ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

The triage of low-grade cytological abnormalities by the immunocytological expression of cyclin-dependent kinase inhibitor p16^{INK4a} *versus* Human Papillomavirus test: a real possibility to predict cervical intraepithelial neoplasia CIN2 or CIN2+

RUXANDRA STĂNCULESCU[1)], ELVIRA BRĂTILĂ[1)], VASILICA BAUŞIC[2)], TEODORA VLĂDESCU[3)]

[1)]*Department of Pathological Gynecology*
[2)]*Department of Molecular and Cellular Biology*
"Carol Davila" University of Medicine and Pharmacy, Bucharest
"St. Pantelimon" Clinical Emergency Hospital, Bucharest
[3)]*Department of Pathology,*
"St. Pantelimon" Clinical Emergency Hospital, Bucharest

Abstract

Objective: Assessing the hypothesis that p16^{INK4a} immunocytochemistry (ICC) has better relevance than Human Papillomavirus (HPV) testing at detecting high-grade cervical intraepithelial neoplasia (HGCIN) upon histopathological diagnosis in women with abnormal cytologies such atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesions (LSIL). *Materials and Methods*: A retrospective study of 63 selected cases (22 with ASC-US and 41 with LSIL) was performed at "St. Pantelimon" Clinical Hospital, Bucharest, Romania, using p16^{INK4a} ICC and Linear Array HPV Genotyping Test. All cases have been followed-up by colposcopy and biopsies. The sensitivity and specificity of p16^{INK4a} and HPV were analyzed by *chi*-squared test. *Results*: LSIL cytologies were more likely to be p16^{INK4a} positive than those with ASC-US: OR=3.1, 95% CI (1.06–9.11). The processed data show that in women with LSIL the sensitivity of p16^{INK4a} is 37.5% higher than that of high-risk(hr)-HPV (p=0.0050), whereas in ASC-US it is 44.5% higher (p=0.0577). In ASC-US, p16^{INK4a} has a higher specificity (84.62%) than hr-HPV (53.85%); for LSIL cytologies, this difference is less steep: 58.82% for p16^{INK4a} as compared to 47.06% for HPV. *Conclusions*: The p16^{INK4a} is significantly more sensitive than hr-HPV in both low-grade abnormal cytologies and has higher specificity than HPV testing to detect HGCIN, mainly in women with ASC-US cytologies. Only women with ASC-US and LSIL cytologies who test positive for p16^{INK4a} should be directed to colposcopy and/or biopsy. p16^{INK4a} is a suitable immunocytochemical marker which increases the accuracy of diagnosis at women with low-grade cytologic abnormality.

Keywords: Atypical Squamous Cells of Undetermined Significance (ASC-US), Low-grade Squamous Intraepithelial Lesions (LSIL), Human Papillomavirus (HPV), cyclin-dependent kinase inhibitor (p16^{INK4a}), Cervical Intraepithelial Neoplasia grade 1, 2, 3 (CIN 1, 2, 3), Liquid Based Cytology (LBC).

Introduction

According to the *International Agency for Research on Cancer* (*IARC*) and *World Health Organization* (*WHO*), every year 3402 Romanian women are diagnosed with cervical cancer and 2005 die from this disease [1, 2]. It is the most common cancer in Romanian women aged 15–44, with an annual crude incidence rate of 24.4 out of 100 000 women [1, 2]. In Romania, the primary cancer prevention measure supported by the Public Health Ministry is Human Papillomavirus (HPV) vaccination. Unfortunately, parents are reluctant to accept the vaccination of their girls.

In the summer of 2012, the Romanian Ministry of Health has initiated a screening program for cervical cancer with the support of the European Union. One of the goals is to facilitate the implementation of such management protocols on a wider scale in clinical practice. The Romanian cervical screening program uses the Papanicolaou (Pap) cytology.

National government-funded research programs investigating cervical cancer have existed in Romania since 2007. One of these grants was awarded to the Departments of Gynecology and Histopathology at the "St. Pantelimon" Clinical Hospital, Bucharest, and resulted in the development of a research center. The main purpose of the research grant was to translate into clinical practice novel biotechnologies able to identify suitable biomarkers for selecting abnormal cytologies that correspond to cervical intraepithelial neoplasia.

