Cervical Cancer Control in the Era of HPV Vaccination and Novel Biomarkers

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Abstract

Infections with human papillomaviruses (HPV) are a necessary, but not sufficient cause of cervical cancer. Cervical cancer develops over a long time through precursor lesions that can be detected by cytological screening. The majority of these lesions regress spontaneously without treatment. The challenge of cervical cancer screening is to detect the lesions that have a high risk of progression. Since the recently introduced vaccination against HPV cannot provide 100% protection, cervical cancer screening programs must continue. It is assumed that the reduction of precancers related to vaccination will have a negative impact on the efficiency of current screening programs. Therefore, participation rates need to be increased and current screening modalities should be improved. Several promising biomarkers have been described that might improve cervical cancer screening, but currently, high-quality studies on their efficiency are lacking.

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Epidemiology and Pathogenesis of Cervical Cancer

Cervical cancer is the second most common cancer among women worldwide. The incidence ranges from 10/100,000 women in industrialized countries to 60/100,000 women in some developing countries [1, 2]. The differences in incidence are mainly related to the introduction of cytological screening (exfoliative cytology) in many industrialized countries during the second half of the 20th century [3].

Human papillomaviruses (HPV) are necessary for the development of cervical cancer [4–9] (fig. 1). There are more than 100 known HPV types, of which 13–15 carcinogenic types (HR-HPV) have the ability to transform genital mucosal cells and trigger the formation of invasive cervical cancer (fig. 1) [5, 10]. In a recent US-based study on HPV prevalence, HPV was detected in 27% of women aged between 14 and 59 years (15% HR-HPV) and in 45% of women aged 20–24 years (29% HR-HPV) [11]. It is estimated that 70% of all women carry HPV at some point in their lives [12]. Ninety percent of the infections regress spontaneously after a few months without treatment [13]. The risk of development of a precancerous lesion increases with viral persistence [14]. However, more than half of the cervical intraepithelial neoplasias (CIN) regress spontaneously [15, 16].

An important step of viral transformation is the overexpression of the viral proteins E6 and E7 from carcino-

Fig. 1. Progression model of cervical carcinogenesis. Morphologic (top row) and functional (middle row) progression model. Representative biomarkers for the disease stage transitions are indicated.

genic types in basal epithelial cells. E6 leads to the degradation of the tumor suppressor protein p53 and abrogation of apoptosis, while E7 disrupts the binding of pRb tumor suppressor protein and E2F which leads to uncontrolled cell cycle activation through released E2F [17, 18]. This results in uncontrolled cell proliferation and chromosomal instability with accumulating DNA damage and may facilitate viral integration [19, 20]. In the last decade, a functional progression model including the following steps has been established: (1) HPV infection, (2) HPV persistence, (3) transformation with formation of precancerous lesions, and finally (4) invasion to cervical cancer [13] (fig. 1).

Cervical Cancer Screening

Cervical cancer screening using cytology according to Papanicolaou is based on the identification of precancerous lesions which, if necessary, can be removed curatively [21]. A smear encompassing the whole transformation zone is taken from the endo- and ectocervix, fixed on a microscope slide, stained, and microscopically examined for abnormal cells. The effect of the cytological smear on the incidence of cervical cancer has never been investigated in randomized studies. However, there are many indirect proofs of the efficiency of cytological-based screening. Since the introduction of the cytological smear, the

incidence of cervical cancer has decreased by over 60% in many countries [3, 22–24]. In spite of its success, cervical cancer prevention needs to be improved. Each year, almost 500,000 new cases are detected worldwide. Different studies have shown that 16–24% of the cancers occur in women who participated in screening at least once in the last 3–5 years before diagnosis [25, 26]. As the development of cervical cancer generally takes around 7–10 years, these data suggest a low sensitivity of cytological screening [13]. The reasons mainly lie in insufficient sample material and processing, as well as in erroneous interpretation of cytology specimens [27]. Not only false-negative results, but also those with no clear clinical consequences are problematic, e.g. cases with low-grade changes due to HPV infection (ASCUS/LSIL in the Bethesda classification). Internationally, the sensitivity of a single smear is about 50–60%. The reliability of the screening is achieved by repeated testing [13]. With frequent testing, the chance of false-positive results increases, leading to unnecessary treatment of lesions which would normally spontaneously regress. The histological examination of conization series has shown that 23–28% of patients did not have any histological alterations, and that 12–51% of the conizations were done on the basis of false-positive cytology [28]. The indication for conization should be made with caution as it can be associated with bleeding, infections and complications during subsequent pregnancies [29].

