REVIEW ARTICLE

Early Detection of Cervical Carcinomas

Finding an Overall Approach

Nicolas Wentzensen, Stefanie J. Klug

SUMMARY

Background: Infection with human papillomavirus (HPV) is a necessary, but not sufficient condition for the emergence of cervical cancer. Cervical cancer develops over several years through a series of precursor lesions that can be detected by cytological screening. The majority of these lesions, however, regress spontaneously. The challenge of cancer screening is to detect those patients who are at high risk of tumor progression.

Methods: Selective literature review on cervical cancer screening in light of current guidelines and recommendations.

Results and conclusion: Since the recently introduced vaccination against HPV does not provide full protection against cervical cancer, screening programs must continue. HPV vaccination and early detection of cervical carcinomas should be organized into a combined prevention program with systematic documentation, quality control, and active invitation to participate. It is assumed that the reduction in prevalence of precancerous lesions as a result of vaccination will have a negative impact on the efficiency of cytological early detection. Therefore, the existing screening procedures should be optimized and complemented by new techniques. Already available for screening is the detection of HPV DNA. Further promising biomarkers are currently being investigated in international studies, but no conclusions on their potential efficacy can yet be drawn.

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Key words: cervical cancer, HPV, cancer screening, cytology, biomarker, HPV vaccination

Cervical cancer is the second most common cancer in women worldwide, with an incidence ranging from 10 per 100 000 women in industrialized countries to 60 per 100 000 women in some developing countries (1, e1). This uneven distribution is attributable primarily to the introduction of cytologic screening (i.e. exfoliative cytology) in many industrialized nations during the second half of the twentieth century (e16).

Infection with human papillomavirus (HPV) is a necessary cause of cervical cancer (*figure 1*) (2–3, e3–e6). Of the more than 100 different HPV types that have been identified to date, approximately 13 are high-risk types (HR-HPV) that can transform cells in the genital tract and lead to invasive cancer (*figure 1*) (2, e7). In a recent American study on HPV prevalence, HPV DNA was detected in 27% of women aged 14 to 59 years, 15% of whom harbored HR-HPV. Among women aged 20 to 24, these figures were even higher, with HPV DNA being detected in 45% of women in this age group, 29% of whom had HR-HPV (e8). It has been estimated that 70% of women will experience an HPV infection during their lifetime (e9). 90% of infections are cleared spontaneously after several months (8). Although the risk of developing precancerous lesions (cervical intraepithelial neoplasia, or CIN) increases with the persistence of HPV infection (e10), more than half of these lesions regress spontaneously (4, 5).

The overexpression of viral E6 and E7 oncogenes in basal epithelial cells is an important step in the transformation process (6, 7). This leads to uncontrolled activation of the cell cycle, inhibition of apoptosis, and, finally, to chromosomal instability with viral integration (e11, e12). A functional model for the development of cervical cancer (*figure 1*) (8) has been proposed and includes the following steps:

- HPV infection
- HPV persistence
- HPV transformation and development of precancerous lesions
- Invasive cancer.

Cytologic screening

The aim of cervical cancer screening is to detect precancerous lesions and, if necessary, to remove them before they can progress. The cytology test used for screening was developed by Papanicolaou and is known as a Pap smear or cervical smear (e13). A sample is collected from the endocervix and ectocervix, ensuring that cells

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FIGURE 1

Model of cervical cancer progression

a) Morphological and b) functional correlates in cervical cancer progression

are obtained from the entire transformation zone. The sample is then fixed on a glass slide, stained, and checked for abnormalities using light microscopy. After cytologic screening was introduced to West Germany, the incidence of cervical cancer fell by more than 60% (e2). Similar decreases have been reported for other countries (e14–e17). Nevertheless, prevention efforts still need improvement. Each year in Germany approximately 6200 new cases of cervical cancer are diagnosed, many of which involve younger women (e2). In recent years, the incidence of cervical cancer in Germany has remained virtually constant.

