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THE ASSOCIATION BETWEEN SERUM GALECTIN-3 LEVEL AND ITS PLACENTAL PRODUCTION IN PATIENTS WITH PREECLAMPSIA

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Galectin-3 is β-galactoside-binding lectin, used in cardiology as a biomarker of heart failure. Available research suggest galectin-3 may play a role in the development of preeclampsia. Seventy seven women were included in the study: 39 with preeclampsia and 38 with uncomplicated pregnancy. Patients underwent blood sample analysis (galectin-3, N-terminal pro-brain natriuretic peptide (NT-proBNP), soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), cystatin C, creatinine) and echocardiographic examination. After delivery, placental tissue samples were obtained for immunohistochemistry evaluation. In patients with preeclampsia, serum galectin-3 levels (11.8 versus 9.5 ng/ml; $p = 0.004$) and galectin-3 expression in placental tissue (immunoreactive score (IRS) in extravillous trophoblasts: 9 versus 5; $p = 0.002$; in syncytiotrophoblasts: 6 versus 2, $p < 0.001$) were significantly higher than in the control group. Serum NT-proBNP and sFlt-1 levels, sFlt-1/PlGF ratio, serum creatinine and cystatin C levels were significantly higher, whereas serum PlGF levels and estimated glomerular filtration rate (eGFR) were significantly lower in preeclamptic patients than in uncomplicated pregnancy. On echocardiography, preeclamptic women had significantly greater thickness of interventricular septum (IVS) and left ventricle posterior wall (PW) and significantly worse left ventricle diastolic function (higher E/e' values). Serum galectin-3 level did not correlate with any other biochemical parameters, as well as the vast majority of echocardiographic parameters. Significant correlation between serum galectin-3 and its placental expression in syncytiotrophoblasts (STB) was revealed. Renal function parameters and NT-proBNP correlated with antiangiogenic state. This study demonstrated increased serum galectin-3 levels and placental galectin-3 production in preeclamptic patients, in comparison to women with uncomplicated pregnancy. Myocardial dysfunction and worse renal function parameters in patients with preeclampsia were not related to galectin-3. The main source of galectin-3 in maternal blood was its placental production. In the development of preeclampsia, galectin-3 may act as a compensatory mechanism to impaired placentation in early pregnancy.

Key words: *galectin-3, preeclampsia, placenta, myocardial dysfunction, echocardiography, N-terminal pro-brain natriuretic peptide, placental growth factor, uncomplicated pregnancy*

INTRODUCTION

Galectins are known as a family of β-galactoside-binding lectins. At least 15 different galectins have been identified thus far, and all contain carbohydrate-recognition domains (CRDs) in charge of carbohydrate binding. Due to their structure, galectins are classified into three groups: 1) prototype galectins with a single CRD (galectins -1, -2, -5, -7, -10, -11, -13 and -14); 2) tandem repeat galectins with two distinct CRDs (galectins -4, -6, -8, -9 and -12); and 3) chimeric galectins with the presence of an N-terminal domain adjacent to the CRD (galectin-3) (1-3).

Galectin-3 is expressed in human tissues, including epithelial, endothelial, and all types of immune cells, and it serves important functions in numerous biological processes, such as inflammatory response and fibrosis, intercellular adhesion, angiogenesis, cell differentiation and apoptosis (4). Galectin-3 overexpression is associated with myocardial fibrosis and leads to cardiac dysfunction and development of heart failure (HF) (5, 6). Thus, galectin-3 is widely used in cardiology, primarily as a biomarker of acute and chronic HF, both with preserved or reduced ejection fraction (EF). It is also related to myocardial remodelling in arterial hypertension, different types of cardiomyopathy, atrial fibrillation and risk of death from cardiovascular disease. Galectin-3 may be used in the prediction of renal function in the general population and, as well in the diagnosis of chronic kidney disease (CKD) (2, 7-9). Additionally, galectin-3 and galectin-9 play important roles in the pathogenesis of chronic inflammatory bowel disease (10).

The role of galectin-3 during pregnancy has not been well explained. It is expressed on the surface of trophoblast cells and its distribution in normal trophoblasts, as well as in malignant trophoblasts in gestational trophoblastic disease, is well known (11-13). Experimental research of human placental cell line BeWo confirmed galectin-3 as one of the hypoxia-induced

factors (14). Kolla *et al*. performed proteomic analysis of blood samples obtained from patients with high risk of developing preeclampsia during the first trimester and identified 10 proteins up-regulated in women who developed preeclampsia later in pregnancy, in comparison to those with uncomplicated pregnancies. One of these proteins was galectin-3 binding protein (15). Sattar *et al*. demonstrated elevated serum galectin-3 levels in patients with preeclampsia that correlated with insulin resistance and dyslipidaemia (16). On the contrary, Nikolov *et al*. revealed no significant differences between serum galectin-3 levels in preeclamptic patients and women with uncomplicated pregnancy (17). Another study revealed significantly increased serum galectin-1 and galectin-3 levels in patients with preterm premature rupture of membranes (pPROM) (18).

There is evidence that other galectins are involved in the development of preeclampsia. Than *et al*. identified a gene cluster on chromosome 19 that expresses a subfamily of galectins, including galectin-13, -14 and -16. These galectins are expressed by the placenta, induce apoptosis of activated T lymphocytes and contribute to a disturbed maternal immune balance in early pregnancy (19). Placental expression of galectin-13 and -14 is down-regulated in preterm preeclampsia associated with fetal growth restriction (FGR) (19, 20). Moreover, galectin-13 is secreted from the STBs into the maternal circulation and decreased galectin-13 blood concentration in the first trimester was found in women who subsequently develop early-onset preeclampsia (3, 21, 22).

According to the Guidelines of European Society of Cardiology (ESC), preeclampsia is classically defined as gestational hypertension with significant proteinuria (> 0.3 g/24 h or albumin-to-creatinine ratio ≥ 30 mg/mmol) (23). In other definitions, *i.e*., the International Society for the Study of Hypertension in Pregnancy (ISSHP) or American College of Gynaecologists and Obstetricians (ACOG) suggest that new-onset proteinuria is not necessary to diagnose preeclampsia; instead, hypertension can be accompanied by one of the following: signs of liver or renal failure, cerebrovascular or cardiovascular incidents and FGR (24, 25). The underlying mechanism for preeclampsia is thought to be impaired placentation due to inadequate trophoblastic invasion of the maternal spiral arteries. Cardiovascular complications and renal injury are often observed in patients with preeclampsia (26).

The aim of the present study was to explain if galectin-3 in women with preeclampsia is a marker of subclinical HF or if its role in the disease is related to placental production of this protein. The study hypothesis was that abnormal serum galectin-3 levels can be detected in the course of preeclampsia and that these levels most likely result from impaired placentation - a basic process in the pathomechanism of preeclampsia - and not from early myocardial or renal dysfunction.

