Short Thesis for the Degree of Doctor of Philosophy (PhD)

Screening for preeclampsia in the first trimester of pregnancy

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University of Debrecen
Doctoral School of Clinical Medicine

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The Examination will be held 11:30 am, October 30, 2020

Head of the Defence Committee:	Árpád Illés MD, PhD, DSc
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The Defence will be held 13 pm, October 30, 2020

Live online access will be provided. If you wish to take part in the discussion, please send an e-mail to laszlo.orosz.dr@gmail.com not later than 12 pm on the day before the discussion (October 29, 2020). After the deadline, for technical reasons, it is no longer possible to join in to the defence.

1. Introduction

With a prevalence of 2-4% of all pregnancies preeclampsia (PE) is one of the major contributors of feto-maternal morbidity and mortality, and is also responsible for 10% of stillbirths and 12-16% of preterm births. Due to its high prevalence, this disease has one of the highest health economic costs.

The most important etiological factor of preeclampsia is impaired placentation, which results in an inflammatory response that causes damage to the maternal endothelium. The symptoms include high blood pressure, oedema and proteinuria.

Preeclampsia is irreversible and exclusively related to the second half of the pregnancy (after week 20). Maternal symptoms can be treated with antihypertensive drugs but at the moment, apart from terminating the pregnancy, there is no definitive cure for this disease.

From etiological and clinical points of view, preeclampsia can be separated into two groups:

1. Early-preeclampsia: requiring delivery before 34 weeks
2. Late-preeclampsia: requiring delivery after 34 weeks

The prevalence of early-preeclampsia is 0.5 %, and that of late-preeclampsia is 2%.

1.1. Preparation for the study

Over the last 20 years, several first trimester studies have proved that most late-onset pregnancy related diseases can be predicted earlier in pregnancy. By highlighting high risk groups, we can provide customized health care for each high risk patient.

Most of the first trimester screening methods of these late-onset pregnancy related diseases were developed by Kypros Nicolaides, and published in 2011 in „Turning the Pyramid of Prenatal Care". The screening methods were based on maternal previous history, maternal and fetal biophysical measurements, and maternal serum biomarkers.

Between 2012 and 2014 I spent two years on a fellowship with Professor Kypros Nicolaides. During these years I was able to join his research team and learn most of his study designs.

After these two years, I received the "Diploma in Fetal Medicine" from him. I would like to use the knowledge gained at

his department to promote the care of Hungarian pregnant women.

1.2. Literature overview

1.2.1. First trimester screening for preeclampsia

Traditional screening methods for preeclampsia are based on maternal previous history and have a detection rate of 35% at a false positive rate of 10% (NICE guidelines).

In the last couple of decades, many studies have proved that in the second and third trimesters of pregnancy several biomarkers have altered serum levels in preeclampsia compared to healthy controls. Some of these differences have been described to be present even in the first trimester of pregnancy.

Pregnancy associated plasma protein A (PAPP-A), placental growth factor (PlGF), adiponectin, endoglin, pentraxin-3, P-selectin, activin-A, inhibin-A and urinary orosomucoid are the most often used biomarkers in the first trimester of pregnancy.

FMS-like tyrosine kinase (sFLT-1) is responsible for the binding of angiogenic factors such as vascular endothelial growth factor (VEGF) and PlGF. The important role of the other two

angiogenic biomarkers as placental protein 13 (PP13) and soluble endoglin also highlights the importance of vascular remodelling in the pathogenesis of preeclampsia.

In recent years several studies have been published with the aim of developing a reliable and cost effective first trimester screening method for preeclampsia. Combined first trimester screening methods based on maternal previous medical history, body mass index (BMI), mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI), and maternal serum-biomarkers (PAPP-A and PLGF) proved to have a detection rate of 90% for early-PE, 80% for preeclampsia between 34-37 weeks and 60% for PE developing after 37 weeks at a false positive rate of 5-10%.