The quest to determine the most accurate biomarker for the detection of the abnormal cytologies most likely to evolve into cervical cancer is ongoing, particularly in cases with abnormal cytologies such as atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesions (LSIL).

One of the most important emerging biomarkers in the characterization of precancerous cervical lesions is the cyclin-dependent kinase inhibitor $p16^{INK4a}$, a tumor suppressor protein. The superiority of the $p16^{INK4a}$ immunocytochemistry assay ($p16^{INK4a}$ ICC) over high-risk Human Papillomavirus (hr-HPV) DNA detection resides in the fact that the overexpression of $p16^{INK4a}$ indicates the presence of oncogenic transformations within the cervical cells. Conversely, hr-HPV detection cannot distinguish between transient and transforming HPV infections [3].

The present study evaluates the hypothesis that $p16^{INK4a}$ immunocytochemistry (ICC) test is more effective than hr-HPV at identifying women with ASC-US and LSIL who are subsequently diagnosed with cervical intra-epithelial neoplasia (CIN) equal or higher grade 2 (CIN2+) upon histopathological diagnosis. This study investigated the possibility of a statistical correlation between the immunoexpression of $p16^{INK4a}$ and the existence of high-grade precancerous cervical lesions.

Simultaneously, we checked, on a statistical basis, the superiority of cyclin-dependent kinase inhibitor $p16^{INK4a}$ test as compared to HPV test in differentiating those abnormal cytologies underlying CIN2+ at histopathological diagnosis.

The addition of this immunocytochemical marker to the actual diagnostic panel may influence the clinical management of $p16^{INK4a}$-positive patients with the afore-mentioned abnormal cytologies.

Materials and Methods

The data were collected between 2009–2010 from the archives of Gynecology and Pathology Departments, "St. Pantelimon" Clinical Hospital, Bucharest, Romania

The methodology of the current research focuses on three types of investigations represented by immuno-cytological expression of cyclin-dependent kinase inhibitor $p16^{INK4a}$, HPV genotyping of cervical cells included within liquid medium and histopathological diagnosis by HE (Hematoxylin–Eosin) staining of cervical biopsies tissue.

From a total amount of 1820 Papanicolaou (Pap) cytologies, only 63 cases were selected according to the following criteria: ASC-US or LSIL cytologies, age between 25 and 65 years, specimens collected in liquid medium (LBC), genotyped for hr-HPV and immunocyto-chemically stained for $p16^{INK4a}$. All the selected cases have been followed-up by colposcopy with biopsies from suspected zones, from each quarter portion of the cervix with at least minimum 2 mm large diameter and histo-pathological diagnosis.

The eligible specimens were obtained using a well-established investigation and research protocol. Cervical cells were collected by cytobrush, which was then introduced in a liquid-based collection medium (Cytofast). The interpretation of the cytological results was done according to *Bethesda System Criteria* established in 2001.

The cyclin inhibitor kinase $p16^{INK4a}$ was identified by immunocytochemistry staining using a CINtec $p16^{INK4a}$ ready-to-use cytological kit (clone E6H4) manufactured by the MTM Laboratories AG (Heidelberg, Germany). Following a deep analysis of nuclear staining criteria (Wentzensen N *et al.* [4]) – such as increased nuclear size or increased nuclear/cytoplasmic ratio, irregular nuclear shape, granular or hyperchromatic chromatin, variable cellular morphology – and cytoplasmic staining, we classified samples as $p16^{INK4a}$ negative and $p16^{INK4a}$ positive, when positivity was observed for at least two of afore-mentioned nuclear criteria.

The Linear Array HPV Genotyping Test was used for detecting Human Papillomavirus (HPV). The used methodology allowed to detect 37 distinct HPV genotypes classified according to their oncogenic risk (Muñoz N *et al.*, 2003) [5]. Samples were selected if they were positive for one or more of high-risk of oncogenic transformation genotypes (hr-HPV).

