The value of new immunocitologic marker p16^{ink4a} and citologic, colposcopic and histopathological correspondence for diagnosis of cervical lesions

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Grant support:
PNII PC; DC: Biotehnologii; contract 61-44

Abbreviations:
scuamous cervical cancer (SCC), cancer in situ (CIS), histopathology (eg HP), cytology in liquid medium (LBC), colposcopic score value (CSV)

Abstract

Objective: *The study's objective is to analyze the correlation between colposcopic score, the histopathological weighted score, the p16^{ink4a} immunomarker investigated by immunocytochemistry method and the cytology as determined from liquid based cytology.* ***Material and method:*** *A prospective study was performed over 60 women with different types of cervical lesions, between September 2008 to September 2009, by using a colposcopic score, a p16^{ink4a} intensity detection by immunocytochemical method and a histopathological weighted score. Data were processed in SPSS 15.0 using the correspondence analysis.* ***Results:*** *The results of correspondence analysis showed a correlation between the results of abnormal cytological categories and colposcopic score, hystopathologic score and also with the intensity score of p16^{ink4a} immunomarker. The study identified the presence of a correlation between the p16^{ink4a} immunomarker score and histopathologic weighted score too.* ***Conclusions:*** *Classical investigations used to assess lesion severity are not mutually exclusive; based on our made out correlations, the current study revealed the presence p16^{ink4a} immunomarker within dysplastic cervical cells and that its overexpression is related to an increased histopathological score.*
Keywords: *imunomarker p16^{ink4a}, liquid based cytology, colposcopic score, histopatologic weighted score*

Introduction

Current trends on the content of programs aimed at detecting early stages of cervical lesions are primarily guided by smear screening, colposcopy, detection of p16^{ink4a} tumor marker by immunohistochemistry methods, detection of E6/E7 mRNA oncoproteins.

The purpose of the study is to examine the correlation between the colposcopic score, weighted histologic score p16^{ink4a} tumor marker detected by immunohistochemistry method and results of cytological examination in accordance with the Bethesda system.

Liquid based cytology allows the harvesting of a larger number of cervical cells compared to conventional cytology on dry medium and at the same time creates the possibility of detection from the same sample of p16^{ink4a} immunomarker by advanced bio-

technology. The relationship between the presence of p16^{ink4a} immunomarker and severity of cervical lesion is explained by specialists in virusology, genetics. Persistent infection over 6 months[1] with oncogenic HPV strains is able to induce the process of carcinogenesis. The presence of HPV infection in the intermediate and superficial layer characterizes the stage of "replicative infection". In the event that the control of viral genes in the basal cells is lost, viral oncogenes E6 and E7 are highly expressed leading to chromosomal instability with the transformation of affected cells. This last stage may be called "transforming infection". Integration of the viral genome is not responsible for the sudden disruption of viral gene expression model because the integration process occurs later in the progression of high-grade dysplasia (integration is often undetectable even in invasive cancers) and to the fact that it clearly follows the induction of chromosomal instability and aneuploidy. Sudden disturbance of viral gene expression model leads to early expression of E6-E7. p16^{ink4a} marker is a cyclin dependent kinase inhibitor that affects cell cycle by suppressing cyclins dependent kinases(CDKs) CDKs which regulates retinoblastoma gene product (pRB) phosphorylation. Viral E6 and E7 oncoproteins interacts with p53 and pRB[7]. Increased expression of E6 and E7 in cervical dysplasia may be reflected by p16^{ink4a} growth. In high risk oncogenic HPV types, E6 si E7 have affinity for p53 and pRB and can give rise to an increased proliferation and genomic instability leading to cellular transformation. pRB inactivation by E7 oncoprotein induces expression of p16^{ink4a} in cervical lesions. Recent studies have shown that the transition from replicative stage to the transforming HPV infection is accompanied by a overexpression of ciclin dependent kinase inhibitor - p16^{ink4a}. The study points out to clinician gynecologist the correlation between the reviewed parameters and cytological classification as a primary screening method.