Studies in Germany and the UK have shown that between 16% and 42% of cervical carcinomas occur in women who have been screened at least once in the three to five years before their cancer diagnosis (e18, e19, e71, e72). Because cervical cancer usually develops over a period of more than 10 years, these figures point to weaknesses related to the sensitivity of cytologic screening (8). False negative results can be traced to errors in specimen sampling and preparation, or to deficient examination of the specimens (e73). However, test results of uncertain clinical significance are also problematic, such as in cases of only mild abnormalities, usually due to HPV infection (PapIIw/IIID in the Munich Classification System, or ASCUS/LSIL by Bethesda criteria).

Internationally, the sensitivity of a single Pap smear ranges from approximately 50% to 60%; the success of screening programs depends on repeated testing (8, e73). However, as the number of Pap smears increases, so too does the risk of false positive results. Excessive interventions – i.e. in cases of harmless changes that would regress spontaneously – are not uncommon. Indeed, histological investigations of cone biopsies have found no histological changes in 23% to 28% of cases, and that 12% to 51% of cone biopsies were performed based on

false positive cytologic results (e20, e21). The indication for cone biopsy should be assessed carefully, as the procedure has been associated with post-operative bleeding, infections, and complications in subsequent pregnancies (e22).

Frequent testing, follow-up examinations, and excessive treatment due to equivocal findings make cervical cancer screening an important cost factor in most healthcare systems (5).

The role of HPV vaccination

To date, two prophylactic vaccines against infection with HPV have been developed, both of which are aimed at HPV types 16 and 18. One of the vaccines also provides protection against HPV types 6 and 11. A recent overview of HPV vaccination can be found in a review by Löning and colleagues (13).

Currently, HPV vaccination is not a replacement for cervical screening. It will presumably take many years to achieve high vaccine uptake, and long-term data on the durability of vaccine protection and its efficacy in preventing cervical cancer are lacking. As a result, vaccinated women should continue to undergo routine cervical screening.

Nevertheless, HPV vaccination will have a major impact on cervical screening programs, even in the medium term. Data from the published HPV vaccination studies indicate that the vaccinated population will experience a decrease in the incidence of lesions associated with HPV 16 and 18, but that the incidence of lesions caused by other HPV types will remain very much the same (14). This will lead to an overall decrease in the number of precancerous lesions, and thus to a decline in the positive predictive value of the test used (14). There is also some indication that HPV vaccination will have a negative impact on Pap cytology.

The distribution of HPV 16 and 18 varies considerably depending on the severity of the cervical lesions in question (5, e29, e30). Approximately 70% of carcinomas are positive for HPV 16/18 compared to 50% of medium- to high-grade intraepithelial lesions, and only some 20% of low-grade intraepithelial lesions and abnormalities. Although the total number of cytologic abnormalities will be lower in a vaccinated population, the distribution will shift to low-grade lesions, which are the most problematic to assess from a diagnostic point of view (14). Low frequency of precancerous lesions leads to habituation and a consequent loss of vigilance among laboratory screeners, and therefore to a lower recognition rate for abnormal smears (14).

Situation in Germany

In West Germany, annual cytologic screening in women aged 20 years and older was added in 1971 to the catalogue of benefits covered by statutory health insurance. The screening is opportunistic - i.e. eligible women are offered the test, but are not invited systematically (9). Uptake is currently 50% (10, e74). Studies have shown that young women of higher social status are particularly likely to take advantage of annual cervical screening (e23).

There are few systematic investigations of primary cervical cancer screening in Germany. In one study that recruited women attending routine cervical cancer screening offered by private-practice gynecologists in two regions (Hanover and Tübingen), the majority of smears were negative (Pap I/II, 96.9%) (e24). Lesions requiring treatment (Pap IV+V) were found in only a small proportion of women (0.1%). On the other hand there were many unclear results with no direct clinical consequences (Pap IIw-IIId, 3.1%). The sensitivity of a single Pap smear for the detection of CIN2/CIN3 was 43.5% in the Hanover-Tübingen study and only 20% in another study, conducted in the German state of Thüringen (11, e25). In the two study populations, the prevalence of HPV was 6.4% and 7.9% (e24, e25). In the Hanover-Tübingen study, the most frequently detected HPV types in women with histologically confirmed CIN2 or higher-grade lesions were HPV 16, followed by HPV 45, 58, and 52 (12).