Most of these studies have been validated on numerous Anglo-Saxon patients with similar research settings and outcome results.

Other studies performed on South-European-, and Middle-East pregnant populations using the same screening algorithms as those Anglo-Saxon studies could not reproduce their results.

Screening for late-preeclampsia has lower detection rate (31-45%) than for early-preeclampsia since this subgroup of preeclampsia has a different etiology. Unlike in early-

preeclampsia, in late–preeclampsia most of the fetuses are appropriate (AGA)-, or even large for gestational age (LGA). This finding highlights the fact that this form is not related to placental insufficiency; instead, it shows a close connection to maternal cardiovascular status.

The efficacy of first trimester screening for preeclampsia depends on the maternal "a priori risk", which consists of maternal previous medical history and biophysical measurements such as blood pressure and body mass index (BMI). Most of the first trimester screening algorithms have been built using research data from studies performed in Anglo-Saxon patients, with their characteristic "a priori risk". Therefore the screening efficacy of these publications from the United Kingdom was similar to and comparable with each other. Recently many publications have emphasized a need for new validation studies in pregnant women different from those of the Anglo-Saxon populations.

By adapting maternal "a priori risk" to the examined population the sensitivity and specificity of the tests are expected to increase.

Moreover, 20-30 years later cardiovascular morbidity is higher among women whose pregnancies were complicated either

with early-, or late-preeclampsia, so in addition to lowering feto-maternal morbidity, these screening tests might also lower the long term cardiovascular morbidities of these patients.

1.2.2. Novel biomarkers

Several molecular changes can be detected in the maternal serum even before the onset of preeclampsia. One of the most promising areas is proteomics. Many proteomic studies have shown that in the first trimester of pregnancy expression of several proteins changes in women with early-, or late-preeclampsia.

Differences can be observed in the renin-angiotensin system, immune system, complement system and coagulation cascade.

From a genetic point of view the metilation status of some placental genes such as ZNF554, BCL6 and ARNT2 can also be observed in preeclampsia.

In a collaboration study performed in Israel, 19 altered proteins were identified in first trimester serum samples from patients with early-preeclampsia complicated with small for gestational age (SGA) fetuses when compared with healthy

controls. This study used two dimensional differential gelelectroforesis (2D-DIGE). Most of these 19 proteins have a major regulatory role in the immune-, coagulation-, and complement- cascade, while some others are responsible for lipid transport, blood pressure regulation and angiogenesis.

The same Israeli group also reported on 14 altered proteins in the first trimester when samples from patients with late-preeclampsia with appropriate for gestational age (AGA) fetuses were compared with controls. Most of these (14) proteins have the same biological role as the previously described 19 proteins.

These findings highlight the fact that most of the etiological factors of both early-, and late-preeclampsia are present even in the first trimester of pregnancy.

2. Objectives

2.1. Adaptation of the Fetal Medicine Foundation (FMF) algorithms to the Hungarian pregnant population

Our primary goal was to test the feasibility of first trimester screening for preeclampsia using FMF algorithms in an unselected pregnant population under routine clinical settings in

Eastern-Hungary. Our secondary aim was to examine the role of different fetal or maternal parameters in risk calculation.

2.2. Research for novel biomarkers – proteomics

In the second part of our study our aim was to identify a characteristic first trimester proteomic pattern in serum samples from patients with early-preeclampsia. On the one hand, this pattern might help to develop a new screening method for preeclampsia based on proteomics only. On the other hand, it might help to increase the detection rate of FMF algorithms.

Out of the previously described 19 altered proteins in the Israeli study, 10 immune proteins were selected as candidates for the Hungarian first trimester validation study.