Table 1 Cytological category

Cytology	Frequency	Percent	Valid Percent	Cumulative Percent
ASC-H	6	10.0	10.0	10.0
L-SIL	21	35.0	35.0	45.0
AGC-NOS	6	10.0	10.0	55.0
AGC FAV NEO	4	6.7	6.7	61.7
H-SIL	17	28.3	28.3	90.0
CC	6	10.0	10.0	100.0
Total	**60**	**100.0**	**100.0**	

Material and method

The purpose of this investigatory prospective study conducted in Emergency Hospital of St. Pantelimon on a group of 60 women, between September 2008-September 2009, is to find the correlation between abnormal cytology categories >= ASC-H and colposcopic score value (CSV), p16^{ink4a} immunomarker score and outcome of histopathology exam(HP). Cytological examination consisted in describing the appearance of cervical cells collected in liquid medium (cytofast) with interpretation in Bethesda reporting system. Colposcopy examination was carried out using conventional techniques, visualization after application of normal saline, 5% acetic acid and Lugol solution. Interpretation of the captured colposcopy images was performed using Copplesson-Reid index, last revised in 2004, and signified basis for colposcopic score calculation. Colposcopic score assessment relies on the interpretation of lesion features on colposcopy regarding the edges/surface, acetic acid coloration and iodofilia appearance vessels. Determination of p16^{ink4a} was made in the 60 cytology samples in liquid medium. For the detection of p16^{ink4a}, CINtecp16^{ink4a} Cytology Kit (clone E6H4, Dako, ready-to-use, Glostrup, Denmark) was used. p16^{ink4a} tumor marker value was evaluated relative to the intensity immunocytology distribution. The intensity staining in nuclei was used to describe the presence of p16^{ink4a} imunomarker (absent staining in nuclei at high magnification [intensity=0; score=negative]; visible only magnified [intensity=1, score=weak positive]; easy visible at low magnification [intensity=2, score=moderate positive]; mostly positive at low magnification [intensity=3, score=intense positive]).

Weighted histopathological score was assessed according to a scale in relation to the severity of complex histopathological diagnosis of lesions.

Each diagnose and its score assignment has been performed by the same specialist in colposcopy, cytology and histopatology who worked individually in three different laboratories. Correspondence analysis was conducted for each abnormal cytological diagnosis, which required biopsy examination, with colposcopic score values, marker intensity and weighted histopathological score. Data was processed in SPSS using correspondence analysis. For this processing the results of cytological examination were assimilated according to a working scale and histopathology results were introduced by the aggregation of weighted histologic score according to histopathologic lesion severity. Interpretation of the information obtained through lot analysis targeted the correlation between different diagnostic methods complementary to cytological diagnosis.

Results

From the data analysis we observe a distribution of the results with the predominance of cytology class LSIL (35%) and HSIL (28.3%) (Table 1).

Distribution of colposcopic score follows a model in which values 4 and 5 are prevailing (together accounting for 46.6%) and also the value of 10 (13%) (Table 2).

Regarding the distribution of p16 ink4a intensity immunomarker - the most common value is 1 - 40%. (Table 3).

Histopathology weighted score results showed an almost uniform distribution with two peaks for value 1 - 28.3% and for value 100 - 20%.

The correspondence analysis generates a variety of points with graphical distribution in a two-dimensional table showing the relationship between outcomes of cytology category and studied variables: colposcopic score, p16^{ink4a} score and weighted score of ex-HP. The graphic two dimension representation output shows the correlation and demonstrates the connection between cytological category and studied variables (Figure 1-3).

Thus, it is noted in graphic representation in correspondence analysis of the cytology and colposcopic score, the cytological AGC NOS category appears to be closer to colposcopic score having values 3, and also 4 and 5, being much more distant from any other values of the CSV.

Instead cytology classes LSIL, ASC-H are very close to the values 4 and 5 of CSV, AGC FAV NEO and HSIL are close to the values 6, 7, 8 and 9 of CSV. In terms of cervical cancer - unlike the other categories it is closer to the high values of CSV.

