REVIEW ARTICLE

Significance of Biomarkers in Primar Detection of Cervical Intraepithelial Neoplasia Lesions – Review of the Literature

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ABSTRACT

Cervical Intraepithelial Neoplasia (CIN) represents human papillomavirus (HPV) infection within epithelial cells. It consists potentially precancerous entity in which abnormal cells grow on the cervical surface. Depending on the cellular abnormality level and affected cervical tissue, three grades of CIN have been established (CIN I to CIN III). Classification key is strongly related to the proportion of cervical epithelium exhibiting dysplastic cells low-grade infection (CIN I) which involves the lower 1/3 or less of the cervical epithelium. On the other hand, high-grade infection (CIN II and CIN III) includes the entire thickness of the cervical epithelium. According to the current bibliography, many biomarkers have been established to primar diagnose cervical intraepithelial neoplastic lesions. Serum autoantibodies against tumor-associated antigens, circulating serum miRNA, Ki-67, $p16^{INK4a}$, BD ProEx C, Cytoactiv HPV L1., and urinary biomarkers are being evaluated for the primary screening of CIN. The aim of our study reflects an assiduous literature review of such biomarkers strongly related to primar diagnosis and treatment of CIN lesions.

Key words: biomarkers, cervical cancer, cervical intraepithelial neoplasia, human papillomavirus

INTRODUCTION

Cervical cancer represents the second most common tumor in the women population worldwide.[1] The role of sexually transmitted human papillomavirus (HPV) virus in the etiology of invasive cervical cancer is widely entrenched. It is found that at least 13 viral genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) reflect most commonly cervical cancer. HPV16 consists of most prevalent genotype with increased incidence in both squamous cell carcinoma (59.3%) and adenocarcinoma (36.3%) worldwide.[2] Cervical intraepithelial neoplasia (CIN) consists of a possibly precancerous entity. CIN arises from cells of the ectoendocervical squamocolumnar junction of the cervix, on the condition that they are infected by the HPV virus. Depending on the proportion of the cervical epithelium exhibiting dysplastic cells, CIN is classified into three stages. CIN I represents the low-grade infection, whereas CIN II and III the high grade. The duration required concerning high-grade lesions to become invasive cancer remains controversial. The majority of sexually active people will experience HPV infection at some point in life, with an estimated lifetime risk of approximately 80% for any oncogenic type.[2] Almost 90% of CIN I cases regress within 2 years, while 5% of CIN II and 40% of CIN III cases develop into invasive cervical cancer. The 5-year survival rate of cervical cancer reaches 90% in cases of primary detection and appropriate treatment. However, in further stages will be diminished dramatically, approximately 15–35%.[3] On account of that, the need of identifying biomarkers in the primary detection of CIN is of uppermost importance.

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Serology tests seem most mandatory because they are easy to collect, non-invasive, and allow high throughput screening. Serum autoantibodies receive much attention as potential biomarkers. Such proteins are autoantibodies against cancer Antigen 15–3 (CA15-3), carcinoembryonic antigen (CEA), Cancer Antigen 19-9 (CA19-9), c-Myc, p53, heat shock protein (Hsp) 27, and Hsp70. Increases of anti-CA15-3 and anti-CEA immunoglobulin G (IgG) in cases of cervical cancer were more pronounced than other markers. In addition, the level of anti-CA19-9 IgG in cases of CIN III stage accounted higher than in cases of CIN I, CIN II, or cervical cancer [Figure 1]. Furthermore, a serum miRNA panel (miR-9, miR10a, and miR20amiR196a) has been established that could effectively detect CIN patients in the primary stage.[4] MicroRNAs as non-coding RNAs regulate gene expression, demonstrating a crucial role concerning the molecular process of tumorigenesis. At the same time, biomarkers in both cervical cytology and histology have been recommended to increase the detection of women at greatest risk for developing cervical cancer, including Ki-67, $p16^{INK4a}$, BD ProEx C, and Cytoactiv HPV L1 [Table 1].[5]

MATERIALS AND METHODS

All scientific parameters have been collected through assiduous databases such PubMed and Cochrane database.

