# *PREVENTION OF CERVICAL CANCER -A REVIEW.*

*Diploma work in Preventive Medicine*

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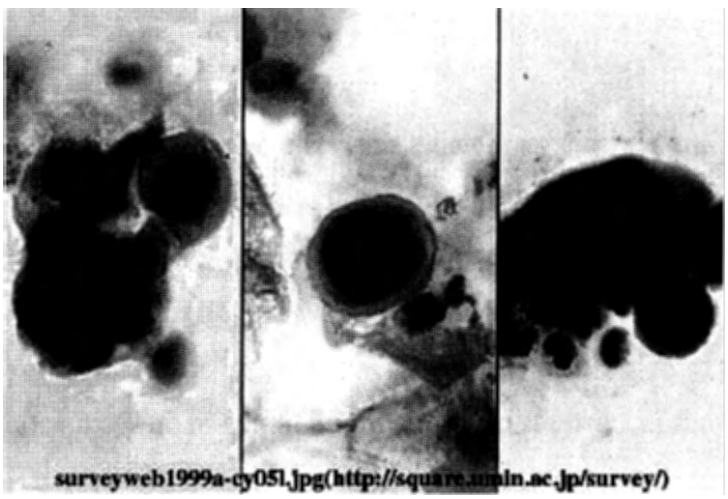



***Cervical smear after the Papanicolaou method.***

**Cervical cancer is now the second most common cancer among women worldwide. As a result of advances in molecular biology, the association between Human papilloma virus (HPV) infection and cervical cancer has been firmly established, and the introducion of a new vaccine against HPV infection may significantly decrease the incidence and mortality from cervical cancer in the years to come.**
**This article will review the etiology and prevention of cervical cancer.**

## Introduction

Cervical carcinoma, despite dramatic improvements in early diagnosis and treatment, continues to be one of the major causes of cancer related deaths in women. Worldwide, it is the second leading cause of cancer death in women, each year an estimated 500 000 cases are newly diagnosed. More than 80 % of these occur in developing countries. Latin America and the Caribbean, along with Sub-Saharan Africa and SouthEast Asia exhibit some of the highest incidence rates worldwide.[1]
Among populations, there are large differences in incidence rates, this reflecting the influence of environmental factors, screening papanicolau tests, and treatment of pre-invasive lesions. [1]
In industrialized countries, mortality from cervical cancer has shown a dramatic decrease over the past decades, mainly due to periodic cytologic screening by Papanicolau (Pap) smear, which was introduced in the 1950`s. However, programs for the prevention of cervical cancer have been unsuccessful in the developing world, even in countries where cytology has been available for many years and where organized health care systems exist. [3]

According to the institute of health information and statistics of the Czech Republic, the incidence of cervical carcinoma in the Czech Republic, has remained at a steady state of 21/100 000 from 1990 up till today, despite screening programs. In other European countries, for example Norway, there has been a significant decline in the incidence of cervical carcinoma over the past decade. According to the Cancer registry of Norway the incidence has decreased from 21/100 000 cases in the mid 1970ies till 10/100 000 in the year 2001.

## Basic pathology of cervical carcinoma.

There are two pathologic types of invasive cervical carcinoma; squamous cell carcinoma and adenocarcinoma. About 80 % to 95% are squamous cell carcinomas, which generally evolve from precursor cervical intraepithelial neoplasia (CIN). It is important to emphasize that most (probably all) invasive cervical squamous cell carcinomas arise from CIN. However, not all cases of CIN progress to invasive cancer, in fact CIN may persist without change or even regress. [4]

Cytologic examination using the Pap smear can detect CIN long before any abnormality can be seen grossly. The follow-up of such women have made it clear that precancerous epithelial changes may preceed the development of an overt cancer by as long as 20 years.

CIN generally begin with minimal cellular atypia and progress over time to more marked dysplastic changes with varying degrees of disordered maturation. The severity and extent of the cellular atypia has been graded as mild, moderate and severe dysplasia labelled CIN I, II and III respectively. Because of difficulties in segregating one grade from another, cervical lesions are classified as either

(i) Low-grade squamous intraepithelial lesions(LSIL), corresponding to CIN I and

(ii) High-grade squamous intraepithelial lesions(HSIL), corresponding to CIN II/III. (CIN III was formerly called carcinoma in situ).