Histopathological results were classified into two groups.

The first group contains cases with histopathological diagnosis (HPD)<CIN2, that is cases which are negative for dysplasia (ND) or positive for cervical intraepithelial neoplasia grade 1 (CIN1).

The second group contains cases with HPD≥CIN2+, that is cases positive for cervical intraepithelial neoplasia with grade equal or higher than 2 (CIN2+).

The ability of $p16^{INK4a}$ and hr-HPV testing to predict CIN2+ was evaluated by computing the sensitivity (Se), specificity (Sp), and positive predictive value (PPV) of these two markers in the selected specimens.

The statistical relevance of the obtained data was computed with a *chi*-squared test. For this, we employed the statistical software GraphPad Prism 6.0b, Macintosh Version (©1994–2012, Software MacKiev).

Results

From the 63 selected cytological specimens, 41 were typical for LSIL and 22 were characteristic of ASC-US. The case distribution as regards the association of abnormal cytologies with hr-HPV positive infection and the possibility of CIN2+ histopathological diagnosis result showed the following aspects. Therefore, according to mentioned selection criteria, 41% of the 22 cases with ASC-US were positive for hr-HPV. The statistical analysis shown that 33.3% of these HPV-positive cases were diagnosed with CIN2+ after histopathological diagnosis. Within the same group of ASC-US cases, 41% were positive for $p16^{INK4a}$. A more deep evaluation indicated that 77.7% of these $p16^{INK4a}$-positive cytologies were obtained from patients diagnosed with CIN2+ after biopsy (Table 1).

The present study reveals the same significant issues concerning the association between HPV high-risk infection, positivity of $p16^{INK4a}$ and CIN2+ in cases with LSIL cytology. Therefore, hr-HPV infection was demonstrated in 51.2% of the 41 specimens with LSIL (Figures 1–3).

The data obtained shown that from these cases 50% were HPV-positive and were proved to be CIN2+ at histopathological diagnosis. Within the same group of LSIL cases, 68% were positive for $p16^{INK4a}$ and from these $p16^{INK4a}$-positive cytologies a high percentage, 87.5%, was obtained from women who were diagnosed with CIN2+ after biopsy (Table 1).

The sensitivities and specificities of these investigated markers were compared in order to establish if one of the two markers was superior to the other with respect to detecting CIN2+ (Figure 4).

Table 1 – ***The distribution of hr-HPV infection and p16^{INK4a} positivity in a study group of 63 women, correlated with their cytological and histopathological diagnosis***

Cytology results (*n*=63)		ASC-US (*n*=22)		LSIL (*n*=41)	
Histopathological diagnosis (*n*=63)		*<CIN2 (n=13)*	*≥CIN2+ (n=9)*	*<CIN2 (n=17)*	*≥CIN2+ (n=24)*
p16^{INK4a}	Positive % (*n*)	15.3% (2)	77.7% (7)	41.17% (7)	87.5% (21)
	Negative % (*n*)	84.6% (11)	22.2% (10)	58.82% (10)	12.5% (3)
hr-HPV	Positive % (*n*)	46.15% (6)	33.3% (9)	52.94% (9)	50% (12)
	Negative % (*n*)	53.8% (7)	66.6% (8)	47.05% (8)	50% (12)

p16^{INK4a} – Cyclin-dependent kinase inhibitor p16^{INK4a}; Hr-HPV – High-risk human papillomavirus; ASC-US – Atypical squamous cells of undetermined significance; LSIL – Low-grade squamous intraepithelial lesion; <CIN2 – Negative for dysplasia or cervical intraepithelial neoplasia grade 1; ≥CIN2+ – Cervical intraepithelial neoplasia grade 2 or 2+; n – No. of cases.

The Se, Sp, and PPV of p16^{INK4a} and hr-HPV for a subsequent diagnosis of cervical intraepithelial neoplasia were calculated and analyzed with the *chi*-square test in order to determine their relevance (Table 2).

