timing of risk of invasion, if precancers were left untreated, will remain unknown because contemporary cohort studies, in which treatment of precancer is mandated, cannot study invasion ethically.[78] Crude estimates from early studies of large precancers suggested a 20–30% risk of invasion over a 5–10-year time frame.[79,80]

Apart from age, risk factors for invasion are unknown except for viral type; in particular, HPV16, HPV18, and HPV45 are found in a higher fraction of cancers than in precancers than are other HPV types.[18] The integration of the HPV genome into the host genome is associated with invasive cancer and might be an important biomarker distinguishing HPV infection from precancer.[81] However, integration might not be necessary to cause invasion, since not all women with invasive cancers have measurable integration.[82,83] Continued transcriptional activity of the HPV oncogenes is needed to maintain the cancer.[84]

Prevention of cervical cancer

Risk as a guiding principle of prevention strategies

The steps in cervical cancer pathogenesis can guide prevention and management. Short-term risk of CIN3 is a scientifically valid, ethically justified surrogate for long-term cancer risk, and can be estimated in prospective studies and clinical trials. To base clinical decisions on knowledge of risk of such lesions makes sense; the clinical response should be uniform irrespective of what clinical test is used to define risk[85] (panel 1). For example, finding HPV16 on an HPV DNA test conveys slightly higher risk of subsequent CIN3 than does cytologicial identification of low-grade squamous intraepithelial lesions.[44] The best way to predict individual risk is to use the risk estimate from a large stable group of women with similar characteristics.

Panel 1: **Replacing clinical protocols with risk stratification**

Total cost and total benefit are the key statistics needed to assess and compare old and new technologies for screening and diagnosis. For public-health programmes like screening and management of very common abnormalities, the costs and benefits should be described on a population basis: for example, the number of cases of disease or death averted and the total cost—ie, financial and iatrogenic medical consequences—summed over all women receiving the test. These integrated costs and benefits, and therefore prioritisation of who will receive the tests, will vary with the indication for the screening and with geography, age, and sexual behaviour. Sensitivity and specificity without weighting by frequency of disease are not enough to capture integrated costs and benefits adequately. By contrast, positive predictive value, which is the probability of disease among women testing positive, and negative predictive value, which is a measure of the reassurance of no disease among women testing negative, are expressed in terms of population risks and are helpful.

There are several corollaries of thinking in terms of risks. First, there should be no distinction between a clinical and a molecular test. For example, the utility of a colposcopic examination, a Pap test, and an HPV test should be compared on an even footing on the basis of performance and cost. Second, for a test with fixed sensitivity and specificity, the clinical or population subset with the higher risk of disease should have higher priority for intervention, given equal cost. Third, no one-dimensional comparison of tests or programmes, like an odds ratio or even a risk difference, can capture the information needed to choose between them. Finally, the cost effectiveness of a programme is measured by comparison with alternative programmes, including no programme. Thus, visual inspection with acetic acid could be cost effective in a resource-poor area where there is no alternative programme in place, despite its low accuracy.

Adoption of a-priori thresholds based on risk for deciding who needs closer surveillance, colposcopy, and treatment will aid clinicians in making decisions that maximise the health benefit to women. For example, a society might decide that women at less than 2% absolute risk of precancer within the subsequent 2–3 years are normal and can stay in regular interval screening, women with a 2–9% risk should be re-screened in a year, women with a 10–39% risk need intensive colposcopic assessment immediately, and women with 40% or greater risk need immediate treatment. The choices of such cutoff points, once accepted, could guide management as new risk biomarkers are validated.[85]

There are a few implications of strategies based on absolute risk. First, no screening strategy is efficient among young women, who have very high prevalence of HPV infection and of its cytological signs, both of which are very likely to clear without intervention. Strategies to prevent the rare but sometimes fatal rapidly invasive cancers among young women require screening and aggressive management of huge numbers of ultimately normal young women; prevention strategies in regions seeking a nearly perfect level of prevention can be so expensive that the entire programme is no longer cost effective. Second, using HPV testing to detect very low viral loads and marginally carcinogenic types (eg, HPV53) should be avoided to preserve the predictive value of a positive test.[86]

Panel 2: **Important questions regarding the new HPV16/18 vaccines**

1. What is the duration of protection and the total effect on cancer incidence?
2. Are boosters safe and effective if needed?
3. Do the vaccines provide cross-protection against a few related types, as previously suggested?
4. What is the efficacy of fewer than three doses of vaccine, as will sometimes occur in vaccination programmes?
5. Does the vaccine prevent infection in men, and reduce the transmissibility of HPV from men to their partners?
6. When immunity wanes and incident infections occur, are the natural history of HPV16 and HPV18 infections and the related risks for precancer and cancer the same as in unvaccinated women who typically acquire infections by these types as young women?
7. What will be the effect of HPV vaccination on compliance with screening programmes, which are needed for prevention of the 30% of cancers against which the vaccines do not provide protection?
8. How great will the negative effect be of the reduced prevalence of HPV16 and HPV18 in post-vaccinated populations on the clinical performance and cost-effectiveness of screening assays and diagnostic procedures?
9. Will prevention of infection with HPV16 or HPV18 alter the natural history of other carcinogenic types and the number of cervical cancers they cause?
10. Do these vaccines protect against other HPV-related cancers such as oropharyngeal and anal cancers?
11. In developing countries, where 80% or more of cervical cancer occurs, who can afford to get vaccinated, even with tiered pricing, in view of competing health priorities?

