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ELE HANSON

Clinical and biochemical markers for the prediction and early diagnosis of pregnancy related complications

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Clinical and biochemical markers for the prediction and early diagnosis of pregnancy related complications

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications and unpublished data.

I. Hanson, Ele; Rull, Kristiina; Ratnik, Kaspar; Vaas, Pille; Teesalu, Pille; Laan, Maris (2022). Value of soluble fms-like tyrosine kinase-1/placental growth factor test in third trimester of pregnancy for predicting preeclampsia in asymptomatic women. Journal of Perinatal Medicine, 1−8. DOI: 10.1515/jpm-2022-0127.

II. Ratnik, Kaspar; Rull, Kristiina; Hanson, Ele; Kisand, Kalle; Laan, Maris (2020). Single-Tube Multimarker Assay for Estimating the Risk to Develop Preeclampsia. The Journal of Applied Laboratory Medicine, 5 (6), 1156−1171. DOI: 10.1093/jalm/jfaa054.

III. Hanson, Ele.; Ringmets, Inge.; Kirss, Anne.; Laan, Maris; Rull, Kristiina (2022). Screening of Gestational Diabetes and Its Risk Factors: Pregnancy Outcome of Women with Gestational Diabetes Risk Factors According to Glycose Tolerance Test Results. Journal of Clinical Medicine, 11(17), 4953. DOI: 10.3390/jcm11174953

My contribution to the publications:

Paper I: Participated in the study design, data collection, analysis and interpretation of the outcome. Prepared the first draft of the manuscript and contributed significantly to its revision and finalization.

Paper II: Participated in the data collection, clinical data analysis and reviewing the manuscript.

Paper III: Participated in the study design, collection and analysis of the data. Prepared the first draft of the manuscript and contributed significantly to its revision and finalization.

ABBREVIATIONS

ACOG	American College of Obstetricians and Gynecologists
ADA	American Diabetes Association
ADAM 12	a disintegrin and metalloproteinase 12
AOR	adjusted odds ratio
ASA	acetylsalicylic acid, aspirin
BMI	body mass index
C-section	cesarean section
CI	confidence interval
d.	days
DM	diabetes mellitus
DR	detection rate
FGR	fetal growth restriction
FPR	false positive rate
GDM	gestational diabetes mellitus
g.d.	gestational days
GH	gestational hypertension
g.w.	gestational weeks
HbA1c	hemoglobin A1C
IADPSG	International Association of Diabetes and Pregnancy Study Group
IL	interleukin
ISSHP	The International Society for the Study of Hypertension in Pregnancy
IVF	*in vitro* fertilization
LGA	large for gestational age
LMWH	low molecular weight heparin
MAP	mean arterial pressure
NICE	National Institute for Health and Care Excellence
OGTT	oral glycose tolerance test
OR	odds ratio
PAPP-A	pregnancy-associated plasma protein A
PCOS	polycystic ovary syndrome
PE	preeclampsia
PlGF	placental growth factor
PPV	positive predictive value
PTB	preterm birth
PTX3	pentraxin 3
sFlt-1	soluble fms-like tyrosine kinase 1
SGA	small for gestational age
sENG	soluble endoglin
SLE	systemic lupus erythematosus
Tregs	T-regulatory cells
WHO	World Health Organization

1. INTRODUCTION

Most of the pregnancies proceed without problems and result with the delivery of a healthy neonate. However, complications endangering the health of the mother and the baby during pregnancy and delivery occur in approximately one in four pregnancies (tai.ee). The complications may have long-term impact on both the mother and the offspring.

One of the leading causes of maternal and perinatal mortality and morbidity worldwide are pregnancy related hypertensive disorders affecting 5–10% of pregnancies (Mathew et al., 2023; Poon et al., 2019). Preeclampsia (PE) is a multisystem syndrome originating in the maternal–fetal interface. Hypertension is the cornerstone of the syndrome, accompanied by end-organ dysfunction. PE can result in renal, cardiac, pulmonary, hepatic, and neurological dysfunction, hematologic disturbances, fetal growth restriction, stillbirth, and/or maternal death (Ives et al., 2020). PE is responsible for more than 500,000 fetal and neonatal and over 27,000 maternal deaths worldwide (Poon et al., 2019; Wang et al., 2021; Magee et al., 2022). Although the known treatment for PE is delivery, the damage related to PE is not reversed by ending the pregnancy and continues to affect the long-term health of the mother (Melchiorre et al., 2022). Fortunately, the prevalence of PE is decreasing worldwide, especially in developed countries due to well-established antenatal care (Wang et al., 2021).

The other common pregnancy complication, gestational diabetes mellitus (GDM), is becoming more prevalent, complicating one in sixth births (Yuen et al., 2019). GDM, hyperglycemia detected in pregnancy, is related to several obstetric and neonatal complications such as increased birthweight, shoulder dystocia, stillbirth, 4^{th} grade perineal tear, etc. but also long-term health problems like type II diabetes and cardiovascular diseases for both the mother and the baby (Sweeting et al., 2022).

A lot of research is devoted to better understanding the causes and development of PE and GDM, so the antenatal care can shift from handling the consequences to prevention.

The main aim of the thesis is to assess the prevalence of PE, GDM and their risk factors. Also, to evaluate risk factors, biomarkers and oral glycose tolerance test in the early prediction and diagnosis of the pregnancy complications.

2. LITERATURE REVIEW

2.1 Preeclampsia

Preeclampsia (PE) is one of the most known and well-studied pregnancy complications due to its unpredictable course and serious complications. However, the etiology of this heterogenous disease remains unclear, earning the title of "disease of theories" (Melchiorre et al., 2022).

PE, a multisystem disorder, is characterized by new onset hypertension (systolic ≥ 140 mmHg and/or diastolic ≥ 90 mmHg) at or after 20 weeks of pregnancy accompanied by maternal end-organ dysfunction (Magee et al., 2022). Traditionally significant proteinuria has been the additional symptom referring to PE. Since 2018 according to International Study for the Study of Hypertension in Pregnancy (ISSHP) guidelines the definition was expanded including also signs of uteroplacental dysfunction, liver involvement (elevated liver enzymes), neurological (headache, eclampsia) and/or hematological (thrombocytopenia, hemolysis) complications (Brown et al., 2018; Poon et al., 2019).

PE can be subclassified by the gestational age at clinical presentation. The early onset form is characterized by the preterm delivery due to PE before 34 gestational weeks (g.w.) often accompanied by fetal growth restriction (FGR), while in late onset PE (delivery after 34 g.w.) the weight of the newborn is usually normal or even large for gestational age (LGA). Also classification of preterm (delivery ≤ 37 g.w.) or term (delivery ≥ 37 g.w.) can be used (Dimitriadis et al., 2023; Magee et al., 2022; Poon et al., 2019). Since 2018, the classification as mild or severe PE is not recommended anymore, as mild symptoms of PE can rapidly deteriorate (Brown et al., 2018).

2.1.1 Patophysiology of PE

Preeclampsia is a pregnancy specific disorder and is thought to be caused by the abnormal development and/or function of the placenta (Huppertz, 2018). PE is diagnosed only in humans as the placenta is more invasive compared to other species (Varas Enriquez et al., 2018).

During normal implantation, the trophoblast invasion leads to myometrial spiral arteries transformation into high-volume, low resistance, thin-walled vessels with large lumens, making the increased blood flow to the placenta possible regardless of maternal vasomotor changes (Cross et al., 2002; Sojka et al., 2019; Lyall et al., 2013). Inadequate trophoblast invasion and incomplete myometrial spiral arteries remodeling causes malperfusion of the placenta (Sircar et al., 2015; Kaufmann et al., 2003; Lyall et al., 2013) **(Figure 1).** Apart from PE, placentation problems can result also as other "placental syndromes" including isolated FGR, spontaneous preterm birth (< 37 g.w.), abruption of placenta or intrauterine fetal death (Staff, 2019).

