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Prognostic Significance of Soluble Intercellular Adhesion Molecule Serum Levels in Women with Threatened Abortion

Ocena wartości prognostycznych stężeń międzykomórkowych cząsteczek adhezyjnych w surowicy krwi u kobiet z poronieniem zagrażającym oraz u pacjentek, u których doszło do utraty ciąży

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Abstract

Objectives. Determining the potential prognostic significance of soluble intercellular adhesion molecule serum concentrations in women with threatened abortion and patients whose pregnancies ended in miscarriage.

Material and Methods. Serum samples were collected from 30 patients with threatened abortion with subsequent normal pregnancy outcome, 30 women with miscarriage, and 20 women during the first trimester of normal, successful pregnancy. Concentrations of soluble intercellular adhesion molecules (sICAM) 1, 2, and 3 were measured using commercially available ELISA kits. Sensitivity, specificity, analytic accuracy, and positive and negative predictive values were assessed with receiver operating characteristic (ROC) curves.

Results. Analysis of the area under the ROC curves showed that serum sICAM-1 levels had the best prognostic value. The highest values of the area under the ROC curves were 0.92 in patients with threatened abortion and 0.99 in those who miscarried. Serum levels of sICAM-1 had the best diagnostic value in discriminating women with both threatened abortion and pregnancy ending in miscarriage (88% and 94%, respectively).

Conclusions. Serum sICAM-1, but not sICAM-2 and -3, levels may be of prognostic value in women with threatened abortion (*Adv Clin Exp Med* 2006, 15, 3, 485–490).

Key words: sICAM, threatened abortion, miscarriage.

Streszczenie

Cel pracy. Ocena wartości prognostycznych stężeń międzykomórkowych cząsteczek adhezyjnych ICAM (*intercellular adhesion molecule*) w surowicy krwi w różnicowaniu kobiet z poronieniem zagrażającym oraz pacjentek, u których doszło do utraty ciąży.

Material i metody. Próbkę surowicy krwi zostały pobrane od 30 kobiet hospitalizowanych z powodu poronienia zagrażającego z późniejszym prawidłowym przebiegiem ciąży, 30 pacjentek ze spontanicznym poronieniem dokonanym oraz od 30 kobiet w pierwszym trymestrze ciąży niepowikłanej. Stężenia międzykomórkowych cząsteczek adhezyjnych sICAM 1, 2 i 3 oceniono metodą immunoenzymatyczną ELISA z użyciem zestawów dostępnych komercyjnie. Czulość, swoistość, dokładność analityczna oraz pozytywne i negatywne wartości predykcyjne oceniono z użyciem krzywych ROC (*receiver operating characteristic curves*).

Wyniki. Analiza krzywych ROC wykazała, że stężenie sICAM-1 ma największą wartość prognostyczną w różnicowaniu ciąży z poronieniem zagrażającym oraz dokonanym. Najwyższe wartości pola pod krzywymi ROC wynosiły 0,92 u kobiet z ciążą zagrożoną poronieniem oraz 0,99 w ciążach poronionych. Poziom sICAM-1 w surowicy krwi miał największą wartość w dyskryminacji ciąży z poronieniem zagrażającym (88%) oraz dokonanym (94%).

Wnioski. Stężenie sICAM-1 w przeciwieństwie do sICAM-2 i -3 ma największą wartość w różnicowaniu ciąży z poronieniem zagrażającym i dokonanym (*Adv Clin Exp Med* 2006, 15, 3, 485–490).

Słowa kluczowe: sICAM, poronienie zagrażające, poronienie dokonane.

Various factors have been implicated in the physiological development of pregnancy. Implantation and early embryonic development involve the activation of cell adhesion processes and transmembrane signaling [1]. Adhesion molecules participate in embryo development from the time of ovum fertilization and are present on the membranes of most cells which play roles in implantation and early trophoblast development [2, 3]. Intercellular adhesion molecules (ICAM) types 1, 2, and 3 are the ligands for integrins, which are responsible for maintaining cellular and tissue structure as well as for transmitting signals between cells [3, 4]. ICAM 1, 2, and 3 have been identified on the surfaces of trophoblast, decidual stroma cells, and the endothelium of the implantation site as well as on leukocytes migrating from the maternal circulation to the decidua [3, 5]. Intercellular adhesion molecules also play a role in the regulation of the maternal immune response to blastocystic antigens, and their localization on blood vessels facilitates decidual leukocyte migration [5]. ICAMs, produced by the stroma cells, bind NK cells in the decidua and, following exfoliation, they exert an immunosuppressive effect by blocking the LFA1-dependent cellular interactions [6].