Table 2 – ***Sensitivity and specificity of p16^{INK4a} and HPV testing in ASC-US and LSIL cytologies***

Cytology		p16^{INK4a} (95% CI)	HPV test (95% CI)	*P*
ASC-US cytology	Sensitivity	0.77 (0.39–0.97)	0.33 (0.074–0.70)	0.05
	Specificity	0.84 (0.54–0.98)	0.53 (0.25–0.80)	0.08
LSIL cytology	Sensitivity	0.87 (0.67–0.97)	0.50 (0.29–0.70)	0.005
	Specificity	0.58 (0.32–0.81)	0.47 (0.220.72)	0.49

HPV – Human papillomavirus; p16^{INK4a} – Cyclin-dependent kinase inhibitor p16^{INK4a}; ASC-US – Atypical squamous cells of undetermined significance; LSIL – Low-grade squamous intraepithelial lesion.

Women with LSIL were three times more likely than those with ASC-US to have p16^{INK4a}-positive cervical test are (Odds Ratio, OR=3.1; 95% CI: 1.06–9.11). Concurrently, hr-HPV positive tests in LSIL cytologies were eleven times more likely than those in ASC-US cytologies to be p16^{INK4a}-positive (OR=11.8; 95% CI: 1.66–84.56).

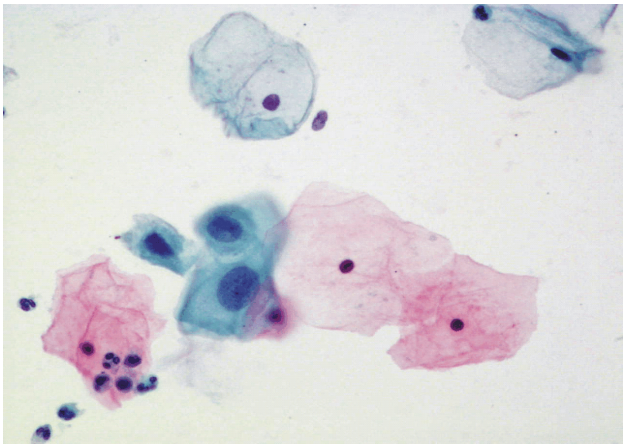

Figure 1 – ***Cytology: ASC-US versus LSIL (before p16^{INK4a} test). Pap test, ob. ×20 (photo collection of own research grant).***

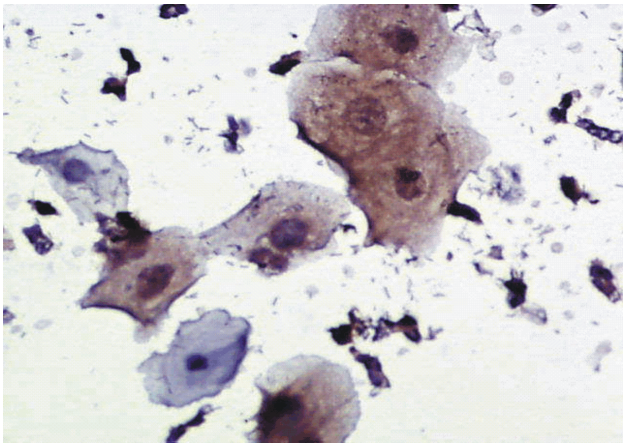

Figure 2 – ***Immunocytochemistry: p16^{INK4a} test. LSIL: Intermediate squamous cells p16^{INK4a} positive (nuclear and in cytoplasmic staining), increased nuclear size irregular nuclear shape, granular or hyperchromatic chromatin and nuclear pleomorphism, ob. ×20 (photo collection of own research grant).***