When p16^{ink4a} immunomarker values are correlated with cytology was observed that the absence of p16^{ink4a} correlation is close to cytological AGC NOS category and ASC-H. LSIL category is closer to the value 1 of p16 and is far distant from the higher values 2 and 3 of p16.

Value 2 of p16 score is closer to cytological AGC FAV NEO category and value 3 is closer to HSIL and cervical cancer categories.

When cytology results and weighted histopathology score are correlated, the categories AGC NOS, LSIL, ASCH, are very close to the lowest scores, being less correlated than the value of histological lesion severity. As for AGC FAV NEO, this category seems to be associated with high rating of HP exam - 70, 83 and 96 being distant from the minimum values. Categories HSIL, ASC-H are in close proximity to the low values of HP exam - 11, 26, 28, 30, but surprisingly also to the very high - 100, which suggests that abnormal cytology results show a large variability at histopathological confirmation. When p16 immunomarker intensity is correlated with the scoring of ex-HP (Figure 4) it is observed that there is a proportional correlation trend - the score 0 and 1 being close to low values of ex-HP, value 2 was closer to average values of ex-HP weighted score, the highest intensity - value 3, of p16 is closer to higher values of HP exam 70 and 100, being far distant from the lower values.

The results obtained so far in the study suggest that abnormal cytology results showed a large variability compared with histopathological diagnosis. At the same time was observed a slight trend of directly proportional correlation between p16^{ink4a} immunomarker and histopathology which emphasizes the value of p16^{ink4a} as a surrogate marker which shows the activation of HR HPV- oncogenes expression in dysplastic cervical cells.

Photos from the collection of pathology department of Clinical Hospital "Sf Pantelimon" sustain the statistical data processed (Figure 1, Figure 2, Figure 3, Figure 4).

Table 2 Colposcopic Score

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	3	2	3.3	3.3	3.3
	4	14	23.3	23.3	26.7
	5	14	23.3	23.3	50.0
	6	7	11.7	11.7	61.7
	7	5	8.3	8.3	70.0
	8	2	3.3	3.3	73.3
	9	4	6.7	6.7	80.0
	10	8	13.3	13.3	93.3
	11	3	5.0	5.0	98.3
	12	1	1.7	1.7	100.0
	Total	**60**	**100.0**	**100.0**	

Table 3 Distribution of p16^{ink4a} intensity

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	12	20.0	20.0	20.0
	1	24	40.0	40.0	60.0
	2	8	13.3	13.3	73.3
	3	16	26.7	26.7	100.0
	Total	**60**	**100.0**	**100.0**	

Discussions

Correspondence analysis of CSV reported to cytological exam justifies the value of colposcopic investigation in the primary screening to identify cervical