MATERIALS AND METHODS

Study population and protocol

In the present case-control study conducted between August 2015 – April 2018, women > 18 years of age in singleton pregnancies admitted to the Department of Gynecology and Obstetrics, Institute of Mother and Child in Warsaw, with the diagnosis of preeclampsia, as defined by the 2011 ESC Guidelines (27), were included. The results obtained in the group of preeclamptic patients were compared to those obtained in healthy pregnant volunteers. Gestational age < 22 weeks, multiple pregnancies, history of CKD, antiphospholipid syndrome, congenital and acquired heart defects, congenital or acquired coagulopathies (haemorrhagic diathesis or thrombophilia), pregestational diabetes and symptoms of infectious diseases (including suspected chorioamnionitis) were exclusion criteria for both groups. Both preeclamptic patients and healthy controls underwent blood sample biochemical analysis and echocardiographic examination. After delivery, placental tissue samples were obtained for immunohistochemistry evaluation. The definition of HELLP syndrome was elevated liver enzymes (ASPAT > 70 U/l), haemolysis (LDH > 600 U/l) and low platelets (< 100,000/a). FGR was defined as an estimated intrauterine weight below the 10^{th} percentile after gestational age had been confirmed by an first trimester ultrasound. Early- and late-onset preeclampsia were defined as ≤ 34 weeks and > 34 weeks, respectively. Body mass index (BMI) was calculated on the day of inclusion to the study.

The study was approved by the Local Bioethics Committee at the Institute of Mother and Child, and written informed consent was obtained from all participants. The study was performed in accordance with the guidelines described in the Declaration of Helsinki (28).

Blood sample preparation and biochemical analysis

Whole blood samples were collected on the day of patient inclusion to the study, then were centrifugated at 25°C for 20 min at 2000 × g and obtained sera were stored at –80°C until further analysis.

Serum galectin-3 levels were assessed using automated enzyme-linked fluorescent assay (VIDAS® Galectin-3, BioMerieux, France). Serum NT-proBNP levels were assessed using automated electrochemiluminescence method (Elecsys® NT-proBNP, Roche Diagnostics, Germany). Serum sFlt-1 and PlGF levels were assessed using fully automated immunoassays (Elecsys® sFlt-1 and Elecsys® PlGF, Roche Diagnostics, Germany) and then, based on the obtained values, sFlt-1/PlGF ratio was calculated. Serum creatinine levels were assessed using automated kinetic colorimetric assay based on the Jaffe method in alkaline solution, with picrate (Creatinine Jaffe Gen.2®, Roche Diagnostics, Germany). Serum cystatin C levels were assessed using automated particle enhanced immunoturbidimetric assay (Tina-quant Cystatin C®, Roche Diagnostics, Germany). Estimated glomerular filtration rate (eGFR) was calculated using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation: $GFR = 141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1,209} \times 0.993^{age} \times 1.018$ (if woman) × 1.159 (if black race) where: Scr is a serum creatinine level, α is –0.329 for women and –0.411 for men, κ is 0.7 for women and 0.9 for men.

Echocardiographic examination

All patients underwent standard two-dimensional and Doppler transthoracic echocardiography performed according to the guidelines of European Association of Cardiovascular Imaging and American Society of Echocardiography within at most seven days after inclusion to the study.

Using M-mode in the parasternal long-axis view, we measured left ventricle (LV) end-diastolic and end-systolic diameters (LVDD and LVSD, respectively), thickness of the interventricular septum in systole and diastole (IVSs and IVSd, respectively), thickness of the posterior wall in systole and diastole (PWs and PWd, respectively) and, also, left atrium and ascending aorta width (LA and AO, respectively). In the short-axis view, we measured width of pulmonary trunk and right ventricle (PP and RV, respectively).

Left ventricle systolic function was assessed using EF and values > 54% were considered normal for women of reproductive age. Right ventricle systolic function was assessed using the following parameters: tricuspid annular plane systolic

excursion (TAPSE) and tricuspid lateral annular systolic velocity (S'). Left ventricle diastolic function was assessed using the following parameters: E/A, the ratio of peak velocity blood flow from left ventricular relaxation in early diastole (the E wave) to peak velocity flow in late diastole caused by atrial contraction (the A wave), and E/e', the ratio between early mitral inflow velocity and mitral annular early diastolic velocity.

Placental tissue examination

Placental tissues were collected from 67 women immediately after delivery. Samples were fixed in 4% neutral buffered formalin, paraffin-embedded and sectioned (4 μm). The immunohistochemistry protocol included: pre-treatment in High Grade TRS in 96°C for 20 minutes, incubation with galectin-3 (9C4) Mouse Monoclonal Antibody by Cell-Marque (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) in 37°C for 16 minutes and then incubation in Flex liner (DACO) for 10 minutes and in Flex Polymer (DACO) for 20 minutes. Staining visualisation was gained using DAB (DACO). Positive and negative controls were also performed. Human colon mucosa and thyroid papillary cancer samples, which are known to be positive for galectin-3, were used as positive control for galectin-3 expression.

The intensity and distribution patterns of the staining reaction were evaluated by an experienced pathologist using the semi-quantitative IRS. The IRS score was calculated by multiplication of optical staining intensity (0: no, 1: weak, 2: moderate, 3: strong staining) and the percentage of positively stained cells (0: no staining, 1: < 10%, 2: 10 – 50%, 3: 51 – 80%, 4: > 80%). The pathologist was blinded to any clinical data, including the attachment of the patient to preeclampsia or control group. Galectin-3 expression in placental tissue was evaluated separately for extravillous trophoblasts (EVT), STBs and placental stroma.

Statistical analysis

All statistical analyses were carried out using IBM-SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA). For variables with normal distribution, average values were compared using the t-student's test, whereas for variables with skewed distributions, significance was assessed with the Mann-Whitney U test. For categorised data, between-group comparisons were performed using chi-square test or Fisher's exact test. For more than two group comparisons, variance analysis or Kruskal-Wallis test were carried out. Where appropriate, Pearson or Spearman correlation coefficients were calculated to evaluate the relationships between biochemical and echocardiographic parameters and the expression of galectin-3 in placental tissue. In the analysis of data from groups paired by duration of pregnancy, Wilcoxon's test (for continuous variables) or McNemar's test (for dichotomous variables) were used. $P \leq 0.05$ was considered to indicate a statistically significant difference.

RESULTS

In total, 77 patients were included in the study: 39 with preeclampsia and 38 with uncomplicated pregnancies. The demographic and clinical characteristics of the study participants are presented in *Table 1*.

Serum galectin-3 and other biochemical parameters

Serum galectin-3 levels were significantly higher in patients with preeclampsia than in those with uncomplicated pregnancy (11.8 versus 9.5 ng/ml; p = 0.004). Significant between-group differences were also found in all other studied biochemical parameters. Serum NT-proBNP and sFlt-1 levels, sFlt-1/PlGF ratio, serum creatinine and cystatin C levels were significantly higher, whereas serum PlGF levels and eGFR were significantly lower in preeclamptic patients than in the control group (*Fig. 1a*, *Table 2*).

Echocardiographic parameters

When compared to the control group, patients with preeclampsia presented some abnormalities in myocardial structure and left ventricle diastolic function. They had significantly greater thickness of interventricular septum (IVSs: mean ± standard deviation, 13.8 ± 2.14 mm versus 12.6 ± 2.01 mm; p = 0.034; IVSd; 10.19 ± 1.92 mm versus 8.9 ± 1.58 mm; p = 0.008) and left ventricle posterior wall in systole and diastole (PWs: 14.31 ± 2.24 mm versus 12.93 ± 1.53 mm; p = 0.013; PWd: 9.89 ± 1.4 mm versus 8.63 ± 1.19 mm; p = 0.002). Left ventricle systolic function was preserved and there were no differences between the groups in EF (*Fig. 1b*). Left ventricle diastolic function was significantly worse in preeclamptic women than in the control group and it manifested as higher values of E/e' (9.58 ± 2.37 versus 7.59 ± 2.6; p = 0.042). Among indicators of right ventricle systolic function, S' was also significantly higher in patients with preeclampsia than in uncomplicated pregnancy (15.3 ± 3.77 cm/s versus 12.7 ± 1.92 cm/s; p = 0.003). Other studied echocardiographic parameters did not differ between the groups (*Fig. 1b*).

