

Diagnostic methods and techniques in preventing cervical carcinoma Part I: Conventional cytology and new cytological methods

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ABSTRACT

Cancer of the cervix is one of the most predictable and preventable types of cancer, however, still one of the most common malignancies. Due to a lack of information available to women about the causes of the disease, accessibility of screening programs, and limitations to the existing screening techniques, cervical cancer is the second most common type of cancer in women worldwide. Detection and follow-up of pre-cancer stages of the disease are based on the Pap test, which is now well established as a basic method of secondary prevention. Relative low sensitivity of the Pap test has initiated the development of additional technologies and methods towards enhanced screening quality and error elimination not only in the process of sample taking and analysis but also in screening and interpretation. Immunocytochemical methods and liquid based cytology are the new diagnostics possibilities in secondary prevention. In order to decrease morbidity, thus mortality too, it is necessary that the primary prevention (vaccination) be also implemented.

Key words: Pap test, immunocytology, liquid based cytology, HPV vaccines

INTRODUCTION

Even nowadays, cervical cancer still remains one of the leading causes of death in women and presents one of the most common malignancies affecting women worldwide (1,2). Since the introduction of the Pap test, incidence and mortality of cervical cancer have dramatically declined. Cervical cytology has become one of the most valuable cancer prevention interventions with significant diagnostic accuracy. In the countries where organized screening program is absent, the incidence of cervical cancer continues to rise, while in countries with adequate screening program prevention is shifted towards detection of precursor lesions of cancer (1). In countries of the developing world up to 80% of cases of cervical cancer and deaths occur because of the poor or absence of screening programs. One of the most established and well known

screening methods is cervical smear popularly called Pap smear.

Conventional Pap test

Conventional Pap test includes three diagnoses: microbiological (detection of microorganism that can be identified either directly or indirectly by cytopathic effects on the cells), hormonal (quantitative analysis of estrogen effect on the cells depending on patient's age or/and history) and finally analysis of malignant changes of the cells.

In Croatia a classification named „Zagreb 2002“, which is a slightly modified classification of the „NCI Bethesda System 2001“, has been used in cytological analysis. By this classification squamous cell lesions are divided into three groups: 1) atypical squamous cells subdivided into ASC-US (atypical squamous cells of undetermined significance), ASC-H (atypical squamous cells where high grade lesion cannot be excluded); 2) low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL) lesion and 3) squamous carcinoma. A parallel terminology and terms of cervical intraepithelial neoplasia – (CIN), and carcinoma in situ (CIS) has been retained to leave an opportunity for cytological and colposcopic follow-up. Glandular lesions are also classified into three groups as follows: atypical glandular cells with subdivisions as probably reactive changes, intraepithelial and invasive changes; adenocarcinoma in situ (AIS) and adenocarcinoma with noted origin of atypical cells. Conventional Pap test is also used as a differential diagnostic method predicting histological diagnosis and in that way generates diagnostic and therapeutic procedures (3).

Since 1953, when the first laboratory for cytology was established in Croatia, a network of laboratories has been developed. High number of cytological experts and their education, as well as education of cytotechnologists presents a force adequate to cover the female population if screened once in three years (3). As the result, 68% of the target population in Croatia was covered so incidence and mortality of cervical cancer has decreased (4).

Cytology as part of the screening program has some shortfalls, like an increasing number of taken Pap smears as a result of over-screening of

the population (usually well educated women with higher socio-economic status). On the other hand, women mainly from the rural parts make a part of population that is still un-screened. Besides, cytology results depend on quality of sampling and knowledge and experience of the observer. Being a subjective method, cytology and its potential interpretative mistakes resulted in a wide range of sensitivity and specificity rates. Sensitivity of cytology varies from only 30% - 90% and specificity is better and varies from 86 – 98.7 %. (3, 5)

Screening program is successful only if it covers as much female population as possible, and when the coverage is achieved, sensitivity of the screening test becomes very important (6).

Liquid-based cytology

Liquid based cytology (LBC) or thin layer cytology is a new technique for transferring exfoliated cells from cervix to the microscope slide. The sampling device, usually cervical broom, is immersed in a container with special transport medium. For conventional smears most of the sample is discarded with the sampling device while in LBC almost all collected cells are rinsed into the liquid and the entire sample is preserved. The liquid is aspirated through a membrane that detains the cells which are stamped onto a slide as a monolayer. There are several advantages of LBC: immediate liquid fixation prevents artifacts, smaller screening area makes screening easier, and clean background because of the reduced debris, blood or granulocytes which lead to significantly fewer unsatisfactory cases. Beside, various molecular tests (e.g. testing for human papilloma virus - HPV) and immunocytochemistry can be performed from the residual material without recalling the patients.