The peak age incidence of CIN is around 30 years, whereas that of invasive carcinoma is about 45 years.[4]

## Etiology

Risk factors for the development of CIN and invasive carcinoma are:

(i) Persistent infection with human papilloma virus(HPV)

(ii) Sexual history; early age at first intercourse, multiple sexual partners and a male partner with multiple previous sexual partners.

(iii) Cigarette smoking

(iv) Contraceptive pill (?)

(v) Immunosuppression ( due to organtransplantation or HIV)

(vi) Low socioeconomic status

Probably, many of these risk factors are directly related to HPV infection, and persistent HPV infection is indeed nowadays regarded as the most important risk factor.
Cigarette smoking is a risk factor in that nicotine is degraded to carcinogeninc nitrosamines which can be found in the cervical secret of women smokers. In addittion, a study on the effect of passive cigarette smoking in relation to cervical cancer proved that the risk of detection of high-grade squamous intraepithelial lesion increased by 4,6% for every stick of cigarette the spouse of the woman smoked.[12]
The role of the contraceptive pill in relation to cervical cancer is highly controversial, but some have suggested that it promotes malign transformation of normal cervical epithelium because of the steroids(gestagens?) conatined in the pill.

Research in relation to the aetiology of cervical cancer has made substantial progress in the past two decades. For decades, the epidemiological profile of women with cervical cancer was recognised as suggestive of a sexually transmitted process, and several infectious agents were proposed over the years including syphilis, gonorrhoea and type 2 herpes simplex virus.

The development of technology to test for the presence of HPV DNA in cellular specimens in the early 1980s made possible the establishment of a definite aetiological role for HPV in cervical cancer.[5]
The link between genital HPV infection and cervical cancer was first demonstrated by Harold zur Hausen, a German virologist. Since then, the link has become well established. The magnitude of the association between HPV and cervical squamous cell carcinoma is in fact, higher than that for the association between smoking and pulmonary cancer. [7]

*Human papillomavirus (HPV)*

HPV is a double stranded, nonenveloped DNA virus, and is one of the most common causes of sexually transmitted disease in both men and women worldwide.
HPV`s can infect basal epithelial cells of the skin or mucosal lining of tissues, and are categorized as cuteanous or mucosal types. Cutaneous types of HPV target the skin of the hands and feet causing cutaneous warts. Mucosal types infect the lininig of the mouth, throat, respiratory tract, or anogenital epithelium.

More than 200 types of HPV have been recognized on the basis of DNA sequence, approximately 30 types of HPV infect the genital tract. [7]

Based on their association with cervical cancer and CIN lesions, HPVs can be grouped into high-risk and low-risk HPV types. The high risk HPV subtypes 16,18,31,33 and 51 have been recovered from more than 95% of cervical carcinomas.[1]

The effects of HPV infection on the oncogenesis of cervical carcinoma can be explained to a large degree by the regulation and function of the two viral oncogenes, E6 and E7.[6]

HPV genome is usually present in an episomal (cicular and non-integrated) configuration in CIN, whereas in invasive cervical cancer the genome is commonly integrated into the host DNA. Genes E6 and E7 are regulated by the E2 gene product. A characteristic event in malignant transformation is the integration of cicular viral genome into the patient`s genome. Gene E2 is often the site for integration, resulting in disruption of the E2 gene and subsequent de-repression of E6 and E7. [6]

The proteins encoded by viral genes E6 and E7 interfere with the growth-inhibitory activity of the proteins encoded by the p53 and Rb tumor supressor genes.

The HPV E6 gene product binds to p53 and targets it for rapid degradation. This degradation has the same effect as an inactivating mutation. As a consequence, the normal activities of p53 which govern G1 arrest, apoptosis, and DNA repair are interrupted. [7]

The HPV E7 gene product binds to the hypophosphorylated form of the RB family of proteins. This binding disrupts the complex between pRB and the cellular transcription factor E2F-1, resulting in the liberation of E2F-1, which allows the transcription of genes whose products are required for the cell to enter the S phase of the cell cycle. The outcome is stimulation of cellular DNA synthesis and cell proliferation. [7]

In addition to this, the HPV E5 gene product induces an increase in mitogen-activated protein kinase activity, thereby enhancing cellular responses to growth and differentiation factors. This results in continous proliferation and delayed differentiation of the host cell.[7]