Primary prevention of HPV infection

There is some evidence that health education programmes that promote abstinence, conscientious condom use, or both, could reduce the risk of cervical cancer at the population level.[87] However, mutual abstinence until marriage is far from universal, and even strict condom use is not completely protective against HPV transmission because the male anogenital skin is not completely covered.[88] Thus, the development of HPV L1 virus-like-particle (VLP) vaccines is a potentially major advance in prevention of cervical cancer. These vaccines are based on the self-assembly of recombinant L1 protein into non-infectious capsids that contain no genetic material.[89] Intramuscular injection of the vaccine induces high titres of neutralising antibody, more than 50 times the titres induced by natural infection.[90] Protection at the cervix against the same types in the VLP vaccine is probably mediated by antibodies transudated into the secretions that bathe the epithelium, serum antibodies directly exudated at the site of microscopic trauma thought to be involved in transmission, or both[91] (Schiller J, National Cancer Institute, Bethesda, MD, USA; personal communication).

Two VLP vaccines have been developed for primary HPV vaccination. Gardasil (Merck and Co, Bluebell, PA, USA) has gained regulatory approval in several countries. Cervarix (GlaxoSmithKline, Rixensart, Belgium) has been approved in Australia, is pending approval in the European Union, and applications for approval have been submitted to regulatory agencies in the USA and other countries.[92] Both vaccines target HPV16 and HPV18; Gardasil, which includes a standard alum adjuvant, also targets HPV6 and HPV11.[92] Cervarix uses a new proprietary adjuvant intended to boost immunogenicity. In populations of young adult women without known exposure to the target types, both vaccines have shown near perfect efficacy against HPV infection and related cytological and histological endpoints for up to 5 years.[93–97]

Important questions remain (panel 2). For example, the regulatory approval for Gardasil was predicated on rather short-term efficacy data for 15–26-year-old women and data showing good antibody titres after vaccination for 9–15 year olds. At present, girls aged 11–12 years are being targeted in the USA, before entry to middle school. Several jurisdictions are considering mandatory vaccination for 11–12-year-old girls, but this is controversial, especially regarding cost-effectiveness analyses (table 1).[98] Ideally, to ensure that a vaccination programme will protect young women through the age of greatest risk of HPV exposure, we would know that durability will be 10–15 years or greater or that boosting will be safe and effective; waiting for certainty, however, would reduce the benefit for the cohorts of girls born between about 1995 and early 2005.

The value of universal vaccination in the upper age range, 19–26 years, is even more controversial.[99,100] Women who have had several sexual partners and have already been exposed to the target types are at least partly immune and cannot be distinguished from unexposed women by DNA testing, because both groups will be negative and serology is not reliable or accurate as a biomarker of past exposure and protective immunity.[101,102] As women age, they are more likely than younger women to have established monogamous relationships that reduce future risk. The vaccines do not treat existing infections or lesions,[103,104] and cross-protection against other HPV types is partial[95] or non-existent.[103] Therefore, the current HPV vaccines are most certain to yield the greatest public-health benefit (population effectiveness) in girls at an age before most have begun sexual activity. At a certain, still-undetermined age that might vary by region, screening might be more cost effective than vaccination if a trade-off is considered. We believe that broad recommendations for widespread vaccination in adult women should await independent, population-based effectiveness trials and cost-utility assessments.

