Placental growth factor in pregnancies complicated with high blood pressure

Ph.D thesis

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Introduction

Hypertension during pregnancy is one of the most common and severe disorders related to pathological pregnancy. Diseases associated with hypertension influence both the maternal and fetal morbidity and mortality. In the developed countries every fifth maternal death is related to complications due to hypertension during pregnancy.

Considering the maternal and fetal outcome it is crucial to admit the pregnant woman in time to an adequate medical centre ensuring the further management of the pregnancy. This can happen if we identify in time those pregnant women for whom complications due to hypertension during pregnancy and premature birth can be anticipated.

Growth factors are naturally occurring, typically protein or steroid hormone structure molecules, which stimulate cell division, differentiation, they have their physiological role primarily in growth and regeneration. History of the angiogenesis' factors goes back to the first half of the 20th century. At the end of the thirties it has been proposed that in malignant tumours some substance might stimulate vascular generation. In 1956 Melwin and Algire described that there are substantial differences in vasoproliferative responsiveness between normal and tumour tissues. In 1971 Folkman et al. reported the isolation of the first regulator molecule of angiogenesis. After homogenisation of malignant tumour tissue a 100 kDA glycoprotein with pronounced angiogenic activity was identified. It was named tumour angiogenesis factor (TAF).

At the beginning of the 1990s more VEGF isoforms, additionally three VEGF receptors were identified. In 1991 in the Institute of Genetics and Biophysics at the University of Naples the group of Maria Graziella Persico discovered a new ligand binding to VEGFR-1 (Flt-1) receptor, which was isolated from full term human placenta. The newly discovered molecule has an amino acid sequence of 53% identical to VEGF and is less related to PDGF (platelet derived growth factor). The protein was named placental growth factor (PlGF).

The placental growth factor (PlGF) is synthesised during the entire pregnancy, and the main source is the trophoblast cell. Beside the placenta high amount of PlGF is produced in the thyroid gland and the endothelial

cells of the bronchi, but it is produced in a number of different cell types in lower amount, e.g. in certain subgroups of lymphocytes, in smooth muscle cells, in the pharyngeal tonsil, in the testis or prostate cells too.

Since sFlt-1 is a soluble receptor of the placental growth factor it binds the PlGF in the maternal circulation, decreases the plasma level of freely circulating PlGF. Thus the plasma level of sFlt-1 and PlGF changes in different directions. Numerous scientific results confirmed that in case of pregnancies complicated with preeclampsia the plasma level of proangiogenic PlGF is lowered, while the plasma level of its antiangiogenic endogen inhibitor, the sFlt-1 is increased compared to physiological pregnancy.

In 2004 Levine et al. in the frame of the Calcium for Preeclampsia Prevention Trial found that in the last two months of the normal pregnancy the plasma level of sFlt1 is significantly increased and the plasma level of PlGF is significantly decreased in the circulation of pregnant women. In those pregnant women who later had preeclampsia, the plasma level changes occurred earlier and were more pronounced. During their trial they detected that the sFlt1 plasma level increased on the average 5 weeks before the first onset of preeclampsia symptoms. The significance of this finding is further supported by the fact that very early, in the first half of the pregnancy, between weeks 13 and 16, the plasma level of PlGF was significantly lower in the circulation of those pregnant women who had later preeclampsia. The biggest difference between the PlGF values of pathologic and physiologic pregnancy bearing women occurred in the period right before the development of the disease, in parallel with the increase of sFlt1 plasma level in pathologic cases. The difference was more pronounced in case of severe, early onset preeclampsia and in preeclampsia combined with intrauterine growth retardation too.

Aims

As an aim of my scientific work I looked for the answers on the following questions:

1. Whether there is a difference in PlGF plasma levels in the circulation of gravid women suffering from different types of hypertension and healthy pregnant women.

2. What kind of relationship could be found between the measured PlGF level in the maternal blood plasma and the obstetric outcome, in particular the premature birth rate as well as the gestational age at premature birth?