Galectin-3 expression in placental tissue

Significantly higher galectin-3 expression in placental tissue was revealed in preeclamptic patients, in comparison to uncomplicated pregnancy, both in EVT and STB. In EVT, galectin-3 was expressed most strongly, with median IRS scores of 9 in the preeclampsia group and 5 in the control group (p = 0.002). In STB, the median IRS score in preeclamptic patients was 6, and 2 in uncomplicated pregnancy patients (p < 0.001). There were no between-groups differences in galectin-3 expression in placental stroma, where median IRS score was 1 for both groups (p = 0.217; *Fig. 2*).

Correlation between studied parameters and gestational age

In patients with preeclampsia, no correlation was found between serum galectin-3 level and gestational age (r = –0.227; p = 0.164), whereas in healthy controls, on the contrary, serum galectin-3 level was related to gestational age and it was a negative correlation (r = –0.320; p = 0.050). In preeclamptic patients, we found significant negative relationships between gestational age and serum NT-proBNP level (r = –0.328; p = 0.041), serum sFlt-1 level (r = –0.447; p = 0.005) and sFlt-1/PlGF ratio (r = –0.548; p < 0.001) and a positive correlation between gestational age and serum PlGF level (r = 0.509; p = 0.001). Similar correlations were not found in the control group (*Fig. 3*). In both groups, renal function parameters did not correlate with gestational age, except for a significant relationship with eGFR in the preeclampsia group (*Fig. 3*). Among echocardiographic parameters, only in the control group, significant correlation was found between gestational age and EF (r = 0.363; p = 0.049), E/e' (r = 0.497; p = 0.019), LA (r = 0.468; p = 0.009) and RV (r = 0.398; p = 0.029). Galectin-3 expression in placental tissue in both groups did not correlate with gestational age.

Due to the significant difference between groups in gestational age at study enrollment (almost three weeks), we

Table 1. Demographic and clinical characteristic of study participants.

	Preeclampsia group (n = 39)	**Control group** (n = 38)	**p**
Maternal age (years)	32.974 ± 4.49	30.895 ± 5.44	0.071
Gestational age at enrollment (weeks)	33.718 ± 3.46	36.882 ± 2.11	**< 0.001**
Nulliparity	26 (66.7%)	21 (55.3%)	0.69
Maternal medical history: Hypothyroidism Other concomitant disease*	 7 (17.9%) 8 (20.5%)	 9 (23.7%) 4 (10.5%)	 0.535 0.347
BMI (kg/m^2)	31.927 ± 6.85	27.893 ± 4.03	**0.002**
PREGNANCY			
Pregnancy after IVF	2 (5.1%)	2 (5.3%)	1.0
FGR	15 (38.5%)	1 (2.6%)	**< 0.001**
HELLP syndrome	6 (15.4%)	0	**0.025**
Oligohydramnion	3 (7.7%)	2 (5.3%)	1.0
Polyhydramnion	0	1 (2.6%)	0.494
Placental abruption	1 (2.6%)	0	1.0
Gestational diabetes	8 (20.5%)	7 (18.4%)	0.817
Premature rupture of membranes	0	1(2.6%)	0.494
Fetal congenital anomalies	3 (7.7%)	2(5.3%)	1.0
LABOUR			
Gestational age at delivery (weeks)	35.28 ± 3.48	39.08 ± 1.12	**< 0.001**
Preterm birth	15 (38.5%)	1 (2.6%)	**< 0.001**
Cesarean section	32 (82.1%)	9 (23.7%)	**< 0.001**
NEONATAL OUTCOME			
Birth weight (g)	2344.1 ± 900.86	3377.24 ± 448.45	**< 0.001**
Birth weight percentile	31.18 ± 27.5	53.95 ± 27.12	**0.001**
Apgar score in 1st minute: < 4 pkt 4 – 6 pkt ≥ 7 pkt	 2 (5.1%) 4 (10.3%) 33 (84.6%)	 0 1 (2.6%) 37 (97.4%)	 0.123
Apgar score in 5th minute: < 4 pkt 4 – 6 pkt ≥ 7 pkt	 0 1 (2.6%) 38 (97.4%)	 0 0 38 (100%)	 1.0
Admission to ICU	8 (20.5%)	0	**0.005**
Death	1 (2.6%)	0	1.0

BMI, body mass index; FGR, fetal growth restriction; ICU, intensive care unit; IVF, *in vitro* fertilization.
* other concomitant diseases in patients included to the study were: asthma. sarcoidosis. ulcerative colitis. juvenile arthritis. rheumatoid arthritis. methylene tetrahydrofolate reductase (MTHFR) mutation

performed additional statistical analysis, pairing patients from both groups by pregnancy duration on the day of inclusion to the study. This analysis confirmed the primary obtained results: higher serum levels of galectin-3, NT-proBNP and sFlt-1, higher values of sFlt-1/PlGF ratio, lower serum levels of PlGF, greater thickness of PW and higher values of S' in echocardiography and also higher galectin-3 expression in placental tissue in women with preeclampsia compared to healthy controls. These findings allowed the exclusion of the possible influence of differences in gestational age on the obtained results at study enrollment.

Correlation between serum galectin-3 level and myocardial and renal function

In both groups, serum galectin-3 level did not correlate with any other biochemical parameter and had only negative correlation with PWd in preeclamptic women (r = –0.404; p = 0.040).

Correlation between serum galectin-3 level and its expression in placental tissue

In preeclamptic patients, a significant correlation between serum galectin-3 and its placental expression was revealed. This relationship was found with respect to STB (r = 0.407; p = 0.023). Similar correlations were not found in the control group (*Fig. 4*).

Additionally, in preeclamptic women placental galectin-3 expression in EVT was related to serum sFlt-1 level (r_s = 0.425; p = 0.017), whereas galectin-3 expression in STB was negatively correlated with serum NT-proBNP level (r_s = –0.359, p = 0.048).

Table 2. Biochemical parameters in preeclampsia versus control group.