In high-risk HPV types, the E6 and E7 proteins have a high affinity for p53 and pRB. Binding disrupts the normal function of these cellular proteins and can give rise to an increased proliferation rate and genomic instability. As a consequence the host cell accumulates more and more damaged DNA that cannot be repaired. Eventually, mutations accumulate that lead to fully transformed cancerous cells.[7]

In addition to the effects of activated oncogenes and chromosome instability, potential mechanisms contributing to transformation include methylation of viral and cellular DNA, telomerase activation, and hormonal and immunogenetic factors.
Several lines of evidence suggests the importance of the host`s immuneresponse, especially the cellular immune response, in the pathogenesis of HPV associated cervical lesions.[2] Furthermore, there has been some suggestion that sexually transmitted viruses may serve as cofactors in the development of cervical cancer. It has been postulated that coinfection with herpes simplex virus type 2 (HSV-2) may play a role in the initiation of cervical cancer. Cytomegalovirus(CMV ), human herpesvirus 6 (HHV-6) and HHV-7 have also been detected in the cervix. However, recent studies using PCR to detect these viruses in women with abnormal cervical cytologic test results inidate that these viruses are only bystanders rather than cofactors in the development of cervical cancer. [7]

As stated above, HPV has been implicated in 95 % or more of cervical squamous cell cancer cases worldwide. Adenocarcinomas of the cervix are also related to HPV, but the correlation is less pronounced and is age dependent. In women younger than 40 years, HPV was present in 89% of adenocarcinomas, whereas in women aged 60 years and older, HPV was observed in only 43%. [7]

## Prevention

Cervical carcinoma is said to serve as the model for controllable/preventable cancer in these three senses:

(i) there is an identifiable precursor lesion (CIN) with a natural history of usually slow progression

(ii) there is a cheap and noninvasive screening test (the Pap smear) and a follow up diagnostic procedure (colposcopy)

(iii) there are simple and effective treatments for the precursor lesions(cryotherapy, laser ablation, cone biopsy) with high cure rates

In addition to this, the development and introduction of a vaccine against HPV infection, will, if given to girls before their coitarche, offer primary prevention of CIN and cervical carcinoma due to HPV.

Prevention of cervical carcinoma can broadly be divided into two main preventive strategies; prevention of HPV infection and prevention of development of invasive cervical carcinoma.

## ***Prevention of HPV infection***

### *Risk reduction*

HPV has been proposed as the first ever identified "necessary cause" of a human cancer. This implies that cancer does not and will not develop in the absence of the persistent presence of HPV DNA.[5]

Primary approaches to prevent HPV infection include both risk reduction and the development of HPV vaccines.

Informing the general population ,and especially the high-risk population, about the risk of HPV infection and the fact that cervical carcinoma is indirectly a sexual transmitted neoplasm should be part of the prevention programme. However, it is not easy to change people`s attitude or behaviour and probably HPV infection can not be avoided only by encouraging the population to change their sexual behaviour.

Use of latex condoms and a spermicide may decrease the risk of contracting HPV. However, HPV is transmitted by skin- to skin contact and can occur even if a condom is used correctly.

### *HPV vaccine*

Vaccines directed against HPV are still not commercially available but are expected to be available within two years. It is universally accepted that cervical carcinoma is a consequence of HPV infection, and it is reasonable to assume that a vaccine against HPV will reduce the incidence of cervical cancer.

The most common oncogenic types of HPV are sbtypes 16 and 18, and most of the prophylactic vaccines are targeted against these two types. The HPV vaccines are usually composed of virus-like particles (VLP`s), which are empty virus capsids containing the major HPV capsid antigen and possibly the minor capsid antigen but lacking viral DNA. The capsid of HPV contain at least two capsid proteins, L1 and L2.

The vaccines are produced by expressing the L1 or L1 and L2 open reading frame protein coding sequences in eukaryotic cells. These proteins then self-assemble into VLPs which are highly immunogenic. [7]

HPV VLPs are immunogens that are able to elicit potent anti-viral/tumor B- and T- cell responses. [6]
Because of the high level of antigenic specificity of HPV capsid antigens, there is no cross-protection among subtypes. Optimal vaccines would contain a cocktail of VLPs of the most common high-risk HPV subtypes.