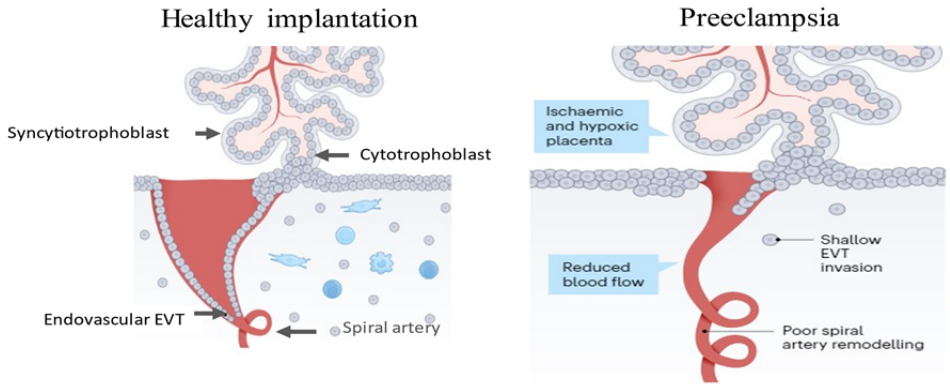

Figure 1. Normal versus defective spiral artery remodelling. Adapted from Dimitriadis et al., 2023. EVT, extravillous trophoblast

For normal implantation, the immune system's ability to adapt to pregnancy and develop tolerance to paternal antigens in the fetus is also crucial (Mor, 2022). In normal pregnancy, the shift in T helper cells phenotype towards the anti-inflammatory Th2 helps to suppress proinflammatory cytokines and angiotensin II type 1 receptor antibodies to support normal placentation. PE, in contrast, is characterized by the T-helper profile shifting towards Th1 therefore promoting the release of proinflammatory interleukins IL-2 and IL-18 and limiting the depth of trophoblast invasion (Ives et al., 2020). Another type of T cells, T-regulatory cells (Tregs), also contribute to development of immune tolerance in pregnancy. Besides their anti-inflammatory, immune-regulatory and vasoregulatory functions, Tregs also promote other leukocytes to contribute to the implantation. In case of PE, the function of Tregs seems to be inadequate and their concentration in the maternal decidua low (Robertson et al., 2018).

Sperm exposure for longer period before conception has shown to be helpful in developing immune tolerance and is protective against PE (Robillard et al., 2022). Shorter period of sexual cohabitation might explain the greater risk of PE in nulliparous women or multigravidas with new partner as the immune system fails to adapt to pregnancy (Staff, 2019). In multiparas with the same partner, long interpregnancy interval seems to be risk factor for PE due to diminishing amount of partner specific memory Tregs cells over time (Skjærven et al., 2002).

On the second half of the pregnancy, the second stage, abnormally developed placenta is unable to cope with the growing needs of the fetus. Stressed placenta starts releasing pro-inflammatory and antiangiogenic proteins into the mother´s circulation (Maynard et al., 2010; Levine et al., 2004; Staff, 2019). Imbalance of factors responsible for regulating angiogenesis is causing endothelial damage (Herraiz et al., 2018).

Not all PE cases could be explained by defective placentation in the beginning of pregnancy. PE is most often diagnosed at term (after 37 g.w.) and is not related to fetal growth restriction, common in early PE. In 2014, Redman et al

suggested that when placenta outgrows the uterine capacity at the end of the pregnancy the uteroplacental ischemia with syncytioblast stress might develop without poor placentation (Redman et al., 2014). In addition to term pregnancies, increased placental volume could explain higher proportion of PE also in multiple pregnancies, and gestations with large for gestational age newborns Staff, 2019).

It is speculated that late PE is a failure of maternal cardiovascular system to adapt to pregnancy related stress (Melchiorre et al., 2022; Staff, 2019) (**Figure 2**).

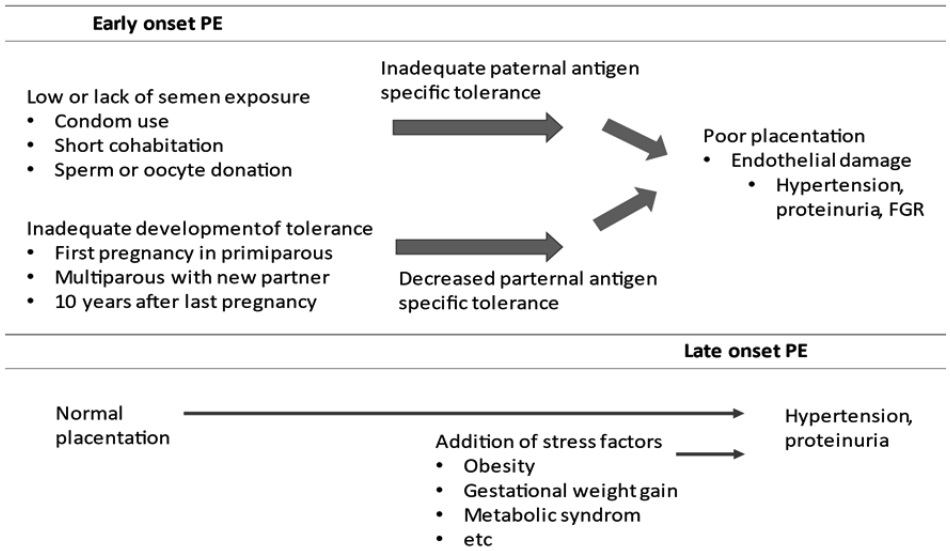

Figure 2. Pathogenesis of early and late PE. Adapted from Robillard et al., 2022.
FGR, fetal growth restriction; PE, preeclampsia.

Pre-pregnancy generalized maternal vascular inflammation present in chronic hypertension, diabetes, obesity, and some autoimmune diseases can affect the spiral arteries and its remodeling during implantation resulting in abnormal placentation but could also predispose maternal cardiovascular system to sensitivity to placenta-produced factors without excessive placental stress (Staff, 2019). In addition, recent study showed different hemodynamic function in normotensive pregnancies compared to women with subsequent PE, even if the latter were not clinically diagnosed with prior cardiovascular disease. Patients with PE or FGR had higher resistance circulation, increased cardiac output and higher blood pressure in comparison with normal gestations (Garcia-Gonzalez et al., 2020).

Morphologic and functional cardiovascular changes seen in PE persist years after birth increasing the risk of development of chronic hypertension later in life by 3 to 6-fold and therefore considered as an independent risk factor for cardiovascular disease in women (Lykke et al., 2009; Melchiorre et al., 2022; Mosca et al., 2011).

2.1.2 Screening for preeclampsia

2.1.2.1 PE risk screening based on maternal medical history

Traditionally, the screening for PE has been based on maternal medical history. The well-known risk factors are chronic illnesses (hypertension, diabetes, kidney disease), previous or family history of PE, obesity, IVF, long interpregnancy interval and nulliparity (**Table 1**). Several guidelines have highlighted a number of risk factors to be considered to start prophylaxis with acetylsalicylic acid (ASA) (Brown et al., 2018; Espinoza et al., 2020; NICE guideline, 2019; Rull et al., 2021).

Table 1. Risk factor-based preeclampsia screening recommended by different guidelines[a]

Risk factor	NICE guideline [b]	ISSHP guideline 2018	ACOG guideline	Estonian guideline
PE in previous pregnancy	++	+	+	+
Chronic hypertension	++	+	+	+
Kidney disease	++		+	+
Pre-pregnancy diabetes	++	+	+	+
Obesity (BMI ≥30kg/m^2) [c]	+	+	+	+
Maternal age ≥40 years [d]	+		+	+
Multiple pregnancy	+	+	+	+
PE in family history	+			+
Nulliparity	+		+	+
Interpregnancy interval ≥10 years	+			+
IVF		+	+	+
SLE	++	+	+	+
Antiphospholipid syndrome	++	+	+	+
Gestational diabetes			+	
Obstructive sleep apnea			+	
Thrombophilia			+	
Race (Afro-Carribean, South Asia)				+

[a] Brown et al., 2018; Espinoza et al., 2020; NICE guideline, 2019; Rull et al., 2021

[b] In NICE guidelines ++ indicates high risk, + a moderate risk; two or more moderate risk factors combined indicates also high risk

[c] In NICE guidelines BMI ≥ 35 kg/m^2 is considered as a risk factor

[d] ACOG guidelines consider maternal age ≥ 35 years as a risk factor

BMI, body mass index; CI, confidence interval; IVF, *in vitro* fertilization; multip, multiparity; nullip, nulliparity; PE, preeclampsia; RR, relative risk; SLE, systemic lupus erythematosus

However, O´Gorman et al showed in 2017 that screening in the first trimester by risk factors according to NICE guidelines was able to detect 41% (95% CI, 18–67%) of PE cases manifesting < 32 g.w., 39% (95% CI, 27–53%) of PE < 37 g.w. and 34% (95% CI, 27–41%) of PE ≥ 37 g.w., at the false positive rate (FPR) of 10.2%. Screening women by ACOG recommendations was able to identify 94% (95% CI, 71–100%) of PE < 32 g.w., 90% (95% CI, 79–96%) of PE < 37 g.w., and 89% (95% CI, 84–94%) of PE ≥ 37 g.w., but at the expense of a very high FPR of 64.2% (O'Gorman et al., 2017). Low detection rate (DR, sensitivity) and high FPR by maternal history-based screening has pointed out the need for more precise screening methods.

