RESEARCH ARTICLE

Scoring System and Management Algorithm Assessing the Role of Survivin Expression in Predicting Progressivity of HPV Infections in Precancerous Cervical Lesions

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Abstract

Background: To identify the risk factors and assess the role of survivin in predicting progessivity precancerous cervical lesions. Materials and Methods: This case-control study was conducted from October 2009 until May 2010. We obtained 74 samples, classified according to the degree of cervical intraepithelial neoplasia (CIN): 19 samples for CIN 1, 18 samples for CIN 2, 18 samples for CIN 3, and 19 samples as controls. Demographic profiles and risk factors assesment, histopathologic examination, HPV DNA tests, immunocytochemistry (ICC) and immunohistochemistry (IHC) staining for survivin expression were performed on all samples. Data was analyzed with bivariate and multivariate analysis. Results: Multivariate analysis revealed significant risk factors for developing precancerous cervical lesions are age <41 years, women with ≥2 sexual partners, course of education ≥13 years, use of oral contraceptives, positive high-risk HPV DNA, and high survivin expression by ICC or IHC staining. These factors were fit to a prediction model and we obtained a scoring system to predict the progressivity of CIN lesions. Conclusions: Determination of survivin expression by immunocytochemistry staining, along with other significant risk factors, can be used in a scoring system to predict the progressivity of CIN lesions. Application of this scoring system may be beneficial in determining the action of therapy towards the patient.

Keywords: Precancerous cervical lesions - cervical intraepithelial neoplasia - survivin - immunocytochemistry

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Introduction

Cervical cancer is the second most frequent cancer in women throughout the world, and contributes to most deaths caused by gynecological cancers. The age-standardized incidence rate of cervical cancer in Indonesia is 12.7/100.000 women per year and is the second most frequently encountered cancer in women following breast cancer (WHO, 2008).

Cervical cancer is still a major issue in Indonesia as most (62%) of the patients present in an advanced stage. Although screening for lesions by the Papanicolau test (Pap test) has dramatically reduced the incidence of cervical cancer, this test has various limitations, such as low sensitivity for detecting cervical intraepithelial neoplasia 2-3. Other modalities are needed to enhance the accuracy of screening for cervical cancer (Boone, 2012).

Human papilloma virus (HPV) is a well-known etiology of cervical cancer. Recent findings in the molecular carcinogenesis by HPV has expanded new areas of study, such as potential biomarkers. These biomarkers may be used to detect precancerous lesions, to enhance diagnostic sensitivity, to predict the prognosis, and/or to be considered when choosing the mode of therapy (Tan, 2010).

Survivin is an inhibitor of apoptosis expressed in the embryonic period; it is not expressed in differentiated adult tissues. Survivin is expressed during the G2/M phase of the cell cycle, and supressed when the cell cycle stops. Consequently, exfoliated epithelial cells will not express survivin. Malignancy is related to disruptions in the cell cycle and may therefore express survivin (Li, 2005). Another mechanism for survivin upregulation in cervical carcinogenesi is its normal transcriptional repression by wild-type p53 being eliminated by oncoprotein E6 in high-risk HPV (Branca, 2005).

Materials and Methods

This case-control study was conducted in Gynecologic

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Oncology Division, Department of Obstetrics and Gynecology, Cipto Mangunkusumo National Referral Hospiral, Jakarta, from October 2009 until May 2010. Samples were selected from women visiting the Colposcopy Clinic. Women eligible for this study were aged 18-50 years, or above 50 years without cytological findings of atrophy, and were not pregnant nor menstruating during the visit. Seventy-four women were included in this study and were divided into case and control groups. All samples agreed to participate in this study and an ethical clearance was issued by the Committee of Medical Research Ethics, Faculty of Medicine University of Indonesia. The case group was further classified as CIN 1, CIN 2, or CIN 3. We obtained 19 samples for CIN 1, 18 samples for CIN 2, 18 samples for CIN 3, and 19 samples as controls.