3. Materials and methods

3.1. Recruitment of patients for the preeclampsia screening study

Patients for the studies were selected from a prospective study performed at two centres between 2010 and 2013:

Department of Obstetrics and Gynaecology, University of Debrecen Medical and Health Science Centre, Debrecen, Hungary

Department of Obstetrics and Gynaecology, Andras Josa County and Teaching Hospital, Nyiregyhaza, Hungary

Patients were enrolled in the study at their routine first trimester ultrasound screening for major chromosomal-, and fetal structural abnormalities. The protocol of the prospective study was approved by the local ethical committee (TUKEB license: DEOEC RKEB/IKEB 3092-2010).

After fulfilling informed consent forms, patients with a gestational age between 11 and $13^{+6\ days}$ weeks (crown-rump length-CRL=45-84mm) were recruited.

Patients were given a questionnaire to complete, recommended by FMF, on previous medical history and maternal characteristics. Maternal age, height, weight, race, parity, method of conception, smoking status, chronic hypertension, diabetes mellitus, antiphospolipid syndrome, systemic lupus erythematosis and previous maternal or family history of preeclampsia were recorded.

Blood pressure (BP) measurements and first trimester ultrasound scans were performed based on FMF guidelines by FMF licensed and audited physicians. During the first trimester scan CRL, ductus venosus pulsatility index (DV-PI), fetal heart rate (FHR), uterine artery pulsatility index (UtA-PI), biparietal diameter (BPD), and nuchal translucency (NT) were measured.

After the first trimester scan blood and urine samples were taken and processed for further research purposes. The samples were stored at -80^{o}C.

Samples collected from one patient: 8 serum, 8 plasma, 2 white blood cells, 3 urine and 1 urine precipitate.

3.1.1. Follow-up of recruited patients

Outcomes of the recruited pregnancies were collected from the database of the University of Debrecen (MedSol) and the database of Andras Josa County and Teaching Hospital (MedWorks). In some cases outcomes could only be acquired through telephone calls or outcome letters. All the data were summarized in an excel file. Preeclampsia was defined by the ISSHP (2014) guidelines, and all files of patients diagnosed with preeclampsia were double checked by two doctors.

3.1.2. Selecting patients for study 2.1.

As described in the objectives (2.1.), patients with preeclampsia (n=82) and matched healthy controls (n=82) were selected for the comparison of first trimester screening efficacy for preeclampsia. Controls were selected based on maternal age, parity, BMI and gestational age at the time of recruitment.

First trimester preeclampsia risk assessing FMF algorithms (Astraia programs) required the following biochemical markers for risk calculation:

1. Maternal serum beta human choriogonadotropin (B-hCG)
2. PAPP-A
3. PlGF

These biomarkers were measured from frozen blood samples using FMF audited BRAHMS Kryptor analyzer (ThermoFisher).

FMF software automatically converts the biochemical levels of these biomarkers to multiples of the expected normal median (MoM). MoM conversion is based on maternal body mass index (BMI), ethnicity and gestational age. Based on these, 1 MoM serum concentration is the expected median value of a biomarker at a certain gestation for a certain patient.

In the first part of our study, our goal was to test in the first trimester of pregnancy the FMF based screening method for preeclampsia in high risk patients and compare their results with the healthy control group. The study was performed in routine clinical practice.

3.1.3. Programs used for first trimester preeclampsia risk calculation

The following two types of FMF software were used for first trimester risk calculation:

1. Astraia 2.3.2 – (A1)
2. Astraia 2.8.1 – (A2)

A1 software

A1 software calculated "a priori risk" based on:

1. Maternal demographic data: ethnicity, method of conception, BMI, parity, smoking habits, family history of preeclampsia
2. Maternal medical history: chronic hypertension
3. Previous history of maternal preeclampsia

This algorithm divided preeclampsia risks into two subgroups for each patient:

1. Risk of early-preeclampsia (X<34 weeks)
2. Risk of late-preeclampsia (X>=34 weeks)

A2 software

Apart from those maternal characteristics used by A1 software for the "a priori risk" calculation, A2 software took into account four additional risk factors, including: maternal diabetes mellitus (DM), systemic lupus erythematosis (SLE), antiphospholipid syndrome (APS) and maternal previous history of delivering SGA newborn.