Koutsky et al. [8] performed a controlled trial of an HPV 16 vaccine. In a double-blind study, 2392 randomly assigned youg women received three doses of placebo or HPV 16 VLP vaccine. Among those enrolled in the study, 1194 received vaccine and 1198 received placebo. Altogether, 1533 women were included in the primary analysis. The most common reason for exclusion was evidence of HPV 16 infection at enrollment.
Koutsky et al. showed evidence of a highly efficacious prophylactic vaccine against HPV infection. All 41 cases of new HPV 16 infection including 9 cases of HPV 16 related CIN occured among placebo recipients (vaccine efficacy of 100%).

HPV 16 L1 VLP vaccines have also been shown to be generally well tolerated and generate high levels of antibodies against HPV 16. [6] Since ~50% of cervical cancers are associated with HPV 16 infection, the administration of this type of vaccine to young girls could reduce the incidence of HPV 16 infection and thus also of cervical cancer.

According to Merck & Co., the pharmaceutical company behind the first experimental HPV vaccine called Gardasil, a large international study conducted from 2002 to 2004 suggested that the vaccine was dramatically effective, and the company is now awaiting the approval from the Food and Drug Administration (FDA) to market the new vaccine.The vaccine was sendt for approval in the end of 2005. This may take 12 months but hopes are that the vaccine will be comercially available during the year 2006.

## ***Prevention of development of invasive cervical carcinoma***

### *Screening for cytology changes-The Pap smear*

Death from cervical cancer is preventable with early detection and treatment.

The primary method for detection of high-risk HPV is still the Papanicolau-stained (Pap) smear. This method was named after its inventor pathologist George Papanicolau, who introduced the method in the 1930s.[9]
Since its introduction, the Pap smear has helped reduce cervical cancer incidence and mortality rates by roughly half to two-thirds.[7]
The conventional Pap smear is obtained by using a spatula and cytobrush to collect cells from the transformational zone and endocervical canal. The specimen is then smeared onto a slide and a fixative agent is applied. Although this is a relatively simple and inexpensive way of preparing a specimen it can result in smears of insufficient quality or the test may be inconclusive or incorrectly interpreted.The smear collect cervical cells for microscopy for dyskaryosis, abnormalities which reflect CIN. A smear therefore identifies women who need cervical biopsy. The degree of dyskaryosis observed on the Pap smear approximates to the severity of CIN. ~50 % of CIN I lesions return to normal but most CIN III lesions progress to invasive carcinoma.

Due to the fact that the conventional Pap smear has a high rate of both false-negative and false-positive results, reserach towards better technology of cervical screening lead to the development of the liquid based Pap smear. The first liquid based system was approved by the FDA in 1996. Compared to samples from conventional Pap smears, liquid based Pap test samples are thinner, more adequate, and cleaner and therefore may allow better interpretation by cytopathologists.[9]
The newer technology also allows convenient adjunctive use of HPV testing, which may eliminate the need for colposcopy in many patients with atypical squamous cells of undetermined significance. The cost-effectiveness of routine use of liquid-based Pap has not been determined. However, initial studies suggest that the higher nominal cost of liquid-based Pap may be offset by reduced follow-up and longer screening intervals. [9]

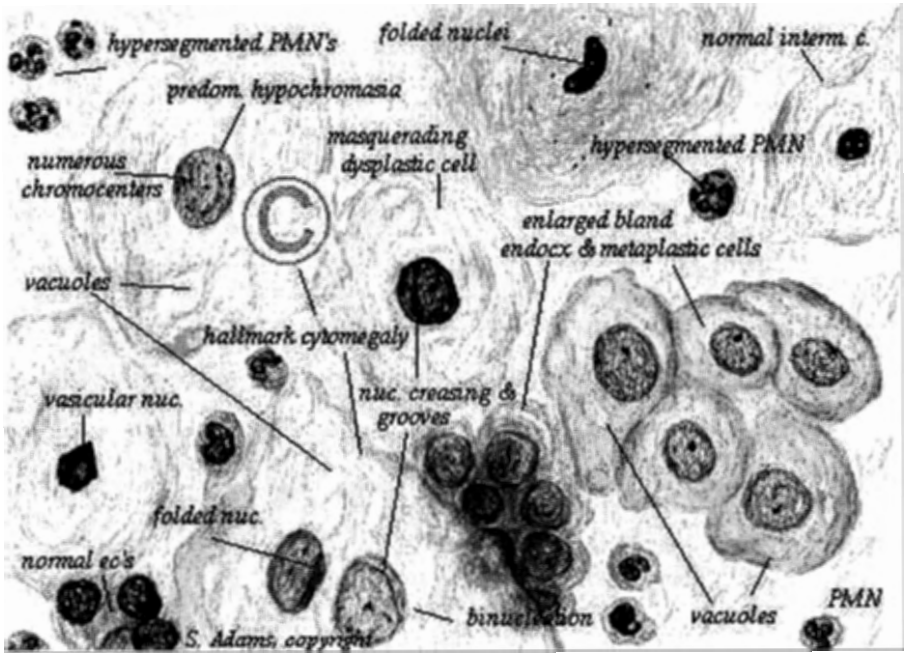



Fig. 1. Cell changes on Pap smear.