Group	Galectin-3 ng/ml	NT-proBNP pg/ml	PLGF pg/ml	sFlt-1 pg/ml	sFlt-1/PlGF ratio	Cystatin C mg/l	Creatinine µmol/l	eGFR ml/min/1.73 ^{2}
Preeclampsia:								
N	39	39	38	38	38	39	39	39
Mean	11.838	367.66	90.442	11501.73	247.491	1.363	58.026	113.57
SD	3.352	639.761	70.883	6408.514	292.345	0.288	13.517	16.571
Median	11.8	147.7	65.1	10796.5	129.4	1.33	55.0	119.2
Minimum	5.1	5.0	7.2	2140.0	7.5	0.79	30.0	69.9
Maximum	19.4	2991.0	375.1	29506.0	1303.3	2.2	91.0	146.5
Control:								
N	38	37	35	35	35	36	36	36
Mean	9.837	70.926	326.651	3813.571	25.537	1.032	47.222	127.15
SD	4.139	64.343	456.525	1672.06	24.003	0.162	6.808	8.297
Median	9.500	52.48	176.5	3645.0	19.0	0.985	48.5	124.7
Minimum	3.3	5.0	72.0	1460.0	0.7	0.78	29.0	115.8
Maximum	29.0	282.5	2765.0	7570.0	94.0	1.44	58.0	145.0
p	**0.004**	**0.009**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

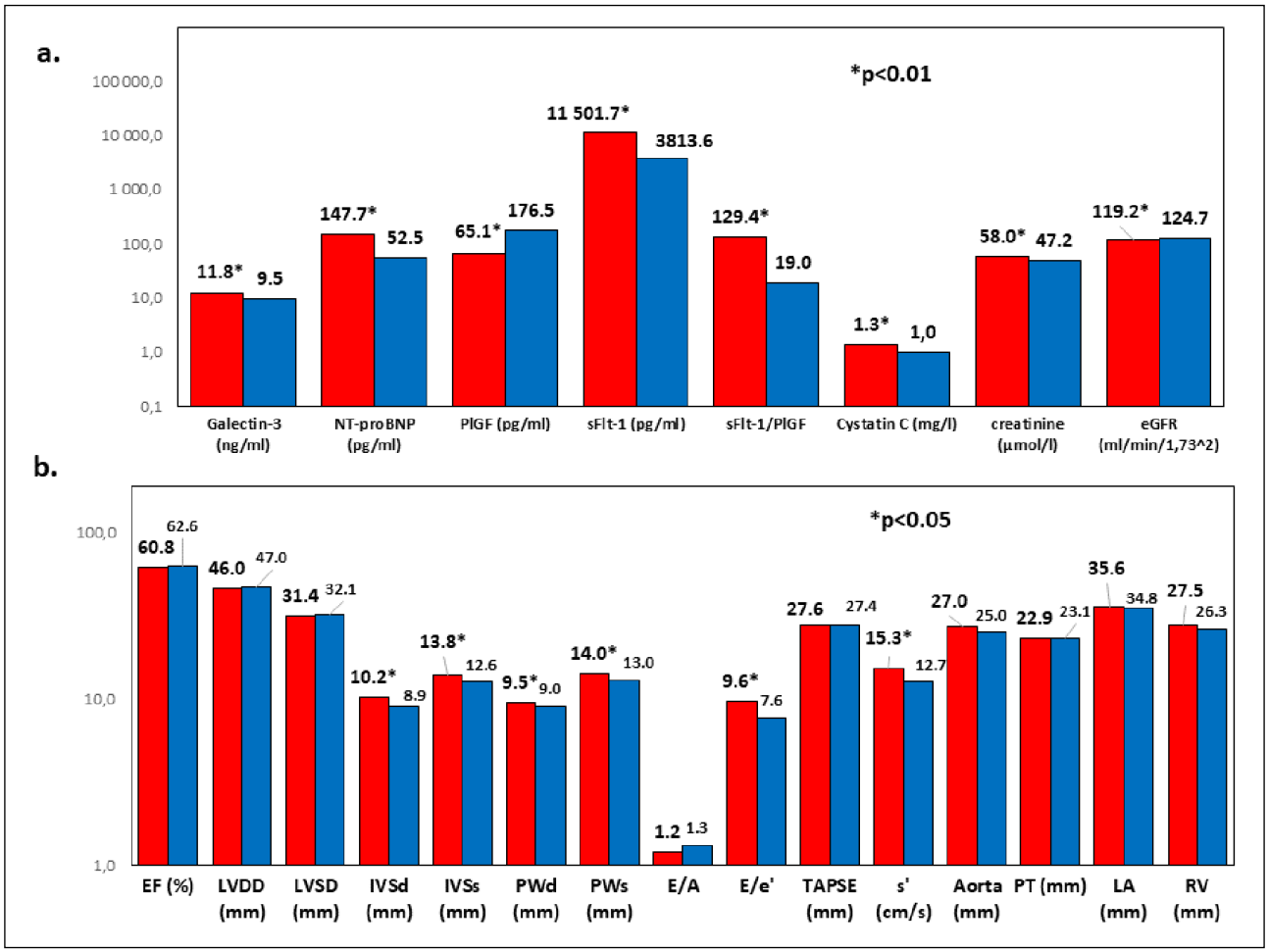

Fig. 1. Between-group differences in biochemical parameters (*1a*) and echocardiography (*1b*).
Abbreviations: E/A, peak velocity of early diastolic transmitral flow to late transmitral flow; E/e', peak velocity of early diastolic transmitral flow to peak velocity of early diastolic mitral annular motion; EF, ejection fraction; IVSd, interventricular septum in diastole; IVSs, interventricular septum in systole; LA, left atrium; LVDD, left ventricle end-diastolic dimension; LVSD, left ventricle end-systolic dimension; PT, pulmonary trunk; PWd, left ventricle posterior wall in diastole; PWs, left ventricle posterior wall in systole; RV, right ventricle; s', tricuspid lateral annular systolic velocity; TAPSE, tricuspid annular plane systolic excursion.