The cancer early detection system in Germany differs considerably in a number of ways from the approaches adopted in other countries (e28, e81). Many countries have implemented organized, invitation-based screening programs, some of which have much longer screening intervals (e26) and have led to a markedly lower incidence of cervical cancer (e27). In the United States, the Hybrid Capture 2 (HC2) test was approved as an adjunct to cytology in primary screening and has become

the standard in evaluating equivocal cytologic smears. Colposcopically directed biopsies are also an important component of cervical cancer screening in many countries. Germany, however, does not have enough certified colposcopy clinics in which experienced assessors could investigate abnormal cervical smears. It should nevertheless be kept in mind that colposcopy is a subjective procedure with heterogeneous results (8).

Ways to improve cytologic screening

The European Guidelines for Quality Assurance in Cervical Cancer Screening have been revised and were recently published (e75, e76, e80). The guidelines recommend organized screening programs in which eligible women are invited on a systematic basis; restrictive quality assurance; and regular reports. A larger number of women can be reached through targeted invitations. An ongoing pilot project in the German city of Mainz (MARZY study) is investigating a screening model that meets these criteria (e31).

Quality assurance can be improved as part of an organized screening program. In many countries, participation in interlaboratory comparison and the systematic rescreening of negative cervical smears are an important quality assurance requirement. Rapid rescreening of all negative smears can help identify false-negative results (e33).

Regulations pertaining to quantitative standards, such as those in effect in operative medicine (e79) and

TABLE 1

Commercial biomarkers

Marker	Detection method	Uses	Evidence	Assays	Comment
High-risk HPV DNA	<ul style="list-style-type: none"> DNA hybridization (Hybrid Capture 2) PCR (Amplicor™) 	Clarification of equivocal cytologic findings; adjunct in primary screening	Large population-based studies; randomized studies (Hybrid Capture 2)	Hybrid Capture 2 has been studied intensively for many years; Amplicor™ is currently under regulatory review	Hybrid Capture 2 is FDA approved
HPV genotyping	<ul style="list-style-type: none"> PCR and specific hybridization (Linear Array, SPF 10-LiPa) DNA chip (Papillo Check™) 	Risk stratification according to HPV type; follow-up care; evaluation of viral persistence	Only few studies on different assays; large comparative studies are ongoing	Many different certified assays, some of which have just been launched onto the market	The various assays differ considerably; some require great expertise
HPV RNA	RNA testing using nucleic acid amplification (PreTect™ Proofer, APTIMA™ HPV)	Identification of high-grade dysplasia; clarification of equivocal cytologic findings	Some clinical studies published; population-based studies ongoing	Two different certified assays: PreTect™ Proofer has been available for some time now; APTIMA™ is currently under regulatory review	
p16	Protein detection (CINtec™ Histology, CINtec™ Cytology)	Identification of high-grade dysplasia; clarification of equivocal cytologic findings; primary screening (?)	Many clinical studies published; population-based studies ongoing	Certified tests have been available for several years	
Replication markers	Protein detection (ProExC™)	Identification of high-grade dysplasia	Very little published data	Certified assay; only recently introduced	
HPV capsid protein	L1 protein detection (Cytoactiv™)	Clarification of equivocal cytologic findings	Very little published data	Certified kits for screening of high-risk HPV L1	Not a replacement for HPV DNA testing

TABLE 2

Non-commercial assays

Marker	Detection method	Uses	Evidence
Proliferation markers MYC, Ki-67	Protein detection, IHC	Identification of high-grade dysplasia	Some feasibility studies
Telomerase, 3q	In situ hybridization	Identification of high-grade dysplasia	Some feasibility studies
Aneuploidy	Flow cytometry or cytology	Identification of high-grade dysplasia	Few studies
HPV integration	(RT)-PCR, molecular cloning, sequencing	Follow-up care, clonality analysis	Functional analyses, few studies

RT-PCR, reverse transcription polymerase chain reaction; IHC, immunohistochemistry

nationwide mammography screening (e78) in Germany, are also used internationally in the area of cytology (e34, e35). Promising efforts are underway in Europe to harmonize quality assurance procedures and to introduce cervical cytology registries (15). In fall 2007, the National Association of Statutory Health Insurance Physicians in Germany published a new set of guidelines for cytologic testing; these represent a further step towards improving the quality of cancer screening (specification of formal training requirements; implementation and quality assurance with external audits) (e77).