2.1.2.2 PE risk screening based on maternal serum biomarkers

Traditional screening by maternal risk factors is often replaced by combined screening (Mosimann et al., 2020) **(Table 2).**

Table 2. Detection rate of different combined tests in first trimester PE screening at fixed FPR of 10%

	Detection rate		
Method of screening	< 34 g.w	< 37 g.w	≥ 37 g.w
Maternal factors	48.3%	41.5%	30.2%
Maternal factors+MAP	65.0%	70.0%	38.7%
Maternal factors +MAP+UtA PI	88.3%	73.9%	43.5%
Maternal factors +MAP+PlGF	73.3%	68.3%	39.6%
Maternal factors +MAP, UtA PI+PlGF	90.0%	81.7%	42.6%
Maternal factors + MAP+UtA PI+ PlGF+PAPP-A	89% (<32 g.w)	75%	48%

FPR, falce positive rate; g.w, gestational week; MAP, mean arterial pressure; PE, pre-eclampsia; PlGF, placental growth factors; UtA PI, uterine artery pulsatility index. Modified from Dimitriadis et al., 2023 and O'Gorman et al., 2016.

Most first trimester combined screening algorithms are based on combinations of biomarker values, measurement of uterine arteries pulsatility index by ultra-sound and/or mean arterial pressure in addition to maternal history identifying up to 82% of patients developing PE therefore doubling the DR compared to screening by risk factors alone (Macdonald et al., 2022; Mosimann et al., 2020) **(Table 2 and 3).**

Table 3. Description of biomarkers used in PE screening

Biomarker	Function	Association with pregnancy complications
PlGF	Stimulates trophoblast growth/ differentiation and contributes to angiogenesis and vasculogenesis	Low levels in early pregnancy in PE patients. Used in III trimester alone or in combination with sFlt-1 to detect PE. Low levels in case of SGA. Marker of placental insufficiency. (Chau et al., 2017)
sFlt-1	Antagonizes the action of proangiogenic proteins (VEGF, PlGF)	Increased on III trimester in PE patients. Used in combination with PlGF.(Levine et al., 2004)
sENG	Affects normal migration and proliferation of endothelial cells	Increased levels in III trimester are associated with severity of PE (Leaños-Miranda et al., 2017)
ADAM 12	Involved in trophoblast migration and invasion into the uterus during placental development	Decreased in pregnancies complicated by PE or SGA (Andres et al., 2022)
PAPP-A	Affects placental development, contributes to trophoblast proliferation, hormone secretion and reduces apoptosis	Low levels are associated with impaired placental development and function related to PE and SGA (Christians et al., 2016)
Leptin	Regulates energy intake and expenditure. Acts in placental nutrient transfer.	Dysregulated leptin is involved in SGA and PE development (de Knegt et al., 2021)
Adiponectin	Mediates anti-inflammatory effects, improves lipid metabolism and insulin sensitivity. Metabolic adaptation in pregnancy.	III trimester level is increased in women with PE (Pheiffer et al., 2021)
PTX3	Involved in the pathogenesis of inflammation and ischemia-related diseases. Activates and interacts with multiple components of the complement system	Increased level in PE, especially in early and severe cases.(Xiong et al., 2020)

ADAM 12, disintegrin and metalloproteinase domain-containing protein 12; PAPP-A, placenta associated plasma protein A; PE, pre-eclampsia; PlGF, placental growth factor; PTX3, pentraxin-related protein 3; sENG, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase-1, SGA, small for gestational age.

However, the limitations for currently applied combined screening tests are low DR (48%) for PE manifesting after 37 g.w. and need for expensive tools such as ultrasound machine for uterine arteries testing, encouraging continuing research for more accurate and easier to use risk assessment methods (Macdonald et al., 2022).

2.1.2.3 III trimester PE screening

After 20 weeks of pregnancy, PE is screened at every antenatal visit by measurement of blood pressure and protein in the urine sample.

In 2016, Zeisler et al proposed that sFlt-1/PlGF < 38 can predict short term (1 week) absence of PE in symptomatic women (Zeisler et al., 2016). Additionally, gestational age specific "rule-in" cut-offs (≥85 before 34 g.w and ≥110 after 34 g.w.) to diagnose PE were developed (Verlohren et al., 2014).

In normal course of pregnancy, the sFlt-1 levels are low during the first two trimester and start rising after 33 to 36 g.w. The free PlGF concentration, in contrary, is high in the second trimester promoting angiogenesis and decreases steadily at the end of the pregnancy. It has been speculated that at term the placental vascular growth is suppressed by an increase of antiangiogenic factors as a normal physiological course of pregnancy (Levine et al., 2004). In PE, the sFlt-1 concentration starts increasing earlier in pregnancy and reaches higher values, while the level of free PlGF remains low throughout the gestation, probably due to a result of diminished placental production or increased receptor binding (Taylor et al., 2003; Levine et al., 2004).

The change in the sFlt-1/PlGF ratio indicating to PE can be detected already five weeks before the onset of symptoms, making this also a possible screening test for PE in the third trimester.

Some national guidelines (e.g., Germany, Stepan et al., 2015) consider sFlt-1/PlGF test useful for the aid to diagnose PE, however, international guidelines ISSHP and NICE remain cautious, stating that further studies in different populations are needed before including sFlt-1/PlGF testing into daily clinical practice (Brown et al., 2018; NICE guideline, 2019).

In addition to biochemical markers, different Doppler indices representing changes in maternal cardiovascular system have been studied for the PE prediction. Sarno et al showed measurement of ophthalmic artery PSV ratio as a predictive tool when measured at 35–37 g.w. (Sarno et al., 2020).

2.2 Gestational diabetes mellitus

GDM is the most common complication in pregnancy. Despite different screening protocols, the prevalence of GDM is increasing worldwide (Sweeting et al., 2022).

GDM is traditionally defined as any degree of glycose intolerance with the first recognition during pregnancy (Sweeting et al., 2022). However, the definition of GDM has been changed as in many cases GDM represents the first

diagnosis not the onset of hyperglycemia in pregnancy (ADA, 2022). “Diabetes in pregnancy” refers to pre-pregnancy diabetes or diabetes first detected during pregnancy and is diagnosed when hyperglycemia at any stage of pregnancy meets the criteria of diabetes of non-pregnant population such as fasting plasma glycose ≥ 7 mmol/l; 2-hour oral glycose test (OGTT) value ≥ 11.1 mmol/l, or random plasma glycose ≥ 11.1 mmol/l associated with symptoms characteristic to diabetes. “Gestational diabetes mellitus” is diagnosed when hyperglycemia is detected during routine testing in pregnancy, usually after 20 weeks of gestation (Hod et al., 2015).

The main aim of the GDM management is achieving maternal normoglycemia to reduce maternal and fetal complications. The first step is lifestyle modifications including diet and moderate exercise. If the maternal glycose levels remain elevated despite of diet, the treatment with medication (metformin or insulin) is recommended (Paschou et al., 2022; Sweeting et al., 2022).

2.2.1 Patophysiology of GDM

Changes in maternal glycose metabolism are needed to provide adequate nutrition for normal fetal growth. In the beginning of pregnancy higher sensitivity to insulin stimulates glycose uptake to adipose stores to prepare for growing energy demands during the second half of pregnancy. As pregnancy advances, levels of local and placental hormones (growth hormone, human placental lactogen and prolactin) increase promoting maternal insulin resistance (Plows et al., 2018). As a result, maternal postprandial glycose and free fatty acid levels are rising and stimulate maternal and fetal growth (Plows et al., 2018; Sweeting et al., 2022). To respond to the increased insulin demand and maintain glycose homeostasis maternal pancreatic β-cells proliferate and become hypertrophic which allows to double insulin secretion (Plows et al., 2018; Sweeting et al., 2022).

When this normal metabolic adaptation to pregnancy fails, GDM develops. Pancreatic β-cells fail to proliferate and therefore are unable to compensate the increased insulin demand as a response to insulin resistance in pregnancy. Glucose uptake decreases leading to hyperglycemia in the maternal and fetal circulation (Sweeting et al., 2022). GDM has been shown to develop more likely in women with preexisting impaired β-cells function and/or insulin resistance e.g., women with pre-pregnancy obesity or polycystic ovary syndrome (PCOS) (Sweeting et al., 2022; Plows et al., 2018). Increasing incidence of obesity among women in childbearing age explains the rising numbers of women with GDM diagnosis (Sweeting et al, 2022).