The frequent examinations needed for subsequent clarifications and unnecessary treatment of unclear results all add to the expenses presented by cervical cancer screening to the health systems internationally [16].

Currently, in most screening settings, the gold standard of the disease is defined by the histological evaluation of colposcopy-guided biopsy specimens. Clinical management is based on these results, and new screening assays are benchmarked against this gold standard. However, there is a lot of evidence that disease ascertainment based on current colposcopy-biopsy standards is limited. The visual assessment of the cervix in colposcopy has a high interobserver variability and frequently fails in detecting the worst lesion [30]. It has been demonstrated that the sensitivity of colposcopy is not related to the experience of the colposcopist, but to the number of biopsies taken [31]. Even in randomly taken biopsies from normal appearing regions on the cervix, substantial disease was identified [32]. Currently, studies are underway that aim at analyzing better colposcopic procedures and at determining how many biopsies are necessary to improve disease ascertainment. Meanwhile, a combined gold standard including histology and cytology results can improve the situation [33].

Relevance of HPV Vaccination for Cancer Screening

Two vaccines are available which were demonstrated to protect against the HPV types 16 and 18, with one of them also protecting against HPV 6 and 11 [34]. At present, the available vaccination does not replace cervical cancer screening. First, it will presumably take a long time until a high vaccination rate has been achieved in the general population. Second, there are no long time follow-up data on either the duration of the vaccine protection or on the prevention of cervical cancer. Hence vaccinated women must continue to participate in screening programs. Currently, there are few feasible vaccination strategies for developing countries that carry the largest burden of disease.

In countries with widespread use, the vaccination will have a high impact on cervical cancer screening. From the data of HPV vaccine studies, it can be estimated that the number of lesions associated with HPV 16 and 18 in a vaccinated population will decrease, while lesions associated with other types would be hardly affected [35]. In general, a reduction of the incidence leads to a lower positive predictive value of any test procedure used. There is evidence that cytology will be additionally affected by

HPV vaccination. The distribution of HPV 16 and 18 differs across disease stages [16, 36–38]. About 70% of the carcinomas are HPV 16/18 positive, 50% of mid- to high-grade dysplasia, but only about 20% of the low-grade abnormalities. In a vaccinated population the total number of abnormal findings will be lower, but the distribution will shift towards low-grade lesions which pose the largest problem diagnostically [35]. As a result of the reduced incidence of high-grade lesions, precancers will be expected less and the ability to detect changes in cytological smears may suffer [35].

Possible Ways of Improving Cytology-Based Cervical Cancer Screening

An update of the European guidelines on cervical cancer screening was recently published [39]. Among other things, the guidelines recommend well-organized screening programs that incorporate systematic invitation procedures, restrictive quality assurance and regular evaluation. Overall, more women can be reached through a well-organized screening program with systematic invitations to participate. Quality control can be improved within an organized program. In many countries the participation in collaborative assessment experiments and the systematic partial rescreening of specimens is already a mandatory, important part of quality control. Fast rescreening of all normal specimens can effectively reduce false-negative results further [40].

Some quality guidelines require that cytology centers evaluate a certain number of specimens per year [41, 42]. Currently, there are promising European efforts to harmonize quality assurance and introduce cytological registers [43].

Technical Advancements of Cytology

Liquid-based cytology is supposed to produce a more uniform cytology specimen that may improve the diagnostic procedures and has been introduced in some countries. While these procedures can be considerably more expensive than conventional cytology, it has not been clearly demonstrated that liquid-based cytology is more sensitive or cost-effective than conventional cytology [44, 45]. It is undisputed that liquid-based cytology can be better standardized than the conventional smear. Additionally, the liquid material can be used for further investigations with biomarkers.