In the A2 software there were some small but significant changes in the calculation of MAP, MAP MoM and UtA-PI MoM.

The results of preeclampsia risk assessment were divided into three subgroups for each patient:

1. Preeclampsia before 34 weeks (PE < 34 weeks)
2. Preeclampsia between 34-37 weeks (PE 34-37)
3. Preeclampsia after 37 weeks (PE > 37)

First group = early-preeclampsia
Second + third groups = late-preeclampsia

The first trimester risks for preeclampsia were assessed for PE and control patients by both A1 and A2 programs.

We used three types of parameter settings for the preeclampsia risk calculation in each program.

The three types of parameters for the settings were:

1. Maternal characteristics, biophysical measurements
2. Maternal characteristics, biophysical measurements + PAPP-A
3. Maternal characteristics, biophysical measurements + PAPP-A + PlGF

Only A2 program could use PlGF values in risk calculation.

The risk calculation of the 82 PE and 82 controls were performed by both A1 and A2 software with all the three settings. Then their preeclampsia risks were assessed and compared.

3.1.4. Statistical methods

For the statistical analysis R-project v.3.5., unpaired student's t-test, one-way analysis of variance (ANOVA), Tukey-HSD posthoc test and ROC curve analysis (pROC package) were used. A $p<=0.05$ probability significant levels were accepted.

3.2. Recruitment of patients for study 2.2.

3.2.1. Protocol of the proteomics study

For the Hungarian validation study of the Israeli altered immune proteins, patients and samples were selected from our first trimester sample collection.

Five cases of early-severe preeclampsia complicated with SGA fetuses and 10 matched controls were selected. We applied the definition of severe preeclampsia by Sibai et al. The definition of SGA was: newborn weight below the 10^{th} centile.

Of the 26 altered proteins described in the Israeli study 10 immune proteins were selected. In contrast to the Israeli study (2D-DIGE method) liquid chromatography mass spectrometry

multiple reaction monitoring (MRM) was used in our study for the examination of altered proteins.

3.2.2. Sample preparation

For the analysis HPLC grade solution was used (Sigma-Aldrich, St. Louis, MO, USA). The frozen serum samples were prepared by denaturating puffer (Biognosys AG; Schlieren, Switzerland). For the alkylation of the samples Biognosys solution was used. Samples were digested with tripsin (Promega; Madison, WI, USA). For the sample preparation for the mass spectrometry, C18 columns were used (Nest Group Inc.; Southborough, MA, USA). SpeVac system was used for peptide drying. Dried up peptides were resolved using LC-A solution (water + 1% acetonitrile + 0.1% formic acid). PlasmaDive™ (Biognosys) reference peptides were used for identification.

3.2.3. LC – MRM

Peptides were injected into a C18 column (Woburn, MA, USA). ThermoScientific TSQ Vantage triple quadrupole mass

spectrometer was used for LC-MRM assay measurements. Biognosys PlasmaDive™ MRM panels were used to measure the 10 immune proteins. SpectroDive™ 8.0—Biognosys software was used for data processing, using 1% q-value filter.

3.2.4. Statistical method for study 2.2.

Due to the low number of patients multiple permutation testing had to be used to predict the power and significance of this pilot study. Significant levels between the groups were calculated by paired t-test and Mann Whitney test. A $p < 0.05$ value was used as a cut-off for successful simulation. Successful simulations were repeated with different quantities (n=10 or n=100 and n=100 or n=1000). $P < 0.05$ values were accepted if the percentage of successful simulations was more than 25.

4. Results

4.1. Outcome of the follow up of the prospective sample collection

Out of the 2,545 patients, outcome of pregnancy was available in 2,251 cases. A total of 2,223 pregnancies ended in live birth. There were 9 terminations due to fetal malformation or major chromosomal abnormalities, 11 miscarriages and 8 stillbirths.