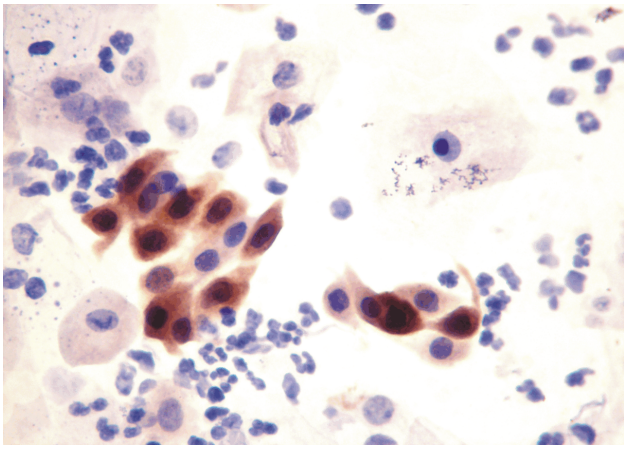

Figure 3 – ***Immunocytochemistry: p16^{INK4a} test. HSIL: p16^{INK4a} positive squamous cells, hyperchromatic nuclei, irregularity of nuclear margins, cytoplasmic positivity of p16^{INK4a}, ob. ×20 (photo collection of own research grant).***

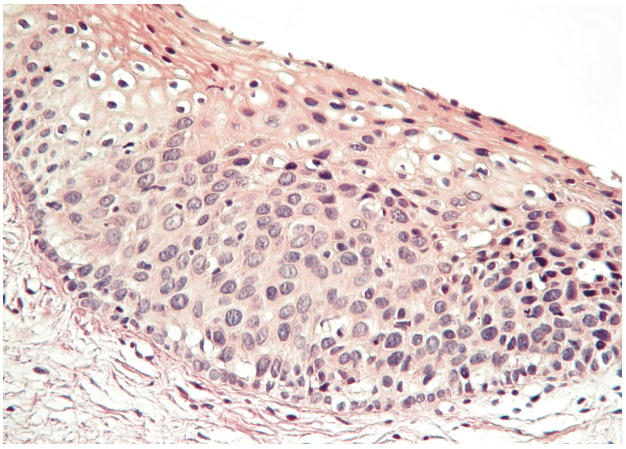

Figure 4 – ***Histopathology: CIN2 (high-grade cervical intraepithelial neoplasia), medium size cells with enlarged, hyperchromatic nuclei, nuclear pleomorphism and irregular nuclear margins, in 2/3 of thickness of squamous epithelium, HE staining, ob. ×20 (photo collection of own research grant).***

For women with ASC-US cytologies, the hr-HPV test had a Se of 33.33%, a Sp of 53.85% and a PPV of 33.33% for the identification of CIN2. In cases with LSIL samples, the hr-HPV test had a Se of 50%, a Sp of 47.06 % and a PPV of 57.14%.

In ASC-US cytologies, the $p16^{INK4a}$ immunocytochemical assay had a Se of 77.78%, a Sp of 84.62% and a PPV of 77.78% for the identification of CIN2+. In LSIL samples, the $p16^{INK4a}$ ICC had a Se of 87.50%, a Sp of 58.82% and a PPV of 75%.

In cases with ASC-US cytologies, the analysis of the Sp of the hr-HPV test *versus* that of $p16^{INK4a}$ ICC demonstrated that the latter led to an almost significant increase by 30.77% in the detection of cases negative for dysplasia or positive for CIN1 in comparison to the former (p=0.0891).

A comparison between the PPV of hr-HPV detection *versus* that of $p16^{INK4a}$ ICC in ASC-US cytologies shown another almost significant increase by 44.5% in favor of $p16^{INK4a}$ (p=0.0577).

For women with LSIL cytologies the analysis of the Sp of the hr-HPV test *versus* that of $p16^{INK4a}$ ICC demonstrated that the latter led to a statistically insignificant rise by 11.76% in the detection of cases negative for dysplasia or positive for CIN1 in comparison to the former (p=0.4921).

The relational aspects between the PPV of hr-HPV detection *versus* that of $p16^{INK4a}$ ICC in LSIL cytologies demonstrated another statistically insignificant rise by 17.8% in favor of $p16^{INK4a}$ (p=0.1870).

A comparison between the Se of $p16^{INK4a}$ ICC *versus* that of hr-HPV highlighted a significant increase in the identification ratio for CIN2+ positive cases in favor of $p16^{INK4a}$: 37.5% in LSIL cytologies (p=0.0050) and 44.45% in ASC-US cytologies (p=0.0577).