Table 1. Selection of commercially available biomarkers

Marker	Test format	Application	Data	Certification	Comment
Carcinogenic HPV DNA	– DNA hybridization (Hybrid Capture 2) – PCR (Amplicor)	Clarification of unclear cytology, additional test in primary screening	Excellent, large population-based studies, randomized studies (Hybrid Capture 2)	Hybrid Capture 2 intensively tested for many years, Amplicor currently undergoing licensing procedure	Hybrid Capture 2 is FDA-licensed
HPV typing	– PCR and specific hybridization: Linear Array; SPF10-LiPa – DNA-Chip: Papillo-Check	Risk estimation according to HPV type, aftercare, estimation of persistence	Few studies with different test methods, large comparison studies running	Many different certified methods just introduced onto the market	Greatly different tests, to some extent high level expertise required
HPV oncogene mRNA	RNA test through nucleic acid amplification: – PreTect Proofer – Aptima	Identification of high grade dysplasia, clarification of unclear cytology	Some clinical studies published, population-based studies running	2 different methods certified, PreTect Proofer available for some time now, Aptima about to be introduced	
HPV capsid protein	L1 Protein test	Clarification of unclear cytology	Hardly any data published	Certified kits for broad-range high risk – HPV L1	Does not replace an HPV DNA test
p16	Protein test CINtec histology, CINtec cytology	Identification of high-grade dysplasia clarification of unclear cytology, primary screening?	Many clinical studies published, population based-studies running	Certified tests available for a few years	
Replication markers	Protein test ProExC	Identification of high-grade dysplasia	Hardly any data published	Certified methods, recently introduced	

Application of Biomarkers in Cervical Cancer Screening

HPV DNA

A variety of biomarkers to investigate cervical lesions are available (tables 1, 2). The HPV test has a high sensitivity for the detection of precancerous lesions and invasive cancer (above 90%). However, an HPV DNA test cannot distinguish between a transient infection and an invasive carcinoma. As the peak of HPV infection in young women is below the age of 30 years, a single HPV test in this age group only has limited significance [46–48].

The Hybrid Capture 2 test (HC2) is currently the only HPV test licensed by the FDA (United States Food and Drug Administration). This test shows whether 1 of 13 high-risk types is present, but does not give information on individual HPV types.

The risk of developing a precancerous lesion is between 10 and 15% for an infection with HPV 16 or 18, while it is

below 3% for all other high-risk types combined [49]. Thus, a better risk stratification could be achieved by using a type-specific test. Individual genotyping procedures are currently not sufficiently validated, expensive, and not common in routine laboratories. Brink et al. [50] provide an overview of the different HPV testing options.

The HC2 HPV test was initially evaluated as a management option for inconclusive cytology results. In a US study it was shown that the HPV test allows a more effective triage of ASCUS cytology than a repeated cytological examination [51]. These results were confirmed in a meta-analysis [52].

In the meantime large randomized studies on the use of HPV testing in primary screening programs have been conducted and were recently published [53–55]. The studies showed that 50–70% more precancers can be diagnosed initially when HPV testing is incorporated in primary screening. Since a negative HPV test can exclude the risk of disease in the subsequent years with high cer-

Table 2. Selection of noncommercial test methods

Marker	Test format	Application area	Data situation
Proliferation markers MYC, ki67	Protein test, IHC	Identification of high-grade dysplasia	Some pilot studies
Telomerase, 3q amplification	In situ hybridization	Identification of high-grade dysplasia	Some pilot studies
Aneuploidy	Flow cytometry or cytology	Identification of high-grade dysplasia	Few studies
HPV integration	(RT) PCR, cloning, sequencing	Aftercare, clonality test	Functional analyses, few studies

tainty, it is possible to run screening intervals of 3–5 years without compromising safety. Apart from that, an HPV-based screening offers the possibility of extracting specimens during self-examination at home, a fact which could lead to increased screening participation rates [56].