3. What kind of relationship exists between the finding of the pathological fetal circulation and the perinatal outcome in the investigated patient population?

4. What kind of relationship exists between the measured maternal PlGF blood plasma level and the perinatal outcome in the investigated patient population?

5. Whether the measured PlGF level in the maternal blood plasma or the findings of the fetal Doppler examination predicts with greater precision the poor pregnancy outcome?

Patients and methods

The diagnosis of pregnant women was set up according to the American College of Obstetricians and Gynecologists (ACOG) and the National High Blood Pressure Education Program's Working Group (NHBPEP) diagnostic criteria between May 2008 and October 2010.

All involved patients were of Caucasian origin and stem from approximate identical geographic area.

Multiple pregnancies, pregnant women with foetus having developmental disorder or genetic disease had been excluded.

The study was conducted according to the rules laid down in the Declaration of Helsinki and was approved by the Medical Research Council National Scientific and Ethical Committee at No. "TUKEB 52/2008".

Indications for termination of pregnancy

The indication for caesarean section had been set up according to the internationally approved guidelines. Caesarean section had been performed irrespective of the gestational age in case of the development of HELLP syndrome, severe, with conventional measures uncontrollable, therapy resistant hypertension, in the presence of higher than 10 g/ 24 hours proteinuria, higher than 70 mIU/mL SGOT/SGPT with epigastrial pain, lower than 100 000 G/L thrombocyte count, pulmonary oedema, severe oliguria, persistent headache, visual disturbance, imminent eclamptic seizure, as well as in case of fetal distress: sever oligohydramnios, abnormal uteroplacental circulatory conditions measured, pathological CTG-findings or sever intrauterine growth retardation observed. If the above conditions were not present, pregnancy was terminated after completed gestational week 37.

Data collection, sampling

After reading and signing the detailed information leaflet and consent form on the aim of the research as well as blood sample storage and utilization, the questionnaires were filled out at first time by the time of diagnosis, and 12 weeks after delivery, mostly via telephone contact.
Blood sampling occurred at the time of clinically necessary blood sampling at the earliest time point after inclusion in case of both complicated

pregnancy bearing and control healthy gravid women. Every blood sampling occurred between gestational weeks 22 and 35. Right after sampling the EDTA anticoagulated sample was centrifuged at 4°C temperature for 10 minutes with 3000 G and the supernatant was pipetted to Eppendorf tubes and stored frozen till sample analysis.

Measurement technique

For the measurement of placental derived growth factor (PlGF) the Triage® PlGF Assay device by Alere (San Diego, USA) company was used. The Triage® device binds the free PlGF molecule to fluorescent tracer bound monoclonal antibody. From the melted, room temperature blood plasma 250 microlitre was pipetted on the disposable test stick belonging to the device which was placed in the device and the PlGF concentration of the blood plasma appeared on the screen of the device 15 minutes later in pg/mL unit. The measurement range of Triage® PlGF Assay is between 12 and 3000 pg/mL. If the result detected by the device was lower than the measurement range, label '<12 pg/ml' appeared on the screen. The test result was deemed positive by using two different cut-off values: 1) if the measured PlGF concentration was immensely low, i.e. lower than 12 pg/mL, 2) if the PlGF concentration was lower than the 5 percentile value of the same gestational age healthy control gravid women.

Considering that the average plasma concentration of the placental growth factor, measured in the maternal circulation, changes with gestational age in physiological case too, the 5 percentile values by gestational ages on the enclosed information sheet by the manufacturer was considered as positive test cut-off value in different gestational ages.