Demographic profiles and risk factors assesment, histopathologic examination, HPV DNA tests, immunocytochemistry (ICC) and immunohistochemistry (IHC) staining for survivin expression were performed on all samples. Samples were screened for sexually transmitted infections by a dermatovenereologist, and included tests for Chlamydia, genital herpes, trichomoniasis, gonorrhea, bacterial vaginosis, syphillis, and candidosis. HPV DNA was tested with hybrid capture 2 (HC2), detecting 13 types of high-risk HPV (HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 68).

Polyclonal rabbit anti-survivin antibody (Abcam® ab8228) was used for ICC and IHC staining. Stainings were conducted in Pathologic Anatomy Laboratorium, Hasan Sadikin Hospital, Bandung, and the slides were interpreted by us and a pathologist. Expression of survivin was measured with immunocytochemistry (ICC) and immunohistochemistry (IHC) staining. A sample is positive if the nucleus with/without cytoplasm is stained dark brown. Intensity is graded on a scale of 0-3: 0: no staining, 1: low intensity, 2: moderate intensity, and 3: high intensity. Distribution is the amount of stained cells. Expression is calculated by multiplying intensity and distribution. Expression of this marker was either low (ICC <70, IHC <110) or high (ICC ≥70, IHC ≥110).

Demographic profiles, risk factors, and test results were analyzed with Stata 9.2, using bivariate analysis and multivariate analysis. Data were also fit to a prediction model and probability scoring to obtain a scoring system.

Results

After data input, we determined a cut-off point for each variable by using the ROC curve. The cut-off was the point yielding maximum sensitivity and specificity. Cut-off points for each of the variables are: age <41 years, course of education ≥13 years, parity ≥2, amount of sexual partners ≥2, age at first sexual intercourse <22 years, ICC expression of survivin: ≥70, and IHC expression of survivin: ≥110.

Demographic profiles analyzed in this study were age, education, and parity. Risk factors studied were amount of sexual partners, age at first intercourse, use of oral contraceptives, smoking, and presence of sexually transmitted infections (STIs). Bivariate analysis of these factors (Table 1) revealed that age <41 years significantly increases the risk of developing CIN 1, 2, and 3. Women with more than two sexual partners, women who had their first sexual intercourse before 22 years, and women with positive STIs have statistically significant risks of developing CIN 3. Other factors were not statistically significant.

HPV DNA was examined with hybrid capture 2 (HC2) and results were classified according to the degree of CIN. Women infected with high-risk DNA had a significantly

Table 1. Odds Ratio and Significancy of Demographic and Risk Factors for Developing CIN

Factors	CIN (-) N (%)	CIN 1 n (%)	OR(95%CI OR) P value	CIN 2 n (%)	OR(95%CI OR) P value	CIN 3 n (%)	OR(95%CI OR) P value
Age <41 years	4 (21.05)	13 (68.42)	8.13 (1.87-35.23) 0.005	15(83.33)	18.75 (3.57-98.54) 0.001	13 (72.22)	9.75 (2.15-44.14) 0.003
Course of education ≥13 years	9 (47.37)	12 (63.16)	1.90 (0.52-6.96) 0.33	12(66.67)	2.22 (0.59-8.41) 0.24	7 (38.89)	0.71 (0.19-2.61) 0.603
Parity ≥2	10 (52.63)	11 (57.89)	1.24 (0.34-4.45) 0.744	10(55.56)	1.13 (0.31-4.10) 0.858	6 (33.33)	0.45 (0.12-1.70) 0.24
Sexual partners ≥2	1 (5.26)	5 (26.32)	6.43 (0.67-61.46) 0.106	6(33.33)	9.00 (0.96-84.49) 0.054	9 (50.00)	18.00 (1.96-164.97) 0.011
First sexual intercourse <22 years	9 (47.37)	9 (47.37)	1.00 (0.28-3.57) 1	10(55.56)	1.39 (0.38-5.07) 0.619	15 (83.33)	5.56 (1.20-25.71) 0.028
Using oral contraceptives	7 (36.84)	6 (31.58)	0.79 (0.21-3.03) 0.733	7(38.89)	1.09 (0.29-4.12) 0.898	11 (61.11)	2.69 (0.71-10.18) 0.144
Positive STIs	0 (0.00)	5 (26.32)	6.43 (0.67-61.47) 0.106	5(27.28)	6.92 (0.72-66.50) 0.094	7 (38.89)	11.45 (1.24-106.04) 0.032