In spite of their structural similarity, ICAM-1 and ICAM-2 share only 35% homology and their actions are therefore different [7]. Soluble forms of intercellular adhesion molecules (sICAM-1, sICAM-2, and sICAM-3) have also been identified. Changes in the serum concentrations of these molecules have been connected with immune system activation and have been observed in various pathological states [8–11]. Schust and Hill [12] investigated the role of sICAM-1 in women with recurrent abortions in an attempt to correlate serum sICAM-1 levels with successful pregnancy outcome. They failed to demonstrate any statistically significant change in serum sICAM-1 concentration not only between non-pregnant and pregnant women, but also between successful pregnancies and those ending in miscarriage. The expression of ICAM-1 on the endometrial surface in patients with recurrent abortions seems to be normal; however, the level of sICAM-1 exfoliated to the circulation has been found to be lower in these women [13].

Many factors seem to be responsible for pregnancy loss in the first trimester, and most of them remain enigmatic. Therefore, further investigations are needed to clarify the role of adhesion molecules in the development of a normal pregnancy and in the pathogenesis of spontaneous abortion. The aim of the study was to determine the potential prognostic significance of the serum concentrations of soluble intercellular adhesion molecules (sICAM-1, sICAM-2, and sICAM-3) during the first trimester of pregnancy in women with

threatened abortion and in patients whose pregnancies were ended in miscarriage.

Material and Methods

Eighty women in the first trimester of pregnancy were enrolled in this study. The mean (\pm SD) age of the studied patients was 23.2 ± 7.4 years (range: 16 to 42 years). Physical and ultrasound examination revealed no pathology in 20 women. These patients, with subsequent normal pregnancy development and outcome, were classified to the control group. Patients with vaginal spotting or bleeding with or without concomitant uterine contraction were assigned to the group with threatened abortion (60 patients). Transvaginal (6.5 MHz) or transabdominal (3.5 MHz) ultrasound examinations confirmed the presence of live embryos as well as the approximate gestational age. These latter women were divided into two subgroups: 1) patients successfully treated with progesterone supplementation and spasmolytic drugs, with subsequent normal pregnancy outcome ($n = 30$); 2) patients treated with progesterone supplementation and spasmolytic drugs, with pregnancy loss within 48 hours of hospitalization ($n = 30$).

Blood serum samples were collected from these patients before treatment, and in the control group samples were obtained during routine control tests. The study was approved by the Lublin University School of Medicine Ethics Committee.

Concentrations of sICAM-1, sICAM-2 and sICAM-3 were measured using the commercially available kits: sICAM-1 ELISA high sensitivity, sICAM-2 ELISA, and sICAM-3 ELISA (Bender MedSystems, Austria). sICAM-1 and -3 levels were expressed in ng/ml and the sICAM-2 concentration was expressed in U/ml.

All data were expressed as means, medians (*Me*), standard deviations (*SD*), and ranges (Min, Max). Statistical significance was determined using the Mann-Whitney test for independent samples. To estimate correlations between sICAMs levels and gestational age, Pearson's coefficient of correlation was calculated. A *p* value less than 0.05 was considered statistically significant. Sensitivity, specificity, analytic accuracy, and positive and negative predictive values were assessed with receiver operating characteristic (ROC) curves.

