IMPROVING CYTOLOGICAL SCREENING. NEW ADDITIONAL METHODS OF CO-TESTING

GEORGETA GÎNFĂLEAN[1]

[1] *"Lucian Blaga" University of Sibiu*

Keywords: *cervical cancer, HPV vaccination, secondary prevention, cytooncological screening*

Abstract: *Cervical cancer is the second type of cancer diagnosed in the hierarchy of genital cancers and the third cause of death due to cancer among the female population. Although important progress has been made in cervical cancer in the etiological, diagnostic and treatment areas, incidence and epidemiology factors have not been influenced to the extent of expectations, with a remarkable occurrence and mortality, both in our country and globally. Prevention and fight at population level involve complex measures included in the global epidemiological, clinical surveillance and laboratory screening (cytological, immunological, serological, radiological). Starting from these prerequisites, primary prevention programs (HPV vaccination) and secondary prevention (cervical cytology screening, classically or liquid-based) have been implemented, to which co-testing methods have been added in order to improve the sensitivity of screening. Since the interpretation of classical cytology on smear shows a number of limitations, new additional methods of co-testing have been developed as follows: primary HPV-DNA genotyping of highly oncogenic subtypes; immunocytodiagnostic co-testing - with biomarker detection panel - p16 and Ki67; co-testing with immunohistochemical biomarkers - on biopsy pieces, respectively detection of overexpression of p16 protein.*

Cervical cancer is the second type of cancer diagnosed in the genital hierarchy of cancers and the third cause of death by cancer among the female population.(1) According to the World Health Organization (WHO), world wide, 500.000 women are newly-diagnosed with cervical neoplasm, of whom 200.000 are dying from this disease.(2)

Cervical cancer is considered a major public health problem, as approximately 90% of deaths due to this type of neoplasia occur in the developing countries, with a clear geographical variation between the developed and the developing countries. The variation is explained by the presence of three common elements in the developing countries: the increased prevalence of Papilloma Human (HPV) virus due to a liberal attitude regarding the sexual practices (HPV infection being considered a sexually transmitted disease) and lack of implementation, low accessibility or ineffective screening (diagnosis, therapeutic sanction and suboptimal follow-up of precancerous cervical lesions).(3)

The European Society for Medical Oncology (ESMO), in the clinical guide on cervical cancer, also points to this variation, which is based on the complication of the three factors mentioned above, and also shows a 5-year survival rate for the women diagnosed with cervical cancer in Europe during 2006-2007, of 62% on average with the following mention: in the North of Europe, the 5-year survival rate was of 67% (in Norway even of 71%), while the Eastern Europe is diametrically opposite with a survival rate of 57% (particularly low <55% in Bulgaria, Poland, Latvia).

Unfortunately, in Romania, the incidence of cervical cancer and the mortality caused by this type of cancer is increased.(3) Globocan reported in 2008 that the incidence of cervical cancer in Romania was 23.9 per 100.000 women and the mortality rate was 11.8 per 100.000 women.(4)

Therefore, primary prevention programs (ani HPV vaccination, immunization) and secondary prevention - cervical cytology screening, classically or liquid-based to which there have been added: primary HPV phenotypes and immunocytosis -/-histochemical detection of some biomarkers from the cytoscopies and biopsies obtained as a result of colposcopy, direct biopsy of a suspected lesion or on pieces obtained after surgical treatment.

After the implementation of vaccination programs of approximately one decade with licensed bivent/quadrivalent/new-valent vaccines, there were significant decreases in the incidence of genital warts, in cytological results such as - high-grade intraepithelial precursor lesions, HPV-associated cancers in both women and men. Mention must be made of the fact that in the US, a prophylactic anti HPV vaccination program has been initiated in the male population as well, since the age of 16.

Post-immunization, there were reported annual reductions in the incidence of the following types of HPV related neoplasia: 90% - cervical cancer, 92% - anal cancer, 87% - vulvar cancer, 85% - vaginal cancer, 85% - penile cancer. These significant decreases, with a strong impact on the reduction in the incidence of cancer associated with HPV infection, show that vaccination has resulted in good primary prevention through the vaccination program, respectively by immunization (with either prophylactic or therapeutic valence).

Currently, it is widely accepted and demonstrated by virology and molecular biology studies that HPV infection is the main cause (99%) of cervical tumors and neoplastic precursor lesions. We mention that of 99% of HPV infections, 80% are transient (especially in the 24-year-old females, adolescents), and the rest are persistent (especially with high oncogenic strains of type 16 and 18 of all 14 oncogenic strains), which

[1]Corresponding author: Georgeta Gînfălean, Str. Moreni, Nr. 19, Sibiu, România, E-mail: rezultatemedicina@yahoo.com, Phone: +04723 254897
Article received on 17.01.2018 and accepted for publication on 29.05.2018
ACTA MEDICA TRANSILVANICA June 2018;23(2):69-71

create the premise of the lesional continuity theory with dislocation of dysplastic lesions towards neoplasia, especially in the female population aged 30 years and over, which often also presents accumulations of genomic alterations in the somatic cells (which is a factor contributing to tumorigenesis) that make them more susceptible to infection, respectively to malignant transformation.