*CIN: cervical intraepithelial neoplasia, STIs: sexually transmitted infections

Table 2. Odds Ratio and Significancy of Survivin Expression for Developing CIN

Survivin expression	CIN (-) N (%)	CIN 1 n (%)	OR(95%CI OR) P value	CIN 2 n (%)	OR(95%CI OR) P value	CIN 3 n (%)	OR(95%CI OR) P value
ICC (High:≥70)	3 (15.79)	10 (52.63)	5.93 (1.29-27.28) 0.022	15 (83.33)	26.67 (4.64-153.22) 0.0001	16 (88.89)	42.67 (6.26-290.7) 0.0001
IHC (High:≥110)	1 (5.26)	7 (36.84)	10.50 (1.14-96.58) 0.038	13 (72.22)	46.80 (4.87-449.58) 0.001	15 (83.33)	90.00 (8.46-957.60) 0.0001

*CIN: cervical intraepithelial neoplasia, ICC: immunocytochemistry, IHC: immunohistochemistry

by a target biopsy, as treatment modalities are choosed based on histopathological results. ASCUS LG-SIL Triage Study (ALTS) stated that borderline atypical squamous cell of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesions (LSIL may be subsequently managed with a follow-up Pap test after 4-6 months, HPV DNA examination, or colposcopy examination. The survivin biomarker may have a role in deciding whether to manage the patient conservatively (follow-up) or to directly perform a colposcopy.

Table 3. Prediction Model of Developing CIN with Survivin ICC Examination

Variable	Coef	SE Coef	Coef/SE Coef.	OR	(95%CI OR)	P value	Score
CIN 1							
Survivin	2.816	1.347	2.09	16.71	0.18-5.46	0.037	30
High-risk HPV	2.674	1.504	1.78	14.5	-5.89	0.075	25
Sexual partners	2.982	1.755	1.7	19.72	-6.88	0.089	24
Age	3.157	1.258	2.51	23.49	0.69-5.62	0.012	36
Education	1.478	1.063	1.39	4.39	-4.17	0.164	20
Oral contraception	-0.231	1.158	-0.2	0.79	-4.53	0.842	0
Constant value	-3.713	1.336	-2.78				
CIN 2							
Survivin	4.713	1.487	2.81	64.93	1.26-7.09	0.005	40
High-risk HPV	3.674	1.595	2.3	39.4	0.55-6.80	0.021	33
Sexual partners	3.305	1.9	1.74	27.26	-7.45	0.082	25
Age	4.006	1.445	2.77	54.91	1.17-6.84	0.006	40
Education	1.825	1.253	1.46	6.2	-4.91	0.145	21
Oral contraception	0.086	1.293	0.07	1.09	-5.07	0.947	1
Constant value	-6.218	1.697	-3.66				
CIN 3							
Survivin	4.702	1.696	2.77	110.17	1.38-8.03	0.006	40
High-risk HPV	6.944	1.974	3.52	1037.89	3.07-10.81	0.000	150
Sexual partners	4.276	2.007	2.13	71.99	0.34-8.21	0.033	31
Age	2.498	1.613	1.55	12.16	-6.32	0.122	23
Education	0.294	1.377	0.21	1.34	-5.4	0.831	3
Oral contraception	1.623	1.405	1.15	5.07	-5.51	0.248	17
Constant value	-8.533	2.24	-3.81				

*CIN: cervical intraepithelial neoplasia, HPV: human papillomavirus

Table 4. Probability of Developing CIN with Survivin ICC Examination

	CIN (-)	CIN 1	CIN 2-3
0	100	0	0
1-53	2	57	41
>54	2	10	88

*CIN: cervical intraepithelial neoplasia

increased risk of developing CIN 2 (OR 22.50, 95%CI 2.45-206.74, p=0.006) and CIN 3 (OR 306, 95%CI 17.70-5289.84, p<0.0001), but not CIN 1 (OR 8.31, 95%CI 0.89-77.57, p=0.063).