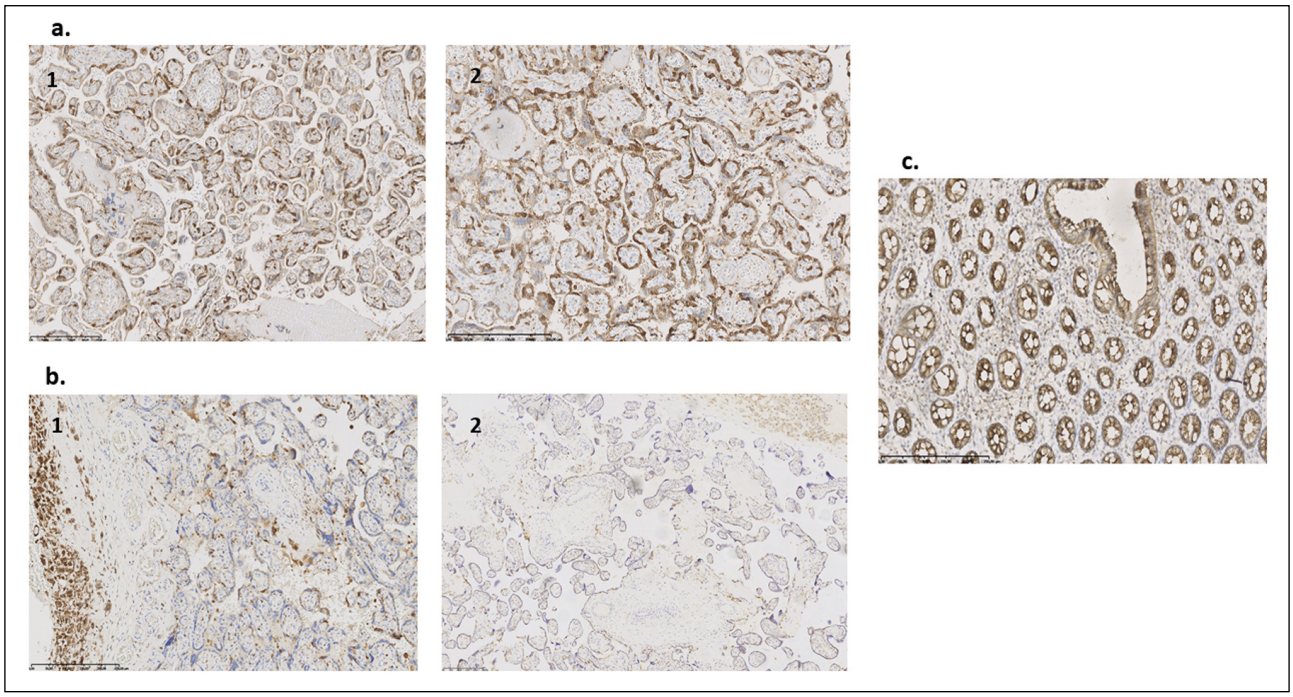

Fig. 2. Placental tissue immunohistochemistry galectin-3 specific staining - examples. Placental tissue samples (full thickness, approx. 2 × 2cm) were obtained from central part of the placenta after delivery:
(*a*) preeclampsia group, (*a1*): (patient no. 51) - EVT percentage of positive stained cells 4, staining intensity 3, IRS score 12, STB percentage of positive stained cells 4, staining intensity 3, IRS score 12; stroma percentage of positive stained cells 1, staining intensity 2, IRS score 2; (*a2*): (patient no. 59) - EVT percentage of positive stained cells 3, staining intensity 3, IRS score 9, STB percentage of positive stained cells 4, staining intensity 3, IRS score 12; stroma percentage of positive stained cells 1, staining intensity 3, IRS score 3; (*b*) control group, (*b1*): (patient no. 16) - EVT percentage of positive stained cells 2, staining intensity 2, IRS score 4, STB percentage of positive stained cells 1, staining intensity 2, IRS score 2, stroma percentage of positive stained cells 1, staining intensity 1, IRS score 1; (*b2*): (patient no. 23) - EVT percentage of positive stained cells 2, staining intensity 2, IRS score 4, STB percentage of positive stained cells 1, staining intensity 1, IRS score 1, stroma percentage of positive stained cells 1, staining intensity 1, IRS score 1.
(*c*) positive control in human colon mucosa.

Correlation between myocardial and renal function and antiangiogenic state

In the preeclampsia group, we found significant correlations between serum NT-proBNP level and serum PlGF level (r = –0.372; p = 0.021), serum sFlt-1 level (r = 0.426; p = 0.008), as well as sFlt-1/PlGF ratio (r = 0.353; p = 0.030). No similar relationships were revealed in the control group. Nevertheless, in healthy controls, we found significant relationships between some echocardiographic parameters and the antiangiogenic state: PWs correlated with serum sFlt-1 level (r = 0.443; p = 0.021), E/e' correlated with serum sFlt-1 level (r = 0.582; p = 0.007) and sFlt-1/PlGF ratio (r = 0.505; p = 0.023).

In both groups, significant relationships between studied renal function parameters and the antiangiogenic state was revealed. In preeclamptic patients, serum creatinine level and eGFR correlated with serum sFlt-1 level (r = 0.499; p = 0.001 and r = –0.386; p = 0.017, respectively), whereas in uncomplicated pregnancy patients, serum creatinine and cystatin C levels correlated with serum sFlt-1 (r = 0.510; p = 0.002 and r = 0.362; p = 0.033, respectively), as well as serum creatinine and cystatin C levels and eGFR with sFlt-1/PlGF ratio (r = 0.418; p = 0.012 and r = 0.420; p = 0.012 and r = –0.396; p = 0.019, respectively).

Comparison between early- and late-onset preeclampsia

Patients with preeclampsia were divided into two subgroups: early-onset disease (20 patients) and late-onset disease (19 patients). Serum galectin-3 levels did not differ between the subgroups (12.9 ng/ml versus 11.61 ± 2.52 ng/ml; p = 0.728). However, serum NT-proBNP and sFlt-1 levels, as well as sFlt-1/PlGF ratio, were significantly higher (472.29 ± 601.46 pg/ml versus 257.52 ± 676.2 pg/ml; p = 0.011; 13908.55 ± 7199.76 pg/ml versus 8827.5 ± 4130 pg/ml; p = 0.033; 365.98 ± 357.71 versus 115.84 ± 90.2, p = 0.009, respectively) and serum PlGF levels were significantly lower (78.9 ± 81.36 pg/ml versus 103.26 ± 56.63 pg/ml; p = 0.022) in early-onset preeclampsia (*Fig. 5*, *Table 3*). We found also no differences between early- and late-onset disease in renal function, echocardiographic parameters and placental galectin-3 expression.

Separately, we revealed an interesting observation after the extraction in patients with HELLP syndrome (six women). Galectin-3 expression in EVT varied between the groups and was undermost in women with HELLP syndrome (*Table 4*).

DISCUSSION

Galectin-3 has been previously studied primarily as a marker of HF. The presented study is one of only a few available research analyses evaluating the role of galectin-3 during pregnancy, especially complicated by preeclampsia. To our best knowledge, this is the first study evaluating serum galectin-3 levels in preeclamptic women, in relation to myocardial and renal function, antiangiogenic state and placental production of galectin-3. The results obtained from this research fully