Figure 1. Colposcopic score value and cytology correspondence

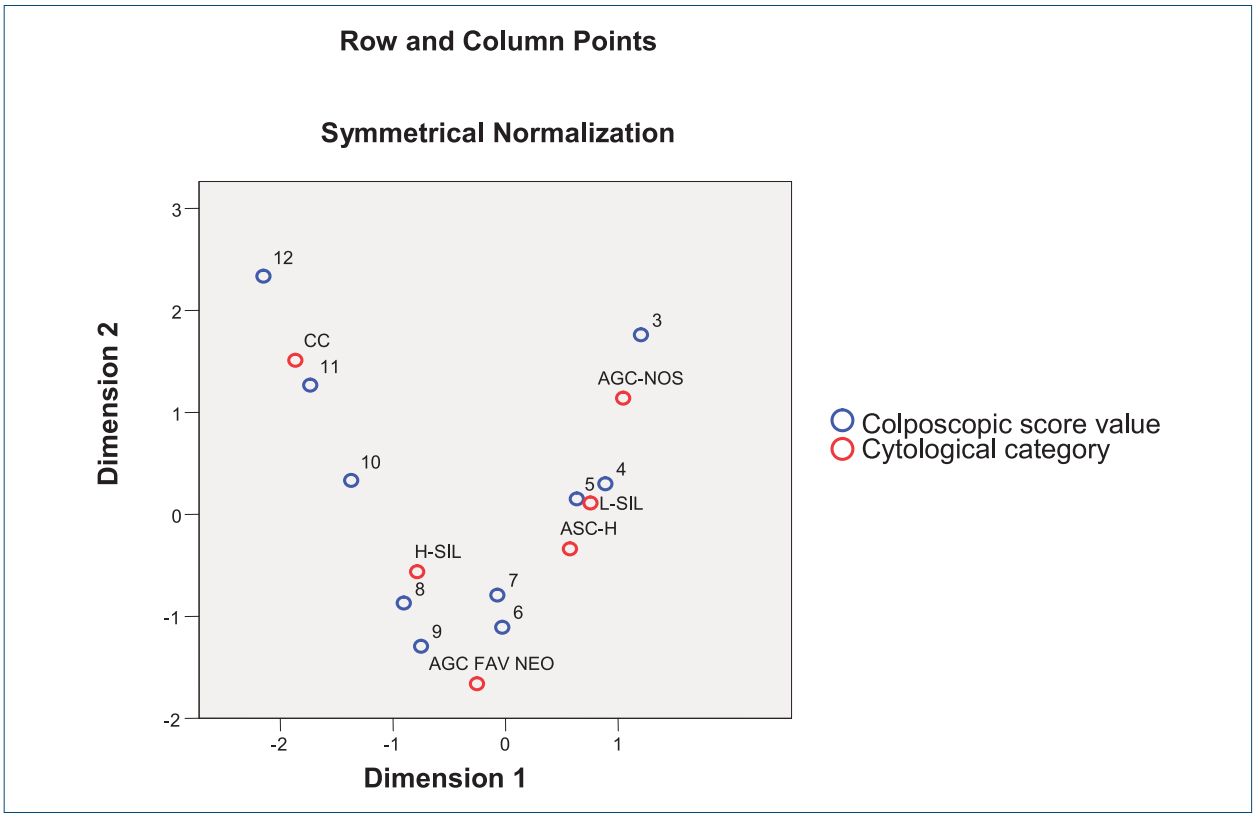
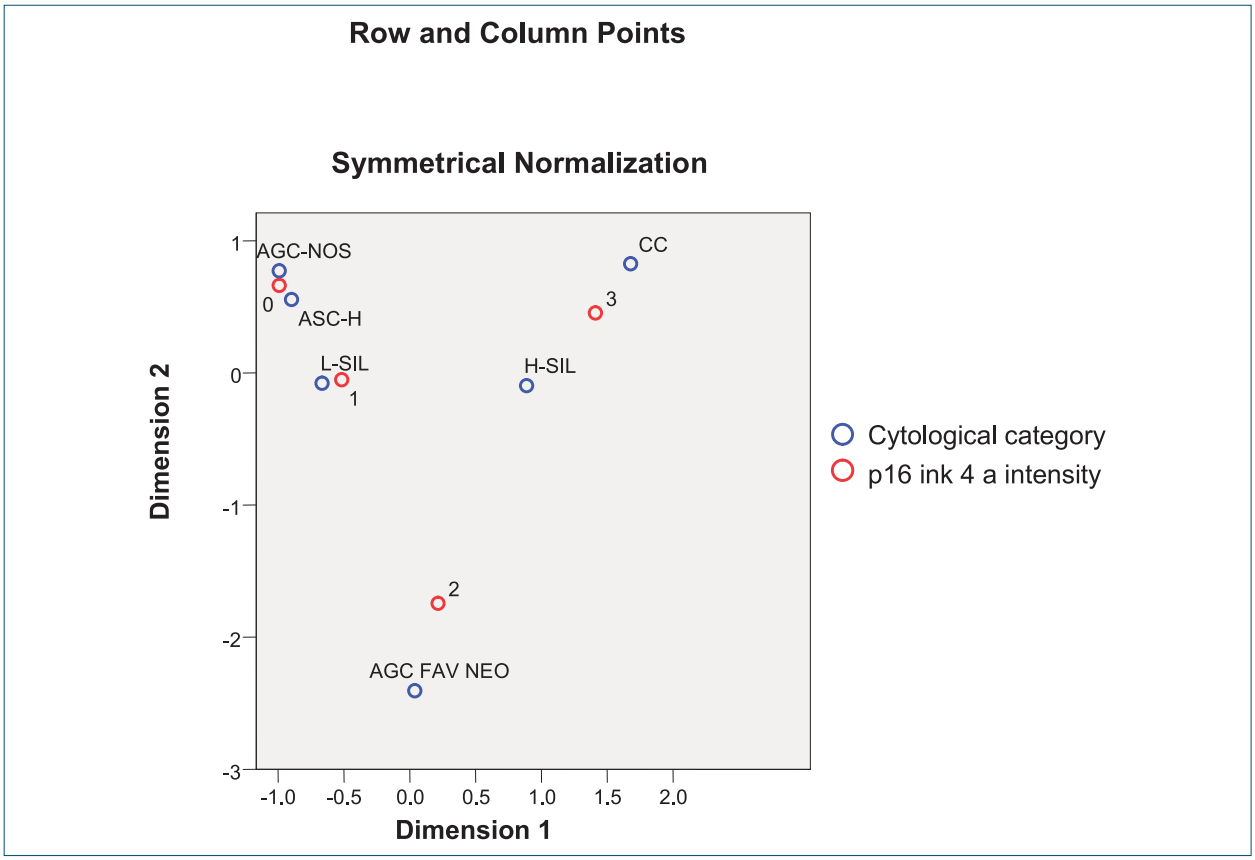