A high survivin expression significantly increases the risk of developing more severe grades of CIN (Table 2).

All of the factors with p value less than 0.25 were included into the multivariate analysis. A stepwise backward elimination was performed, factors were excluded if the exclusion did not significantly change the maximum likelihood ratio. Factors from the full model were then fit into a prediction model (fit model). A score was determined by dividing the coefficient by SE coefficient (Table 3).

The probability of developing CIN by survivin ICC examination is shown in Table 4.

Discussion

It is a common consensus that women with abnormal Pap smears should undergo colposcopy exams followed by a target biopsy, as treatment modalities are choosed based on histopathological results. ASCUS LG-SIL Triage Study (ALTS) stated that borderline atypical squamous cell of undetermined significance (ASCUS) and low-grade squamous intraepithelial lesions (LSIL may be subsequently managed with a follow-up Pap test after 4-6 months, HPV DNA examination, or colposcopy examination. The survivin biomarker may have a role in deciding whether to manage the patient conservatively (follow-up) or to directly perform a colposcopy.

In our study, women <41 years had significantly increased risks of developing all grades of CIN. Higher grades of CIN are found in accordance with increasing age. This finding is consistant with the natural history of precancerous cervical lesions, as CIN 1, 2, and 3 needs 5, 3, and 1 years to develop to carcinoma in situ (CIS). Kim (2012) found that age was significantly and inversely associated with HPV clearance. Therefore, women ≥41 years will more likely have CIS or cervical cancer, rather than precancerous lesions.

We found that course of formal education is not a significant factor for developing CIN, although the odds are increased in women with education ≥13 years. Other studies on course of education have been inconsistent. Castle (2008) found that CIN 1-3 and CIS were more frequently found in women with <12 years of formal education, owing to lack of compliance during therapy. Poomtavorn (2011) also supported these findings. On the other hand, Kim (2012) found that women with higher level of education were associated with high-risk HPV infections. Although we found no significancy in bivariate analysis, this factor was included in the multivariate analysis as the p value was <0.25, and it was included in the fit model.

Parity is not a significant factor in our study. This finding is consistent with Belinson (2008), who found no significant correlation between parity and CIN. On the contrary, Jensen (2013) found the risk of developing CIN 3+ in women with persistent HPV infection who had given birth was 1.78 (95%CI 1.07-2.94). Kim (2012) found parity was a significantly associated with impaired HPV clearance. Alterations in the immune system owing to hormonal changes during pregnancy may increase the susceptibility of HPV infections and/or malignant changes (American Cancer Society, 2009).

We found that women with ≥2 sexual partners have a significantly increased risk of developing CIN 3. Our finding was consistant with de Boer (2006), who discovered the OR of cervical cancer in women with >1 sexual partner was 5.83 (95%CI 2.98-11.36). Almonte (2011) found the age-adjusted odds ratio in women with ≥5 sexual partners was 2.1 (95%CI 1.4-3.2). The risk is also increased if the woman had sexual intercouse with a male with multiple partners. This sexual behaviour may introduce infections with different types of HPV; high-risk HPV increases the risk of dysplasia.

Bivariate analysis revealed that women who have had their first sexual intercourse before 22 years have a significantly increased risk of developing CIN 3. Ruiz (2012) found the odds for high-grade lesions was 3.55 higher in women who had a short interval (<3 years) between menarche and first sexual intercourse. Almonte (2011) found the OR in women who had their first sexual intercourse before 18 years compared to ≥20 years was 1.5 (95%CI 1.2-2.0). Infection with high-risk HPV in the maturation period of the genitalia may induce atypical

transformations.