Glycose transport to the placenta and fetus is mediated by glycose transporter 1 (GLUT1) not insulin (Augustin, 2010). Hence, maternal hyperglycemia means also high glycose levels in fetus leading to fetal hyperinsulinemia, accelerated growth and therefore fetal macrosomia as well as complications accompanying it (Sweeting et al., 2022) (**Figure 3).**

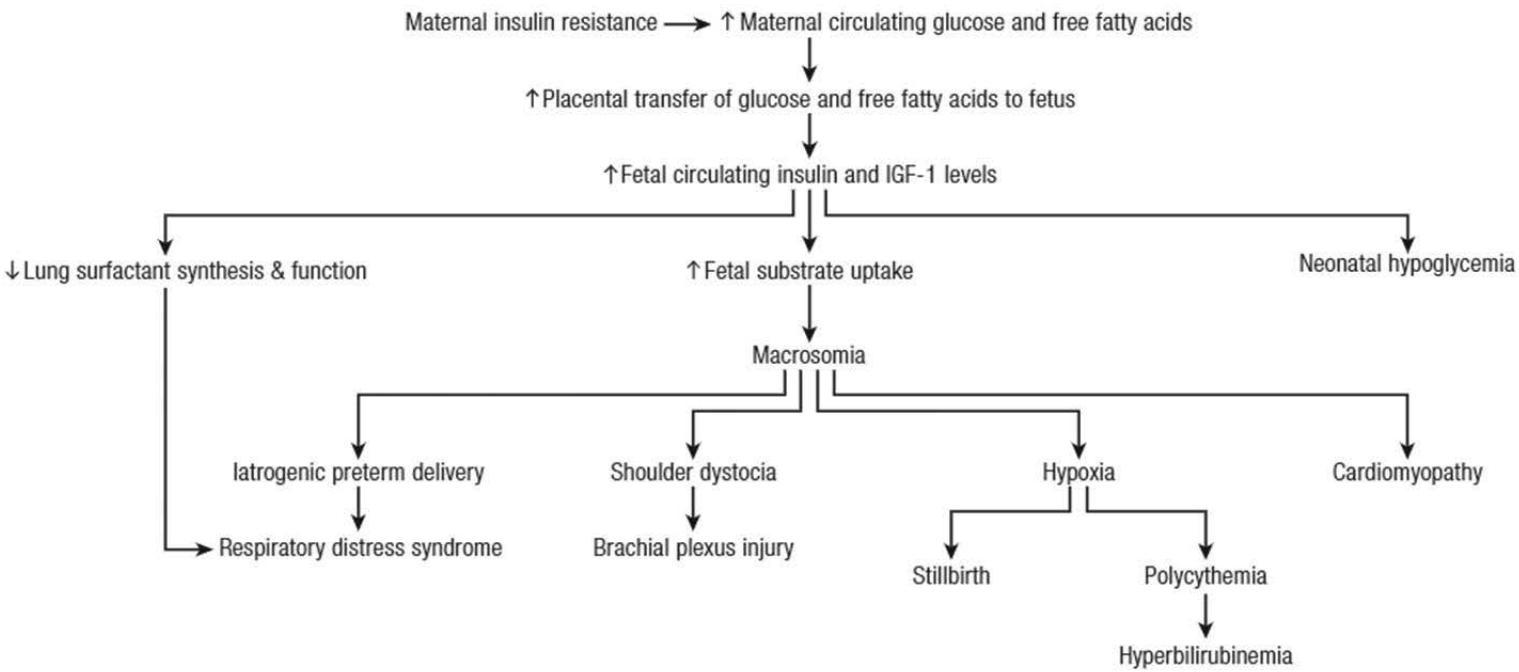

Figure 3. Pathway to perinatal complications related to GDM (Sweeting, et al, 2022).

In short-term, women with GDM have increased risk of induction of labor, operative delivery, and adverse pregnancy outcome such as hypertensive disorders of pregnancy (Anderberg et al., 2010; Sweeting et al., 2022). Additionally, women diagnosed with GDM in pregnancy are nearly 10-times more likely to have type 2 diabetes mellitus later in life compared to normoglycemic pregnancies (Vounzoulaki et al., 2020). Also, the risk of developing cardiovascular disease is increased (Tobias et al., 2017).

2.2.2 Screening for GDM

Currently there is no worldwide consensus who belongs to risk group for GDM or "gold standard" test to define the disease status of GDM (WHO, 2013).

Since 2010, the most widely accepted test for the diagnosis of GDM is 75g oral glycose tolerance test (OGTT) applied at 24–28 g.w. with a cut-off of fasting venous plasma glucose ≥5.1 mmol/L and/or after 1 h and 2 h level of ≥10.0 mmol/L and ≥8.5 mmol/L, respectively (IADPSG, 2010). These thresholds were selected based on the results of HAPO (Hyperglycemia and Adverse Pregnancy Outcomes) study. Based on fully adjusted logistic regression models the average glycose values were selected when the odds of delivering neonate with birthweight, cord C-peptide and body fat > 90th percentile reached 1.75 times the estimated odds of these outcomes at mean glucose values (Metzger et al., 2008; IADPSG, 2010). To make the diagnosis of GDM, at least one of these values must be equaled or exceeded. By IADPSG recommendations all or high-risk women should have fasting or random plasma glycose or hemoglobin A1C (HbA1c)measured at first prenatal visit and 2h 75g OGTT at 24–28 g.w. (IADPSG, 2010).

Most European countries (52%) including Estonia recommend referral to OGTT for GDM screening only for women with risk factors (Benhalima et al., 2016; Vaas et al., 2018). Well-known risk factors are body mass index (BMI) ≥30 kg/m^2, family history (1st degree relative) of diabetes, previous macrosomic baby ≥4.5 kg, previous GDM and PCOS (Benhalima et al., 2019). At the moment GDM is a laboratory and not clinical diagnosis. Risk-based screening is dependent on healthcare worker's ability to notice risk factors and makes the referral to testing. High risk untested pregnant women include those with possible undiagnosed GDM and prone to poor gestational outcome (Avalos et al., 2013).

In addition to OGTT, the HbA1c as a diagnostic marker for GDM has been studied. Although the sensitivity and specificity of isolated HbA1c measurement as a diagnostic test are not sufficient, this could be useful rule-in test in association with traditional OGTT (Minschart et al., 2021).

Furthermore, plasma glycated CD59 (pGCD59), has been suggested as a biomarker for GDM. Measurement of this marker before 20 g.w. accurately predicted the OGTT results (AUC 0.9 (95% CI, 0.81–0.87) and was associated

with 36% of increased odds of the birth of the LGA newborn (Ma et al., 2020; Minschart et al., 2021).

2.3 Antenatal screening in Estonia

In Estonia, according to Medical Birth Registry, 93.1% of women have their visit to the midwife or gynecologist before 12th weeks of pregnancy, therefore several screening test can be applied already early in pregnancy (tai.ee) **(Table 4).**

Table 4. Screening of pregnancy complications according to Estonian antenatal follow-up guideline (Vaas et al., 2018)

Complication	Selective vs universal	Timing (trimester)	Method of testing	Management
GDM	Selective	II and III	OGTT	Diet, medication
Preeclampsia	Universal	I, II, III	Maternal history, ultrasound, MAP, biomarkers, urine	Prevention with ASA I trimester
Anemia	Universal	I, II and III	Blood sampling	Iron or vitamin supplements
Preterm birth	Selective	II	Ultrasound	Early interventions
Thrombosis	Universal	I, II and III	Medical history	Prevention with LMWH
Fetal growth restriction	Selective	I, II and III	Maternal history, PAPP-A, ultra-sound	Monitoring and timed birth

ASA, acetylsalicylic acid; GDM, gestational diabetes; LMWH, low molcular weight heparin; MAP, mean arterial pressure; OGTT, oral glycose tolerance test; PAPP-A, placenta associated plasma protein A.

Traditionally, PE risk prediction in Estonia has been based mainly on the risk factor assessment in the first antenatal visit as cheap and easily available method (Vaas et al., 2018). Combined screening for PE, as a more accurate method for risk prediction, was started in Tallinn and Tartu in 2018 and 2020, respectively. Prophylaxis with acetylsalicylic acid is recommended to the high-risk women as recommended by international and Estonian guidelines(Brown et al., 2018; Rolnik et al., 2017; Rull et al., 2021). The third trimester monitoring for PE includes the routine blood pressure measurement and assessment of proteinuria in every visit. The biomarkers (sFlt-1/PlGF or PlGF) are not recommended for a routine use (Rull et al., 2021).