Secondary prevention is obtained by performing the screening - by sampling and interpreting cytology within the Babes-Papanicolau cytological exam (classically or liquid-based).

Interpretation of cytology presents a series of limitations given by the following aspects: subjective interpretation of cellular morphological alterations, errors in interpretation, false-negative results 30-50%, incorrect sampling of cell material without intercepting the transformation area (for example, squamo-cylindrical junction migrates in perimenopause towards the inside of the cervical canal).

The singular interpretation of the cyto-oncological examination as a whole has an increased specificity of about 98%, low and variable sensitivity of about 55-80% (especially in the case of adenocarcinomas). Therefore, suspicious lesions will be submitted to biopsy per primate as a diagnostic approach, so as not to risk a false-negative result.(5)

Lately, additional methods of co-testing have been developed in triage and in order to increase the sensitivity of the cervical cytological screening as follows:

a) in the case of HPV-DNA primary screening of high oncogenic subtypes (mainly HPV 16 and 18 plus additional testing of 31, 33, 35, 45, 55, 58 oncogenic strains) approved by the Food and Drug Administration (FDA) in 2003, the sensitivity of cytological screening increased from 50% to 85% to almost 100% for high-grade precancerous lesions. This method is indicated in the 30-year-old female population and in older women at risk of persistent HPV infection. If both results are negative, the co-testing will be repeated every 3 years, the incidence of cervical cancer during this time being very low, of about 1/1000.(6)

b) Immunocytodiagnostic co-testing - the proposed test is CINtecPLUS Cytology with simultaneous dual detection panel of two biomarkers - p16 and Ki67 on the collected cell material.

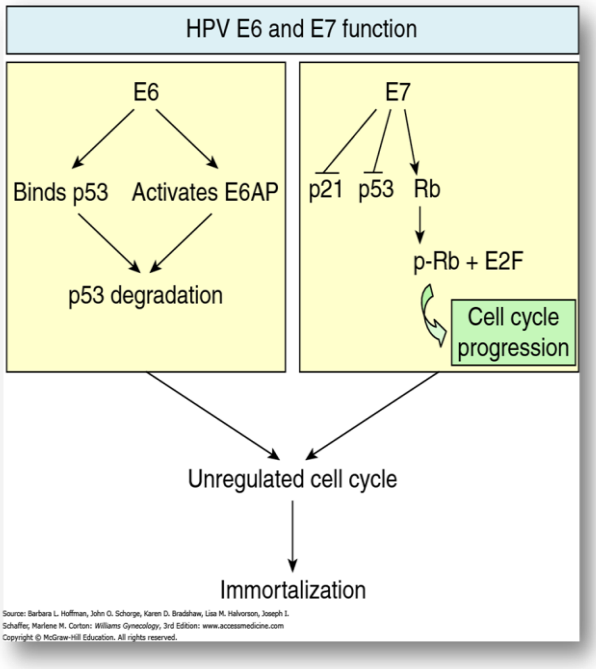
The test identifies HPV-related secondary transformations by providing an objective triage in the following situations: abnormal cytology on non-HSIL Pap test (ASC-US, LSIL) as a result of the high-risk HPV DNA genotyping for primary cervical cancer screening primary HPV-DNA genotype positive screening for high grade oncogenic strains, within the screening program of high-grade cervical lesions or for establishing a type of colposcopic or therapeutic diagnostic method.(7) This test has a specificity of 97.5% and a high sensitivity of 94% regarding the risk of progression to high-grade intraepithelial cervical lesions.

c) Immunohistochemical biomarkers co-testing regarding the biopsy pieces (CINtec Hystology) - detects the overexpression of p16 protein. The method is used to confirm the presence of a precancerous cervical lesion.(7) It also indicates cell cycle disruption on the background of cellular genomic alterations, being a direct marker of the oncogenic HPV activity. Overexpression p16 represents the moment that marks the oncogenic transformation of HPV infected cells.

In HPV infection, the HPV E7/E6 viral oncogenes interfere with and accelerate the degradation of p53/p21 tumor suppressor genes (resulting in genomic instability) and the Retinoblastoma suppressor factor (p Rb), causing the overexpression of p16, which marks the oncogenic transformation of HPV infected cells. p16 is used as a biomarker for precancerous and cancerous lesions.(8)

By inactivating the tumor suppressor factors, HPV infected cells become vulnerable to malignant transformation due to the loss of cell cycle regulation, uncontrolled cell proliferation, and accumulation of DNA mutations (these accumulations of genomic alterations at somatic cells level are a factor contributing to tumorigenesis; therefore, cells are more susceptible to infection, respectively to malignant transformation).