Discussion

Randomly controlled trials have demonstrated that HPV-based screening is more efficient than cytology-based screening for the detection of cervical neoplasia [6].

Recently published data states that cervical cytology exams may prove useful in the triage of HPV-positive cases [7]. Current research is focused on the detection of biomarkers suitable for the triage of abnormal Papanicolaou cytologies and which can be translated into daily clinical practice (*e.g.*, $p16^{INK4a}$ and $p16^{INK4}$–Ki67 double staining) [8].

A meta-analysis performed by Jolien Roelens in 2012 showed that $p16^{INK4}$ is more accurate than hr-HPV at the triage of ASC-US cytology samples, as it demonstrates a similar sensitivity and a higher specificity. In LSIL samples, $p16^{INK4}$ was more specific, but less sensitive than hr-HPV in the detection of CIN equal to or greater than 2 (CIN2+) [9]. These findings have a high degree of similarity with other studies: Holladay EB *et al.* (2006), Wentzensen N *et al.* (2007), Denton KJ *et al.* (2010), Izaaks CD *et al.* (2011) [10–13] (Table 3).

The results of our research are able to show the significance of the investigated markers such as the role of hr-HPV detection and $p16^{INK4a}$ ICC in the selection of those cases of ASC-US and LSIL, which are diagnosed as CIN2+ at the histopathological diagnosis.

The sensitivities and specificities of $p16^{INK4a}$ and HPV test were compared in order to establish if one of the two markers was superior to the other with respect to detecting CIN2.

Table 3 – ***Sensitivity and specificity of $p16^{INK4a}$ in ASC-US and LSIL cytologies as illustrated in other studies***

Study	$p16^{INK4a}$ – ASC-US		$p16^{INK4a}$ – LSIL	
	Se	*Sp*	*Se*	*Sp*
Holladay EB *et al.* (2006) [10]	89%	68.4%	75%	59.1%
Wentzensen N *et al.* (2007) [11]	95%	84%	95%	84%
Denton KJ *et al.* (2010) [12]	92.6%	71.1%	92.2%	53.3%
Izaaks CD *et al.* (2011) [13]	71.4%	50%	90.12%	85.7%

$p16^{INK4a}$ – Cyclin-dependent kinase inhibitor $p16^{INK4a}$; ASC-US – Atypical squamous cells of undetermined significance; LSIL – Low-grade squamous intraepithelial lesion; Se – Sensitivity; Sp – Specificity.

We compared our research results with other up-to-date worldwide-published results on the same interesting subject.

The present study notices that the specificity of $p16^{INK4a}$ is high in both ASC-US and LSIL cytologies, particularly in ASC-US. The results of our work show that the specificity of HPV is similarly increased in ASC-US and LSIL cytologies, but is significantly lower than the specificity of $p16^{INK4a}$.

A study by Samarawardana P *et al.* [14] exposed similar conclusions. Their work states that the sensitivity and specificity of $p16^{INK4a}$ for the detection of underlying CIN≥2+ are 81.7% and 83.3% respectively (p=0.81). The same study determined that the Se and Sp of hr-HPV are lower than those of $p16^{INK4a}$, albeit statistically significant: 78.1% and 50.9% respectively (p<0.01). Other recent studies recommend the use of $p16^{INK4a}$ as a supplemental triage biomarker for ASC-US and LSIL cytologies, which have already been assigned as "high-risk" after hr-HPV detection [15, 16].

Our results on the sensitivity of the two biomarkers partly differ from those of other studies, as $p16^{INK4a}$ proved to be significantly more sensitive than hr-HPV in both ASC-US and LSIL samples. This result may have been generated by the fact that some of the cases included in the study group were positive for at least one of the fifteen high-risk HPV genotypes instead of being positive only for genotypes 16 and 18, which are responsible for the majority of cervical cancers. Another particular fact is that in the present study the hr-HPV test was detected with the Linear Array HPV Genotyping Test. Therefore, the results must be interpreted in light of the fact that there are more specific tests such as the APTIMA RNA assay or Hybrid-Capture-2, which increase the specificity of the detection with a slight-to-inexistent loss in cross-sectional sensitivity [17, 18].