In 10 years (2012–2022) the prevalence of PE in Estonia has decreased from 2.2% to 1.5% according to Estonian Pregnancy Registry data (tai.ee) **(Figure 4).**

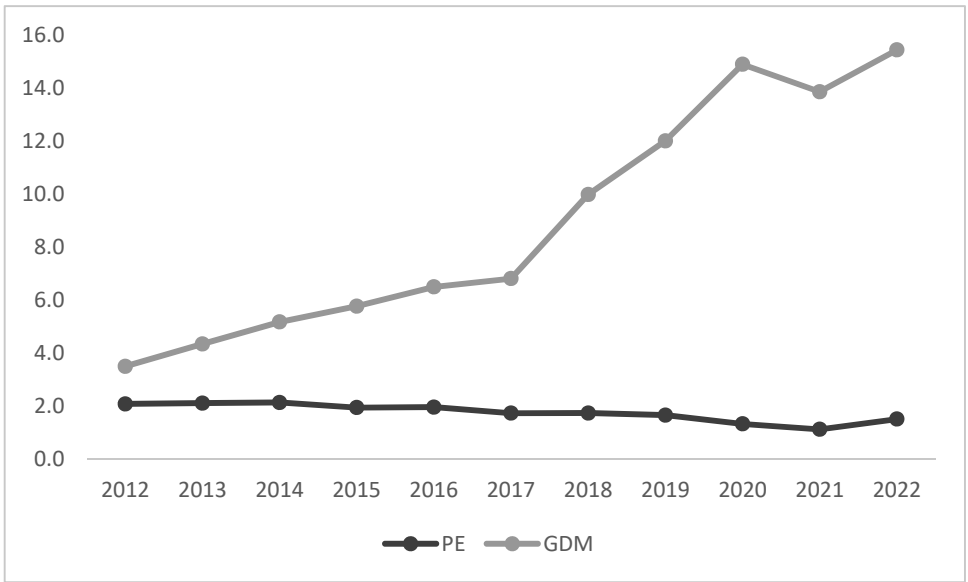

Figure 4. Changes in PE and GDM prevalence in Estonia over 10 years (2012–2022) (tai.ee). PE, preeclampsia; GDM, gestational diabetes

Inverse trend can be seen in the prevalence of gestational diabetes with the growing number of women receiving the diagnosis (from 3.5% in 2012 to 15.5% in 2022) (tai.ee.)**.**

Estonia implemented new GDM screening guidelines following IADPSG recommendations in 2011 (IADPSG, 2010; Kirss et al., 2015) **(Figure 5)**. In Tartu University Hospital Women´s Clinic adaption of new guidelines increased the prevalence of GDM from 1.5% to 6% (Kirss et al., 2015).

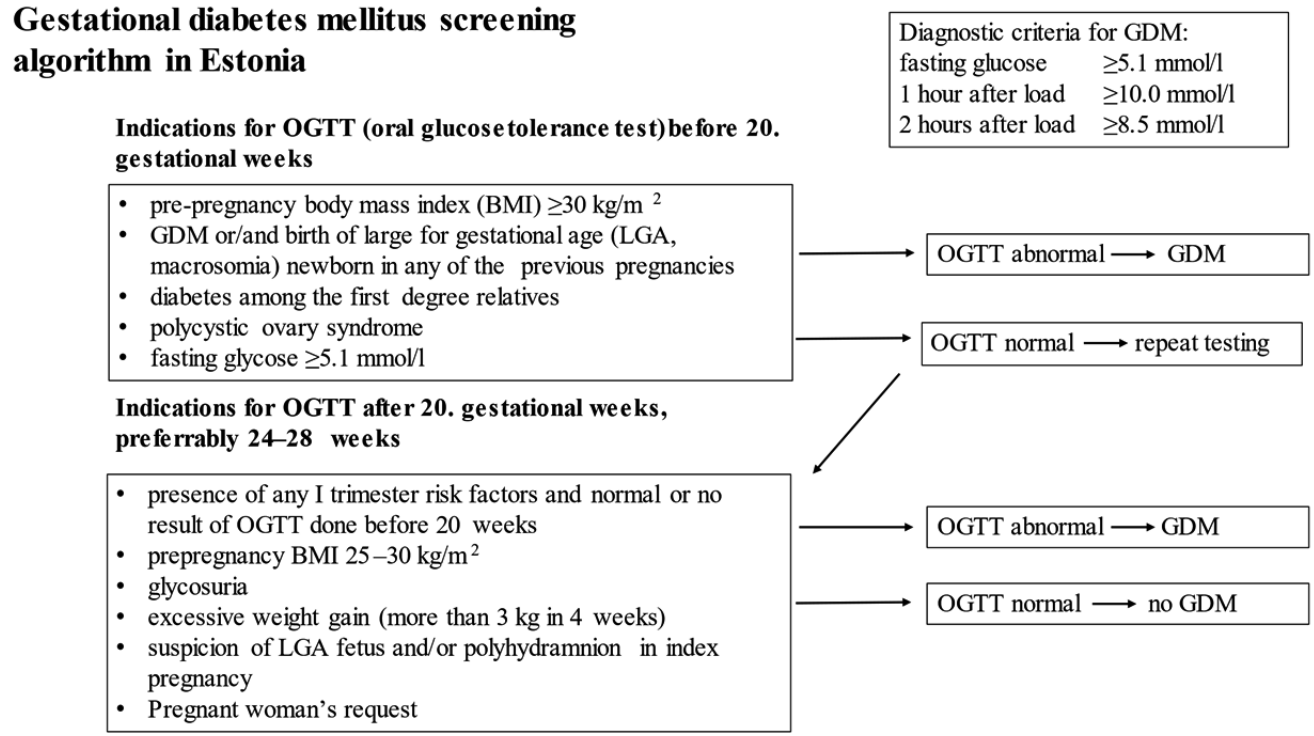

Figure 5. Gestational diabetes mellitus screening algorithm in Estonia. Adapted from Hanson et al., 2022a.

Women with GDM diagnosis are referred to midwives specially trained for GDM monitoring and internal medicine specialist/endocrinologist when necessary. Additional ultrasound to determine estimated fetal weight is offered at 37–38 g.w. and induction of labor at 39 g.w. when LGA is suspected. Women with normal OGTT result continue routine pregnancy follow-up.

2.4 Pregnancy as a predictor of future health

Individuals' health is influenced by different factors (biological, behavioral or social) throughout life (WHO, 2018). Understanding the effect of pregnancy to the female organism and addressing the problems can be beneficial in long term.

Pregnancy is like a stress test, revealing the weakest sides of woman´s organism (Williams, 2003). Many studies have demonstrated that women experiencing hypertension in pregnancy have increased risk of chronic hypertension later in life (Bhattacharya et al., 2012; Drost et al., 2013; (Lykke et al., 2009). The risk is dependent on the severity of the hypertension, the onset during pregnancy, the association with fetal growth restriction, number of pregnancies complicated with hypertension and the need of iatrogenic preterm delivery (Melchiorre et al., 2020).

In preeclamptic pregnancies cardiac dysfunction and left ventricular hypertrophy have been described. These changes are not totally reversed after delivery and contribute to the development of essential hypertension (Melchiorre et al., 2011). Following GDM, the long-term risk of developing cardiovascular disease is twice as high as in women without GDM irrespective of subsequent development of type II diabetes (Kramer et al., 2019).

GDM is also a well-known risk factor for the development of type II diabetes, increasing the risk 10-fold compared to normoglycemic pregnancies (Bellamy et al., 2009; Vounzoulaki et al., 2020). Similarly, women with PE are 2-times more likely to develop diabetes even in the absence of GDM (Feig et al., 2013; Weissgerber et al., 2015). GDM and PE as important risk factors for future cardiovascular disease and type 2 diabetes have been recognized by international organizations (Magee et al., 2022; (Mosca et al., 2011)

The association of chronic diseases and pregnancy complications is at least partly explained by the high BMI. During the antenatal follow-up women are in good and frequent contact with healthcare personnel. This provides the opportunity to counsel about risks in future life and direct towards healthier lifestyle. In addition, after experiencing pregnancy complications, collaboration, and referral to other specialists (cardiologists, nephrologists etc) gives a good opportunity to affect lifetime morbidity (Brown et al., 2018). Strict follow-up within the first weeks after delivery due to pregnancy complication has shown promising results to lower long-term complications (Kitt et al., 2021).