Results

Serum sICAM-1 levels were significantly lower in women with normal, successful pregnancies (controls) than in patients with threatened abortion

($Z = -4.99$, $p = 0.000001$) and women with pregnancy loss ($Z = -5.78$, $p < 0.000001$). The authors found higher mean serum levels of sICAM-1 in women with aborted pregnancies than in patients with threatened abortion but with successful outcome ($Z = -2.67$, $p < 0.008$). sICAM-2 concentrations in the serum of women with threatened abortion were significantly lower ($Z = 2.22$, $p = 0.03$) than in controls. However, the serum levels of this molecule in the group of women with pregnancy loss were similar to those observed in both the controls ($Z = 1.41$, $p = 0.16$) and patients with threatened abortion ($Z = -1.06$, $p = 0.29$). Levels of sICAM-3 did not differ significantly between patients with threatened abortion and women with miscarriage compared with the control group ($Z = 0.64$, $p = 0.52$ and $Z = -1.69$, $p = 0.09$, respecti-

vely). sICAM-3 serum concentration was significantly lower in women with threatened abortion than in patients with miscarriage ($Z = -2.32$, $p = 0.02$) (Table 1). No significant correlation between serum sICAM-1, -2 and -3 levels and gestational age was found in the studied groups (Table 2).

ROC curves analysis was used to test the prognostic significance of sICAM-1, -2, and -3 serum concentrations and to find the best discriminating threshold value between the studied groups. In Tables 3 and 4, sensitivity (SENS), specificity (SPEC), analytic accuracy (ACC), and positive and negative predictive values (PPV, NPV), estimated with 95% confidence levels, are shown. Serum levels of sICAM-1 were found to have the best diagnostic value in discriminating between women with both threatened abortion and pregnancies end-

Table 1. Serum concentrations of sICAM-1, -2, and -3 in women during the first trimester of pregnancy

Tabela 1. Stężenia sICAM-1, -2, -3 w surowicy krwi kobiet w pierwszym trymestrze ciąży

		Controls (Grupa kontrolna)	Threatened abortion (Zagrażające poronienie)	
			successful outcome (prawidłowy przebieg ciąży)	miscarriage (poronienie dokonane)
N		20	30	30
sICAM-1 (ng/ml)	Mn	243.7	794.6	1037
	Me	207	732	1079
	SD	188.6	357.5	290.4
	Min-Max	36–694	185–1374	453–1454
sICAM-2 (U/ml)	Mn	868	705.4	769.8
	Me	868.3	707.5	736.6
	SD	267.4	173.4	215.5
	Min-Max	411–1464.8	320.6–1021.6	386.8–1479.8
sICAM-3 (ng/ml)	Mn	84	84.4	104.5
	Me	93.8	85.3	101.3
	SD	30.9	21.1	35.4
	Min-Max	17.5–115.5	47.5–129.5	41.5–188.5

N – number of patients.

N – liczba pacjentek.

Table 2. Correlation between serum sICAM-1, -2, -3 levels and gestational age

Tabela 2. Korelacje między stężeniami sICAM-1, -2, -3 w surowicy krwi oraz wiekiem ciążowym

Gestational age (Wiek ciążowy)	sICAM-1 (ng/ml)		sICAM-2 (U/ml)		sICAM-3 (ng/ml)	
	R	p	R	p	R	p
Controls (Grupa kontrolna)	-0.12	0.62	-0.14	0.55	-0.16	0.5
Threatened abortion (Zagrażające poronienie)						
Successful outcome (Prawidłowy przebieg ciąży)	0.29	0.12	0.12	0.53	-0.01	0.95
Miscarriage (Poronienie dokonane)	-0.06	0.74	-0.15	0.42	-0.13	0.48

R – correlation coefficient, p – level of significance.

R – współczynnik korelacji, p – poziom istotności.

ing in miscarriage (88% and 94%, respectively). ROC curve analysis showed significant differences in the prognostic values of sICAM-1, -2, and -3 levels in discriminating threatened abortions and normal, successful pregnancies ($p = 0.01$, $p = 0.000001$, $p = 0.047$, respectively) (Fig. 1).

Analyzing the ROC curves, significant differences for sICAM-1, -2, and sICAM-1, -3 levels were also found in discriminating women with normal pregnancies and those with miscarriage ($p = 0.00002$, and $p = 0.000013$, respectively). However, no significant difference was observed for sICAM-2 and -3 concentrations ($p = 0.83$) (Fig. 2).

Analysis of the area under the ROC curves showed that serum sICAM-1 levels had the best prognostic value. The highest values of the area under ROC curves were 0.92 in patients with threatened abortion and 0.99 in those with miscarriage.