These were the following:

Before the gestational week 19:	56.2 pg/mL
Between gestational weeks 19 and 23^{+6}:	62.9 pg/mL
Between gestational weeks 24 and 28^{+6}:	130 pg/mL
Between gestational weeks 29 and 31^{+6}:	128 pg/mL
Between gestational weeks 32 and 34^{+6}:	70.4 pg/mL

Fetal Doppler examination

In the study period the fetal Doppler examinations (i.e. flowmetry) were performed at the Semmelweis University, 1st Department of Obstetrics and Gynaecology. The examiner had appropriate qualification and good proficiency in fetal flowmetry. The method of ultrasound examinations followed the professional position reported by the Hungarian Society of Ultrasound in Obstetrics and Gynaecology. The type of ultrasound machines used for the examinations were HD11 and Samsung Medison X8. The flow parameters were deemed abnormal if diastolic block or reverse flow had been detected in umbilical artery or in the descending aorta, as well as if the signs of centralisation of the fetal circulation were observed.

Oligohydramnios, IUGR, abnormal CTG graph

Oligohydramnios was diagnosed when amniotic fluid index (the sum of the biggest vertical amniotic fluid well sizes detected in the four quadrants of the uterus) was below 5 cm. Intrauterine growth retardation was diagnosed by fetuses below 10 percentile of estimated weight for the gestational age. The evaluation of CTG graphs was performed visually on an at least 20-minute recording by an appropriately qualified professional for CTG analysis at the Semmelweis University, 1st Department of Obstetrics and Gynaecology. The CTG graph was deemed abnormal, if the basic frequency was permanently below 110 per minute or above 160 per minute, if the variability was reduced, or if chemoreceptor mediated decelerations were detected on the recording.

Statistical calculations

The clinical characteristics of the involved gravid women and the healthy, control pregnant women were evaluated by descriptive statistics. Women with positive PlGF test were grouped separately based on the diagnosis and the gestational age calculated at delivery. The correlation of negative and positive PlGF analysis with the gestational age at delivery of patients having gestational hypertension was presented on a Kaplan-Meyer graph. The associated hazard ratio was calculated by uni- and bivariate Cox regression analysis. Within the groups, based on the diagnosis and gestational age, the PlGF plasma level median, lower and upper quartiles were analysed by boxplot diagram, p-values were determined by Wilcoxon rank correlation probe. For the technical implementation of the statistical analysis MATLAB 8.0 version software was used.

Results

Mean maternal age was 31 years (29-34) in the healthy control group, 34 years (29-35) in the group of gestational hypertension, chronic hypertension and superimposed preeclampsia, 33 years (30-37) in cases with preeclampsia, while it was 30 years (28-33) in case of HELLP-syndrome. The highest systolic and diastolic blood pressure values were measured in the group of preeclampsia, superimposed preeclampsia and HELLP-syndrome at the time of blood collection. Considering that based on the ACOG and the NHBPEP classification, blood pressure above 160/110 mmHg suggests severe hypertonic disease, majority of my patients with preeclampsia can be considered to have severe preeclampsia. In addition, the onset of the disease was typically at the gestational week 30 in all the three disease groups (preeclampsia, superimposed preeclampsia and HELLP-syndrome), thus the majority of patients with preeclampsia suffered from early onset preeclampsia.

According to the above, the median value of the gestational age at delivery were 31 and 32 weeks in the groups of preeclampsia and HELLP-syndrome, respectively. Preterm birth occurred most frequently in the HELLP-syndrome group (20/20 cases, 100%) and in the preeclampsia group (22/23 cases, 96 %); in the superimposed preeclampsia group preterm delivery rate was 14 out of the 17 (82%), respectively. The rate of preterm birth was 7/18 (39%) in the gestational hypertonic group and 11/25 (44%) in the chronic hypertonic group.

Intrauterine fetal growth retardation occurred in 3/18 patients with gestational hypertension (17%), in 2/25 (8%) cases in the chronic hypertension group, in 10/23 (44%) pregnancy bearing women with preeclampsia, in 5/17 patients (29%) in the superimposed preeclampsia group, and in 6/20 patients (30%) in HELLP-syndrome group.