Thus, prediction and active management of pregnancy complications can give the health system a tool to possibly prevent or postpone the manifestation of chronic diseases.

2.5 Rationale of current thesis

A lot of research is dedicated to the early detection of pregnancy complications to improve pregnancy outcomes.

Currently applied first trimester screening test for PE combining maternal history, biomarkers, measurement of blood pressure and ultrasound has shown to have high DR (90%) for early onset PE. However, the limitations such as low DR for late PE, which constitutes most of the cases and the need for expensive tools (ultrasound) are encouraging more research of different predictive tests throughout pregnancy that could be applicable before onset of PE symptoms.

Opposite to PE, the aim of GDM screening is to find and treat the disorder instead of prediction. The diagnosis is based on laboratory measurements and dependent on the referral to the test. Therefore, the outcome of pregnancies predisposed to GDM with and without OGTT referral or not exceeding the thresholds for GDM diagnosis could be subject of further attention.

3. AIMS OF THE RESEARCH

The aim of this thesis was to assess, evaluate current and develop novel prediction and assessment approaches to the common pregnancy complications, PE and GDM, by clinical and biochemical markers.

Specific objectives:

1. To assess the prevalence of PE risk factors and their effectiveness in prediction of PE development
2. To assess the effectiveness of biomarker-based tests in the PE risk prediction
3. To assess the prevalence of GDM, its risk factors and their effectiveness in the GDM prediction
4. To find association between the results of oral glycose tolerance test and fetal macrosomia

4. SUBJECTS AND MATERIALS

4.1 Study population and data collection

The pregnant women for the studies were included from Tartu University Hospital (TUH), Women´s clinic during three different years: 2012, 2013–2015 and 2018 **(Table 5 and 6).** The first cohort (Cohort I) incorporated data for 1,373 women starting antenatal care between January 2012 and December 2012. The second set of participants (n=2,334; Cohort II) took part of monocentric prospective observational Happy Pregnancy (HP) study (full name 'Development of novel non-invasive biomarkers for fertility and healthy pregnancy': PI: M. Laan). The third cohort (Cohort III) was formed of all women whose antenatal follow-up in TUH was started between January and December 2018 (n=2,028).

Table 5. Datasets included in studies from different cohorts.

Parameter	Datasets		
	Cohort I 2012	Cohort II Happy Pregnancy study	Cohort III 2018
Recruited pregnant women (n)	1,373	2,334	2,028
Excluded (incomplete data, miscarriage, or termination)	296 (21.6%)	80 (3.4%)	226 (11.1%)
Eligible participants (n, %)	1,077 78.4%)	2,254 (96.6%)	1,802 (88.9%)
Studies			
PE risk factor study		2,254	1,802
S-Flt/PlGF study (asymptomatic/ symptomatic) (Paper I)		178/16	
Multiplex biomarker study (Paper II)		53	
GDM screening study (Paper III)[1]	1,073	2,176	1,772

[1] Pregnant women with pre-gestational diabetes and multiple pregnancy were excluded
GDM, gestational diabetes; PE, preeclampsia; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase1

Pregnant women with miscarriage, termination of pregnancy for medical reasons before 22 g.w. or with missing delivery data were excluded (n=602). Final dataset consisted of 1,077; 2,254 and 1,802 eligible women from the I, II and III cohort, respectively. All participants were of white European ancestry and Estonian residents.

Table 6. General characteristics of three cohorts included in the studies

Parameter	Datasets		
	Cohort I 2012 n=1,077	Cohort II Happy Pregnancy study n=2,254	Cohort III 2018 n=1,802
Basic characteristics			
Maternal age (years)	28 (20–38)	28 (20–38)	30 (22–39)
Pre-pregnancy BMI (kg/m^2)	22.5 (18.0–32.8)	22.0 (18.0–31.0)	22.7 (18.5–33.7)
Primiparity	NA	1,028 (45.6%)	670 (37.2%)
Multiple pregnancy	NA	39 (1.7%)	33 (1.8%)
Individuals with GDM risk factor [a]	467 (43.5%)	1,227 (56.4%)	1025 (57.8%)
Individuals with PE risk factor [b]	NA	1,269 (56.3%)	937 (52%)
ASA consumption	NA	16 (0.7%)	NA
Pregnancy outcome			
Preeclampsia	NA	61 (2.7%)	29 (1.6%)
Gestational hypertension	NA	52 (2.3%)	54 (3.0%)
GDM	56 (5.2%)	128 (5.7%)	243 (13.7%)
Preterm birth	50 (4.7%)	137 (6.1%)	111 (10.2%)
Gestational age at delivery (days)	280 (259–287)	280 (256–293)	279 (255–291)
Birthweight (grams)	3,596 (2,680–4,360)	3,569 (2,605–4,366)	3,582 (2,596–4,300)
Caesarean section	181 (16.9%)	388 (17.2%)	363 (20.1%)
LGA[c]	202 (18.8%)	341 (15.7%)	286 (16.1%)
SGA[c]	17 (1.6%)	56 (2.5%)	50 (2.8%)

Data are given as median (5th–95th percentiles) or number (percentage) when appropriate.

[a] Individuals with risk factors exhibited one or more of the following risk factors for GDM: pre-pregnancy overweight/obesity (BMI 25–29.9 />30 kg/m^2), GDM and/or birth of a newborn >4500g during any of the previous pregnancies, DM among first-degree relatives, PCOS, fasting glucose >5.1 mmol/L, "other" risk factors (glycosuria, excessive weight gain (more than 3 kg in 4 weeks) suspicion of LGA fetus in index pregnancy).

[b] Individuals with risk factors exhibited one or more of the following risk factors for PE: PE or GH in previous pregnancy, chronic hypertension, pre-pregnancy diabetes mellitus, maternal obesity (BMI ≥30 kg/m^2), multiple pregnancy, IVF pregnancy, maternal age ≥40, nulliparity

[c] For the assignment of LGA diagnosis, the fetal growth calculator based on INTERGROWTH-21st Project was applied to convert the newborn birthweight into gestational age and sex-adjusted centiles (Villar et al., 2014). Newborn was categorized as LGA in case the sex-and gestational age adjusted birth centile was more than 95 and SGA in case the sex-and gestational age adjusted birth centile was less than 10. centiles.

ASA, acetylsalicylic acid; BMI; body mass index; GDM, gestational diabetes mellitus; LGA, large for gestational age; NA, not available; SGA, small for gestational age

The information about the pregnancy risk factors, course, comorbidities, diagnoses during pregnancy and perinatal outcome were collected by midwives and/or extracted from electronic hospital medical records. In addition, women in Cohort II filled three questionnaires during pregnancy about their symptoms, diet and physical activity habits, vitamin, and medication use. Serum sampling for research purposes in Cohort II was performed across gestation in parallel with regular clinical visits and blood-draw based tests according to pregnancy monitoring **(Table 7).**

Table 7. Data acquisation for the study in three time periods

	Time period		
Parameter	2012 [a]	2013–2015 [c]	2018 [d]
Maternal age	Calculated by birthdate	Calculated by birthdate	Calculated by birthdate
Pre-pregnancy BMI	Documented by midwife	Self-reported and hospital database	Hospital database
GDM risk factors, maternal history	Documented by midwife	Self-reported and hospital database	Hospital database
Fasting glycose >5.1mmol/L	Laboratory database	Laboratory database	Laboratory database
Number of previous deliveries	NA	Self-reported and hospital database	Hospital database
Excessive weight gain	Documented by midwife as excessive or NA [b]	Calculation based weight gain and pre-pregnancy BMI	Calculation based weight gain and pre-pregnancy BMI
GDM	Laboratory and hospital database	Laboratory and hospital database	Laboratory and hospital database
Hypertensive disorders	NA	Hospital database	Hospital database
Pregnancy outcome, information about delivery and newborn	Hospital database	Hospital database	Hospital database
Additional blood sample for research	NA	Yes	NA

[a] Detailed information about previous pregnancies and total weight gain during pregnancy is not available for this cohort.
[b] Midwives completed GDM risk assessment check-list including excessive weight gain (yes/no),
[c] Self-reported data originate from questionnaires filled by themselves during the pregnancy. Data were revised and completed when discrepancies between pregnant woman's report and medical data were detected.
[d] Data from hospital database derived directly from electronic records and no manual data entry was performed.
BMI, body mass index; GDM, gestational diabetes; NA, not available
Modified from Hanson et al 2022a.