Discussion

In spite of extensive investigation, studies to date are still far from introducing a precise mechanism for the pathophysiology of many cases of ear-

ly pregnancy loss. Serial measurement of serum β -HCG, progesterone, and estradiol levels has proven helpful in ascertaining whether a live intrauterine pregnancy is present; however, new prognostic markers are still under investigation [14–16].

Intercellular adhesion molecules are present on the cell membranes of the trophoblast and decidua and may therefore play a role in implantation and early trophoblast development [3]. ICAMs may also be responsible for the regulation of the maternal immune response to the blastocystic antigens [6, 17]. However, the exact role of the extracellular ICAMs released by proteases remains enigmatic. Since dynamic angiogenesis has been observed in early pregnancy, the measurement of serum soluble intercellular adhesion molecules -1, -2, and -3 may be helpful in evaluating endothelial cell activation in successful pregnancies and in those ending in miscarriage. Lisby et al. [9] suggested that serum sICAM-1 levels reflect early activation of immune responses. Therefore the authors decided to evaluate the serum concentrations of these molecules in women during the first trimester of pregnancy.

The authors found that the sICAM-1, -2, and -3 serum levels in women with physiological pre-

Table 3. Prognostic values of serum sICAM-1, -2, and -3 levels in discriminating women with threatened abortion and normal pregnancies

Tabela 3. Wartości prognostyczne stężeń sICAM-1, -2, -3 w surowicy krwi u kobiet w ciąży fizjologicznej i zagrożonej poronieniem

Parameter – optimal liminal value (Wskaźnik)	SENS	95% CI	SPEC	95% CI	ACC	PPV	NPV
sICAM-1 (318 ng/ml)	80	59.5–92.8	93.3	79.9–98.8	88	88.9	87.5
sICAM-2 (825.8 U/ml)	60	39.2–78.3	76.7	60.4–88.4	70	63.2	74.2
sICAM-3 (30 ng/ml)	15	4.2–34.9	100	90.5–100	66	100	63.8

SENS – sensitivity, CI – confidence interval, SPEC – specificity, ACC – analytic accuracy, PPV – positive predictive values, NPV – negative predictive values.

SENS – czułość, CI – przedział ufności, SPEC – swoistość, ACC – dokładność analityczna, PPV – pozytywna wartość predykcyjna, NPV – negatywna wartość predykcyjna.

Table 4. Prognostic values of serum sICAM-1, -2 and -3 levels in discriminating women with normal and with aborted pregnancies

Tabela 4. Wartości prognostyczne stężeń sICAM-1, -2, -3 w surowicy krwi dla kobiet w ciąży fizjologicznej i zakończonej poronieniem

Parameter – optimal liminal value (Wskaźnik)	SENS	95% CI	SPEC	95% CI	ACC	PPV	NPV
sICAM-1 (595 ng/ml)	95	77.5–99.8	93.3	79.9–98.8	94	90.5	96.6
sICAM-2 (961.6 U/ml)	30	14.0–51.1	93.3	79.9–98.8	68	75	66.7
sICAM-3 (74.5 ng/ml)	35	17.8–56.0	86.7	71.7–95.3	66	63.6	66.7

SENS – sensitivity, CI – confidence interval, SPEC – specificity, ACC – analytic accuracy, PPV – positive predictive values, NPV – negative predictive values.

SENS – czułość, CI – przedział ufności, SPEC – swoistość, ACC – dokładność analityczna, PPV – pozytywna wartość predykcyjna, NPV – negatywna wartość predykcyjna.

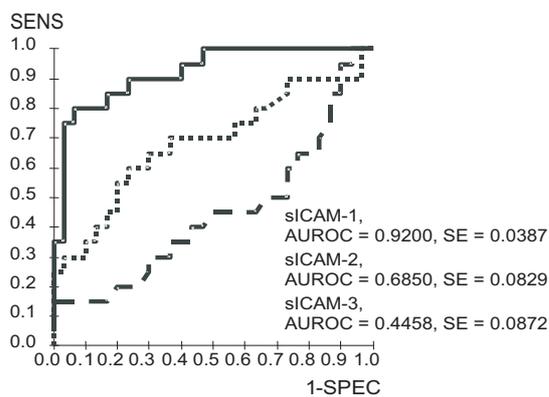


Fig. 1. ROC curve analysis for serum sICAM-1, -2, and -3 levels in discriminating patients with threatened abortion and those with normal pregnancies