Considering the 5 percentiles of the normal range respective to the gestational age (Table 1) as the positive and negative cut-off value of the PlGF test, 22 patients showed positive results out of the examined 23 with preeclampsia (95.7%). PlGF test was found positive in 19 patients out of the 20 cases (95%) with HELLP-syndrome, and 14 out of the 17 cases (82.4%) with superimposed preeclampsia. The result was positive in 8 gravid women out of the 18 with gestational hypertension (44.4%) while in 15 cases out of the 25 (60%) with chronic hypertension.

Table 1. Rate of patients with positive PlGF test among different gestational hypertension groups, using the lower 5 percentiles of the mean PlGF plasma level (relative to the gestational age) of the normal pregnancy as a cut-off value.

	N	N+	N+%	95%LCI	95%UCI
Control	27	1	3.7	0.001	0.190
GHT	18	8	44.4	0.215	0.692
CHT	25	15	60	0.387	0.789
PE	23	22	95.7	0.751	0.999
HELLP	20	19	95.0	0.751	0.999
SIPE	17	14	82.4	0.566	0.962

N: number of participants in a group, N+: number of participants with positive result, N+%: percentage of participants with positive results compared to the total number of participants in the group, 95%LCI: 95% lower confidence interval, 95%UCI: 95% upper confidence interval.

Table 1 shows, that from the groups of preeclampsia, superimposed preeclampsia and HELLP-syndrome, altogether 5 gravid women had negative PlGF test. In case of three patients with superimposed preeclampsia the following PlGF plasma values were detected: 123 (collected at gestational week 33), 149 (collected at gestational week 34) and 265 (collected at gestational week 33). All of them were suffering from the mild form of the disease, and gave birth to a mature weight newborn at gestational weeks 38, 37 and 36. In only one HELLP-syndrome case was the test result negative, where the lower 5th percentile of the PlGF plasma level relative to the gestational age was 70.4 pg/mL, this pregnant woman had a value of 70.9 pg/mL and delivery was induced at gestational week 34. In this case sign of placental failure was not found, only mild hypertension and

proteinuria was present. Similarly, in the group of preeclampsia only one patient had negative PlGF result. In this case PlGF plasma level was well above the threshold limit, however, even the highest blood pressure values were within the normal range, on the upper end (140/90 mmHg), and had only marked proteinuria. Neither in her case was any sign of placental failure, and gave birth to a healthy, mature weight newborn at gestational week 37.

All pregnant women with preeclampsia, who had an induced delivery before completed gestational week 37, had a positive PlGF test.

Table 2 summarizes the plasma levels of the placental growth factor per diagnostic groups.

Table 2. PlGF plasma levels (pg/mL) in different diagnoses (n=130).

	N	Median	Percentile 25	Percentile 75	p*
Control	27	331	163	633	NA
GHT	18	168	28	527	0.0199
CHT	25	64	13	145	<0.001
PE	23	12	12	12	<0.001
HELLP	20	12	12	12	<0.001
SIPE	17	16	12	53	<0.001

* p value relative to the control group. N: Number of pregnant women in the group. NA: Not applicable.

While PlGF concentration was the highest in the healthy control group as predicted previously, compared to the results of the control group, lower values were found in all gestational hypertonic groups. Median value of PlGF plasma level in the control group was 331 (163-633) pg/mL, PlGF

plasma level among patients with gestational hypertension was about half of it, namely 168 (28-527) pg/mL.

In case of patients with chronic hypertension, maternal plasma PlGF value was an average of 64 (13-145) pg/mL. The lowest PlGF plasma level was detected in the groups of preeclampsia and HELLP-syndrome. Most frequently 12 pg/mL values were detected in these groups, but since the lower limit of detection for the device is 12 pg/mL, most probably PlGF plasma levels were even lower than this threshold in these patients.

Table 3. Rate of patients with positive PlGF test grouped based on the gestational ages, using the lower 5 percentiles of the mean PlGF plasma level (relative to the gestational age) of the normal pregnancy as a cut-off value.