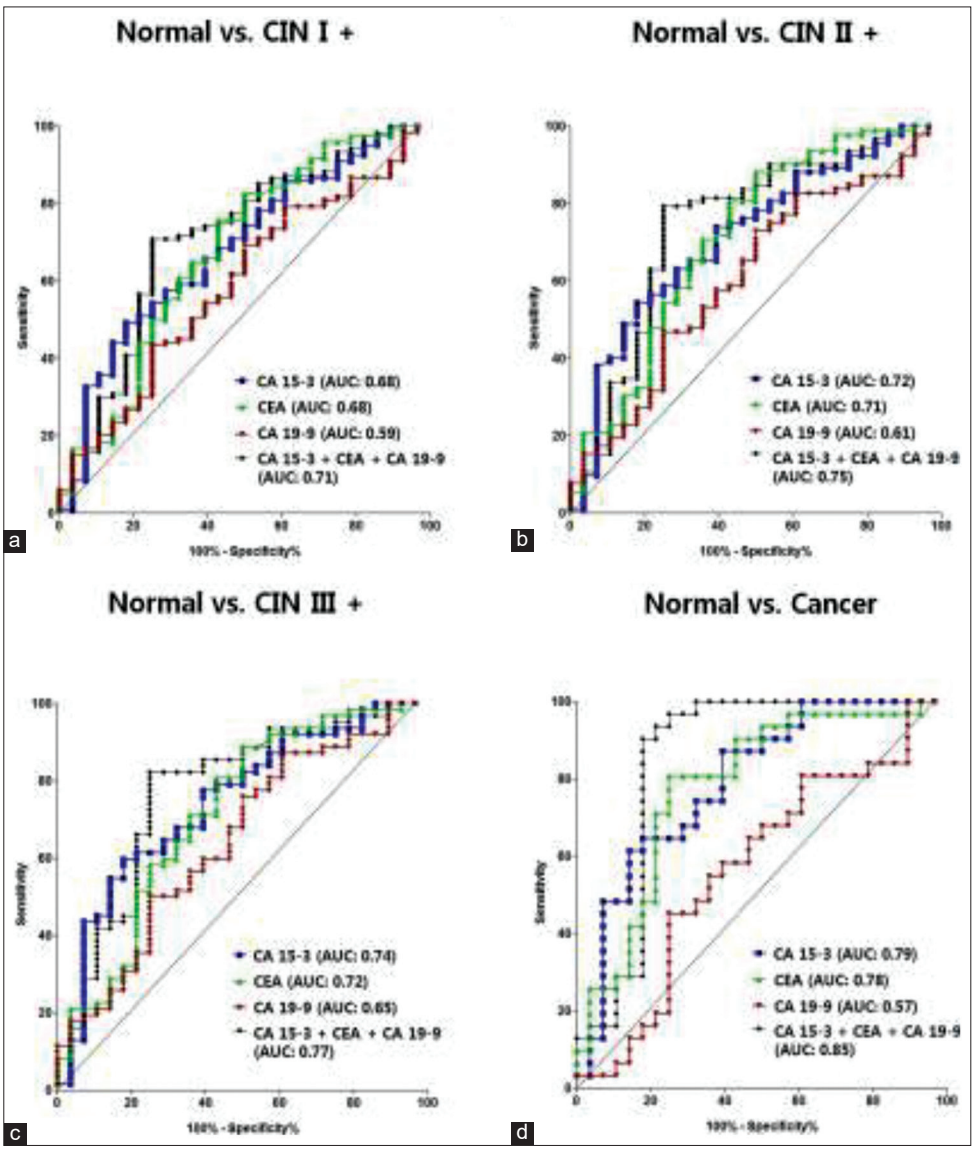

Figure 1: (a-d) Correlation of biomarkers with cervical intraepithelial neoplasia.(CIN) Source: The initials of the mansucript

Focusing on the recent bibliography, the aim of our study remains the reflection and further depiction of biomarkers as diagnostic tools in primary detection of cervical intraepithelial lesions (CIN).

DISCUSSION

The introduction of serum autoantibodies as biomarkers concerning the primary detection of cervical cancer in clinical practice appears to be an attractive possibility. However, there are several controversial issues against this approach. First, elevated autoantibody levels are found in only 10–30% of cancer patients. Second, autoantibodies are not only expressed and amplified in cancer patients but also in other diseases.