Ryc. 1. Krzywa ROC stężeń sICAM-1, -2, -3 w surowicy krwi dla kobiet w ciąży fizjologicznej i zagrożonej poronieniem

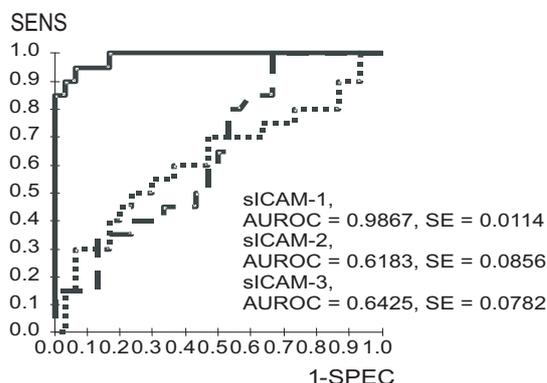


Fig. 2. ROC curve analysis for serum sICAM-1, -2, and -3 levels in discriminating women with miscarriage and those with normal pregnancies

Ryc. 2. Krzywa ROC stężeń sICAM-1, -2, -3 w surowicy krwi dla kobiet w ciąży fizjologicznej i zakończonej poronieniem

gnancies did not correlate with gestational age (between 8 and 12 pregnancy weeks) and were also similar to those observed by other authors in non-pregnant women [18]. Present findings confirm the results of the Gibson et al. study [18] that serum sICAM-1 concentrations did not differ between 8 and 30 weeks of pregnancy. However, the aim of present study was not to explain the exact role of soluble ICAM molecules in the pathophysiology of reproductive failures, but to determine their po-

tential prognostic significance in the evaluation of early pregnancy loss. The authors wanted to compare serum sICAM-1, -2, and -3 levels in the first pregnancy trimester of women with threatened abortion whose pregnancies ended in successful delivery and those whose pregnancies did not.

The authors found higher mean serum levels of ICAM-1 in women with miscarriages compared with patients with threatened abortion but with successful outcome. Therefore the authors hypothesize that serum sICAM-1 concentrations may be used as a prognostic marker for further pregnancy outcome. The prognostic significance of sICAM concentrations was tested with ROC curve construction and analysis. Different cut-off values were used for individual sICAMs to determine the exact specificity and sensitivity. ROC curve analysis showed that only sICAM concentrations might be of prognostic value. The value of 318 ng, with 93.3% specificity, 80% sensitivity, and an area under the ROC curve value of 0.92, was found to be the best discriminating factor between miscarried and successful pregnancies. Sensitivity of 95%, specificity of 93.3%, and an area under the ROC curve value of 0.99 were determined for this liminal value. Analysis of the area under ROC curves showed no prognostic value for sICAM-2 and sICAM-3 concentrations. The degree of placental ablation and time of fetus death was different in individual cases. Therefore, interpretation of the differences between sICAM-2 and sICAM-3 levels in this non-homogenous group of patients was difficult.

In planning present study the authors suspected that the degree of proteolysis would be different for the various types of intercellular adhesion molecules. However, it is surprising that abortion is connected with increases only in the sICAM-1 serum fraction. Further studies are needed to explain the exact role of sICAM-1 in the pathophysiology of early pregnancy loss. Since the authors did not evaluate the concentrations of sICAMs in non-pregnant women, the authors cannot compare their results with data from the Jassoni et al. study [19]. They did not observe any statistically significant difference in serum sICAM-1 levels between pregnant and non-pregnant women. Therefore, further prospective studies are needed to estimate the values of sICAM-1, -2, and -3 levels as prognostic factors for pregnancy outcome.

References

- [1] Enders AV, Schlafke S, Welsh AO: Trophoblastic and uterine luminal epithelial surfaces at the time of blastocyst adhesion in rat. *Am J Anat* 1980, 159, 59–72.
- [2] Blobel CP, White JM: Structure, function and evolutionary relationship of proteins containing a disintegrin domain. *Curr Opin Cell Biol* 1992, 4, 760–765.