	N	N+	N+%	95%LCI	95%UCI
GA < 35^{+0}	63	59	93.7	0.845	0.982
GA 35-37	11	8	72.7	0.390	0.940
GA 36^{+6}	29	11	37.9	0.207	0.577
GA < 37	74	67	90.5	0.815	0.961
GA < 37[1]	71	67	94.4	0.862	0.984

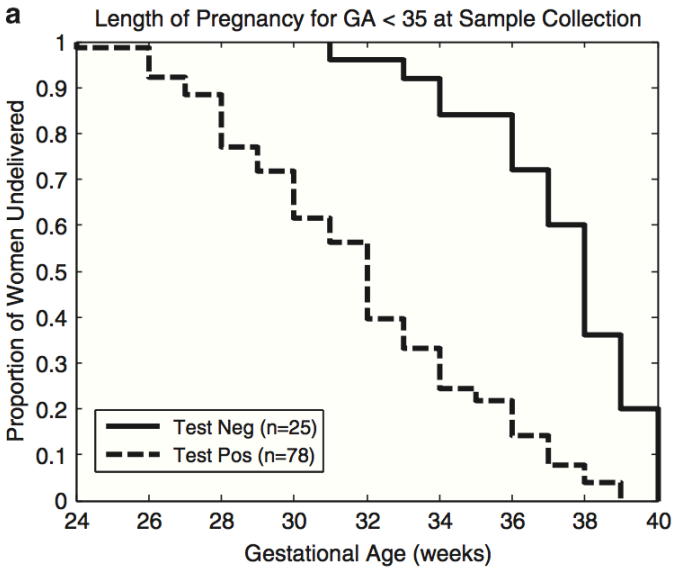
[1] Exclusion of three participants was due to preterm birth as a result of a reason irrespective of the gestational hypertension. GA: gestational age at the time of delivery. N: number of participants in a group, N+: number of participants with positive result, N+%: percentage of participants with positive results compared to the total number of participants in the group, 95%LCI: 95% lower confidence interval, 95%UCI: 95% upper confidence interval.

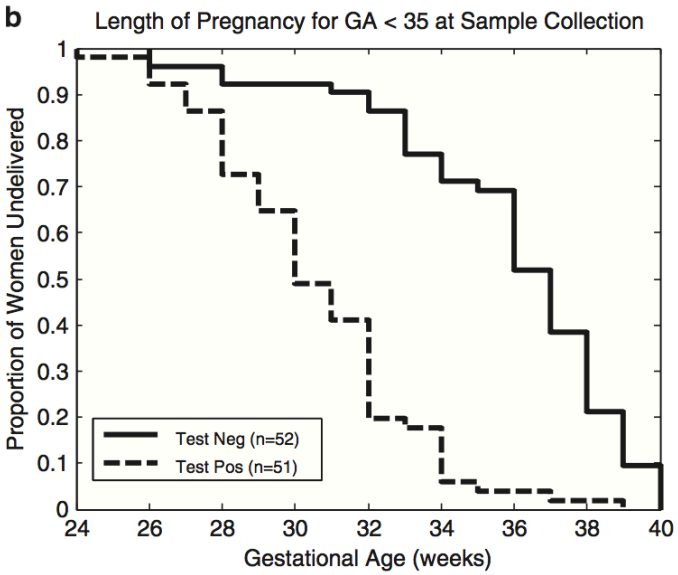
Table 3 shows the relation between the duration of pregnancy and PlGF test, according to the gestational age (GA), using again the lower 5 percentiles of the PlGF plasma levels of the physiological pregnancy as a cut-off value.