Biomarkers for HPV-Associated Transformation

The decisive step in carcinogenesis after HPV infection is the transformation of basal epithelial cells, initiated through the deregulated expression of the viral oncogenes E6 and E7 [17, 18]. A number of markers for this step are currently being analyzed, among them high-risk HPV mRNA, classic proliferation markers (ki67, MYC, telomerase, MCM2, Top2A), as well as p16, an indirect marker for HPV oncogene activity. Other biomarkers show the effects of chromosomal instability caused by the transformation, among them LOH markers (loss of heterozygosity), methylation markers, aneuploidy and HPV integration [57, 58]. The detection of the viral capsid protein L1 aims at differentiating innocuous productive infections from transforming infections [59]. A selection of biomarkers is shown in tables 1 and 2. Currently available data on most of the biomarkers presented does not warrant their routine use yet. However, results of high quality studies on the use of new biomarkers in cervical cancer screening are expected in the near future.

HPV RNA

Oncogenic HPV mRNA detection is supposed to be more specific for the transformation of epithelial cells than detection of HPV DNA [17]. Currently, there are two commercial tests for HPV mRNA. RNA proofer detects HPV oncogene mRNAs of five frequent HPV types, while the Aptima test detects mRNAs of 13 carcinogenic types. Results of studies on the RNA proofer test showed that it

is more specific for high-grade lesions than an HPV DNA test, but has a lower sensitivity [60–63].

Proliferation Markers

Proliferation markers like ki67, MYC, cyclin, MAP kinase pathway markers, and survivin were tested in many small studies [64–73]. Recently, an assay which shows the overexpression of proliferation-associated proteins in histology and cytology has been introduced to the market (ProExC), but up to now there has not been much data available on the clinical relevance of this test [74].

The chromosome amplification 3q, which can be associated with increased telomerase activity, is frequently found in cervical cancer and precancerous lesions and might be a further interesting marker [75, 76].

p16

The overexpression of p16 is a direct result of cell cycle aberrations induced by HPV oncogenes, irrespective of the initiating HPV type [77, 78]. The CINtec tests are based on the histological or cytological detection of p16. Cervical carcinomas and precancers of the cervix are specifically stained with p16 in immunohistochemical studies [79]. The evaluation of the histological specimens of the cervix can be improved by p16 staining [80–82]; many pathologists use p16 to clarify difficult cases. In cytology, staining with p16 can improve the identification of suspicious cells on cytological slides [83]. p16 cytology has been successfully used as a triage marker for ASCUS and LSIL cytology results [84, 85]. Initial data on an ELISA-based p16 test suggest that it could be used as a simple test to evaluate the risk of cervical precancer [86, 87].

Many other biomarker candidates apart from those mentioned here have been described; however, for most of them hardly any clinical or epidemiological data are available [58].

Conclusion

Despite the introduction of vaccines against HPV, it is still a long way to go before a successful control of cervical cancer is achieved globally. The available vaccines do not protect against all tumor-relevant HPV types and it will probably take a long time before a broad vaccine coverage is achieved worldwide. Therefore it is important to continue cervical cancer screening for vaccinated as well as nonvaccinated women. The negative effects of HPV vaccination on the performance of cytology will require a reassessment and modification of current screening programs, especially regarding effectiveness, efficiency and quality control. There are a number of possible starting points how screening can be adapted in a combination with HPV vaccination. It would be beneficial to propagate organized screening programs for vaccinated and nonvaccinated women, which entails systematic documentation of vaccination and screening procedures as well as their results. Existing and new methods have to be strengthened by standardizing the procedures and quality assurance processes. Where appropriate, new biomarkers can be used to support or replace existing ones. Many of the new test methods are complex and can only

be used in specialized laboratories. At present, various ways of reorganizing gynecological cancer screening, mostly based on multistage concepts, are being discussed internationally (e.g. a more sensitive primary test followed by a specific clarification test). Currently, sufficient data for novel approaches in primary screening is available only for HPV testing. Apart from modifying screening procedures, improvement in cervical disease ascertainment is necessary. Revised colposcopy-biopsy protocols with multiple biopsy sampling are currently evaluated.

In future cervical cancer prevention strategies, the aim is to achieve a valid risk estimation using the best methods available. Invitation to participation in screening programs as well as clinical procedures should be oriented on the individual risk profile based on different test results [88]. New biomarkers and tests should only be used after they have been thoroughly validated in high-quality studies.

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