Since in our preeclampsia screening study only singleton pregnancies were enrolled, all 23 multiple pregnancies were excluded. From the remaining 2200 patients 82 ended in preeclampsia (3.7%, 82/2,200).

The rate of early-preeclampsia was 0.5% (11/2200) and that of late-preeclampsia was 3.2% (71/2200). The number of low risk pregnancies selected as matched controls was 82. The risk for preeclampsia was calculated for the 82 preeclampsia and 82 control cases and their results were compared to evaluate the significance of maternal biophysical and biochemical markers recommended to be used by the two types of FMF software.

4.2. Comparison and characteristics: Preeclampsia vs. control groups

There were no significant differences between maternal age, and gestational age at the time of recruitment. BMI was higher in the PE group. Medical problems that increase the risk for PE such as chronic hypertension and insulin dependent diabetes mellitus were represented mostly in the subgroup of PE34-37. There was no difference in parity between the subgroups, while there were significantly higher representations of those women in the subgroup of PE34-37 weeks where previous pregnancies were complicated with PE.

Comparison of A1 and A2 programs revealed small but significant differences in the calculation of UtA-PI MoM, MAP, and MAP MoM. A2 had slightly lower values when the same input data were used.

The largest difference between the expected and observed values was in the PlGF MoMs (1.239MoM).

Maternal weight (BMI), blood pressure MoMs, UtA-PI MoMs were significantly higher while the PlGF MoMs, PAPP-A MoMs were significantly lower in the preeclampsia group

compared to controls. B-hCG MoM levels showed no significant differences.

Despite the fact that PlGF MoMs were lower in the preeclampsia group than in the controls, the FMF algorithm calculated median value (1.239 MoM) of these biomarkers was still above 1 MoM.

The receiver-operating characteristics curve showed that uterine artery PI MoM, blood pressure MoM, body-mass index and maternal weight have a major impact in first trimester risk calculation for preeclampsia.

4.3. Comparison - Preeclampsia subgroups vs. controls

As mentioned before, A2 software divided each patient's individual preeclampsia risk into three subgroups:

1. PE < 34 weeks
2. PE 34-37 weeks
3. PE > 37 weeks

Maternal characteristics, BP MoMs, UtA-PI MoMs, B-hCG MoMs, PAPP-A MoMs and PlGF MoMs were compared

across the three subgroups of preeclampsia and the control group (ctrl).

Maternal weight was significantly lower in the control group (60kg) when compared with PE 34-37 (80kg) and PE>37 (71.5kg) groups.

The BMI of the controls (22kg/m^2) was significantly lower than any of the values of the preeclampsia subgroups.

The BP MoMs of the controls were significantly lower than any of the preeclampsia subgroups, and there was a significant difference between PE>37 (1.135 MoM) and the PE 34-37 (1.19 MoM) groups.

UtA-PI was significantly higher in the PE<34 (0.93 MoM) vs. ctrl (1.09 MoM) group and also between PE>37 (1.045 MoM) vs. ctrl (1.09 MoM) group.

PAPP-A MoMs were significantly lower in the PE<34 group (0.836 MoM) vs. ctrl (1.1755 MoM) group.

PlGF MoMs were significantly lower between PE34-37 (1.086 MoM) vs. ctrl (1.284 MoM) groups only.

4.4. Different input data and detection rates: A1 and A2 programs

We calculated the risk for preeclampsia by both A1 and A2 software as described previously in Chapter 3.1.3.

Based on the three types of input parameters, the results were the following:

1. Maternal characteristics, biophysical measurements
2. Maternal characteristics, biophysical measurements + PAPP-A
3. Maternal characteristics, biophysical measurements + PAPP-A + PlGF

A1 software: of the 11 early-PE cases, 7 (63.6%, 7/11) tested screen positive using the combination of maternal "a priori risk" and the measurements of first trimester BP and UtA-PI and 6 (54.5%, 6/11) when PAPP-A biomarker levels were added to risk assessment. When we calculated the risk for the 71 late-preeclampsia cases, it was found to be elevated in 48 cases (67.6%, 48/71) without PAPP-A biomarker. When PAPP-A was added, only 46 cases (64.8%, 46/71) turned out to be high risk. The false positive rate was 6% (5/82) in both calculations.