	For mandatory vaccination	Against mandatory vaccination
Do we have enough information to promote a major public-health mandate?	Many vaccination programmes are implemented before long-term durability and safety data are available. Implementation will create the needed vaccinated cohort to permit assessment	Because screening is a good alternative to vaccination, no mandatory programme should be promoted until strong supportive data are already available to permit integration of vaccination and screening
Population benefit of vaccination overlaid on screening	Making vaccination mandatory would increase coverage of prevention among the poorly screened, highest-risk population that would benefit most from vaccination	Further reductions in incidence and mortality (already low in the USA) would be difficult to achieve. Adding vaccination to screening might even reduce compliance with screening because women might falsely assume that they are protected
Urgency of mandate	If we delay, cohorts of girls will miss the benefits of vaccination	It is better to proceed slowly, accumulate more data and public acceptance based on voluntary vaccinations, and to move to mandates when public-health benefit is established
Safety	Good safety profile to date	Rare effects can not be ruled out until many more girls are vaccinated
Known durability	Established durability of about 5 years with sustained serotitres for HPV16	Peak risk of sexual exposure lasts for more than 10 years after suggested mandatory vaccination age
Trends in protection in years after vaccination	No evidence for decreasing efficacy over 5 years	HPV18 serotitres fall within 2–3 years of vaccination with Gardasil,[96] which might herald a subsequent decrease in protection
Feasibility of boosting	One unpublished, small study suggests good antibody response to booster among young adult women	General lack of large-scale evidence, and lack of well-formulated strategies regarding how and when boosting could be done. Lack of data on the safety of boosting. No serology assay widely available for monitoring if titres proved predictive
Choice of vaccine	Gardasil is already approved in many countries	Cervarix is approved in Australia and might be approved elsewhere within a year. Weighing its relative benefits might make sense. Are the two vaccines interchangeable and compatible?
Impending development of second-generation vaccines with more types, longer durability, or lower cost	It is best to start now and replace or boost with newer vaccines when available	Especially for lower-resource regions, second-generation vaccines could reduce number of doses, need for boosting, and cost
Ethical and family issues	There is no evidence that vaccination would promote sexual activity. Parents could opt out	Vaccination might encourage onset of sexual activity and deny the parents their right to choose
Cost-effectiveness	Analysis assuming lifelong durability already indicates cost-effectiveness at current prices	Unknowns include durability, need for boosting, integration with screening schedules, and possibly reduced performance of screening tests. Strain on public-health resources

Table 1: **Arguments for and against mandatory HPV vaccination of girls before the average age of sexual debut**

Multivalent vaccines (which target an expanded range of types) and different approaches to produce immunising HPV proteins are being tested.[105] The effect of expanding the number of types on vaccine price and safety are unclear, as is the acceptability of the current vaccine once there is public awareness that a broader-spectrum vaccine is coming. One alternative to achieve broad-spectrum protection that is being explored is vaccination with L2, the minor capsid protein, which has been shown to elicit cross-neutralising protection (albeit weaker responses than the L1 VLP vaccines).[106] The ideal would be a low-cost, single-dose, needle-free pan-HPV vaccine that is stable without refrigeration. Alternatively, there is evidence that carrageenan, an inexpensive polysaccharide used in a wide spectrum of human-use products, including vaginal lubricants, potentially blocks HPV infection in a mouse challenge model.[27]

Screening

Cervical cancer prevention, as practised in high-resource regions, includes: screening; triage of equivocal results; colposcopically guided biopsy of abnormal screening results; decision whether to treat; treatment; and post-treatment follow-up (including eventual return to routine screening intervals if appropriate). Cervical cancer prevention programmes vary widely by country and could be radically improved by new technologies.

Whichever validated screening method is chosen, broad coverage and full follow-up of abnormalities are the key requirements for reducing the incidence of cervical cancer by screening. Screening in the absence of a treatment programme is unethical. The appropriate programme for a given setting (when to begin, the proper intervals between screens, when to stop) depends on affordability, differing societal demands for protection against cancer risk, and desire to prevent iatrogenic complications among women who are at low risk of cervical cancer. However, a few principles follow from the established fact that the cause of cervical cancer is persistence of sexually transmitted HPV infection of the transformation zone. Screening women within 5–10 years of first sexual intercourse, when the risk of finding benign HPV infections is very high but risk of cancer is vanishingly low,[107] cannot be cost effective. Similarly, it is not cost effective to screen women after total hysterectomy for reasons unrelated to cancer[108] or older women with

Panel 3: Why HPV16 deserves individual consideration in prevention programmes

1. HPV16 causes half the cases of cervical cancer worldwide and is the major carcinogenic type in almost every country surveyed[133]
2. HPV16 is the most common carcinogenic type in the general population, accounting for about 20% of infections among cytologically normal women,[134] 20% among women with equivocal lesions,[133] and 26% among those with mild abnormalities[133]
3. Although HPV16 is no more likely to cause cytological abnormalities than other carcinogenic types,[40] it disproportionately causes changes suggesting precancer and accounts for about 45% of those severe interpretations[133]
4. Prospectively, HPV16 persists longer on average than any other type and persistence is highly associated with precancer (about 40% of women with persistent HPV16 are diagnosed within 5 years with precancer)[17]
5. HPV16 is the main HPV type that causes other anogenital and oropharyngeal cancers that are not common enough to merit screening but might be prevented by an effective vaccine[135]

repeated negative cytology, HPV tests, or both, assuming endocervical sampling is adequate.[52]

The development and implementation of organised and effective cytology-based cervical cancer screening—eg, Papanicolaou (Pap) tests—for detection and treatment of precancerous lesions and earlier stage, treatable cancers has led to significant decreases in the incidence and mortality of cervical cancer.[109] The effect of cytology programmes has been best documented from ecological correlations of cervical cancer with screening activities in populations, mainly in Nordic countries. Many early screening programmes targeted the peak ages of cervical cancer risk and produced decreases in incidence confined mainly to women aged 30–70 years.[110] When screening coverage is extended to younger and older women, rates at all ages decrease, although cost per cancer averted rises, as seen in US Surveillance and End Result (SEER) data.[111] A fraction of the earliest cases of cervical cancer are probably rapid onset;[112] their shorter time of development permits fewer rounds of screening that could detect precancerous lesions.

