

NEW METHODS OF IMMUNOHISTOCHEMICAL DIAGNOSIS OF TUMOR GROWTH

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Relevance. Early diagnosis of precancer and cervical cancer should be based on the use of a combination of methods: liquid cytology, HPV testing using the PCR-HB method and ICC with double staining p16/Ki67 - this significantly increases their sensitivity and specificity.

Keywords: human papillomavirus, cervical cancer, cervical intraepithelial neoplasia.

Abstract. To date, questions regarding the frequency of screening, the use of markers, and diagnostic methods for cervical cancer, especially in the case of transient infection, remain open. The American Society for Colposcopy and Cervical Pathology recommends starting a cervical cancer screening program for women at age 21. In Denmark, the starting age for cervical cancer screening is 23, in France - 25, and in Finland - 30. The issue of the specifics of cervical cancer screening in women under 30 and over 30 remains controversial. To conduct an effective screening test and early diagnosis of cervical cancer, it is necessary to find methods with high specificity and sensitivity. In countries with organized cervical cancer screening based on cytological examination, a decrease in morbidity and mortality from cervical cancer has been demonstrated [1,13,14,15].

Immunohistochemistry (IHC) is a method for identifying the precise localization of a cellular or tissue component (antigen) using immunological and histochemical reactions; Immunohistochemistry is used to analyze tissue sections or cytological material while preserving cell morphology. Several primary applications of IHC can be identified in diagnostic practice: in the study of human tumors to determine the histogenesis of undifferentiated tumors and distant metastases, to differentiate various tissue components, and complex tumors; and to assess the prognosis of the subsequent course of the disease and, finally, to prescribe therapy. Immunohistochemistry is particularly valuable when it is difficult to determine the histogenetic identity of a tumor based on routine hematoxylin-stained sections. In these cases, the term "undifferentiated tumors" is often used. Determining tumor histogenesis is essential for determining treatment tactics and prognostic assessment.

Immunohistochemical studies to assess the organ specificity of distant metastases are performed using a set of antibodies characterizing the antigenic properties of cells in various organs: in particular, antibodies to PSA detect prostate cancer; the presence of

estrogen and progesterone receptors in tumor tissue suggests a metastasis from the mammary gland or endometrium; antibodies to thyroglobulin detect tumor cells of follicular thyroid cancer. Antibodies to various cytokeratins are of great help in determining the organ origin of metastases. To prognosticate the disease, predict the biological behavior of the tumor, the appearance of metastases, and the effectiveness of therapy, a study of proliferative activity (Ki-67), the severity of angiogenesis, the determination of growth factor receptors (her-2/neu oncogene products in breast cancer), the identification of steroid hormone receptors, and a study of the degree of cell anaplasia (mutant protein of the p53 gene) are carried out.

Objective of the study. To evaluate the effectiveness of cytological, immunocytochemical, and HPV testing for the detection of cervical precancer and cancer, and to study the pathological and morphological features of cervical repair processes in the presence of HPV infection.

Materials and methods. All 167 women were examined according to a standard protocol: clinical examination, collection of smears in SurePath vials (BD, USA), preparation of Papanicolaou-stained cytological preparations using a TriPath processor (BD, USA), and smear evaluation using the Bethesda system [1,12]. Three groups of women were selected: 1) 91 women to evaluate the effectiveness of HPV testing using the PCR-NV method and liquid-based cytology in detecting HPV-associated cervical pathology in women aged 30 years and older. 2) 148 women to evaluate the effectiveness of a combination of methods: liquid-based cytology, double immunostaining of p16/Ki67 and HPV testing using the PCR-NV method for HPV-associated cervical pathology. 3) 61 women to study the morphological and immunohistological features of different types of SEMS. At the first stage, 91 patients aged 17 to 56 years were included in the study with the following examinations: 1) detection of cervical pathology after liquid cytology; 2) performing colposcopy with targeted sampling of altered or suspicious areas of the cervical canal for histological examination; 3) conducting a molecular biological study to study the expression of p16/Ki-67 markers and HPV DNA genotypes in cytological preparations prepared from residual material.

Study results. Our study also showed that p16/Ki-67 dual staining has a higher efficiency with high sensitivity and high specificity than conventional liquid cytology and HPV DNA testing in detecting high-grade cervical epithelial lesions (CIN \geq II). The number of p16/Ki-67 positive atypical cells is associated with the histological severity of cervical intraepithelial lesion. p16/Ki-67 is a marker of cell cycle dysregulation and uncontrolled cell proliferation and is observed only in dysplastic cells that have undergone HPV-associated transformation. Our findings show the same

high sensitivity and specificity as the dual staining findings of other researchers [1,2,4,6,8,10]. When comparing the efficiency of double immunostaining with HPV testing, a group of researchers [3,5,7,9] found, and we also confirmed, that ICC analysis using CINtec PLUS is more specific, since we see not only the presence of the virus, but also the response of cervical epithelial cells to its invasion. Ki-67 is a reliable indicator of tumor proliferation, depth of invasion, degree of malignancy, progression and recurrence of bladder cancer. In immunohistochemical studies, the percentage of cells positively stained for Ki-67 allowed us to reliably distinguish between non-invasive and invasive tumors, and this indicator increased significantly with increasing depth of invasion. In our studies of bladder cancer, the intensity of nuclear staining using antibodies to the P53 protein allows us to assess the degree of malignancy of tumor cells, and the pathological type of CK 20 expression and a high percentage of nuclei positively stained for the Ki-67 protein characterize invasive urothelial tumors. The use of a panel of antibodies to P53, Ki-67, and CK20 can be recommended for the pathohistological examination of biopsy and surgical specimens of urothelial tumors to objectively diagnose them and predict their clinical course.

It has been shown that as neoplastic processes in the cervical epithelium progress, the expression of p53 gene products and the number of cells with a positive nuclear reaction to Ki-67 increase, while the expression of BCL-2 and the number of estrogen receptors decrease. In this case, almost all neoplastic epithelial cells lacked estrogen receptors.

Thus, during neoplastic transformation of ectocervical cells, their sensitivity to estrogens decreases. Nevertheless, hyperestrogenism can be considered one of the factors contributing to the formation of tumor lesions of the cervix.

Conclusions. Thus, IHC represents a valuable modern diagnostic method that allows us to determine the histogenesis, degree of proliferative activity, and anaplasia of tumor cells, characterize the prognosis, and propose appropriate treatment methods for patients. However, the potential of this method for treating a specific patient should not be overstated. IHC is only a complementary research method, and its results should be interpreted in the context of other examination data, including clinical ones.

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