In studies comparing conventional Pap smears and LBC, cytology abnormalities such as LSIL or less were reported in larger number in LBC than in conventional smears while sensitivity and specificity for HSIL are similar in both techniques (7).

In spite the fact that liquid-based cytology is nowadays widely used in many European countries, evidence for confirming superiority and accuracy of liquid-based cytology to conventional cytology is yet insufficient (8).

HUMAN PAPILLOMA VIRUS AS ETIOLOGICAL FACTOR FOR CERVICAL CARCINOMA

Viral etiology of cervical cancer is firmly established. There are over 100 different types of human papilloma viruses but only dozen are highly carcinogenic for humans. The most frequent high risk types (hrHPV) are 16 and 18 associated with 70% of the cervical cancer, followed by 33, 31, 45, and 52 genotypes. These hrHPV types are responsible for precursor lesions as well as for cervical cancer. Types 16 and 18 were found in 50%-60% of high grade squamous lesions (HSIL) and in 25% of low grade lesions (LSIL) (9, 10).

HPV infection is extremely common and is transmitted between sexually active people. Almost 50% of sexually active young women are infected with HPV (9) but luckily, most high risk infections are subclinical and are spontaneously cleared by host immune response.

Cuzick (11) and coworkers compared sensitivity of cytology and a hrHPV testing showing hrHPV testing much more sensitive than cytology, with more variable specificity. Sensitivity range of hrHPV testing is from 68%-100% while cytology showed much more variations in sensibility depending on authors and country they come from and existence of uniform classification of cervical precancerous lesions in their country (12-16). The International Agency for Research on Cancer (IARC) recommended the following: "There is sufficient evidence, based on surrogate markers, that the efficacy of HPV testing, using a validated system, as the primary screening modality can be expected to be at least as good as that of conventional cytology".(9)

According to that a wide range of countries support the idea of using hrHPV testing as the primary screening test starting at age of 25 (17-19). HPV infections with high risk types are very common in women younger than 25 years of age, but the percentage of persistence of HPV infection is rather low, probably because of the adequate immune answer of young women for dealing with the HPV infection. Kjaer and coauthors (20) estimate the risk of atypia in general population of Danish women whose cervical cytology was negative and hrHPV DNA positive during more than 10 years of follow-up. Younger women

experienced 8% of moderate dysplasia or worse within five years of follow-up, and 13.4% within 10 years. Elderly women had risk of HSIL of 15.6% after five years, and 27.4% after 10 years. But patients with both negative tests had a much lower risk of cervical abnormalities (3.4%) after 10 years regardless of patient's age. These results indicate a high negative predictive value in patients with negative hrHPV DNA test as well as cytology on one hand, and on the other, single positive hrHPV DNA test can stratify patients at a different level of risk for developing cervical abnormalities.

There are lot of data suggesting usage of hrHPV testing in the 1) triage of borderline cytology and proving its better sensitivity than repeated cytology in women with equivocal cytology; 2) as a follow-up test for recurrence after cervical lesion treatment but no sooner than six months. That is the most sensitive way to detect persistent or recurrent lesions (5, 9). Hopefully, the clinicians will benefit from knowing about presence of specific HPV type, so infections with hrHPV with high oncogenic potential can be separated from those that can be less intensively managed (low risk HPV infections). That means that hrHPV positive test identifies those women who have risk of developing cervical lesions as long as the infection persist, but we cannot be sure if that is really going to happen.

TUMOR MARKERS IN PREDICTING OUTCOME OF CERVICAL LESION

To predict an outcome of HPV infection is a major challenge. A persistent high risk HPV type infection can lead towards malignant transformation by complex molecular pathways mediated by specific cellular proteins. These proteins – biomarkers can be categorized into different classes such as oncogenes, tumor suppressor gene, apoptotic markers, metabolic markers, imaging markers etc. (9), each of them is used in a specific way to understand the mechanism of cancer initiation and progression (21). The main event for carcinogenesis is integration of viral oncogenes E6 and E7 into host genom. If these genes are expressed at high levels, they interrupted normal cell cycle regulation and induce genomic instability (22).

