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New molecular markers for cervical precancer detection optimization. Immunocytochemistry test: p16/Ki-67 dual staining (CINtec PLUS test)

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Abstract

The dual immunostaining of cervical cytology p16/Ki-67 which combines a high sensitivity with a high specificity is the solution that has been developed in recent years for cervical precancer detection optimization. The test is a double immunocytochemistry staining performed simultaneously in the same cell for p16 - transforming infection marker and Ki67, a proliferation marker. This immunocytochemistry test is intended to optimize the surveillance or screening of young patients with cytology results: atypical squamous cells of undetermined significance with high risk human papilloma virus or low-grade squamous intraepithelial lesions, thus avoiding colposcopies and any invasive gesture at nulliparous, reducing the anxiety of patients linked to the indication of colposcopy and ultimately reducing diagnosis costs. Each year there are 500.000 new cases of cervical cancer detected worldwide and the average of 5 years survival rate is only 50%, thus including the CINtec PLUS test in cervical cancer screening programs may be seen as an innovative strategy an can provide real benefit to clinicians and patients as it helps identifying underlying disease and establish who should proceed to further procedures.
Keywords: *cervical detection, immunocytochemistry, colposcopy*

Introduction

Cervical cancer is the most common gynaecological cancer that affects women. The majority of cases occur in relation to the infection with human papilloma virus (HPV).

In the evolution of HPV infection to neoplasia there are also involved factors related to the host[(1)].

For women infected with high-oncogenic HPV subtypes, cervical squamous cell carcinoma risk is 189 times higher and cervical adenocarcinoma risk is 110 times higher as compared to women without HPV infection[(1)].

Increasingly more data suggest that HPV oncoproteins have a critical role in continuous cellular proliferation. Unlike other HPV serotypes, the oncogenes have the capacity to integrate in the human genome[(1)].

Consequently, E1 and E2 early replication oncoproteins will facilitate viral replication in the cervical cells. Thus, these proteins synthesized in considerable amounts in early phases of HPV infection, will induce cytological alterations evidenced by Pap Test. These can be followed by the amplification of viral replication and normal cells transformation to tumour cells, a process that involves E6 and E7 viral oncoproteins[(1)].

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The international guidelines for practice in cervical cancer prevention have been improved for the last two decades, at short periods of time, as molecular mechanisms of carcinogenesis are developing, new studies are being conducted and new clinically significant technologies are expanding[(2)].

Because of the HPV infection, which is both widespread and in most cases spontaneous transient, the HPV test, although very sensitive, has a limited specificity for precancer and cancer, being associated with an inevitable number of false positive results[(2)].

The solution that has developed in the last few years consists in the use of more specific molecular markers in secondary for cervical precancer, combining high sensitivity with a high specificity of which the most studied is the p16/Ki-67 dual immunocytochemical staining (Figure 1).

The sensitivity of p16/Ki67 double staining

The published studies show that the sensitivity of p16/Ki-67 double staining is comparable to that of the HPV testing, but at a significantly higher specificity[(2)].

The cervical intraepithelial neoplasia (CIN)tec test is a double immunocytochemical staining simultaneously

in the same cell for p16, a marker of transforming HPV infection and for the Ki-67 proliferation marker. It identifies a disorder of the cell cycle by inactivating the retinoblastoma 1 protein located in the proliferating cell, this mechanism resulting from the E7 oncogene expression of a high-risk HPV. The overexpression of p16 is therefore an indirect means of diagnosing not only high-risk HPV infection, but also the E7 oncoprotein expression related to a transforming infection. P16 is present in almost all forms of grade 2 CIN(2+) and cervical cancers[2].

The immunocytochemical evaluation is performed in cytological slides displayed separately from those stained by Pap standard method for usual morphological evaluation.

They may be sampled conventionally or by liquid medium techniques, or may the residual liquid from the initial cytology may be used[2].

Technically, a kit consists of a cocktail of primary antibodies p16INK4 - mouse specific primary monoclonal antibody (clone E6H4) and Ki - 67, rabbit specific primary monoclonal antibody, followed by the application of two different reagents and two differently stained chromogenic substrates (DAB brown coloured and Fast red, red coloured)[2].

Due to the haematoxylin counterstain of the preparation, sometimes the morphology can also be measured. The presence of one or more double stained cervical epithelial cells defines a positive result of CINtec PLUS test, irrespective of the morphology interpretation[2].