The success of well-established cytology programmes in detecting cervical precancer and treatable cancer is attributable to repeated, fairly insensitive screening of women during the slow progression from incident HPV infection to easily diagnosed precancer (5–10 years) and from precancer to cancer (typically ≥10 years).[113,114] The need for repeated screening cycles makes cytology-based cervical cancer screening programmes expensive.

Further substantial, cost-effective improvements in cytology programmes could be difficult to achieve, although automated screening of liquid-based cytology might someday prove itself.[115] In choosing between cytology techniques, there is no convincing evidence that liquid-based cytology is more accurate than conventional pap smears, especially when adjunctive HPV testing is done.[116,117] Nonetheless, liquid-based cytology might reduce the proportion of inadequate smears,[118,119] especially in settings where conventional smears are prone to air-drying (eg, the tropics) or where widespread cervical inflammation is a problem. Although not abnormal, an inadequate smear can similarly increase anxiety of women.[120]

Assays for HPV have been introduced to improve the efficiency and maximise the sensitivity of cervical cancer screening. There is convincing evidence that testing for carcinogenic HPV DNA is cost effective and sensitive for detection of precancerous lesions in women with equivocal cytology,[114,121,122] is more sensitive but less specific than cytology-based methods for primary cervical cancer screening,[45,114,117,123,124] can be added usefully to the follow-up of women post-colposcopy when precancer is not found,[125] and can guide assessment of cure post-treatment.[126] Most importantly, testing negative for carcinogenic HPV provides greater reassurance against cervical precancer and cancer than does cytology-based methods. The greater reproducibility of current tests for carcinogenic HPV types[127] is an added advantage over cytology. In the USA, HPV testing to triage equivocal cytology is commonly used. HPV is also approved in primary screening in women 30 years and older, who are past the peak of self-limited infections, and in whom the positive predictive value is higher than in younger women.[128] The International Agency for Research on Cancer has

	Proportion of cervical cancers caused	Cumulative total
HPV16	54·6%	54·6%
HPV18	15·8%	70·4%
HPV33	4·4%	74·8%
HPV45	3·7%	78·5%
HPV31	3·5%	82·0%
HPV58	3·4%	85·4%
HPV52	2·5%	87·9%
HPV35	1·8%	89·7%
HPV59	1·1%	90·8%
HPV56	0·8%	92·2%
HPV51	0·7%	92·9%
HPV39	0·7%	93·6%
HPV73	0·5%	94·1%
HPV68	0·5%	94·6%
HPV82	0·2%	94·8%
No type identified	5·2%	100%

Data adapted from reference 18.

Table 2: **Proportion of cervical cancer caused by the carcinogenic HPV types**

endorsed the use of carcinogenic HPV testing alone as an option in primary cervical cancer screening.[109]

At least four assays indicating current infection give roughly similar results when used to assay the major carcinogenic HPV types as a pool: Hybrid Capture 2; the MY09/MY11 primer set and its improvements like PGMY; the GP5+/GP6+ primer set; and SPF10/LiPA PCR-based methods.[123,129,130] Only Hybrid Capture 2 is approved in the USA by the Food and Drug Administration (FDA); it does not provide individual typing information. Two PGMY-based systems have been submitted for FDA approval, one of which is a pooled test like Hybrid Capture 2, while the other provides genotyping. We believe that only completely standardised assays should be used for routine practice because standardisation is, with sensitivity, the major advantage of HPV testing.[131] Without rigorous standardisation, subtleties of HPV tests can greatly affect analytical performance.[132]

HPV16 is by far the most important HPV type worldwide, and its effect is reflected in all aspects of cervical cancer prevention (panel 3). If we could eliminate HPV16 infection or reliably identify and destroy all its cytopathological or colposcopic manifestations, we could prevent up to half of cervical cancer cases.[2] Table 2 shows the relative importance of different HPV types. An important goal is to improve specificity of HPV testing while maintaining its clinical sensitivity. Possible advances include type-specific detection of HPV16 and HPV18, which has been shown to identify women at the greatest risk of developing precancer and cancer.[136] Detecting persistence of these most carcinogenic HPV types would be an even more specific marker of clinically important infections, theoretically, but clinical use of genotyping will require robust assays and workable clinical protocols. Other promising screening assays under development detect carcinogenic HPV E6/E7 mRNA[137] and $p16^{INK4a}$.[138]