4.1.1 Subjects for PE risk assessment (Papers I and II)

The pregnant women for the PE studies were included from the Cohort II and III. Data of 4,056 eligible women was used **(Table 5, 6 and 8).**

Table 8. Maternal characteristics of patients in studies assessing PE risk

	Assessment of clinical risk factors prevalence	Biomarker based assessment		
		sFlt-1/PlGF study		Multimarker study
		Asymptomatic	Symptomatic	
No of patients	4,056	178	16	53
Maternal age (years)	29.5 (21–38.9)	27.8 (20.9–38.9)	29.8 (24.4–42.3)	28.3 (20–38)
Pre-pregnancy BMI	22.1 (18–32.3)	23.8 (18.9–33.8)	30.3 (21.6–42.8)	25.6 (18.3–37.6)
Primiparity	1,698 (41.9%)	101 (56.7%)	6 (37.5%)	33 (62.3%)
GA at delivery (days)	280 (255–292)	280 (251–292)	273 (235–291)	277 (234–291)
PE	90 (2.2%)	24 (13.5%)	8 (50%)	22 (41.5%)

Data are given as median (5th–95th percentiles) or number (percentage) when appropriate.
BMI, body mass index; GA, gestational age; PE, preeclampsia, PlGF, placental growth factor; sFlt-1, soluble Fms like kinase 1.

Women were classified as high risk based on maternal history according to Estonian antenatal care guideline when presenting at least one of the high-risk factors (PE or GH in previous pregnancy, chronic hypertension, pre-pregnancy diabetes mellitus, maternal obesity (BMI ≥30 kg/m^2), multiple pregnancy, maternal age ≥40, nulliparity, IVF pregnancy) (Vaas et al., 2018) **(Table 1).** As all our participants were of white European ancestry, none had chronic kidney disease and only one patient had systemic lupus erythematosus (normotensive pregnancy), these were not considered as risk factors in our study. In addition, interpregnancy interval was not included as a risk factor due to limited data.

For the sFlt-1/PlGF study (Paper I) 178 women were chosen from the Cohort II. Women with subsequent diagnosis of PE (n=24) and with GH (n=12) were selected based on the availability of the third trimester serum samples drawn before 37 g. w. (180–259 g.d.) and absence of symptoms at the blood draw. For control group (n=142), women representing uncomplicated pregnancies (no PE/GH) were chosen matching the cases by maternal age and gestational age at available blood samples. In addition, sFlt-1/PlGF ratio was measured in 16 women with hypertension or proteinuria at sampling (209–257 g.d.) defined as symptomatic.

The third trimester single tube multimarker assay PE prediction study (Paper II) included 61 sera from 53 women from cohort II. The samples were collected

between 180 and 275 g.d. Four samples had been drawn at the clinical diagnosis of PE, 25 samples 4–62 days before PE diagnosis, and 32 samples from controls **(Table 5 and 8).**

4.1.2 Subjects and criteria for GDM risk assessment study (Paper III)

The dataset for GDM screening study included total of 5,021 eligible pregnancies: 1,073; 2,176 and 1,772 women from the Cohorts I, II and III, respectively **(Table 5, 6).**

To assess gestational diabetes and pregnancy outcome in singleton pregnancies, women with pregestational diabetes mellitus (DM) including type 2 DM diagnosed at the 1st trimester (fasting plasma glycose ≥7.0 mmol/l or any plasma glycose above 11.1 mmol/L) and multiple pregnancies were excluded **(Table 5).**

In compliance with current Estonian antenatal guideline OGTT is indicated only in the presence of any of GDM risk factors (Vaas et al., 2018) **(Figure 5).**

Therefore, OGTT was indicated only for those women who were categorized as high risk for GDM

After assessment of GDM risk factors and OGTT results, women were further divided into four groups: 1) group 1/low risk: women without risk factors and no indication to OGTT (n=2,302; 46%); 2) group 2/no OGTT: women with risk factors but no OGTT or only one normal test result before 20 g.w., (n=939; 19%); 3) group 3/normal OGTT: women with risk factors and normal OGTT result obtained after 20 g.w. (n=1,357; 27%); 4) group 4/GDM: women with abnormal OGTT result during the gestation (n=423; 8%).

4.2 Methods

Gestational age at sampling and at the delivery was set according to the first day of last menstrual period. If the menstrual cycle was irregular or the time of last period unknown the pregnancy was dated based on the ultrasound measurement of fetal crown-rump length at 11–14 g.w. IVF pregnancies were dated according to oocyte retrieval date. Deliveries <37 g.w. (<259 g.d.) were considered as preterm. Gestational hypertension (GH) was diagnosed as new onset of isolated hypertension after 20 g.w. The diagnosis of PE followed the international guidelines (ISSHP) at the time of recruitment: a new-onset hypertension (≥140/ ≥90 mmHg) after 20 g.w. accompanied by any additional signs of maternal organ dysfunction (proteinuria, thrombocytopenia, impaired liver function, hematological or neurological complications) (Tranquilli et al., 2014).

GDM was diagnosed when 75g oral glucose tolerance test (OGTT) resulted in fasting venous plasma glucose level of ≥5.1 mmol/l and/or at 1h and 2h later plasma glucose level of ≥10.0 mmol/l and ≥8.5 mmol/l glucose, respectively (Vaas et al., 2018).

For the correct assignment of small/large for-gestational-age (SGA/LGA) diagnosis, a growth calculator based on the INTERGROWTH-21st Project was applied to convert newborn birth weights into gestational age and sex-adjusted centiles (Villar et al., 2014). A newborn was categorized as SGA in case the adjusted birthweight was ≤ 10th centile and LGA as birthweight ≥95th centile.

Total weight gain during the pregnancy was considered excessive when it exceeded the widely accepted recommendations: >9.0 kg for obese (pre-pregnancy BMI 30.0 kg/m^2 or higher); >11.5 kg for overweight (25.0–29.9 kg/m^2), >16.0 kg for normal weight (18.5–24.9 kg/m^2) and >18.0 kg for underweight women (less than 18.5 kg/m^2) (Rasmussen et al., 2010).

For maternal complication during vaginal delivery, only perineal ruptures involving anal sphincter (3rd grade) and/or anal epithelium (4th grade) were analysed.

Adverse pregnancy outcome was defined as diagnosis of PE, SGA, GH, or preterm birth.

4.3 Biomarker assessment

All samples for the biomarker assessment were included from Cohort II (**Table 5**).

After blood draw, serum samples were separated and stored at -80°C until analysis.

Study I included 178 samples from asymptomatic and 16 samples from symptomatic women (hypertension or hypertension and proteinuria). The symptoms alerting to PE (hypertension, proteinuria) were assessed and documented at the time of blood draw.

Concentrations of serum sFlt-1 and PlGF were measured using the BRAHMS sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE ratio test (Thermo Fisher Scientific, Henningdorf, Germany) at the Synlab Germany service laboratory (Leinfelden, Germany). Service provider was blinded for clinical data. In the 3rd trimester sFlt-1/PlGF ratio was used for PE prediction.

Cut-off 38 was used to distinguish between high (sFlt-1/PlGF >38) and low risk (sFlt-1/PlGF ≤ 38). Diagnostic cut-offs for sFlt-1/PlGF by gestational age were ≥85 and ≥110 between 24+0 to 33+6 g.w. and from 34+0. g.w. until delivery, respectively (Stepan, et al., 2015).

One aliquot of 61 samples was subjected also to the analysis on Multiplex assay.

Multiplex biomarker assessment study (Study II) included 61 samples drawn for third trimester PE prediction (180 and 275 g. d.). PE was represented by 29 cases.

Using Luminex® xMAP-based approach multiplex measurement of biomarkers in a single test tube was conducted by co-author K. Ratnik. Biomarker selection in development stage included well established biomarkers (sFlt-1, PlGF, sENG), additional biomarkers potentially involved in PE pathogenesis (leptin, ADAM 12) and novel biomarker PTX3 (**Table 3).**

For GDM study (Study II) plasma glycose was measured in Tartu University Hospital United Laboratories by routine testing using hexokinase method. (*www.kliinikum.ee/yhendlabor/pildid/kasiraamat/Fg/Glykoos_ii_.pdf*, 2021). Data was accessed from electronical medical database.

4.4 Statistical analysis

Summary estimates of the data (median, 5^{th}–95^{th} centile) were calculated and all statistical tests were implemented using the STATA software ver. 13.1 (StataCorp TX, USA) or R3.3.3 language and environment (Free Software Foundation, Boston, MA, US, http://www.r-project. org).

To compare groups Wilcoxon rank-sum test was used for continuous variables and Chi^2 test for categorical variables. Significance level of 0.05 was used. Bonferroni correction for multiple testing was applied according to number of tests performed.