- [3] **Yoshinaga K:** Cellular and molecular events surrounding blastocyst implantation: report on NICHD meeting (Bethesda, MD, USA, 15–16 November 1999). *Trends Endocrinol Metab* 2000, 11, 116–118.
- [4] **Damsky CH, Librach C, Lim K, Fitzgerald ML, McMaster MT, Janatpour M, Zhou Y, Logan SK, Fisher SJ:** Integrin switching regulates normal trophoblast invasion. *Development* 1994, 120, 3657–3666.
- [5] **Xiao J, Garcia-Lloret M, Winkler-Lowen B, Miller R, Simpson K, Guilbert LJ:** ICAM-1-mediated adhesion of peripheral blood monocytes to the maternal surface of placental syncytiotrophoblasts: implication for placental villitis. *Am J Pathol* 1997, 150, 1845–1860.
- [6] **Thomson AJ, Greer MR, Young A, Boswell F, Telfer JF, Cameron IT, Norman JE, Campbell S:** Expression of intercellular adhesion molecules ICAM-1 and ICAM-2 in human endometrium: regulation by interferon-gamma. *Mol Hum Reprod* 1999, 5, 64–70.
- [7] **Gahmberg CG, Valmu L, Tian L, Kotovuori P, Fagerholm S, Kotovuori A, Kantor C, Hilden T:** Leukocyte adhesion – a fundamental process in leukocyte physiology. *Braz J Med Biol Res* 1999, 32, 511–517.
- [8] **De Fougères AR, Stacker SA, Schwarting R, Springer TA:** Characterisation of ICAM-2 and evidence for a third counter-receptor for LFA-1. *J Exp Med* 1991, 174, 253–267.
- [9] **Lisby S, Ralfkiaer E, Rothlein R, Vejlsgaard GL:** Intercellular adhesion molecule-1 (ICAM-1) expression correlated to inflammation. *Br J Dermatol* 1989, 120, 479–484.
- [10] **Meyer DM, Dustin ML, Carron CP:** Characterisation of intercellular adhesion molecule-1 ectodomain (sICAM-1) as an inhibitor of lymphocyte function-associated molecule-1 interaction with ICAM-1. *J Immunol* 1995, 155, 3578–3584.
- [11] **Whalen MJ, Doughty LA, Carlos TM, Wisniewski SR, Kochanek PM, Carcillo JA:** Intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 are increased in the plasma of children with sepsis-induced multiple organ failure. *Crit Care Med* 2000, 28, 2600–2607.
- [12] **Schust DJ, Hill JA:** Correlation of serum cytokine and adhesion molecule determinations with pregnancy outcome. *J Soc Gynecol Invest* 1996, 3, 259–261.
- [13] **Gaffuri B, Airoidi L, Di Blasio AM, Vigano P, Miragoli AM, Santorsola R, Vignali M:** Unexplained habitual abortion is associated with a reduced endometrial release of soluble intercellular adhesion molecule-1 in luteal phase of the cycle. *Eur J Endocrinol* 2000, 142, 477–480.
- [14] **Coumans ABC, Huijgens PC, Jakobs C et al.:** Haemostatic and metabolic abnormalities in women with unexplained recurrent abortion. *Hum Reprod* 1999, 14, 211–214.
- [15] **Reindollar RH:** Contemporary issues for spontaneous abortion. Does recurrent abortion exist? *Obstet Gynecol* 2000, 27, 541–554.
- [16] **Scroggins KM, Smucker WD, Krishen AE:** Spontaneous pregnancy loss: evaluation, management, and follow-up counseling. *Prim Care* 2000, 27, 153–167.
- [17] **Arck P, Dietl J, Clark D:** From the decidual cell internet: trophoblast-recognizing T cells. *Biol Reprod* 1999, 60, 227–233.
- [18] **Gibson JL, Lyall F, Boswell F, Young A, Maccuish AC, Greer JA:** Circulating cell adhesion molecule concentrations in diabetic women during pregnancy. *Obstet Gynecol* 1997, 90, 874–879.
- [19] **Jassoni VM, Buemi M, D’Anna R, Ruello A, Scilipoti A, Leonardi J:** Lack of endogenous estrogens effects on circulating adhesion molecule ICAM-1. *J Endocrinol Invest* 1997, 20, 61–622.

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