A2 software: From the 11 early-PE cases, 7 (63.6%, 7/11) had an elevated risk using the combination of maternal “a priori risk” and the measurements of first trimester BP and UtA-PI, and 8 cases (72.7%, 8/11) when PAPP-A was also used in risk calculation. Using both PAPP-A and PlGF biochemical markers in risk calculation, the detection rate in the early-PE group dropped to 5 cases (45.5%, 5/11).

The same screening pattern could be observed in the subgroup of preeclampsia between 34-37 weeks. In this subgroup, 10 patients tested screen positive (90.1%, (10/11)) both with and without PAPP-A, and the detection rate dropped to 8 cases (72.7%, (8/11)) when both biochemical markers (PAPP-A and PlGF) were used in the risk assessment.

In the subgroup of preeclampsia after 37 weeks, the detection rates were 50% (30/60) without biomarkers, 48.3% (29/60), with PAPP-A and 51.7% (31/60) when both hormones were used.

4.5. Results of the Hungarian validation study of the 10 Israeli immune proteins

The levels of Beta-2-glycoprotein 1, Complement C1q subcomponent subunit B, Complement factor B, Complement C4-A, Histidine-rich glycoprotein, Kininogen-1 and Plasminogen showed significant differences between the preeclampsia and control groups. Of these proteins the direction of the changes in preeclampsia was consistent with the Israeli study in four proteins: Beta-2-glycoprotein 1, Complement factor B, Complement C4-A and Histidine-rich glycoprotein.

5. Discussion

5.1. Population specific “a priori risk”

To our knowledge this is the first Hungarian prospective routine clinical study on screening for preeclampsia in the first trimester of pregnancy. The screening was developed using the data of the Hungarian characteristic maternal previous history and biophysical measurements. In the FMF algorithm the “a priori risk” calculation for preeclampsia is based on biochemical and biophysical measurements of previous studies from the United

Kingdom (UK). In these studies most of the patients had Afro-Caribbean origin and higher cardiovascular risk factors. This observation in agreement with other studies' conclusion highlights the need for setting up population specific "a priori risks" and biophysical measurements in order to increase detection rates in pregnant populations different from those in the UK.

Our Hungarian study proved that using a combination of maternal characteristics, MAP, UtA-PI and PAPP-A, a detection rate of 72.7% is feasible in routine clinical practice. Our detection rate was lower than that of the Anglo-Saxon studies (89.2%) performed under research settings, but similar to south-European studies performed under routine clinical practice.

Compared with the A1 program, the A2 program has a higher detection rate for early-preeclampsia with a combination of maternal characteristics, biometry and PAPP-A due to the development of screening algorithms Our results also confirm that this software is the one recommended to be used for first trimester preeclampsia screening.

Although the detection rate of late-preeclampsia was similar to that in other studies, it is still much lower than the detection

rate of early-preeclampsia. The explanation is the multifactorial etiology of this subgroup of preeclampsia. As late-preeclampsia is influenced by maternal cardiovascular factors, which play an important role in “a priori risk” calculation, adaptation of the “a priori risk” to a specific population is crucial in order to increase detection rate.

5.1.1. The role of biochemical measurements

In accordance with other studies, blood pressure MoM, BMI, maternal weight and UtA-PI MoM were the strongest early-preeclampsia predictors.

B-hCG MoM did not show any differences between the preeclampsia and the control group, a finding consistent with other studies.