Cervical-vaginal self-collection permits the use of molecular testing outside clinical settings with clinical sensitivity for precancer and cancer that seems as good as cervical cytology but lower than clinician-directed specimen collection.[139,140] The degree of sensitivity might be sufficient if self-collection encourages and permits otherwise unscreened women to be screened.[141]

Importantly, HPV testing can be too analytically sensitive. HPV is not a disease per se, and any molecular detection of HPV is worthwhile only as part of a cervical cancer prevention programme. Moreover, benign infection is very common, creating a tension between positive and negative predictive values. The cutoff points of positivity must aim for clinical sensitivity, defined as the detection of CIN3 or worse until the next screening, while attempting to minimise the detection of insignificant infections or traces of viral DNA. The detection of some benign infections is unavoidable, but the types targeted must be restricted to clearly carcinogenic types to prevent non-specificity that could affect thousands of women falsely labelled as being at risk of cancer.

Diagnosis of abnormalities found on screening

Historically, colposcopically directed biopsies have been the clinical reference standard for diagnosing precancer or even to make finer distinctions such as CIN grade 1, 2, or 3.[142] However, the choice of biopsy site and the histopathological diagnosis of resultant biopsies tend to be variable and subjective.[143] Clinicians rely on colposcopy to determine the presence or absence of epithelial lesions, find the area of the cervix with the highest degree of disease, and direct the biopsy for histological diagnosis. Colposcopic assessment also provides information about location and extent of disease, which is important for planning treatment.

Although the sensitivity of screening has improved considerably in the past decade, colposcopy has not advanced, given the weak correlations between visual changes and disease severity and lack of reproducibility among assessors.[144] Even highly experienced assessors have false negative colposcopy rates as high as 20–40% in patients with a histological diagnosis of precancer.[145,146] Two factors affect this false negative rate: first, CIN3 lesions missed by colposcopy are smaller and involve fewer quadrants of the cervix than do lesions that are detected visually;[147] and second, patients with precancer related to non-HPV16 carcinogenic types are more likely to have equivocal visual lesions.[144] Therefore, the use of colposcopy might be even more limited when HPV testing and vaccination become more widely used.

Colposcopic sensitivity increases significantly if more than one non-random biopsy is taken, irrespective of training or expertise of the assessor.[146,148] More studies are needed to determine whether the additional biopsies should be taken only from apparent lesions, from areas of minor epithelial changes, or even from seemingly normal quadrants of the cervical epithelium.

Treatment of cervical precancer and invasive cancer

The effect of behavioural factors on the clearance of HPV or precancer is poorly understood. However, consideration of smoking is always important for reasons of public health. There is some evidence that smoking cessation promotes resolution of HPV-induced cytopathology.[149] Genotoxic smoke constituents are secreted at high levels into the cervical mucus.[150] Enhancement of cellular immunity is also probably involved. In any case, it makes sense to encourage women with precancerous screening abnormalities to stop smoking in the context of a broader programme for prevention of smoking-related cancer and health problems.

At present, the usual practice for treatment of precancers is to treat the entire transformation zone of women diagnosed with equivocal (CIN2) or more definite (CIN3)

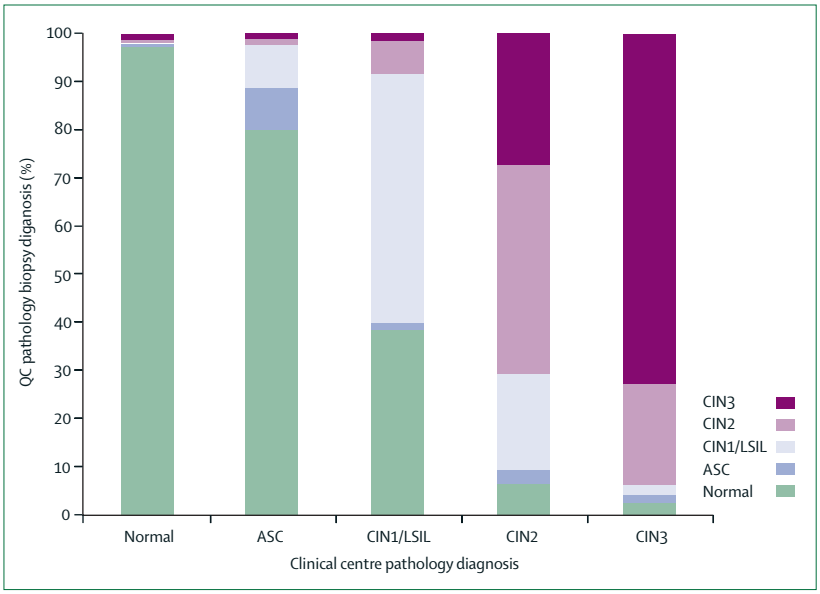

***Figure 7*: A comparison of community pathology biopsy diagnoses to quality control pathology review diagnoses**

Comparison of biopsy diagnoses made by clinical centre pathologists (community diagnosis)[156] with biopsy diagnoses rendered by the expert quality control pathology.[54] Patterned bars highlight the proportion of agreement for a given community diagnosis. Note that many biopsies diagnosed as CIN2 by the clinical centre pathologist, the threshold for excisional treatment in many countries, were not called CIN2 on expert review.