Further technical development of cytology

Liquid-based methods in cervical cytology have been available for some time now and offer a picture of the sampled cells that is more consistent and easier to interpret than conventional smears. Although some of these new methods are considerably more expensive, clear evidence of improved sensitivity or greater cost-effectiveness compared to conventional cytology is lacking (16, e36). Several countries have introduced liquid-based cytology as part of their cancer-screening programs; in Germany the Joint Federal Committee has opposed such a measure to date (e37). It is, however, undisputed that this method is easier to standardize and allows for the sampled material to be used for additional biomarker testing.

Biomarkers in cervical cancer screening

HPV DNA test

HPV DNA testing has high sensitivity (>90%) for detecting precancerous lesions and invasive carcinoma. However, it is unable to distinguish between transient HPV infections and malignancy. Because there is a peak in the prevalence of HPV infections in women younger than 30, a single HPV DNA test in this age group lacks specificity (e38–e40).

The Hybrid Capture 2 (HC2) test is the only HPV DNA test currently approved by the US Food and Drug

Administration (FDA). It shows whether any of 13 high-risk types are present, but does not provide type-specific information. The various HR-HPV types differ in their oncogenic potential. The likelihood of developing pre-cancer ranges from 10% to 15% in women who test positive once for HPV 16 or 18, and below 3% for all other high-risk types combined (8, e41). Although type-specific testing could lead to improved risk stratification, HPV genotyping assays have yet to be validated, are expensive, and are not routinely available. Brink and colleagues provide an overview of the various methods (17).

HPV DNA testing was first investigated as an approach to clarifying equivocal cytologic findings. In an American study, HPV DNA testing was shown to be more effective in this regard than repeat cytology (e42). These results were confirmed in a meta-analysis (18).

Since then, a number of large randomized studies on the use of HPV DNA testing in primary screening have been completed and their findings published. These show that including HPV in primary screening leads to the identification of between 50% and 70% more precursor lesions. A negative HPV DNA test largely rules out the risk of developing disease for several years, allowing for the prolongation of screening intervals (19, 20, e43). A study of HPV DNA testing based on self-sampling of cervical specimens has yielded promising results (e32).

Biomarkers of HPV-associated transformation

The key step in carcinogenesis after HPV infection is the transformation of basal epithelial cells, which is triggered by the deregulated expression of viral E6 and E7 oncogenes (6, 7). A variety of biomarkers for this step are under investigation and include HR-HPV mRNA, classical markers of proliferation (Ki-67, MYC, telomerase, MCM2, TOP2A), and p16, which is an indirect marker of HPV oncogenic activity. Other biomarkers may reflect the consequences of chromosomal instability resulting from transformation; these include loss-of-heterozygosity (LOH) markers, methylation markers, aneuploidy, and HPV integration (21, e44). The aim of testing for HPV L1 capsid protein is to distinguish between harmless productive infections and infections that lead to oncogenic transformation (e45). An overview of selected biomarkers is presented in *tables 1 and 2*, as well as *figure 2*. Although currently available data do not support the routine use of most of the markers presented here, the results from a number of high-quality studies on the use of new biomarkers in cervical cancer screening will soon be available.

HPV RNA

The use of HPV mRNA testing has been shown to be more specific than HPV DNA testing for transformation (6). Currently, there are two commercial assays available for detecting HPV mRNA. The PreTect™ HPV-Proofer assay detects oncogenic mRNAs from five common HPV types, whereas the APTIMA™ test detects mRNAs from 14 high-risk types. Studies of the HPV-Proofer

assay show that it is more specific for detecting high-grade lesions than HPV DNA testing, but at the same time less sensitive (22, e46–e48).