Delivery occurred before gestational week 35 in 63 cases, among which 59 cases (93.7%) showed positive result to the PlGF test. Investigating preterm births before gestational week 37, altogether 74 cases were found. From these, three preterm deliveries were not caused by a reason connected to hypertension (delivery was due to fetal bradycardia in one case, and preterm rupture of the amniotic sac in two cases). These three cases were excluded from the pool of preterm births, and 67 cases showed positive PlGF test out of the 71. PlGF test positivity occurred in 90.5% of the total number of preterm births and in 94.4% of preterm births connected to hypertension.

Graph 1 compares the gestational duration between pregnant women with positive and negative PlGF tests, by using Kaplan-Meier graphs.

Cut-off values between positive and negative tests were 12 pg/mL (graph 1a) or the lower 5 percentiles of PlGF plasma levels relative to the different gestational ages (graph 1b). Graphs show, that gestational duration is shorter in case of patients with positive PlGF test, than in those with negative test, independently from what was considered as a cut-off value.





Graph 1.

Table 4 summarizes the PlGF plasma levels and the Doppler results according to the diagnoses and the perinatal outcome (preterm birth, IUGR).

Maternal PLGF levels in the peripheral circulation were assigned to three groups. The PlGF concentration was categorized as the follows: ≤12 pg/mL: very low, 12-100 pg/mL: low, >100 pg/mL: normal.

In 20 cases out of the 89 (22.5%) the Doppler finding was pathological and in all 20 cases, low PlGF plasma levels (i.e. <100 pg/mL were detected. Concordance between PlGF level and fetal Doppler parameters is significant, p=0.0023.

Mature term delivery occurred in 28 cases. Among them, pregnancy was complicated by gestational hypertension in 11 cases, chronic hypertension in 13 cases, superimposed preeclampsia in 3 cases, and preeclampsia in 1 case.

Table 4. PlGF plasma level and fetal Doppler test result categorized according to the fetal outcome.

	Normal PlGF		Low PlGF		Very low PlGF	
Flow	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
n	22	0	23	3	24	17
n/N	0.247	0.00	0.258	0.034	0.27	0.191
Preterm birth	5 (22.7)	NA	14 (60.9)	3 (100.0)	22 (91.7)	17 (100.0)
IUGR	0 (0.0)	NA	3 (13.0)	3 (100.0)	9 (37.5)	7 (41.2)
GH	10 (45.5)	NA	3 (13.0)	1 (33.3)	2 (8.3)	3 (17.6)
CHT	8 (36.4)	NA	11 (47.8)	0 (0.0)	2 (8.3)	3 (17.6)
PE	1 (4.5)	NA	2 (8.7)	1 (33.3)	10 (41.7)	5 (29.4)
HELLP	0 (0.0)	NA	2 (8.7)	0 (0.0)	6 (25.0)	4 (23.5)
SIPE	3 (13.6)	NA	5 (21.7)	1 (33.3)	4 (16.7)	4 (23.5)

N: 89 pregnant women suffering from gestational hypertension. n: sample size of the group created according to the PlGF plasma level and Doppler result. Normal PlGF plasma level: PlGF > 100 pg/mL, low PlGF level: 12 pg/mL < PlGF < 100 pg/mL, very low PlGF level: PlGF < 12 pg/mL. Numbers show the number of cases, while the percentage of cases related to n are in brackets. NA: not applicable.

In all 28 cases ultrasound examination detected normal Doppler result and even PlGF plasma levels were in the normal range in 9 gestational hypertonic cases out of the 11, in 5 chronic hypertonic cases out of the 13, in 2 superimposed preeclampsia cases out of the 3 and in the only preeclampsia case as well.

In all 22 foetus showing IUGR, low or very low PlGF levels were detected, fetal Doppler was also pathologic in 10 cases out of the 22 (45.5%). In 20 cases out of the 61 preterm deliveries (32.8%), Doppler results were abnormal and in all 20 cases low or very low PlGF values were detected. Doppler results were negative in 41 cases (67.2%), however, 36 pregnant women out of this 41 (87.8%) had PlGF plasma levels in the low or very low category.