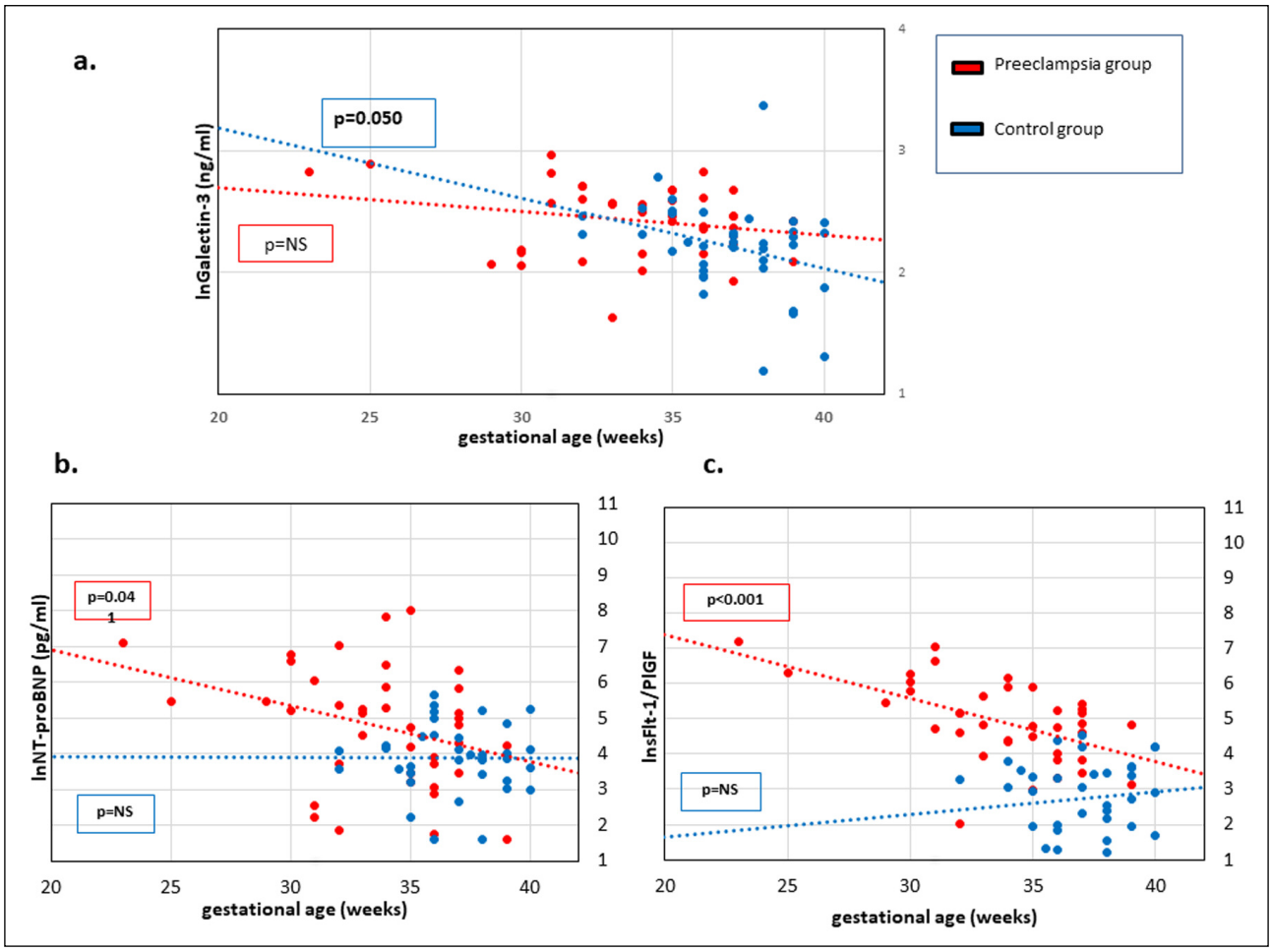

Fig. 3. Correlation between selected biochemical parameters and gestational age in preeclampsia vs control group: (*a*) galectin-3, (*b*) NT-proBNP and (*c*) sFlt-1/PlGF.

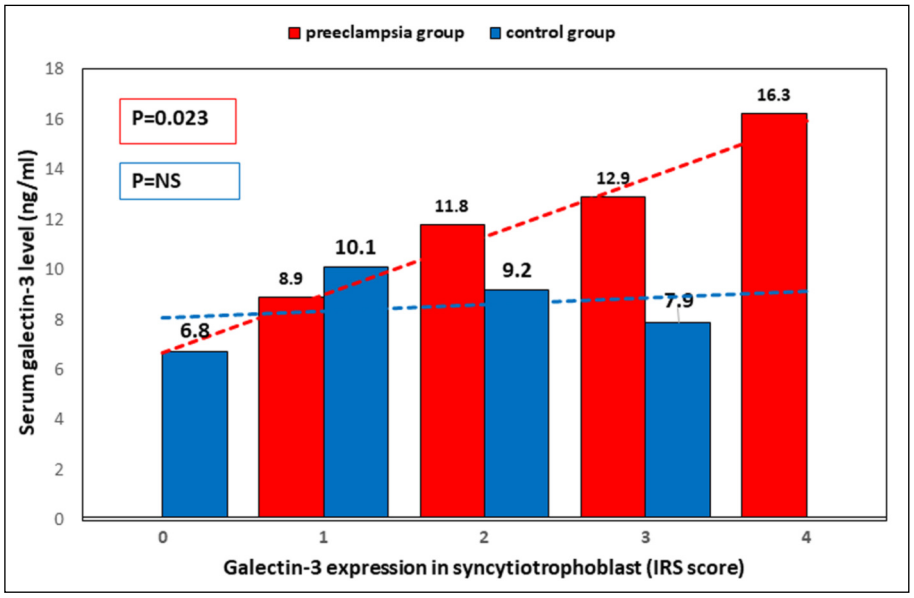

Fig. 4. Correlation between serum galectin-3 level and its expression in syncytiotrophoblast.

confirmed the study hypothesis and provides a starting point for deliberations on the potential role of galectin-3 in the development of preeclampsia.

In our study, serum galectin-3 levels were significantly higher in preeclamptic patients than in uncomplicated pregnancy. So far, there have only been a few studies assessing serum galectin-3 in preeclamptic patients. Sattar *et al*. demonstrated significantly higher serum galectin-3 levels in patients with preeclampsia, in comparison to women with uncomplicated pregnancy. In their study, elevated serum galectin-3 levels in patients with

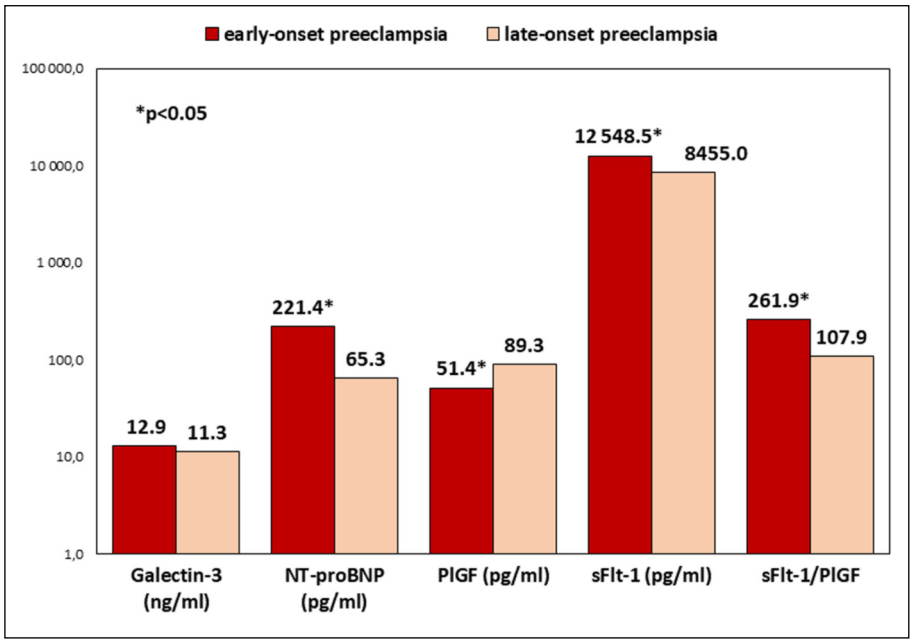

Fig. 5. Selected biochemical parameters in patients with early-onset and late-onset preeclampsia.

Table 3. Biochemical parameters in early- and late-onset preeclampsia.

	Galectin-3 ng/ml	**NT-proBNP** pg/ml	**PLGF** pg/ml	**sFlt-1** pg/ml	**sFlt-1/PlGF** ratio	**Cystatin C** mg/l	**Creatinine** µmol/l	**eGFR** ml/min/1,73 ^{2}
Early-onset preeclampsia								
N	20	20	20	20	20	20	20	20
Mean	12.055	472.29	78.905	13908.55	365.98	1.39	61.4	110.24
SD	4.04	601.46	81.36	7199.76	357.72	0.36	15.75	19.92
Median	12.9	221.4	51.35	12548.5	261.9	1.39	59.5	117.75
Min	5.1	6.45	7.2	2803.0	7.5	0.79	30.0	69.9
Max	19.4	2515.0	375.1	29506.0	1303.3	2.20	91.0	146.5
Late-onset preeclampsia								
N	19	19	18	18	18	19	19	19
Mean	11.61	257.52	103.26	8827.5	115.84	1.34	54.47	117.08
SD	2.52	676.195	56.63	4130.78	90.17	0.19	9.9	11.63
Median	11.3	65.27	89.25	8455.0	107.9	1.32	54.0	119.2
Min	6.9	5.0	34.9	2140.0	19.8	1.02	41.0	82.7
Max	16.8	2991.0	244.6	15559.0	366.40	1.73	83.0	133.1
p	0.728	**0.011**	**0.022**	**0.033**	**0.009**	0.728	0.079	0.258