Figure no. 1. Oncoproteins E6, E7 diagram, suppression of tumour suppression factors (p53,p21, p-Rb) (9)



CINtecPLUS immunocytodiagnostics is based, as I have mentioned above, on the dual, simultaneous and qualitative detection of two biomarkers, p16 (INK4a) and Ki67, on the cytological material. These are plasma proteins, which play a part in regulating the cell cycle.

The p16 protein (INK4a) is involved in cell cycle control, acting as a secondary tumor suppressor, by a mechanism of blocking the uncontrolled multiplication of HPV infected cells, respectively, slowing progression from phase G1 to the S phase in the cell cycle.

The Ki 67 protein is a cell proliferation marker, expressed in the nucleus of proliferative cells in the parabasal layer of the cervical epithelium.

Positive association of p16-Ki67 in the same cell (p16 overexpression is indicated by the positive cytoplasmic staining reaction - brown color, and the Ki67 overexpression is indicated by the nuclear staining immunocytochemical reaction – red colour) indicates cell cycle disruption due to cellular genomic alterations, being a direct marker of HPV oncogenic activity and represents an objective criterion for selecting high-risk patients for high-grade cervical lesions for colposcopic evaluation.

Following multiple molecular studies, the following roles of immunohistochemical markers in cervical cancer have been established: diagnostic and prognostic for both squamous carcinomas and adenocarcinomas (table no. 1).(10) Also, mention must be made of new markers that assess the risk of metastasis or prediction of chemotherapy response in advanced cervical cancer stages.

Table no. 1. Immunohistochemical markers with diagnostic and prognostic role in squamous carcinoma and cervical adenocarcinoma

	Diagnosis	Poor Prognosis	Protection
Cervical squamous cell carcinoma (SCC)	p16(+) Ki-67(+) E-caderina(+) Ciclina D1(+) NF-κB(+) c-IAP2(+) Lgr5(+) SphK1(+) STOML2(+) Th17/Foxp3(+) Caspase-3(-)	p16(+) RIPK4(+) HMGB1(+) Survivina(+) Beclin-1(-) LC3(-)	Galectina-7(+) S100A9(+)
Adenocarcinoma	p16(+) Ki67(>50%) p53 (+) EGFR(+) CEA (+) CA125(-) ER(-) RP(-) Vimentina(-)	LTBP2(+)	-

(+): Positive expression; (-): Negative or weakly positive expression; NF-kB: Nuclear Factor-κB; c-IAP2: Inhibitor Of Apoptosis Protein-2; Lgr5: Leucine-rich repeat-containing G proteincoupled receptor 5; SphK1: esfingosina quinase 1; STOML2: Stomatin-Like Protein 2; RIPK4: Receptor Interacting Protein Kinase 4; EGFR: Epidermal Growth Factor Receptor; ER: Estrogen Receptor; PR: Progesterone Receptor; LTBP2: Latent Transforming Growth Factor Beta-1.

REFERENCES

1. ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up, Cervical Cancer Anals of Oncology. 2017;28 Supplement 4;iv72-iv 83.
2. SMGroup, Immunohistochemistry in Cervical Cancer, Cervical Cancer: Recent Research and Review Studies; www.smgebooks.com. Accessed on 12.04.2018.
3. Tratat de chirurgie editia a II-a, vol.V, Obstetrica si Giencologie, coordinator Gheorghe Peltecu, editura Academiei Romane Bucuresti; 2014. p. 179.
4. Globocan2008, www.iarc.fr; https://www.iarc.fr/en/mediacentre/iarcnews/2011/globocan2008-prev.php. Accessed on 31.04.2018.
5. William's Gynecology, edition 3 rd, section 4 Gynecology Oncology, Chapter 30. Cervical cancer; 2016. p. 667.
6. American College of Obstetricians and Gynecologists, 2005b, Wright 2007a. ACOG; 2005.
7. Improving consistency in the diagnosis of cervical pre-cancers: Roche CIN tec Hystology test receves FDA clearance. Switzerland, Basel April 2017, Media Release. www.roche.com. Accessed on 13.03.2018.
8. Tsutsui T, Kumakura S, Yamamoto A, Kanai H, Tamura Y, Kato T, Anpo M, Tahara H, Barrett JC. Association of p16INK4a and pRb inactivation with immortalization of human cells. Carcinogenesis. 2002;23(12):2111-2117.
9. Diagrama oncoproteinelor E6, E7, supresia factorilor de supresie tumorala (p53,p21, p-Rb) https://accessmedicine.mhmedical.com/content.aspx?bookid=1758&sectionid=118172491&jumpsectionID=118172515. Accessed on 14.04.2018.
10. http://www.smgebooks.com/cervical-cancer/chapters/CC-15-05.pdf. Accessed on 02.02.2018.