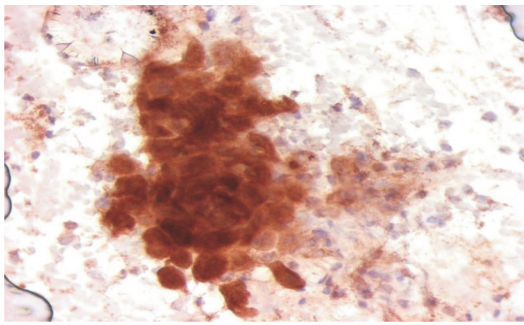


Figure 1. Positivity for p16 INK4a suggesting HSIL (x 450)

There are lots of regulating proteins involved in cell cycle control including p16^{INK4a}, minichromosome maintenance proteins 2, 4 and 5, Cyclin D1, prostaglandin synthetase, TOP2A (topoisomerase 2 alpha). Telomerase overexpression has also been acknowledged as one of the markers of cervical carcinoma and high grade lesions on cervical biopsies (23). Expression of the E7 oncogene of hr HPV leads to functional inactivation of tumor suppressor retinoblastoma protein (pRB), because of hypermethylation or mutation which in turn, results in strong overexpression of the cyclin-dependent kinase inhibitor p16^{INK4a}. This indicates that an active expression of the viral E7 oncogene is present in cancer and dysplastic cells which can be detected throughout the epithelium. Furthermore, p16^{INK4a} expression may help identify low grade lesions that are at an increased risk for progression to a high grade lesion (Figure 1) or cancer (Figure 2) and it has been found to reduce interobserver disagreement compared with the histological diagnosis (24-26).

In certain cervical lesions (mild to moderate) virus replication is evident in the upper part of epithelium, and the viral major capsid L1 antigen is expressed allowing immunochemical detection of an infected cell (Figure 3) This can be used as a tool for triaging early cervical abnormalities

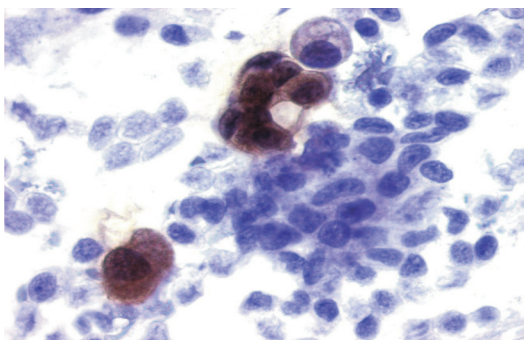


Figure 2. Positive p16INK4a reaction in malignant cells (x 450)

with tendency to progress from those which are more likely regressible. Griesser et al provided evidence that mild dysplasia without immunochemically detectable HPV L1 capsid protein can be expected to progress more likely (26). Reason for that is yet unknown, but probably the loss of L1 expression is due to the abnormalities in transcription pathways and to the process of integration into the genome.

Many molecular markers were evaluated for their role in cervical screening. Some of them like Ki-67, PCNA (proliferating cell nuclear marker) have shown limited potential, while others like CDC6 (DNA replication protein), mini-chromosome maintenance protein (MCM5) and p16INK4a have shown high potential. (9)

A combination of tumor markers with other methods of secondary prevention will increase sensitivity and specificity of the assay (27).

VACCINATION

The development of prophylactic vaccine, based on the L1 capsid protein has started a new era in primary prevention of cervical carcinoma. Presently, there are two available vaccines: bivalent vaccine containing L1 of hrHPV types 16 and 18 ("Cervarix", Glaxo Smith Kline, UK), and quadrivalent including two low risk types (HPV 6 and HPV 11, responsible for most condylomata cases), and two high risk types 16 and 18 ("Gardasil", MSD, USA). Both vaccines have shown cross protection against infection with HPV 45 and 31 because of their genetic relatedness to HPV 18 and 16 respectively (28). HPV vaccination as a primary prevention together with secondary prevention (Pap test) will significantly reduce cervical cancer morbidity and mortality. However, integration of vaccination with screening

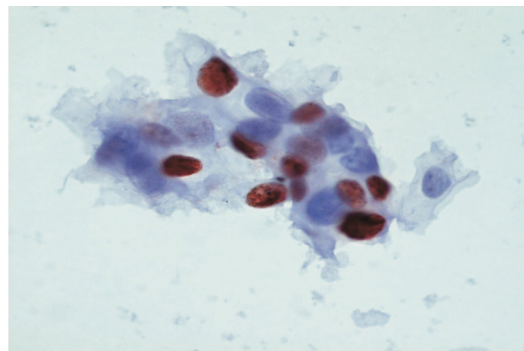


Figure 3. HPV L1 in LSIL lesion (x 450)

ning is a complex issue. Vaccination will likely prevent at least 70% of potential cancer. Protection is limited because of the multiplicity of HPV types, and vaccines contain only two most frequent types (1, 27-29). Other problems include yet uncertain need for booster doses, expensiveness of vaccines, undetermined population for vaccination (boys and girls or only girls), right age of first vaccination and etc. (30).

Besides vaccination, the cervical cancer primary prevention includes health education, and secondary prevention in addition to conventional

Pap test includes a large scale of different techniques, which can improve traditional screening programs. For example, liquid-based cytology and computer assisted cytology can speed up the screening process, and panel of biomarkers of the carcinogenic process and HPV testing can improve sensitivity and specificity of conventional methods.

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