We found no significant risk of developing CIN in women using oral contraceptives. In a systematic review by Gadducci (2011), oral contraceptive consumption increased the relative risk of cervical cancer, and the risk decreased after pill discontinuation. Nevertheless, WHO does not recommend any changes in oral contraceptive practice as the benefit outweighs the risk. Hellberg (2005) stated the immunosupressive nature of progesterone correlated with HPV infections. Progesterone enhances HPV mRNA and stimulates viral replication. In the transformation zone, estradiol is converted to estrone, which is associated with malignant transformation in estrogen-sensitive cells. Although we found no significancy in bivariate analysis, this factor was included in the multivariate analysis as the p value was <0.25, and it was included in the fit model.

Various studies have reported the role of sexually transmitted infections in increasing the risk of CIN. Roeters (2010) found the OR for Chlamydia and Gardnerella in high-grade smears are 7 and 12, respectively, and the OR for Trichomonas was also significantly high. Engberts (2007) reported the OR of Candida is 1.85 for LSIL and 2.0 for HSIL. We found that STIs significantly increases the risk of developing CIN 3. By-products of these pathogens uch as propionate and butyrate may damage epithelial cells, and alterations in the vaginal environment to a pro-inflammatory state may contribute to the development of ervical lesions (Gillet, 2012).

Infection by high-risk HPV significantly increases the risk of higher grades of CIN. We found high-risk HPV DNA in 21.21% of CIN 1 (OR: 6.73), 56.1% of CIN 2 (OR: 31.04) and 70% of CIN 3 (OR: 50). Our findings are similar to previous studies. However, the amount of women infected with high-risk HPV in our study are lower than other similar researches. Belinson (2008) found high-risk HPV DNA in 85.2% of CIN 1, 96.5% of CIN 2, and 97.4% of CIN 3. This discrepancy may be due to different reagens used; our study used HC2 reagen detecting 13 types of high-risk HPV.

Bivariate and multivariate analysis proved the significance of survivin expression in CIN; the odds are higher in higher grade lesions. Similar findings were reported by Barbosa (2011) and Branca (2008), in which survivin expression was reported to be increased in accordance with tumor progressivity. Therefore, higher grade lesions have higher levels of survivin expression.

Oncoprotein E6 in high-risk HPVs has a significant role in the regulation of survivin-gene transcription. P53 supresses the expression of survivin, thus in conditions with loss of p53 function, such as cancer, expression of survivin is enhanced. HPV E6 induces the activity of survivin promoter region and increases endogenous survivin mRNA in human embryonic fibroblasts (Borbely, 2006; Mita, 2008)

We propose a combined scoring system consisting of survivin expression by ICC and factors proved significant from multivariate analysis (Table 5). A biopsy will first be performed on the subject, classified to CIN 1, CIN 2, or CIN 3. Subjects will then be assessed for risk factors and examined for tissue biopsy and survivin expression by ICC staining. The score will then be interpreted by using probability of lesion progressivity. The score will then be interpreted by using probability of lesion progressivity. For example, a patient with CIN 1 and total score of 42, has a 2% chance of regressing, 57% chance of persisting in CIN 1, and 41% chance of progressing to CIN 2-3. This scoring system may help determine whether the patient

Table 5. Combined Scoring System of Developing CIN

Variable		Degree of subject's lesion		
		CIN 1	CIN 2	CIN 3
Subject Evaluation & Scoring				
Survivin ICC	Low (<70)	0	0	0
	High (≥70)	30	40	40
High-risk HPV DNA	Negative	0	0	0
	Positive	25	33	50
Age	>41 years	0	0	0
	<41 years	36	40	23
Sexual partners	<2	0	0	0
	>2	24	25	31
Course of education	<13 years	0	0	0
	>13 years	20	21	3
Use of oral contraception	No	0	0	0
	Yes	0	1	17
Total score				
Score		CIN (-)	CIN 1	CIN 2-3
Probability Of Lesion Progressivity				
	0	100	0	0
	1-53	2	57	41
	>54	2	10	88

*CIN: cervical intraepithelial neoplasia, ICC: immunocytochemistry, HPV: human papillomavirus