Finally, it can also be detected in healthy individuals. Therefore, the use of autoantibodies in cancer screening had created scientific confusion when attempted. Anti-CA15-3 IgG and anti-CEA IgG tend to increase in advanced cervical cancer lesions stage than in cases of CIN I, II, and III. In addition, anti-CA19-9 IgG elevates only in cases of CIN III. There are no significant differences between anti-c-Myc, anti-p53, anti-Hsp27, and anti-Hsp70 IgG level. The resulting anti-CA 15-3 IgG and anti-CEA IgG represent critical parameters concerning the classification of cervical cancer and anti-CA19-9 IgG level critical parameter concerning the classification of CIN III cases, respectively. Moreover, the combination of these three autoantibodies reflects increased sensitivity and specificity of the lesion. A positive correlation of CIN with the existence of the circulating serum miMRA panel (miR-9, miR-10a, miR-20a, and miR-196a) proposes an attractive approach to cervical screening. Expression of these non-coding RNAs was detected increased in CIN cases as compared with normal patients. At the same time, this combination was detected in significantly higher levels in CIN patients with HPV infection. Taking this into account, overexpression of these miRNAs might be closely associated with HPV infection. This expression may occur even before the morphological changes of the cervical epithelium. Numerous protein biomarkers in both cytology and histology have been demonstrated for the detection of cervical disease, including Ki-67, p16 INK4 a BD ProEx C, and Cytoactiv HPV L1 [Table 1]. The aim of this observation remains to overcome issues related to low specificity and positive predictive value (PPV) of cotesting (cytology and HPV DNA testing) to avoid unnecessarily raising patient anxiety levels and overtreatment. These proteins are nominated to have a role in the triage of indeterminate cytology cases, discrimination of true high-grade cervical dysplasia from mimics in histology, and prediction of such lesions that are more likely to progress to invasive cancer.[5] Many of these proteins are involved in cell cycle regulation, signal transduction, DNA replication,

Table 1: Biomarker positivity by the histological grade of cervical intraepithelial lesion

Study	Biomarker	WLN	CIN 1	CIN 2+
Shi 2007 *et al.*	Ki67	2/14 (14%)	29/34 (85%)	14/14 (100%)
	$_{p16}$INK4a	0/14 (0%)	26/34 (77%)	14/14 (100%)
	BD$_{ProEx}$C	0/14 (0%)	32/34 (94%)	11/14 (79%)
	BD$_{ProEx}$C/$_{p16}$	0/14 (0%)	34/34 (100%)	14/14 (100%)
Badr 2008 *et al.*	Ki67	0/37 (0%)	6/22 (27%)	34/37 (91%)
	$_{p16}$INK4a	2/38 (5%)	8/23 (35%)	35/37 (93%)
	BD$_{ProEx}$C	1/38 (3%)	11/23 (48%)	34/37 (92%)
	BD$_{ProEx}$C/$_{p16}$	3/35 (9%)	15/23 (65%)	37/37 (100%)
Pinto 2008 *et al.*	Ki67	11/23 (48%)	ND	34/36 (94%)
	$_{p16}$INK4a	13/35 (37%)	ND	51/61 (84%)
	BD$_{ProEx}$C	10/35 (29%)	ND	52/60 (87%)
	BD$_{ProEx}$C/$_{p16}$	9/23 (39%)	ND	33/36 (92%)
Halloush 2008 *et al.*	Ki67	14/28 (49%)	22/27 (82%)	15/16 (94%)
	$_{p16}$INK4a	19/29 (66%)	25/27 (92%)	19/19 (100%)
	BD$_{ProEx}$C	2/29 (7%)	2/27 (7%)	16/19 (84%)
Zamora 2009 *et al.*	Ki67	6/26 (23%)	10/21 (48%)	76/85 (89%)
	$_{p16}$INK4a	4/28 (14%)	12/19 (63%)	74/85 (87%)
	BD$_{ProEx}$C	3/25 (12%)	10/19 (53%)	70/80 (88%)

Source: Boggers J, Sahebali S, Depuydt C, De Prins F, Malinowski D. Role of protein biomarkers in the detection of high-grade disease in cervical cancer screening programs. J Oncol 2012; 2012:2893

and cellular proliferation. The altered expression of these proteins is a consequence of the binding of the high-risk HPV E6 and E7 oncogenes to host regulatory proteins, resulting in the degradation of the p53 tumor suppressor gene product and the inactivation of the retinoblastoma protein, leading to dysregulation of the cell cycle[5] [Table 2].

Ki-67 represents a nuclear and nucleolar protein expressed normally to the proliferating basal cells.