By using p16/Ki-67 immunocytochemical testing the medical conduct of screening or surveillance of young patients with cytological results such as low-grade squamous intraepithelial lesions (LSIL) or positive high-risk HPV atypical squamous cells of undetermined significance (ASC-US) is optimized, unnecessary colposcopy is avoided (indications for colposcopy are restricted to positive CINtec PLUS cases), thereby limiting nulliparous invasive gestures and negative CINtec PLUS results are monitored every 12 months by cytological and viral testing[3].

The sensitivity of a single cytology test for the detection of CIN2 + or high-grade CIN (HGCIN) is unsatisfactorily low. To improve sensitivity, testing for the presence of high risk HPV has been proposed as an alternative or an auxiliary instrument for cervical cancer screening[4].

Higher sensitivity for HPV testing

Numerous studies have shown that HPV testing offers a high sensitivity for CIN2+[5-7].

However, the specificity of screening women for HGCIN with HPV testing is limited because most HPV infections are transient and only a small percentage of infections persist and may progress into transforming infections and HGCIN (11). Given the high prevalence of HPV infections in younger women, currently, HPV testing is not recommended for screening women younger than 30[8].

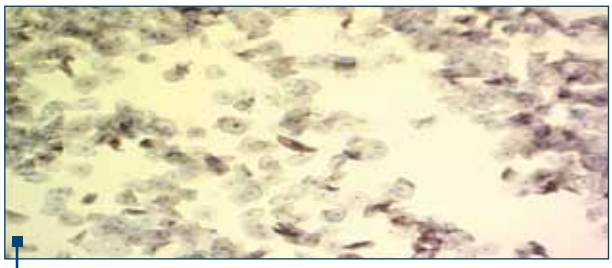

Figure 1. P16/Ki-67 double staining (personal collection, Micomi Clinic)

In order to estimate the sensitivity and specificity of p16/Ki-67 dual staining and to compare it with Pap testing, a study (Primary ASC-US and LSIL Marker Study- PALMS Study) was conducted. The study included a routine screening of European population for cervical cancer in women age 18 or 18+, and HPV testing of women age 30 or 30+[9-11].

A number of 196 women aged 18 or even older, subject to routine cytology - based cervical cancer screening were enrolled in hospital - based screening canters in Belgium, France, Germany, Italy and Spain.

Screening exclusion criteria used: pregnancy and previous hysterectomy. All subjects in the study gave their written informed consent. All women were subject to Pap cytology, p16/ Ki-67 double staining and HPV testing[11].

All subjects with abnormal Pap results (ASC-US or ASC-US +) or a positive p16/Ki-67 dual staining cytology result and/or a positive high-risk HPV test result were referred for colposcopy, except when the HPV was the only positive test in women younger than 30 years. Subjects with negative results in all tests completed the study upon receipt of results[11].

A total of 27.349 women participating in routine screening for cervical cancer were enrolled into the study: 12.226 (44.7%) in Germany, 5.250 (19.2%) in Italy, 4.034 (14.8%) in France, 3.929 (14.4%) in Spain and 1.910 (7.0%) in Belgium. The screened population average age was 39.9 years[11].

Among the 27.248 subjects with all three tests performed, the overall prevalence of positive dual - stained cytology results was 5,4%, similar to ASC-US+ prevalence and half of HPV prevalence (10.7%). Positivity rate was overall higher in women group aged 18-29 years, as compared to women in their 30's. The prevalence rate of positive dual-stained cytology was comparable to ASC-US+ and approximately half of HPV prevalence rate in both age groups[11].

PALMS study data show that dual stained cytology can offer both high sensitivity and specificity for the detection of HGCIN in a single test[12].

The new immunocytochemistry test offers the clinicians an alternative to the classical recommendation of colposcopy, and the possibility to optimize the medical attitude in screening or surveillance of young patients with ASC-US cytology results and high- positive risk HPV or LSIL and of patients of any age with normal cytological smear, associated with high-risk HPV positive test.

The positive test result is a referral for colposcopy evaluation, and the negative test allows for the maintenance of a cytological surveillance[2]. The dual immunostaining of cervical cytology p16/Ki-67 thus selects from the patients who are likely to hide or develop serious illness of the cervix in the future, those who really need colposcopy, allows avoiding invasive gestures to nulliparous, helps reduce anxiety at patients related to the indication of colposcopy and ultimately to lower costs of diagnoses[2].

Conclusions

Each year there are 500.000 new cases of cervical cancer detected worldwide and the average 5 years survival rate is only 50%, thus including the CINtec PLUS test in cervical cancer screening programs may be seen as an innovative strategy an can provide real benefit to clinicians and patients as it helps identifying underlying disease and establish who should proceed to further procedures. ■

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