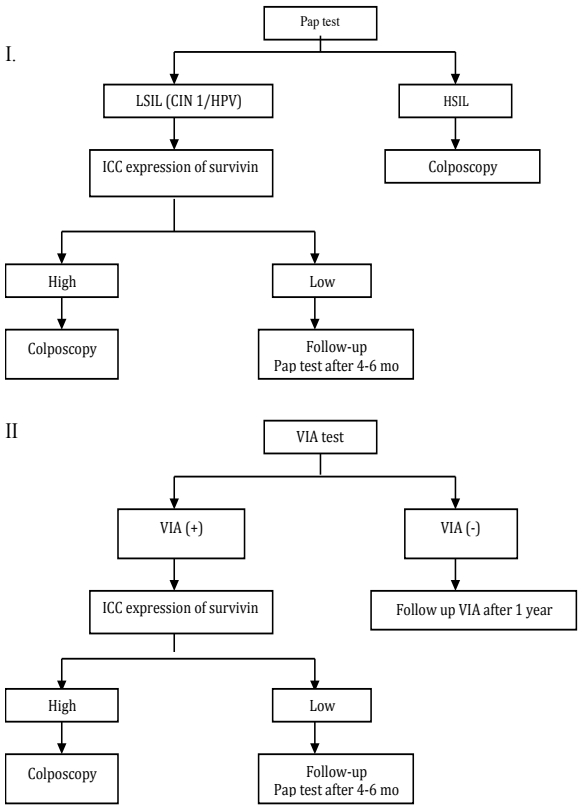

Figure 1. Management Algorithm by Incorporating the Role of Survivin Expression. LSIL: low-grade squamous intraepithelial lesions; HSIL:high-grade squamous intraepithelial lesions; CIN:cervical intrepithelial neoplasia; ICC:immunocytochemistry; HPV:human papillomavirus; VIA:visual inspection with acetic acid

should be managed conservatively or actively.

Evaluation precancerous lesions by measuring survivin expression can be used to determine whether the patient needs active or conservative management. The algorithm is found in Figure 1. Patients with LSIL on their Pap test results, or patients with positive acetowhite lesions on visual inspection with acetic acid, should be evaluated for survivin expression. If the results are low, the patient should return for subsequent Pap tests after 4-6 months. If the results are high, the patient should be examined with colposcopy. This evaluation helps reduce the need for colposcopy, because in Indonesia colposcopy is not readily available with very few experts centralized in major cities. Survivin expression is easier to perform, as speciments can be shipped to laboratories and the price is cheaper than colposcopy.

In conclusion, we found the significant risk factors for developing precancerous cervical lesions are (1) age <41 years, (2) use of oral contraceptives, (3) ≥2 sexual partners, (4) use of oral contraceptives, (5) positive high-risk HPV DNA, and (6) high survivin expression by ICC or IHC staining. These risk factors can be combined to create a scoring system. Detection of survivin expression by ICC staining may be incorporated in a scoring system and management algorithm of precancerous cervical lesions. Application of this scoring system and algorithm may be beneficial in determining the action of therapy towards the patient.

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References

Almonte M, Ferreccio C, Gonzales M (2011). Risk factors for high-risk human papillomavirus infection and cofactors for high-grade cervical disease in Peru. *Int J Gynecol Cancer*, **21**, 1654-63.

American Cancer Society (2009). What Are the Risk Factors for Cervical Cancer? Available at http://www.cancer.org/cancer/cervicalcancer/detailedguide/cervical-cancer-risk-factors. Accessed July 14, 2010.

Barbosa LC, da Silva ID, Correa JC, Ribalta JC (2011). Survivin and telomerase expression in the uterine cervix of women with human papillomavirus-induced lesions. *Int J Gynecol Cancer*, **21**, 15-21.

Belinson S, Smith JS, Myers E, et al (2008). Descriptive evidence that risk profiles for cervical intraepithelial neoplasia 1, 2, and 3 are unique. *Cancer Epidemiol Biomarkers Prev*, **17**, 2350-5.

Bibbo M, Klump WJ, DeCecco J, Kovatich AJ (2002). Procedure for immunocytochemical detection of P16INK4A antigen in thin-layer, liquid-based specimens. *Acta Cytol*, **46**, 25-9.

Boone JD, Erickson BK, Huh WK (2012). New insights to cervical cancer screening. *J Gynecol Oncol*, **23**, 282-7.