preeclampsia correlated with dyslipidaemia and insulin resistance and this finding indicated a potential link between preeclampsia and the risk of metabolic syndrome and cardiovascular diseases in a patient's subsequent life. The second marker investigated in this study was apelin, an adipocyte-specific hormone (16). Recent research indicated that apelin may control placental function by regulating the production of steroid and protein hormones in trophoblast cells (29). In the study performed by Nikolov *et al*. there were no significant differences between serum galectin-3 levels in preeclamptic patients compared to normal pregnant women and the authors concluded that galectin-3 may not be a useful method for prediction of early-onset preeclampsia (17). These results are in contrast with our study, however there were major differences in the study protocols. Our study consisted of both early- and late-onset preeclampsia, and the disease was diagnosed according to ESC guidelines, requiring the presence of proteinuria to confirm the preeclampsia diagnosis and measuring serum galectin-3 levels using standardised, automated enzyme-linked fluorescent assay (VIDAS® Galectin-3, BioMerieux, France). These issues may be of importance in the explanation of the different results of these two studies.

As previously mentioned, galectin-3 is commonly used in cardiology. There is a large body of evidence supporting the role of galectin-3 in myocardial remodelling. Galectin-3 stimulates the release of inflammatory mediators and disrupts the synthesis of different types of collagen in the myocardium, leading to subendocardial fibrosis, diastolic dysfunction and HF (30-32). Noteworthy is that myocardial diastolic dysfunction with subendocardial fibrosis can be observed in preeclamptic women (33-35). Galectin-3 is also strictly related to renal function. Serum galectin-3 levels correlated with elevated creatinine and cystatin C levels and decreased eGFR (36-38).

In the present study, serum galectin-3 levels did not correlate with NT-proBNP or echocardiographic parameters

Table 4. Galectin-3 placental expression in early- and late-onset preeclampsia and HELLP syndrome.

	Extravillous trophoblast			Syncytiotrophoblast		
	% Positive stained cells	**Staining intensity**	**IRS score**	**% Positive stained cells**	**Staining intensity**	**IRS score**
Late-onset preeclampsia (n = 14)						
Mean	3.29	3.00	9.857	2.00	2.71	5.64
SD	0.726	0.000	2.1788	0.679	0.611	2.468
Median	3.00	3.00	9.000	2.00	3.00	6.00
Min	2	3	6	1	1	1
Max	4	3	12	3	3	9
Early-onset preeclampsia without HELLP syndrome (n = 12)						
Mean	2.58	2.58	7.250	2.08	2.67	5.83
SD	1.165	0.669	4.1148	1.165	0.492	3.810
Median	3.00	3.00	9.000	2.00	3.00	5.00
Min	1	1	1	1	2	2
Max	4	3	12	4	3	12
HELLP syndrome (n = 6)						
Mean	2.60	2.60	7.000	2.20	2.80	6.40
SD	0.894	0.548	3.4641	0.837	0.447	2.881
Median	2.00	3.00	6.000	2.00	3.00	6.00
Min	2	2	4	1	2	2
Max	4	3	12	3	3	9
p	0.076	**0.020**	0.057	0.798	0.884	0.788

IRS, immunoreactive score.

(except for correlation with PWd). This implies that despite the discreet abnormalities present on echocardiography and NT-proBNP levels in preeclamptic women, serum galectin-3 was not related to these symptoms. Similarly, serum galectin-3 in our study was not related to renal function parameters, although patients with preeclampsia had significantly worse levels in comparison to patients with uncomplicated pregnancy. This suggests another source of galectin-3 in this group of patients. Moreover, serum galectin-3 levels did not differ between early- and late-onset preeclampsia, which indicates that galectin-3 may play an important role in both types of the disease.

Our study revealed significantly higher placental galectin-3 expression in preeclamptic patients than in uncomplicated pregnancy patients, both in STB and EVT. Galectin-3 placental expression in preeclampsia has thus far been studied in only few studies. Jeschke *et al*. confirmed increased galectin-3 production in EVT in preeclamptic and HELLP placentas in comparison to healthy pregnant women (13). Ruikar *et al*. revealed the increased expression of annexin A1 and galectin-3 in placental tissue of preeclamptic women, suggesting their role in preeclampsia pathophysiology by participating in a systemic inflammatory response (39). In our study, galectin-3 expression in EVT additionally correlated with serum sFlt-1 level. This finding confirms direct relationship between galectin-3 and one of the most important elements in preeclampsia pathophysiology, antiangiogenic state, caused by increased sFlt-1 production. Moreover, this connection applies to the EVT, which is the part of the trophoblast responsible for maternal spiral artery invasion. In turn, galectin-3 expression in STBs in our study correlated with serum galectin-3 level, which implicates the placenta as a source of blood galectin-3. Moreover, this relationship may also indicate the way that galectin-3 is transported from the placenta into maternal circulation. In the course of preeclampsia, excessive release of syncytiotrophoblast microparticles (STBM) can be observed. STBM induce maternal inflammatory response and are one of the factors connecting the two stages of disease (40-42). Evidence exists that sFlt-1 and galectin-13 are transported from placenta into maternal blood with the participation of STBM and in case of galectin-3, this process may be similar (3, 21, 43, 44).

Considering the role of galectin-3 in the development of preeclampsia, we suggest that placental galectin-3 production may serve as a compensatory mechanism for impaired placentation and decreased placental perfusion. There are several arguments for this. First is the relationship between galectin-3 expression in EVT and sFlt-1 serum level, because it shows the direct dependence of galectin-3 placental production on the severity of imbalance between angiogenic and antiangiogenic factors. Additionally, Hu *et al*. in their experimental research on hypoxia-induced responses of human placental cell line BeWo, revealed galectin-3 among 13 proteins with increased production under hypoxia (14). It can therefore be concluded that galectin-3 placental production is an example of a positive stress response. In addition, recent research revealed galectin-3 as a binding partner for endoglin. These proteins may associate with each other in the regulation of endothelial function in vascular-related pathologies (45). Soluble endoglin (sEng) is one of the main antiangiogenic factors involved in preeclampsia and significantly higher serum sEng levels are found in patients with preeclampsia. Gallardo-Vara *et al*. demonstrated that sEng induces the expression of bone morphogenic protein 4 (BMP4) *in vitro* and *in vivo* and suggested that BMP4 is a downstream mediator of sEng (46-48). There is a possibility that galectin-3 may in some way inhibit the antiangiogenic effect of sEng.