A screening programme of at least one smear by the age of 25 and then 3-yearly smears was established in january 1995 in Norway. This is the general screening program also in the rest of Europe, and it is belived that this program could reduce the incidence of invasive carcinoma by 90%.
However, the American Cancer Society now recommends that women younger than 30 be screened for cervical cancer annually with conventional cytology or every two years with liquid-based cytology. The latter approach should ultimately reduce the cost of screening.[9]

The biggest problem with the screening is that those most at risk are also the hardest to reach and persuade to have screening, for examples old women, smokers, and those in inner cities.

### *HPV testing*

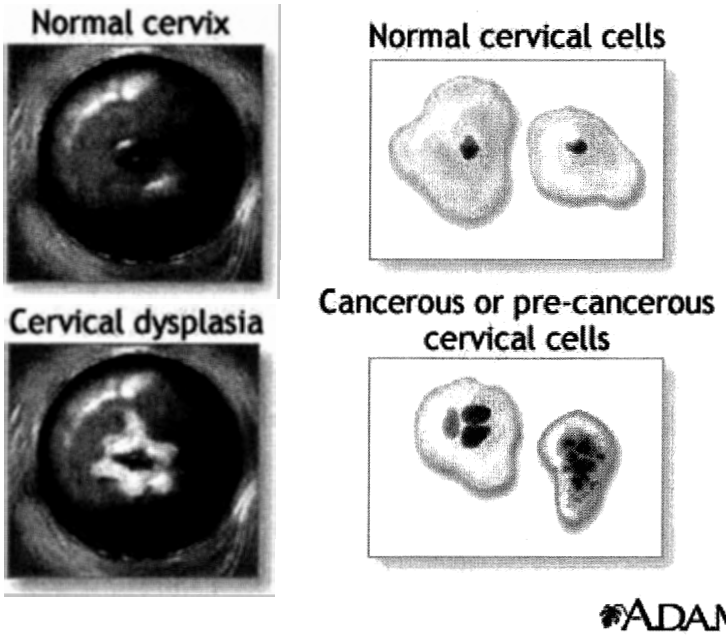
For the last years it has been widely debated wether HPV DNA testing should be a part of the screening for cervical cancer. HPV infection is very common, and the persisting infection with oncogenic subtypes of HPV (eg. 16 and 18) is now considered almost a necessary condition for cervical cancer to develop. However, most women will suffer an asymptomatic infection with HPV without this leading to cancer.

HPV DNA testing relies on detection of viral DNA on exfoliated cell specimens by the so called Hybrid capture test. It has proved to be accurate and reproducible.
It has been shown in a study from Greece by T. Agorastos et al. that HPV testing could be useful in screening women at low risk for cervical cancer, either as an adjunct tool to augment existing cytology programs or as a unique test of its own.[10]
Kyung-Ju Lee et al. conducted a study in Korea to evaluate the sensitivity and efficiency of HPV DNA testing in comparison with the conventional cytology for detection of cervical intraepithelial neoplasia (CIN) and cancer. The study included 593 women between the age of 14 and 88 who were referred for abnormal cytology. After histologic confirmation by either colposcopically guided biopsy or endocervical curettage, the efficiency of the two detection methods for high-grade cervical lesions was evaluated by HPV DNA testing, conventional cytology, and the two tests combined. The results were that the sensitivity and negative predictive values of HPV DNA testing was 92,4% AND 93,2% respectively. This indicates that HPV testing is more sensitive than the Pap smear. When the two tests were combined, the sensitivity was 97,8% and the negative predictive value 97,3%. Lee concluded that HPV DNA testing for detection of high-grade cervical lesions was more sensitive than cytology alone. [13]