Proliferation markers

Proliferation markers such as Ki-67, MYC, cyclins, and survivin have been investigated in many smaller studies (e49–e58). Recently, a novel assay became available that is able to detect overexpression of two proliferation-associated proteins (MCM2, TOP2A) in patient tissue and cytology preparations. Data on the clinical utility of this assay are still limited (e59).

Another interesting marker is chromosomal amplification of 3q, which can result in increased telomerase activity and is found in cervical carcinomas and precancerous lesions (e60, e61).

p16 staining

The overexpression of p16 is a direct consequence of changes in the cell cycle caused by HPV oncogenes, regardless of the causative HPV type (e62, e63).

With the CINtec tests, it is possible to detect p16 in histological specimens and cytology preparations. Immunohistochemical staining allows for the specific identification of carcinomas and precancerous lesions of the cervix, as these show marked overexpression of p16 (23). This can aid in the interpretation of histological material (e64–e66), and many pathologists are now using p16 staining to clarify equivocal cases. Moreover, p16 staining can facilitate the identification of abnormal cells in cytology preparations (e67). Indeed, p16 has been evaluated as a biomarker to triage equivocal cytologic findings (24, e68). In the future, ELISA-based quantification of p16 may represent a simple biochemical approach to risk stratification (e69, e70).

Although there are many other biomarker candidates in addition to those described above, data on their utility is still very limited (21).

Conclusion

Despite the advent of prophylactic vaccination against HPV, we are still a long way from bringing cervical cancer rates under control. The currently available vaccines do not afford protection against all oncogenic HPV types, and it will take many years to achieve high vaccine coverage in the community. As a result, both vaccinated and unvaccinated women must continue to undergo routine cervical cancer screening.

The probable impact of HPV vaccination on cytologic screening means that current approaches need to be reassessed, especially in terms of effectiveness, efficiency, and quality assurance. A variety of recommendations have been made for adapting screening programs to the new situation of coexistence. By including systematic documentation of vaccination, screening visits, and test results, an organized prevention program for vaccinated and unvaccinated women alike would facilitate routine evaluation and the assessment of success in reducing cervical cancer rates. Both existing and new methods should be based on standardized procedures

Figure 2: Examples of new assays: a) HC2 HPV DNA test (a change in color to yellow shows infection with high-risk HPV); b) HPV genotyping with hybridization bands (each column represents an individual specimen, and the different heights of the bands represent different HPV types); c) L1 protein detection in a CIN1 lesion (the darkly stained nuclei are indicative of infected cells, which produce the viral L1 capsid protein); d) p16 protein detection in a CIN3 lesion (the brown staining of the entire cervical epithelium is indicative of cells that have been transformed by high-risk HPV).

and mandatory quality assurance measures. Where appropriate, new diagnostic assays can be used as adjuncts to, or even partial replacements for, existing techniques (*box*).

Internationally, a variety of measures for redesigning gynecological cancer screening are currently under discussion. Most of these are based on multi-level concepts – e.g. primary screening with high sensitivity followed by a specific test to clarify equivocal findings. At present, HPV DNA testing is the only method with

BOX

Key messages

- An organized screening program for vaccinated and unvaccinated women can increase the effectiveness and efficiency of cervical screening.
- Additional improvements are possible in the areas of organization, documentation, and quality assurance.
- In its current form, cervical cancer screening will become less effective as a result of HPV vaccination.
- HPV DNA testing can be employed effectively in primary screening.
- New biomarkers have great potential to improve cervical cancer screening, but have not yet been sufficiently validated.

enough data to support its use as part of primary screening in addition to cytology.

The goal is to use the different diagnostic and screening tests to obtain a valid estimate of risk. Based on clinical data and the results of these tests, the risk profile can serve as the basis for targeted screening invitations (25). New tests or diagnostic procedures should only be used after they have been validated in high-quality studies, and to address clearly defined clinical questions. Finally, many of the new tests are complex and can be performed only in specialized laboratories. Thus, it needs to be kept in mind that there is currently little robust data supporting their utility in routine practice.

Conflict of interest statement

Dr. Klug provides consultancy services to the company Cytoc in the implementation of the Rhein-Saar study. PD Dr. Wentzensen declares that no conflict of interest exists according to the guidelines of the International Committee of Medical Journal Editors.

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