However, in cases of dysplasia and carcinoma, expression extends above the basal one-third of the epithelium resulting in an increase of positive cells, depicting a significant positive correlation between ascending grade of squamous intraepithelial lesion and labeling index.[5] In patients with atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesion (LSIL), Ki-67 immunocytochemistry demonstrated 96% sensitivity, 67% specificity, 49% PPV, and 98% negative predictive value for detection of high-grade CIN.[6] The protein p16 INK4a consists of a cell-cycle regulator, widely accepted as a sensitive and specific biomarker of dysplastic cervical cells and a surrogate biomarker for persistent infection with high-risk HPV. Recent prospective and point studies have shown that p16 INK4a in low-grade lesions demonstrate a higher risk of progression than negative lesions.[7-10] In an ASC-US/LSIL triage study, scoring system resulted in 95% sensitivity and 84% specificity for ASC-US and 100% sensitivity and 82% specificity in LSIL for the detection of biopsy-proven high-grade CIN.[11] Conducting the European Equivocal or Mildly Abnormal Pap Cytology Study, the sensitivity of the dual (Ki-67 and p16 INK4a) stain cytology for biopsy-confirmed CIN2+ was 92.2% for ASC-US cases and 94.2% for LSIL, with specificity of 80.6% and 68.0%, respectively.[12] As a triage for HPV-positive, cytology-negative cases in women ≥30 91.9% sensitivity and 82.1% specificity for CIN2+ on biopsy were observed.[13] HPV L1 is a capsid protein found in mild-to-moderate dysplasia, not observed in higher grade lesions, presented attractively as a possible prognostic marker to identify early dysplastic lesions most likely to progress to high-grade disease. L1-negative HPV high-risk positive mild and moderate lesions have an extremely low probability to regress spontaneously (5%) in contrast to the L1 positive cases showing a low malignant potential[14] BD ProEx C is proposed to distinguish true dysplasia from mimics (reactive/reparative epithelial changes, immature squamous metaplasia, and atrophy) in histology specimens.

Table 2: Biomarkers used in cervical cancer screening and diagnosis

Biomarker	Staining Pattern	Cellular process detected	Reported use of biomarker
Ki-67	Nuclear	Increased Ki-67 staining reflects increased epithelial cell proliferation found in HPV-infected tissues	(i) Measure of cell proliferative capacity (ii) Recognizes tissues involved by HPV and extent of Ki-67 immunostaining generally parallels increasing grades of dysplasia (iii) Predominantly used in histology applications
$p16^{INK4a}$	Nuclear and cytoplasmic	p16 levels increased in response to irregular cell cycle inactivation resulting from the disruption of interaction of pRb with transcription factor E2F in the presence by the HPV E7 oncogene	(i) Detection can serve as a surrogate biomarker for persistent infection with high-risk HPV (ii) Triage of equivocal cytology findings can facilitate identification of abnormal cells in cytology preparations (iii) Aid in interpretation of histological material. Limited evidence for use as a predictor of disease progression in histology specimens
BD ProEx C	Nuclear	Increased cellular levels of MCM2 and TOP2A due to aberrant transcription of S-phase proteins resulting from the interaction of HPV E6 and E7 oncoproteins with cell cycle proteins p53 and Rb	(i) Marker of cells with proliferative capacity (ii) Triage from abnormal cytology to increase PPV over cytology alone or HPV triage for detection of CIN 2+ disease. Can also facilitate identification of abnormal cells in cytology preparations (iii) Use in histology to distinguish true dysplasia from mimics such as reactive/reparative changes, immature squamous metaplasia, and atrophy
Cytoactiv HPV L1	Nuclear	HPV L1 capsid protein found in mild-to-moderate dysplasias, but lost in higher grade intraepithelial neoplasias	Possible prognostic marker to identify early dysplastic lesions most likely to progress to high-grade disease

Source: Boggers J, Sahebali S, Depuydt C, De Prins F, Malinowski D. Role of protein biomarkers in the detection of high-grade disease in cervical cancer screening programs. J Oncol 2012; 2012:2893.

In addition, it may play a role in the triage from abnormal cytology to increase PPV over cytology alone or HPV triage for detection of CIN2+ in women with ASC-US and LSIL cytology results. Goal of cervical screening consists of cervical cancer cases prevention with a risk-based approach, while biomarker research focuses on the identification of cervical lesions with progression to invasive lesions. Treatment mapping will be reserved for those women who are at risk of developing invasive cancer lesions, rather than treating any high-grade lesion. It is expected that the use of these biomarkers can be applied as a primary screen improving the overall accuracy of Pap Smear but also as a reflex test with high specificity to triage the high number of HPV positive tests.

CONCLUSION

Cervical cancer reflects the high incidence among gynecological cancers worldwide. In women of reproductive age, a crucial role in therapeutic mapping consists of fertility preservation and minimally surgical dissection in the early stages. Focusing on specific biomarkers, we can predict primary cervical intraepithelial lesions and establish a proper treatment. A multidisciplinary approach is mandatory to detect such lesions.

DISCLOSURE OF INTEREST

All authors declare any financial interest with respect to this manuscript.

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How to cite this article: Sofoudis C, Peraki A, Moschopoulou S. Significance of Biomarkers in Primar Detection of Cervical Intraepithelial Neoplasia Lesions – Review of the Literature. J Clin Res Oncol 2020;3(1):1-5.