HPV DNA testing may reduce costs by triaging patients into appropriate management strategies and reducing unnecessary colposcopy and less frequent screening in low risk patients, while still maintaining the sensitivity necessary to detect higher-grade squamous intraepithelial neoplasia.[7]

Further, it has also been shown that testing negative for HPV did not decrease the anxiety for having cervical cancer, and testing positive naturally might increase the anxiety. More studies are needed to determine how to avoid some of the anxiety, distress and concern caused by positive results on HPV testing and how to provide some reassurance for women recieving negative results.[11]

***Visual inspection***




In low-resource settings, visual inspection of the cervix with acetic acid could be a possible approach. Interpreting the results of this method relies highly on subjective experience of the physician but would be cheap. However, further research is needed before recommending this for population-based screening.

***Colposcopy***

Colposcopy is a procedure in which the cervix is examined in more detail by using an optic instrument with a powerful light source and 6-10 or 16 x magnification. The colposcope is in fact a binocular microscope.

Colposcopy was introduced by H. Hinselmann in the 1920s.

The main indications for colposcopy are (i) abnormal cervical cytology
(ii)clinical suspicion of invasive disease
(iii) mild/borderline findings on two occasions 6 months apart.

Following the application of a 3% acetic acid solution the cervix is examined. Acetowhitening and the vascular patterns characteristic of dysplasia or carcinoma can be seen. Colposcopy can detect low-grade and high-grade dysplasia but does not detect microinvasive disease. If

no abnormalities are found or the entire squamocolumnar junction can not be visualized, a cervical cone biopsy is done. Biopsy can be used to confirm most diagnoses by observing characteristic pathologic features of HPV infection such as epithelial hyperplasia and degenerative cytoplasmic vacuolization in teminally differentiated keratinocytes with atypical nuclei. Also HPV DNA or RNA can be demonstrated in biopsy tissues. [7]

### *Treatment of CIN*

Cervical intraepithelial neoplasia has long been known as a precancerous stage of invasive cervical carcinoma.

The natural history of CIN I varies somewhat with studies, but generally, the likelihood of regression is about 50% to 60%; of persistence 30%; and of progression to CIN III 20%. Only 1% to 5% of CIN I becomes invasive. The situation for CIN III however is different. With CIN III the likelihood of regression is only 33% and of progression 6% to 74% (in various studies). It is evident from this, that the higher the grade of CIN, the greater is the likelihood of progression.[4]

This also makes evident the fact that screening programs to prevent cervical carcinoma would loose their meaning if the detected lesions were not treated and followed during time. This is a basic requirement for any screening program, and for it to be possible to decrease the prevalence of cervical carcinoma, facilities for treatment of CIN should be present.

Treatment of CIN involves completely removing the abnormal epithelium. This can be done either by an excisional technique or by destroying the abnormal epithelium by techniques such as cold agglutination, cryotherapy and laser vaporization.

K. Nordland et. al presented a study comparing laser conisation with excisional conisation in women in Norway. In Norway, many hospitals replaced excisional conisation by laser conisation during the 1980s. The study concludes that laser conisation represents an improvement in the treatment of precancerous cervical lesions both in relation to post operative complications and use of resources.[14]

## Conclusions

Cervical cancer is a theoretically preventable disease. As for the etiology, it is now universally accepted that cervical carcinoma is a consequence of HPV infection and that HPV infection is the most important etiological factor. In fact, the association between HPV

infection and cervical cancer is stronger than the association between smoking cigarettes and pulmonary cancer. It is reasonable to assume that a vaccine against HPV as a primary prevention, will reduce the incidence of cervical cancer. Vaccines directed against HPV are still not commercially available but are expected to be available within two years. The vaccine would have to be given to young girls before their coitarche to be effective.

Even if the vaccine offers a great hope of reducing the incidence of cervical cancer in the near future, cervical cancer still kills about half a million women, 80% of whom live in developing countries. Programs for the prevention of cervical cancer have been unsuccessful in the developing world, even in countries where cytology has been available for many years and where organized health care systems exist. The main challenge therefore lies in making preventive strategies available also for these women, both the vaccine,the screening Pap smears and the follow up in case of pathological findings. As for the new vaccine, the cost should be acceptable also for these countries.

The prevention of cervical cancer will need further research over many years to be fully answered. However, the recent developments, and especially the introduction of the vaccine against HPV infection in the near future, is very promising for the future work of preventing cervical cancer.

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