Conclusions

Our study reveals that the $p16^{INK4a}$ immunoexpression in ASC-US cytologies produces high values for both sensitivity and specificity. Thus, we straightforwardly make the following recommendation for clinical practice. Women with ASC-US cytologies who test positive for $p16^{INK4a}$ should be directed to colposcopy and/or biopsy, while women who test negative should not. The same reasoning can be applied for the $p16^{INK4a}$ immuno-

expression in LSIL cytologies. In this case, the sensitivity is high, so we expect to miss a low number of positives. However, due to a lower specificity value, one should expect a larger number of patients to be unnecessarily directed to colposcopy and/or biopsy. This study highlights the fact that modern high-fidelity biotechnologies are necessary to ensure the prevention of cervical neoplasia. However, this cannot be done without making these strategies accessible to a large population of women. For Romania, a feasible affordable solution is to screen women with Papanicolaou test and to consequently triage abnormal cytologies using $p16^{INK4a}$ immunocytochemical staining in both ASC-US and LSIL cytologies.

Acknowledgments

The immunocytochemical identification of $p16^{INK4a}$ was funded by The Romanian National Research Program – "St. Pantelimon" Clinical Hospital Research Grant: Project Number 3368/61–44 PN II/2007–2010.

We address our thanks and gratitude to Eugenia Panaitescu, MD, PhD, Lecturer at Department of Medical Informatics and Biostatistics, "Carol Davila" University of Medicine and Pharmacy, Bucharest, for her contribution to statistical processing of our present study data.

References

[1] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (eds), *GLOBOCAN 2008: cancer incidence and mortality worldwide*, IARC CancerBase No. 10, 2010.

[2] ***, *WHO/ICO Information Centre on Human Papilloma Virus (HPV) and Cervical Cancer*, Summary Report Update 2010 September 15, Romania, World Health Organization http://www.who.int/hpvcentre.

[3] Reuschenbach M, Clad A, von Knebel Doeberitz C, Wentzensen N, Rahmsdorf J, Schaffrath F, Griesser H, Freudenberg N, von Knebel Doeberitz M, *Performance of p16INK4a-cytology, HPV mRNA, and HPV DNA testing to identify high grade cervical dysplasia in women with abnormal screening results*, Gynecol Oncol, 2010, 119(1):98–105.

[4] Wentzensen N, Bergeron C, Cas F, Eschenbach D, Vinokurova S, von Knebel Doeberitz M, *Evaluation of a nuclear score for p16INK4a-stained cervical squamous cells in liquid-based cytology samples*, Cancer, 2005, 105(6):461–467.

[5] Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group, *Epidemiologic classification of human papillomavirus types associated with cervical cancer*, N Engl J Med, 2003, 348(6): 518–527.

[6] Franceschi S, Denny L, Irwin KL, Jeronimo J, Lopalco PL, Monsonego J, Peto J, Ronco G, Sasieni P, Wheeler CM, *Eurogin 2010 roadmap on cervical cancer prevention*, Int J Cancer, 2011, 128(12):2765–2774.

[7] Arbyn M, de Sanjosé S, Saraiya M, Sideri M, Palefsky J, Lacey C, Gillison M, Bruni L, Ronco G, Wentzensen N, Brotherton J, Qiao YL, Denny L, Bornstein J, Abramowitz L, Giuliano A, Tommasino M, Monsonego J, *EUROGIN 2011 roadmap on prevention and treatment of HPV-related disease*, Int J Cancer, 2012, 131(9):1969–1982.

[8] Waldstrøm M, Christensen RK, Ørnskov D, *Evaluation of p16(INK4a)/Ki-67 dual stain in comparison with an mRNA human papillomavirus test on liquid-based cytology samples with low-grade squamous intraepithelial lesion*, Cancer Cytopathol, 2013, 121(3):136–145.

[9] Roelens J, Reuschenbach M, von Knebel Doeberitz M, Wentzensen N, Bergeron C, Arbyn M, *p16INK4a immunocytochemistry versus human papillomavirus testing for triage of women with minor cytologic abnormalities: a systematic review and meta-analysis*, Cancer Cytopathol, 2012, 120(5): 294–307.