precancer, not just the identified lesions.[151] There is some international variation in the treatment threshold, and consideration of age and desired family size is common and appropriate. The presence of a lesion indicates that the entire transformation zone is at risk and colposcopists cannot ascertain the site of worst pathology with certainty. In many countries, hysterectomy is no longer acceptable as a primary treatment option for precancers, and fertility-sparing treatments such as cone-shaped excision and cryotherapy are the first options for intraepithelial lesions of the cervix. Loop electrosurgical excision procedure (LEEP) or large loop excision of the transformation zone (LLETZ) has become the most popular procedure because it can be done as an outpatient with local anaesthesia and because it removes only a small amount of cervical stroma. Although taking the minimum amount of tissue is the goal, recent reports show that women who undergo LEEP/LLETZ are at increased risk compared with the general population for premature deliveries.[152] Cold-knife cone is still used when more extended tissue removal is required.

Cryotherapy with nitrous oxide, a low-cost ambulatory treatment, is almost as effective as ambulatory excisional procedures to treat small precancerous lesions.[153] Cryotherapy is widely used in low-resources areas mainly because it can be provided without local anaesthesia or electricity. One noteworthy drawback is the typical weeks of recovery marked by discharge with some possibility of infection. Carbon dioxide is often used as an alternative gas to nitrous oxide because it is less expensive and easier to find in remote areas; however, technical improvements are needed to overcome serious deficiencies such as blockage of equipment[154] and poor depth of tissue necrosis.[155]

While CIN2 is a poorly reproducible diagnosis (figure 7) and the accuracy of colposcopic biopsy itself is in some doubt, we might soon be able to treat the transformation zone based on more exact virological assessment of risk. Specifically, if a carcinogenic HPV type persists for a number of years in an older woman for whom fertility issues are not important, the risk posed by LEEP might be warranted to address the possibility that a precancer is being missed by colposcopy. The use of a molecular test to guide treatment will require extremely careful study (panel 1) and we suggest it for discussion, not immediate adoption.

Cytology and HPV testing are useful to assess cure after treatment by LEEP. Women successfully treated usually test HPV negative. Those testing HPV-negative 4–6 months after LEEP have no appreciable risk of recurrent CIN2 or worse within the subsequent couple of years, although the relevant studies have lasted only around 2 years, so for how long negative tests after LEEP can be interpreted in this way is not clear. Those testing positive must be monitored more closely, although the proper immediate management must be individualised.[126,157]

Any proposed outpatient non-surgical method must work very well, because excisional procedures are 90–95% effective with minimal side-effects. Current HPV vaccines do not treat existent HPV infections or precancerous lesions. A better understanding of the molecular underpinnings of HPV and cervical carcinogenesis could lead to the rational design and development of an array of targeted, lower-morbidity non-surgical treatments such as therapeutic vaccines, topical immunotherapies (eg, imiquimod and resiquimod for treatment of condyloma), and topical chemotherapies (eg, siRNA and apoptosis inducers).

There is a pressing need to educate health professionals and the public regarding the natural history of HPV as we move towards HPV-based prevention strategies. As evidenced by the recent alarmist reaction to a report that HPV is very common in the general US population,[158] many still conflate the one-time detection of HPV DNA with high risk of cervical cancer (unpublished data). Unwarranted psychosocial damage can follow detection of HPV.[159] Many women would probably still prefer to be notified of a mildly abnormal Pap than of an HPV infection because the connection between abnormal Paps and sexual behaviours was previously not well understood, although the two test results address the same biological processes.

We have yet to agree as a health community on the full set of messages that should accompany HPV screening. It is not clear which health professional will have the time, training, and interest to lead the education effort in different regions, especially as messages change following the advent of vaccination. A critical example of an

educational issue is what to tell women with normal cytology and a positive HPV test. We propose that patients should be informed that although HPV exposure is extremely common, almost all infections go away within a year or two; many are gone within 6 months. Patients should make sure that they get retested, and if the infection does not clear, then they will need a full examination (colposcopy with multiple biopsies) and possibly treatment to prevent precancer and later risk of cancer.