Based on previous studies, PAPP-A used to be a useful marker in first trimester preeclampsia risk calculation, although when combined with other biomarkers its role becomes less pronounced. Our data proved that PAPP-A can elevate the detection rate of first trimester PE screening and also lower false positive rate. The role of PAPP-A in the lowering of the false positive rate can be accidental due to the small number of cases,

thus further larger studies are required to investigate this hypothesis. In conclusion, when PAPP-A serum levels are available in the first trimester, as a part of the combined test, it can be a useful biomarker for the risk calculation of preeclampsia as a component of the combined test.

5.1.2. PlGF

In contrast to other studies PlGF decreased detection rates for early-, and late-preeclampsia and increased false positive rate. The most likely reasons for this contradiction are the high MoM levels of PlGF in the preeclampsia (1.215 MoM) and control (1.284 MoM) groups. FMF software converted PlGF values into MoMs, and the algorithm of this conversion is based on the Anglo-Saxon pregnant population. Due to several factors both the normal and abnormal PlGF values have different distributions in the Eastern European population. Similar conclusions were published by Studies performed in the Middle-East and South-European pregnant populations have reported on similar conclusions.

In our opinion, the detection rate of early-preeclampsia in Hungary and in the Eastern part of Europe could be increased by adjusting PlGF MoM levels to the normal distribution of the examined population. Further larger East-European validation studies are suggested to define normal and abnormal values of PlGF.

Our study also highlights the need for novel biomarkers in order to increase detection rates and lower false positive rates. New biomarkers that can independently predict high risk groups without using population specific “a priori risk” would be the optimal choice for screening.

5.1.3. Cost-effective PE screening strategies in Eastern Europe

The socioeconomic status of the eastern European population is not as high as in the Western part of Europe. Not all pregnant women can afford the cost of biochemical markers (as a part of the combined test) in the first trimester of pregnancy. Since the ultrasound screening in the first trimester is covered by the National Health Insurance it can be used in the first trimester risk calculation for preeclampsia. Using the combination of

maternal previous history, blood pressure measurements and first trimester ultrasound markers, A2 software could detect 63.6% (7/11) and 56.3% (40/71) of early-PE and late-PE, respectively. This result highlights the fact that in routine clinical practice first trimester preeclampsia screening, based only on maternal medical history and ultrasound markers, can provide an acceptable screening method for a large proportion of the population at minimal extra cost. To achieve this, FMF standardised ultrasound measurements and algorithms are required to be used by FMF audited practitioners, too.

5.2 New biomarkers – proteomics

Despite the ethnical and methodological differences, the MRM could confirm the results of the Israeli study in 4 out of 10 immune proteins. So far five studies have been able to detect the same protein differences in early-preeclampsia but most of these studies used different methods and examined second and third trimester serum samples.

This highlights the fact that pro-inflammatory changes are present even in the first trimester of pregnancy in early-preeclampsia. Since these altered proteins are present in normal

blood samples as well, the change in the serum level may be detected even earlier than the first trimester.

Proteomic publications on first trimester serum samples from patients with early-, and late-preeclampsia have also described alterations among proteins that play an important role in the renin-angiotensin-aldosterone-, complement-, and coagulation systems.

Based on the results of our Hungarian study we recommend further larger validation studies to investigate the role of the altered immune protein pattern in first trimester screening for preeclampsia. This approach might increase the detection rates of both early-, and late-preeclampsia.

After the development of a cost effective screening method, new pharmacological studies could be set up using acetylsalicylic acid to decrease the prevalence of preeclampsia.

Acetylsalicylic acid has an anti-inflammatory effect and thus can decrease the prevalence of early-preeclampsia. Theoretically, acetylsalicylic acid therapy might be more effective among patients with higher immune protein alterations compared to those patients who have alterations in the renin-angiotensine system. Based on this theory, the results of the largest placebo

controlled acetylsalicylic acid trial (ASPRE) for preeclampsia prevention could be explained, where acetylsalicylic acid therapy was not effective in the subgroup of high risk patients with chronic hypertension.