[10] Holladay EB, Logan S, Arnold J, Knesel B, Smith GD, *A comparison of the clinical utility of p16(INK4a) immunolocalization with the presence of human papillomavirus by hybrid capture 2 for the detection of cervical dysplasia/ neoplasia*, Cancer, 2006, 108(6):451–461.

[11] Wentzensen N, Bergeron C, Cas F, Vinokurova S, von Knebel Doeberitz M, *Triage of women with ASCUS and LSIL cytology: use of qualitative assessment of p16INK4a positive cells to identify patients with high-grade cervical intraepithelial neoplasia*, Cancer, 2007, 111(1):58–66.

[12] Denton KJ, Bergeron C, Klement P, Trunk MJ, Keller T, Ridder R; European CINtec Cytology Study Group, *The sensitivity and specificity of p16(INK4a) cytology vs HPV testing for detecting high-grade cervical disease in the triage of ASC-US and LSIL pap cytology results*, Am J Clin Pathol, 2010, 134(1):12–21.

[13] Izaaks CD, Truter EJ, Khan S, *Correlative analysis of CinteC p16 and detection of HPV DNA by PCR in cervical abnormalities*, Med Technol SA, 2011, 25(2):23–29.

[14] Samarawardana P, Dehn DL, Singh M, Franquemont D, Thompson C, Gaido L, Torkko KC, Homer P, Burke S, Titmus MA, Nayi V, Shroyer KR, *p16(INK4a) is superior to high-risk human papillomavirus testing in cervical cytology for the prediction of underlying high-grade dysplasia*, Cancer Cytopathol, 2010, 118(3):146–156.

[15] Gustinucci D, Passamonti B, Cesarini E, Butera D, Palmieri EA, Bulletti S, Carlani A, Staiano M, D'Amico MR, D'Angelo V, Di Dato E, Martinelli N, Malaspina M, Spita N, Tintori B, Fulciniti F, *Role of p16(INK4a) cytology testing as an adjunct to enhance the diagnostic specificity and accuracy in human papillomavirus-positive women within an organized cervical cancer screening program*, Acta Cytol, 2012, 56(5):506–514.

[16] Nasioutziki M, Daniilidis A, Dinas K, Kyrgiou M, Valasoulis G, Loufopoulos PD, Paraskevaidis E, Loufopoulos A, Karakitsos P, *The evaluation of p16INK4a immunoexpression/immunostaining and human papillomavirus DNA test in cervical liquid-based cytological samples*, Int J Gynecol Cancer, 2011, 21(1): 79–85.

[17] Monsonego J, Hudgens MG, Zerat L, Zerat JC, Syrjänen K, Halfon P, Ruiz F, Smith JS, *Evaluation of oncogenic human papillomavirus RNA and DNA tests with liquid-based cytology in primary cervical cancer screening: the FASE study*, Int J Cancer, 2011, 129(3):691–701.

[18] Rebolj M, Bonde J, Njor SH, Lynge E, *Human papilomavirus testing in primary cervical screening and the cut-off level for hybrid capture 2 tests: systematic review*, BMJ, 2011, 342:d2757.

Corresponding author

Teodora Vlădescu, MD, PhD, Senior Pathologist, Head of Department of Pathology, "St. Pantelimon" Clinical Emergency Hospital, 340 Pantelimon Highroad, Sector 2, 021659 Bucharest, Romania; Phone +4021–255 40 90, e-mail: teodoravladescu@yahoo.com

Ruxandra Stănculescu, MD, PhD, Senior Physician in Obstetric and Gynecology, Lecturer, "Carol Davila" University of Medicine and Pharmacy, Head of Department of Pathological Gynecology, "St. Pantelimon" Clinical Emergency Hospital, 340 Pantelimon Highroad, Sector 2, 021659 Bucharest, Romania; Phone +4021–255 40 90, e-mail: ruxandra_v_stanculescu@yahoo.com

Received: March 25, 2013 *Accepted: December 24, 2013*