Figure 2. The $p16^{ink4a}$ intensity and cytology correspondence



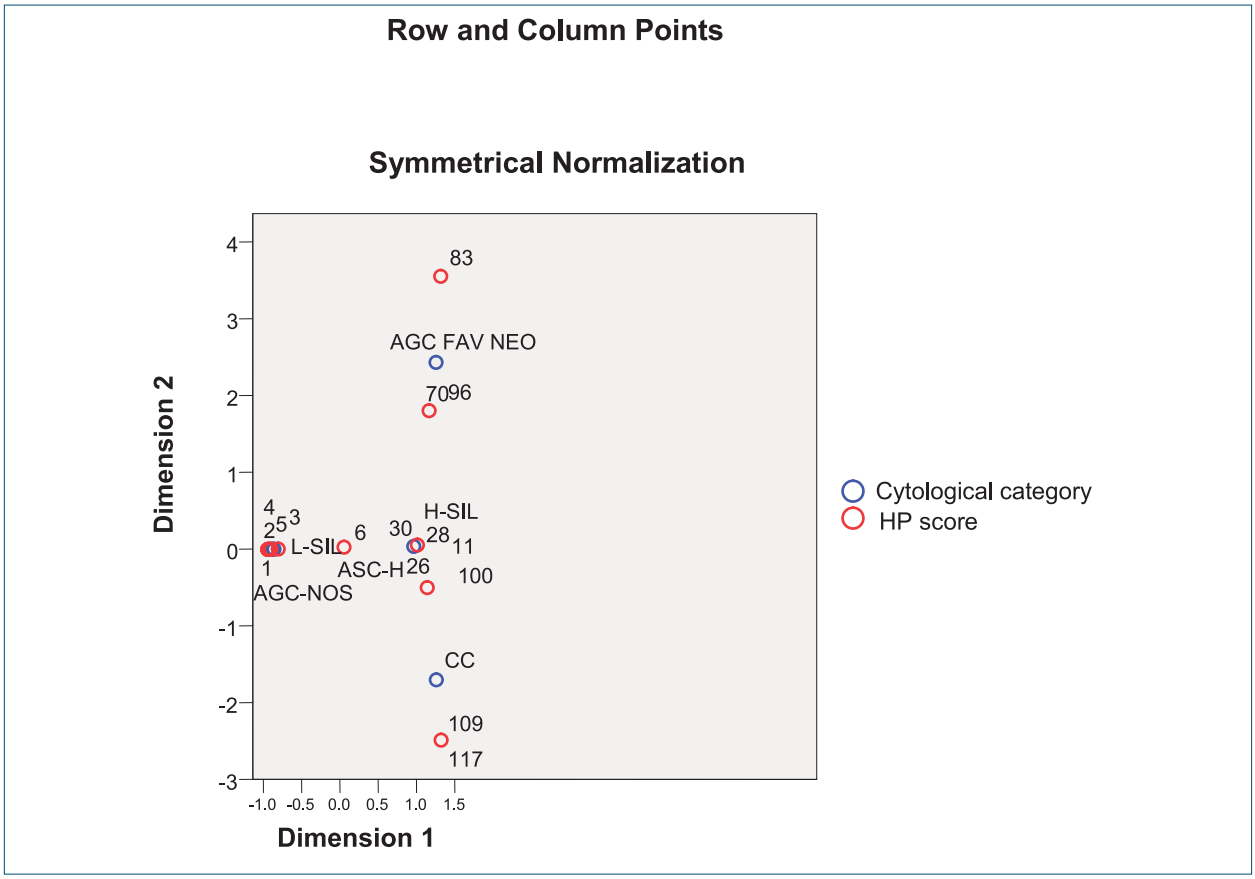


Figure 3.
Histology score and Cytology correspondence

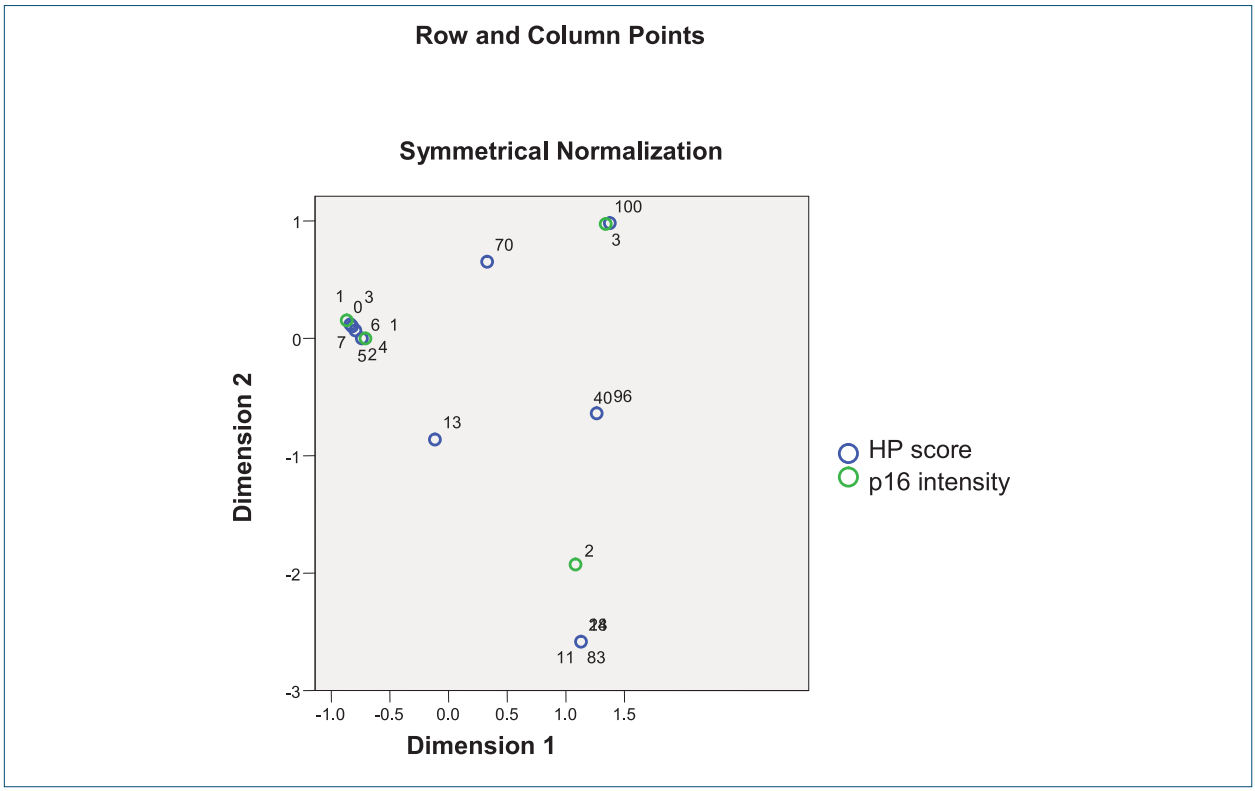


Figure 4.
The correspondence analysis of HP score and p16 immunomarker

lesions. Study of correspondence of p16 ink4a immunomarker and cytological examination values shows a close correlation between the intensity and severity of p16 ink4a and the gravity of cytological diagnosis; this correspondence progressively increasing in cases, L-SIL, H-SIL, CIS, CC. Analysis of correspondence between the CSV and weighted histopathological scores in investigated cases shows that the results of these investigations are closely consistent with the cytology lesion severity[8].

Data from the present study notes the significance of the immunocytological investigations by highlighting p16 ink4a marker in dysplastic cervical cells. Recent studies have shown that the transition from HPV replicative infection to the stage of transforming infection through molecular mechanisms leading to chromosomal instability is accompanied by massive overexpression of ciclin-dependent kinase inhibitor-p16 ink4a. p16 ink4a staining techniques allow the visualization in reproducible way of the cells that have entered the stage of transformation of viral gene expression. These cells have a higher risk of progression to neoplasia than those HPV infected cells that still keep their gene expression[1]. Colposcopy represents a screening method that is part of cervical cancer screening with benefits and limits, but capable to assist in assessing the risk lesion together with immunomarker and histopathological results[3],[5].

Colposcopy examination is a subjective test with low reproducibility intra-interobservars even among experienced colposcopists[2],[4].

The results obtained in this study reinforce the idea that the gynecologist must have control of the health by merging the clinical with biotechnology using advanced diagnostic techniques.