6. Conclusion

Despite the role preeclampsia (PE) plays in perinatal feto-maternal mortality/morbidity, no widely accepted screening method exists for it as yet.

Our primary goal was to test in routine clinical practice the efficacy of first trimester Fetal Medicine Foundation (FMF) screening algorithms for PE and investigate the role of different maternal factors in risk calculation. We also looked for other biomarkers and validate on Hungarian first trimester blood samples the distribution of 10 immune proteins, previously described in Israel, in PE and in healthy controls.

Our retrospective study was performed on a prospectively collected database (82 PE-, and 82 controls) and on samples from 2,545 unselected pregnant women between 2010 and 2013 during routine first-trimester ultrasound screening.

Patient specific individual risks for preeclampsia in the first trimester were assessed using two commercially available risk-assessing FMF algorithms. For the proteomic study 5 PE and 10 control samples were selected and compared.

Combining maternal characteristics and biophysical parameters, Astraia 2.3.1 program had a 63.6% detection rate (DR) for early- and 67.6% for late-PE. Using an additional biomarker such as placenta associated plasma protein A (PAPP-A) in risk assessment lowered DRs to 54.5% and 64.8%, respectively. Combining maternal characteristics and biophysical parameters, Astraia 2.8.2. program, produced a 63.6% and 56.3% DRs for early-, and late – PE. PAPP-A biomarker increased DR to 72.7% in early-PE and decreased to 54.9% in late-PE. Placental growth factor (PlGF) decreased DRs in both PE groups. Despite the different populations and methods, 4 of the 10 immuneproteins showed the same patterns in PE in our study as in the Israeli study.

In conclusion, first trimester screening for PE based on maternal characteristics, biophysical parameters, and PAPP-A is feasible in routine clinical practice. It is the distribution curve of PLGF concentrations that is most likely to be different in Hungarian

patients, compared to Anglo-Saxon populations. This could explain our finding that adding PlGF to risk calculation could not improve screening efficacy. In agreement with previous reports we advise creating new Eastern European distribution curves for PlGF. Since most of the immune-proteomics changes described in the second and third trimesters of pregnancy originate from the first trimester, using this proteomics pattern, the detection rate for early-, and late-preeclampsia in the first trimester might be increased. Further validation studies are required to investigate this theory.

7. Summary of major results and scientific novelties

1. To our knowledge our study was the first in Hungary to prove the feasibility of first trimester preeclampsia screening in routine clinical practice.
2. Combination of the FMF suggested maternal previous history, blood pressure-, and UtA-PI measurements can result in an acceptable screening method during the routine first trimester ultrasound scan.
3. The first trimester biophysical and biochemical measurements of the Hungarian pregnant population is different from those of Western-European pregnant women.
4. Despite the fact that in our study first trimester PlGF MoM levels were lower in pregnancies associated with preeclampsia, mean PlGF levels were still above 1 MoM both in the PE and control groups. Due to this PlGF could not increase the detection rates of preeclampsia screening.
5. Our study proved that the levels of immune proteins are altered in the first trimester of pregnancy in early-preeclampsia compared to controls. In previous studies

most of the altered proteins have been described in the second and third trimesters. Our findings highlight the fact that most pathological pathways, described in the second and trimester of pregnancy, can be observed already in the first trimester in women with early-preeclampsia.

8. Acknowledgments

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9. Publications

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Candidate: László Orosz
Neptun ID: G7GMNY
Doctoral School: Doctoral School of Clinical Medicine
MTMT ID: 10032549

List of publications related to the dissertation

1. **Orosz, L.**, Orosz, G. B., Veress, L., Dósa, D., Orosz, L., Arany, I., Fábián, A., Medve, L., Pap, K., Karányi, Z., Tóth, Z., Póka, R., Than, N. G., Török, O.: Screening for preeclampsia in the first trimester of pregnancy in routine clinical practice in Hungary.
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Total IF of journals (all publications): 13,518
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