Altogether 5 gravid women participated in the study, who had preterm delivery with normal results of both fetal Doppler and PlGF test, although in all 5 cases mature weight newborns were born, out of which in 2 cases the root cause of the preterm birth was premature amniotic sack rupture irrespective of hypertension.

In those 69 cases, when fetal Doppler results were found to be normal, the positive PlGF test (low or very low PlGF plasma levels) strongly correlated with the unfavourable fetal outcome. The correlation with preterm delivery had a p value of <0.001 and correlation with the intrauterine growth retardation had a p value of 0.0069.

Also among pregnant women showing pathological CTG graph and oligohidramnios, the rate of patients with low PlGF plasma level was high. From the 24 gravid women with pathological CTG graph, low or very low PlGF plasma levels were measured in 21 cases (87.5%), from the 27 cases complicated with oligohidramnios, low PlGF concentration was found in 20 cases (74.1%).

Conclusions

1. In all gestational hypertonic groups lower PlGF plasma levels were detected compared to the normotonic gestational group. The lowest placental growth factor plasma levels were found in cases of preeclampsia and HELLP-syndrome, which suggests the role of abnormal placentation in the aetiology of preeclampsia and HELLP-syndrome.

2. Maternal placental growth factor plasma level is lower in pregnancies which resulted in preterm birth, than in those ending up with term delivery. This may contribute to the early identification of pregnancies ending up with preterm birth.

3. In pregnant women, who had preterm delivery before gestational week 35, PlGF plasma level was lower than in those giving birth at gestational week 35-37.

4. In case of normal PlGF plasma level, pathological Doppler result did not occur, consequently normal PlGF plasma level suggests lower risk for poor fetal outcome compared to those with low PlGF plasma level. This may help the judgement of prognosis later on.

5. Preterm birth and intrauterine growth restriction was more accurately predicted by the low or very low PlGF plasma level, than by the results of the fetal Doppler examination, which confirms the relation between placental failure and placental growth factor plasma level.

6. In case of pregnancies with pathological CTG graphs and oligohydramnios, maternal PlGF plasma levels were mostly low or very low.

List of original articles

Publications dealing with the subject of the thesis

1. Gullai N.; Stenczer, B.; Molvarec, A.; Fugedi, G.; Veresh, Z.; Nagy, B.; Rigo, J. (2013) Evaluation of a rapid and simple placental growth factor test in hypertensive disorders of pregnancy, Hypertens Res, 36: 457-462. IF: 2,936

2. Molvarec, A., Gullai, N., Stenczer, B., Fugedi, G., Nagy, B., Rigo, J. (2013) Comparison of placental growth factor and fetal flow Doppler ultrasonography to identify fetal adverse outcomes in women with hypertensive disorders of pregnancy: an observational study, BMC Pregnancy Childbirth, 13: 161. IF: 2,152

3. Alasztics B., Gullai N., Molvarec A., Rigó J. (2014) The role of angiogenic factors in preeclampsia, Orv Hetil, 155: 1860-1866.

Other publications

1. Balázsa T., Bíró J., Gullai N., Ledent C., Sperlágh B. (2008) CB1-cannabinoid receptors are involved in the modulation of non-synaptic [^{3}H]serotonin release from the rat hippocampus. Neurochem Int, 52: 95-102. IF: 3,228

2. Bertalan R., Patocs A., Nagy B., Derzsy Z., Gullai N., Szappanos A., Rigo J., Racz K. (2009) Overrepresentation of BclI polymorphism of the glucocorticoid receptor gene in pregnant women with HELLP syndrome. Clin Chim Acta, 405: 148-152. IF: 2,535

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4. Stenczer B., Molvarec A., Veresh Z., Gullai N., Nagy G., Walentin S., Szijártó J., Rigó J. (2011) Circulating levels of the anti-angiogenic thrombospondin 2 are elevated in pre-eclampsia. Acta Obstet Gynecol Scand, 90: 1291-1295. IF: 1,771

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