Borbely AA, Murvai M, Konya J et al (2006). Effects of human papillomavirus type 16 oncoproteins on survivin gene expression. *J Gen Virol*, **87**, 287-94.

Branca M, Giorgi C, Santini D, et al (2005). Survivin as a marker of cervical intraepithelial neoplasia and high-risk human papillomavirus and a predictor of virus clearance and prognosis in cervical cancer. *Am J Clin Pathol*, **124**, 113-21.

Branca M, Ciotti M, Giorgi C et al (2008). Predicting high-risk human papillomavirus infection, progression of cervical intraepithelial neoplasia, and prognosis of cervical cancer with a panel of 13 biomarkers tested in multivariate modeling. *Int J Gynecol Pathol*, **27**, 265-73.

Castle PE, Cox JT, Schiffman M WC, Solomon D (2008). Factors influencing histologic confirmation of high-grade squamous intraepithelial lesion cytology. *Obstet Gynecol*, **112**, 637-45.

de Boer MA, Vet JN, Aziz MF et al (2006). Human papillomavirus type 18 and other risk factors for cervical cancer in Jakarta, Indonesia. *Int J Gynecol Cancer*, **16**, 1809-14.

Engberts MK, Verbruggen BS, Boon ME, van Haaften M, Heintz AP (2007). Candida and dysbacteriosis: a cytologic, population-based study of 100,605 asymptomatic women concerning cervical carcinogenesis. *Cancer*, **111**, 269-74.

Gadducci A, Barsotti C, Cosio S, Domenici L, Riccardo GA (2011). Smoking habit, immune suppression, oral contraceptive use, and hormone replacement therapy use and cervical carcinogenesis: a review of the literature. *Gynecol Endocrinol*, **27**, 597-604.

Gillet E, Meys JFA, Verstraelen H, et al (2012). Association between bacterial vaginosis and cervical intraepithelial neoplasia: systematic review and metaanalysis. *PLoS One*, **7**, 45201.

Hellberg D, Stendahl U (2005). The biological role of smoking, oral contraceptive use and endogenous sexual steroid hormones in invasive squamous epithelial cervical cancer. *Anticancer Res*, **25**, 3041-6.

Jensen KE, Schmiedel S, Norrild B et al (2013). Parity as a cofactor for high-grade cervical disease among women with persistent human papillomavirus infection: a 13-year follow-up. *Br J Cancer*, **108**, 234-9.

Kim JW, Song SH, Jin CH, et al (2012). Factors affecting the clearance of high-risk human papillomavirus infection and the progression of cervical intraepithelial neoplasia. *J Int Med Res*, **40**, 486-96.

Kim K, Kim JJ, Kim SM, No JH, Kim YB (2012). Prevalence and determinants of high-risk human papillomavirus infection in women with high socioeconomic status in Seoul, Republic of Korea. *Asian Pac J Cancer Prev*, **13**, 269-73.

Li F (2005). Role of survivin and its splice variants in tumorigenesis. *Br J Cancer*, **92**, 212-6.

Mita AC, Mita MM, Nawrocki ST, Giles FJ (2008). Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. *Clin Cancer Res*, **14**, 5000-5.

Poomtavorn Y, Suwannarurk K, Thaweekul Y, Maireang K (2011). Risk factors for high-grade cervical intraepithelial neoplasia in patients with atypical squamous cells of undetermined significance (ASC-US) Papanicolaou smears. *Asian Pac J Cancer Prev*, **12**, 235-8.

Roeters AM, Boon ME, van Haaften M, et al (2010). Inflammatory events as detected in cervical smears and squamousintraepithelial lesions. Diagn Cytopathol, **38**, 85-93.

Tan GC, Norlatiffah S, Sharifah NA et al (2010). Immunohistochemical study of p16 INK4A and survivin expressions in cervical squamous neoplasm. *Indian J Pathol Microbiol*, **53**, 1-6.

WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre) (2010). Human Papillomavirus and Related Cancers in Indonesia. Summary Report 2010. Accessed 5 February 2013.