The correspondence of investigated parameters shows that it is unlikely that the screening method used individually or in combination with other methods to allow an accuracy of 100% in preventing the failure to diagnose cervical cancer[6],[8]. Data published in literature -especially that provided by Gardasil trials, study Stranxi Provita for cervical cancer screening (SPOCCS)- shows only the report of the diagnostic value of colposcopy directed biopsies and histopathological examination result by drawing attention to low sensitivity of colposcopy-guided biopsy for lesions > CIN2.

Assessment of correspondences between cytology, colposcopic score, $p16^{ink4a}$ intensity and ex. HP demonstrates that these investigations are not mutually exclusive in assessing lesion severity, but there is a correlation between $p16^{ink4a}$ intense positive results and severe histopathological lesions. The limits are that the study is in a primary research phase and therefore complex statistical inferences will be applied later.

Conclusions

The study highlights the practical information with clinical application useful to gynecologist and the correspondence between colposcopy investigation, ICC $p16^{ink4a}$, histological and cytological classification as primary method of screening. The correspondence analysis identifies the presence of correlation between $p16^{ink4a}$ imnunomarker and ex. HP noting the similarity of the dependence of $p16^{ink4a}$ immunomarker intensity and increased exam score HP.

The correspondence analysis between colposcopic score, $p16^{ink4a}$ immunomarker and weighted histological score against cytological result opens a multicriteria tackling mode in order to appraise each parameter's significance and to specify the diagnosis and therapeutic approach. Extending the database should continue such research. ■

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