Two important advances in the treatment of invasive cancer deserve brief mention. Radical hysterectomy has been the preferred treatment for stage I cases, but during the past few years, minimally invasive surgery has become an option for young women with small tumours who desire fertility. Currently there are enough data to conclude that radical vaginal trachelectomy with laparoscopic pelvic lymphadenectomy is a safe procedure with an acceptable recurrence rate (4%). In one study, pregnancies occurred in 31 (43%) of 72 treated patients and 36 (72%) of 50 pregnancies reached the third trimester.[160]

Radiotherapy is still the best choice for stage II–IV patients, but several randomised studies have shown improvement of survival with concurrent chemotherapy.[161] Cisplatin seems to be the best drug for advanced squamous carcinoma as a single agent or in combination with other cytotoxic drugs. The optimum treatment of adenocarcinoma is less clear.

Fitting prevention strategies into available resources and existing programmes

New cervical cancer prevention methods must be introduced with consideration of added value and added cost. Otherwise, the rich could easily be over-treated, while the poor at higher risk are neglected. For example, the addition of HPV testing to cytology for screening, if repeated every year, cannot be cost effective and will lead to excessive interventions.[162] Similarly, new preventive vaccines, if adopted with high acceptance, rationally must lead to less frequent screening to be cost effective.[163] With less HPV16 and HPV18, the predictive value of positive screens will fall as the number and relative proportion of important lesions decreases, and the marginal gain in reassurance from negative screens will decrease as well.[113,164] In general, regional planners must decide which prevention strategies do well, and which additions (or replacements) are most worthwhile.

To reach their potential, new cervical cancer prevention methods will need to be accessible and affordable to women who are currently underserved and at the greatest risk. The average cost per year of life saved with cytology-based screening programmes in countries where it has been successful is higher than most resource-limited countries' annual gross per head income.

Cervical cancer screening programmes requiring one to three interventions in a woman's lifetime are the most cost effective; more visits (common in high-resource regions) have a notably reduced cost-effectiveness.[165] High programme coverage and immediate, effective treatment of positive cases (to minimise loss to follow-up) are crucial to achieve a reduction in cervical cancer mortality. Other factors that increase cost include the woman's time requirement, need for transportation and the availability and cost of treatment of cancer cases. The relative importance of each of the different factors varies by country; thus programme selection has to be adjusted accordingly.[166]

One of the least expensive, easiest, and most widely assessed screening approaches is visual inspection with acetic acid or with Lugol's solution. Visual inspection with acetic acid has been better studied, and its sensitivity estimates vary considerably (40–90%), partly due to lack of technique standardisation but also to the use of different gold standard methods.[167–170] Additionally, the technique provides instant results that, if combined with treatment options such as cryotherapy, allows for 1-day see-and-treat schemes that decrease the overall cost of screening programmes substantially. Despite its low sensitivity, specificity, and predictive values when used as a stand-alone test, in scarce-resource areas visual inspection with acetic acid is a realistic screening method when the only alternative is no screening.[171]

We face an important challenge to apply HPV-based technology widely at low cost. The effect on cancer of vaccines against HPV infection will not be felt until about 20–30 years after a countrywide programme is introduced.[163] The introduction of such programmes will probably require the involvement of donors like WHO, the Pan American Health Organisation, the GAVI Alliance, or the Bill & Melinda Gates Foundation to make vaccines available and affordable.

Like HPV vaccines, existing HPV tests are unaffordable and need to be done in specialised laboratories. A new HPV DNA test has been developed for low-resource regions by the Program for Appropriate Technology in Health (PATH) through a grant from the Gates Foundation. This test will provide results within a few hours with sensitivity and specificity similar to current commercially available tests, but at a cost of under US$5.[172] Additionally, there are few infrastructure and reagent requirements, making HPV testing a practical possibility as a stand-alone screening method. Validation studies are currently underway.

We believe that a logical prevention strategy in regions with scarce resources would combine vaccination before sexual debut (if reduced cost or donated vaccine is available) and screening at an optimum age around 35 years with cryotherapy of all HPV-positive women except for those needing expert care—eg, for obvious cancers.[24] Region-specific age rates of HPV prevalence should be considered to guide the ages of vaccination and screening. Combining vaccination and screen-and-treat strategies would reduce overall HPV endemicity and provide lasting population benefit.

Future directions

There are a number of important, active research topics that will soon affect clinical management of cervical HPV and precancer: the average risk and timing of clearance versus persistence of each type of HPV; the risk and timing of diagnosis of precancer given persistence of each of the types; the effect, if any, of age at infection on these rates of clearance, persistence, and progression; the risk, if any, of occasional re-appearance of an HPV type via reinfection or latency, if such a state exists for HPV, following initial clearance; the origin and significance of age-specific HPV prevalence curves that differ by region; the unique carcinogenicity of HPV16, including its molecular mechanism and natural history; the occult nature of HPV18 infection and related lesions and the increased importance of HPV18 in the development of adenocarcinoma; the validation of new molecular markers with better predictive values than CIN2 diagnoses for distinguishing between HPV infections with and without concurrent precancer and, among those without concurrent precancer, distinguishing those that are most likely to become precancer in the next 5 years; the immune response that prevents HPV reinfection and the response that underlies HPV clearance, including genetic influences on the success of these responses; and HPV natural history in immunosuppressed individuals.