Stimulation of angiogenesis and the anti-apoptotic effect, among many biological functions of galectin-3, may play a crucial role in the development of preeclampsia. Galectin-3

stimulates angiogenesis through the vascular endothelial growth factor (VEGF) receptor dependent pathway, which binds VEGF receptor 2 (VEGFR2), prevents its internalisation and increases its sensitivity for VEGF (49, 50). During preeclampsia with significantly reduced VEGF bioavailability, this process may be very important. There is also evidence that galectin-3 located in cellular cytoplasm acts as an anti-apoptotic factor, and decreased galectin-3 expression in trophoblasts in first trimester of pregnancy is related to increased apoptosis in developing placental villi, leading to missed abortion (4, 51). Apoptosis inhibition may be a compensatory mechanism in late-onset preeclampsia, in which the main pathophysiological process is oxidative stress, leading to cellular death not only in the placenta, but also in the endothelium. This process may also be due to a cause of lack of differences in serum galectin-3 levels between patients with early-onset and late-onset disease in our study. Another argument in favor of the compensatory role of galectin-3 during preeclampsia is revealed in our study as a negative correlation between placental galectin-3 expression and the degree of myocardial abnormalities (the higher galectin-3 expression, the lower serum NT-proBNP level and left ventricle walls thickness in echocardiography). The last key finding in our study seems to be the results separately obtained after the extraction patients with HELLP syndrome. In patients with HELLP syndrome, placental galectin-3 expression, in comparison to early-onset preeclampsia without HELLP and late-onset preeclampsia, was undermost. Perhaps, in this case, decreased placental galectin-3 production indicated an inefficient compensation mechanism, leading to the development of the most severe complication, HELLP syndrome. Additionally, Freitag *et al*. demonstrated that galectin-3 deficiency in mouse pregnancy led to compromised placental vascularisation and perfusion, resulting in placental insufficiency and the subsequent development of FGR. Moreover, the development of FGR was accompanied by an altered pattern of circulating galectin-3 levels in humans. These results may also be supportive for our hypothesis (52). Other placental proteins, such as visfatin, may also affect fetal growth (53). The proposed hypothetical model of the compensatory role of galectin-3 during preeclampsia is presented in *Fig. 6*.

The limitations of our study were: lack of information about patients' arterial blood pressure values, pre-pregnancy BMI and the significant difference between the groups in gestational age on enrollment (almost 3 weeks). However, due to the additional statistical analysis resulting from pairing patients from both groups by gestational age on the day of inclusion to the study, we excluded the possible influence of this difference on obtained results. Another limitation of this study may be a lack of serum sEng level measurement, because, as previously mentioned, sEng and galectin-3 recently have been revealed as binding partners and such data could be very supportive in the further explanation of a potential role of galectin-3 in the development of preeclampsia.

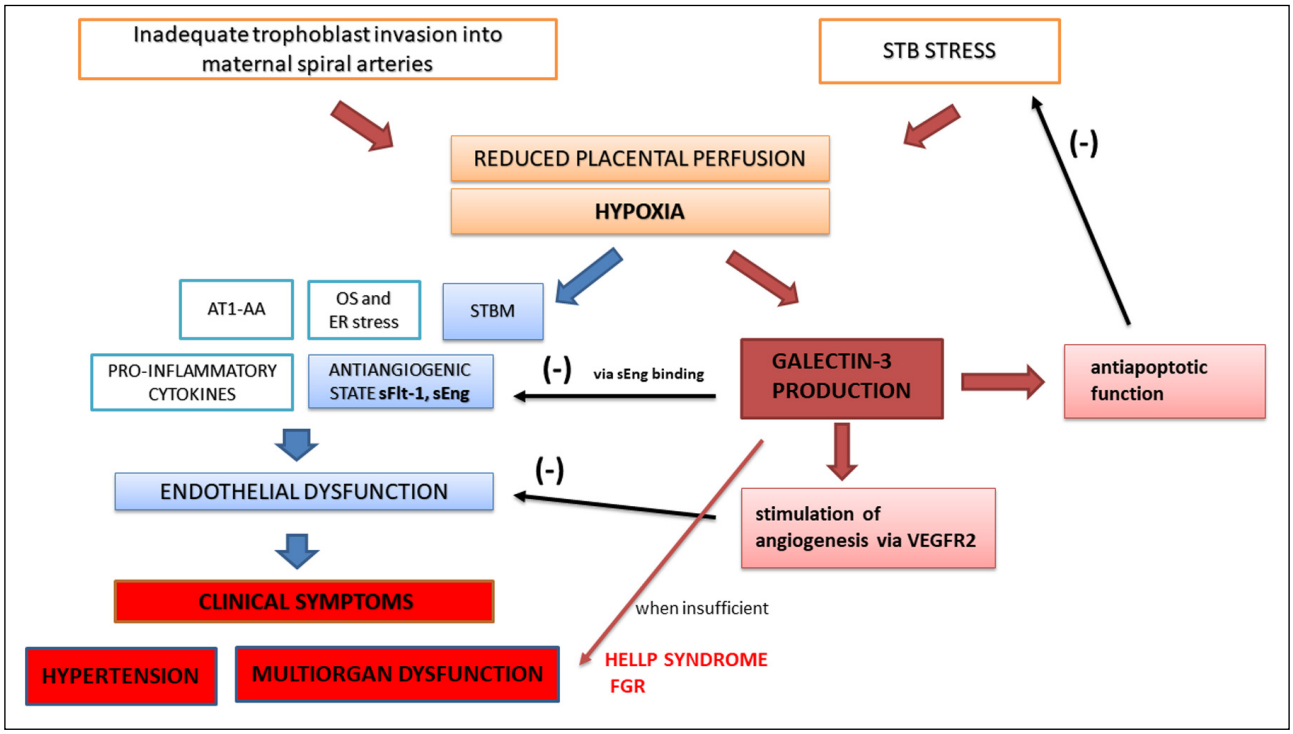

Fig. 6. The proposed hypothetical model of the compensatory role of galectin-3 during preeclampsia. In the development of preeclampsia galectin-3 placental production is a positive stress response to hypoxia occurring in early pregnancy due to placental dysfunction. Galectin-3 is a binding partner for sEng and therefore it may inhibit sEng antiangiogenic activity. Galectin-3 stimulates angiogenesis *via* VEGFR2 and therefore it may decrease the severity of endothelial dysfunction. Galectin-3 has also anti-apoptotic activity and therefore it may inhibit apoptosis in STB cells and decrease STB stress. Insufficient galectin-3 production leads to development of severe complications, such as HELLP syndrome and/or FGR. However, this model is fully theoretical and needs further investigation to be confirmed.

Abbreviations: AT1-AA, angiotensin II 1 receptor autoantibodies; ER, endoplasmic reticulum; FGR, fetal growth restriction; OS, oxidative stress; sEng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase; STB, syncytiotrophoblast; STBM, syncytiotrophoblast microparticles; VEGFR2, vascular growth factor receptor 2.

Strengths of the study were: the wide range of studied parameters (biochemical, echocardiographic and histopathological), and high group homogeneity obtained, *i.e*., by applying narrow inclusion criteria (proteinuria as a necessary component) and exclusion of multiple pregnancies.

In conclusion, our data showed that galectin-3 serum level and placental expression are significantly higher in preeclamptic patients than in uncomplicated pregnancy. The source of galectin-3 in maternal serum was its placental production. Despite women with preeclampsia presenting discreet symptoms of myocardial dysfunction and worse renal function parameters, these symptoms were not related to galectin-3. Moreover, the correlation between serum galectin-3 level and its expression in STB reflects the possible galectin-3-transporting pathway from the placenta into maternal circulation *via* STBM. Additionally, we suggest that during preeclampsia, galectin-3 placental production may serve as a compensatory mechanism for impaired trophoblast invasion into maternal spiral arteries in early pregnancy, followed by placental malperfusion and hypoxia. This hypothesis needs to be experimentally substantiated in future research.

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