The advent of highly efficacious prophylactic vaccines against HPV16 and HPV18 has irrevocably changed the landscape of research into cervical cancer prevention. No-one can predict how quickly we can move towards the goal of a vaccine that protects against all carcinogenic HPV types, with a safe and inexpensive, universally applicable route of delivery. However, each improvement in vaccines will force a reconsideration of the whole prevention effort, relative to resources. Based on the current vaccines, some new clinical directions are already evident.

Although approval of the Merck vaccine has proceeded rapidly in many countries, actual adoption has been irregular by country and, at least in the USA, by state. Mandatory vaccination, catch-up vaccination of older girls and young women, and vaccination of boys remain controversial issues. The protection offered by two rather than three doses of vaccine is being determined, which will affect cost. The trade-offs from the quadrivalent coverage of the Merck vaccine and the novel adjuvant of the GSK vaccine will be considered in cost-effectiveness analyses once more longer-term, type-specific efficacy data are released.

The use of HPV testing for primary screening will certainly increase, but the relative roles of cytology and HPV testing (alone or combined) will vary by country for years to come. We predict that HPV genotyping, first for HPV16, will eventually enter clinical practice as an important prognostic biomarker, and hope that its introduction is preceded by clear validation of reliable typing assays and useful follow-up algorithms. Once genotyping is reliable, type-specific viral persistence will immediately become an appealing and powerful prognostic biomarker. The discussion will shift to proper time intervals for defining persistence. Even if data demonstrate high risk in women with persistent infections in the absence of clearly diagnosed precancer, excisional treatment based on molecular tests alone will probably be very controversial and adopted as policy only in societies favouring aggressive clinical management.

Successful, widespread vaccine programmes will motivate reconsideration of optimum screening techniques and strategies. It is evident that the most clear-cut cytopathological and colposcopic abnormalities are caused by HPV16. The predictive values of screening protocols including HPV testing depend to a major extent on the risks associated with HPV16. Screening protocols will need to change with time as the population prevalence of HPV16 is gradually reduced.

Improvements in diagnosis will need to be made to match the improvements in screening. Colposcopically directed biopsy, in which the clinician targets the most abnormal lesion, is not sufficiently reliable or accurate to diagnose precancer in women referred by the combination of HPV testing and cytology. To foster better diagnosis for clinical practice and as a reference standard of disease, there is an urgent need for clinical trials to assess how biopsies should be taken to improve sensitivity and the reassurance of a negative colposcopic examination. Otherwise, the benefits of improved screening will not be fully realised.

Developing a simple and safe treatment for persistent HPV infection, including small precancerous lesions, would be an important breakthrough of immediate importance worldwide. We can now reliably and sensitively detect infection with carcinogenic types of HPV to identify women at risk of cervical cancer, but viral clearance is too common to justify immediate treatment, especially at younger ages. In some regions, for women past the peak ages of HPV prevalence, the most effective screening-based prevention strategies mandate immediate rather than delayed treatment (which results in losses to follow-up). Immediate treatment requires safe, inexpensive, simple destruction of the transformation zone and surrounding epithelium (or an equivalently safe and simple non-surgical approach). Finding a treatment better than the current forms of cryotherapy and loop excision should be a high priority.

We project that improved vaccines, screening tests, and management strategies will continue to emerge without evident end. New vaccine candidates and molecular biomarkers will supplant the prevention tools we have discussed. How quickly the institutions supporting cervical cancer prevention can react to

evolving opportunities is unclear. For example, recent studies of visual inspection with acetic acid indicate some benefit in detection of cervical cancers and large precancers. Adoption of this technique could reduce the incidence of, and mortality due to, cervical cancer in very low-resource regions. However, any large prevention effort based on this technique should take into account further improvements such as inexpensive HPV testing, which will be available within a few years. To frame and disseminate public-health messages at this pace is difficult, even when the change represents advances.

Conclusions

Much of the cervical cancer problem can be solved with existing or soon-to-be available technology, sufficient will, and modest resources. There is an enlarging repertoire of options for cervical cancer prevention for regions with varying needs and values, based on innovative technology and clear understanding of cervical carcinogenesis. Because of the importance of the problem and the feasibility of ameliorating it, we hope to see a major decrease in the numbers of women affected with this cancer within our lifetimes.

Conflict of interest statement

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