Binary logistic regression analysis was used to examine the association between pregnancy outcome and allocated GDM risk group or sFlt-1/PlGF ratio as well as between risk factors or biomarker measurements and the clinical onset of PE. The odds ratios and adjusted odds ratios with 95% confidence intervals are reported. For GDM study the ORs were adjusted for cohort and previous parities, LGA newborn, pre-pregnancy BMI, maternal age, or gestational age at delivery, depending on pregnancy outcome variable. For PE the ORs were adjusted for BMI, maternal age, parity, or cohort when necessary.

Detection rate was defined as the proportion of true positives among cases. False positive rate (FPR) was defined as ratio of false positives among controls.

For the 3^{rd} trimester multimarker PE prediction, alternative statistical models were built using combinations of measured biomarker data (2–5 biomarkers; 30 alternative tested formulae). Modelling was conducted by co-author K. Ratnik.

4.5 Ethical approval

The data collection and analysis were approved by the Research Ethics Committee of the University of Tartu, Estonia (permissions no. 225/T-6, 06.05.2013; 221/T-6, 17.12.2012, 286/M-18, 15.10.2018; 291/T-3, 18.03.2019 and 322/M-17, 17.08.2020) and study was carried out in compliance with the guidelines of the Declaration of Helsinki.

5. RESULTS

5.1 Screening for preeclampsia

5.1.1 Prevalence of preeclampsia and predictive value of its risk factors for risk assessment (unpublished data)

The study sample to assess the prevalence of PE risk factors included 4,056 women recruited at the Women's Clinic, Tartu University Hospital representing Cohorts II and III (2,254 and 1,802, respectively) **(Table 5, 6 and 8)**. The prevalence of PE in the study population was 2.2% (n=90). Early onset PE (delivery <34 g.w.) developed in 14 women and preterm PE (delivery <37 g.w.) in 37 cases, 15.5% and 41.1% of all PE cases, respectively.

One or more of eight clinical risk factors were detected in approximately half of the study population (n=2,206; 54.4%) **(Table 6 and 9).** In comparison, the number of risk factors carriers was slightly decreased (56.3% vs 52%, P = 0.006) between two cohorts **(Table 6).**

The presence of any of the risk factors increased the odds to develop PE 7.8-fold (95% CI 3.9–15.6) **(Table 9).** In addition to PE other adverse pregnancy outcomes (preterm birth, SGA newborn and gestational hypertension) were diagnosed significantly more frequently in high-risk group compared women without PE risk factors **(Table 10).** Most of the patients (n=81; 90%) diagnosed with PE had at least one risk factor, however, the PPV for PE among high-risk women remains low (3.7%) **(Table 9).**

Table 9. Prevalence of risk factors and odds to develop PE among women diagnosed with PE compared to non-PE women

Risk factors	No PE n=3,966	All PE cases n=90	Adjusted odd ratio (95% CI)	PPV/NPV	PE by the GA at delivery		
					Early PE < 34 g.w n=14	Preterm PE <37 g.w n=37	Term PE ≥37g.w n=53
Individuals with any risk factor [a]	2,125(53.6%)	81 (90%)*	7.8 (3.9–15.6)	3.7%/99.5%	10 (71.4%)	33 (89.2%)	48 (90.6%)
BMI ≥ 30 kg/m^2 [b]	395 (10%)	18 (20%)*	2.8 (1.6–4.8)	4.3%/98%	5 (35.7%)	7 (18.9%)	11 (20.8%)
Primiparity [c]	1,698 (42.8%)	70 (77.8%)*	5.3 (3.2–8.8)	3.9%/99.1%	8 (57.1%)	27 (73%)	43 (81.1%)
Prior PE/GH [d,f]	131 (5.8%)	7 (35%)*	8.3 (3.2–21.7)	12%/98.5%	0	3 (30%)	4 (40%)
Diabetes mellitus [c]	21 (0.5%)	5 (5.6%)*	10.3 (3.9–31.6)	19.1%/97.9%	2 (14.3%)	4 (10.8%)	1 (1.9%)
IVF [e]	167 (4.2%)	9 (10%)*	3.3 (1.6–6.9)	5.1%/97.9%	1 (7.1%)	6 (16.2%)	4 (7.5%)
Chronic hypertension	28 (0.7%)	2 (2.2%)	3.4 (0.8–14.7)	6.6%/97.8%	2 (14.3%)	2 (5.4%)	0
Multiple pregnancy	72 (1.8%)	4 (4.4%)	2.7 (0.9–7.5)	5.2%/97.9%	1 (7.1%)	4 (10.8%)	0
Maternal age ≥40years	150 (3.8%)	3 (3.3%)	0.9 (0.3–2.9)	1.9%/97.8%	1 (7.1%)	2 (5.4%)	1 (1.9%)

Data are given as number (percentage). Groups were compared using Chi^2 test for categorical variables.

[a] Individual with a risk factor exhibited one or more of the following risk factors for PE: PE or GH in previous pregnancy, chronic hypertension, pre-pregnancy diabetes mellitus, maternal obesity (BMI ≥30 kg/m^2), multiple pregnancy, IVF pregnancy, maternal age ≥40, nulliparity; [b] Adjusted for cohort, parity; [c] Adjusted for cohort, parity, BMI; [d] Adjusted for maternal age, BMI; [e] Adjusted for cohort, maternal age, BMI; [f] Proportion of multiparous women; *P<0.05

BMI, body mass index; CI, confidence interval; GA, gestational age; GH, gestational hypertension; g.w, gestational week; IVF, *in vitro* fertilization; NPV, negative predictive value; N.S, non-significant; PE, preeclampsia; PPV, positive predictive value.

Compared to normotensive pregnancies, women with a subsequent PE development were mostly primiparous (77.8% vs 42.8%; AOR 5.3 (95% CI 3.2–8.8)) and obese (BMI≥30 kg/m^2; 20% vs 10%; AOR 2.8 (95% CI 1.6–4.8)). The strongest risk factors referring to PE development were pre-pregnancy diabetes and PE/GH in previous pregnancy; AOR 10.3 (95% CI 3.9–31.6) and 8.3 (95% CI 3.2–21.7), respectively **(Table 9)**. No significant differences were observed in the prevalence of PE risk factors in early, preterm or term cases **(Table 9)**.

Table 10. Prevalence and odds of adverse pregnancy outcomes[a] among women with and without risk factors for PE

Complication	Risk factor carriers[b] n=2,206	No risk factors n=1,851	*P*-value	Odds ratio (CI 95%)
All PE	81 (3.7%)	9(0.5%)	**6.9×10^{-12}**	**7.8 (3.9–15.6)**
Term PE (≥ 37 g.w)	48 (2.2%)	5 (0.3%)	**5.0×10^{-12}**	**2.9 (1.8–4.6)**
Preterm PE (<37 g.w)	33 (1.5%)	4 (0.2%)	**8.5×10^{-6}**	**7.1 (2.5–20.2)**
Early PE (< 34.g.w)	10 (0.5%)	4 (0.2%)	0.18	2.2(0.7–6.9)
Isolated SGA[c]	69 (3.1%)	28 (1.5%)	**4.0×10^{-4}**	**2.2 (1.4–3.4)**
PE+SGA[c]	9 (0.4%)	0		
Spontaneous PTB	120 (5.4%)	74 (4.0%)	**0.011**	**1.5 (1.1–1.9)**
GH	81(3.7%)	24 (1.3%)	**1.5×10^{-8}**	**3.2 (2.1–5.0)**
Uneventful pregnancy course	1,838 (83.3%)	1715 (92.3%)	**4.0×10^{-17}**	**0.4 (0.3–0.5)**

Data are given as number (%). Groups were compared using Chi^2 test. Statistically significant nominal p-values are highlighted in bold (*P*<0.05).

[a] Adverse pregnancy outcome were defined as PE, spontaneous preterm birth, SGA newborn or gestational hypertension

[b] Risk factor carrier exhibited one or more of the following risk factors: PE or GH in previous pregnancy, chronic hypertension, pre-pregnancy diabetes mellitus, maternal obesity (BMI ≥30 kg/m^2), multiple pregnancy, IVF pregnancy, maternal age ≥40 years, nulliparity.

[c] For the assignment of SGA diagnosis, the fetal growth calculator based on INTERGROWTH-21st Project was applied to convert the newborn birthweight into gestational age and sex-adjusted centiles (Villar et al., 2014). Newborn was categorized as SGA in case the sex-and gestational age adjusted birth centile was less than 10. centiles.

GH, gestational hypertension; g.w, gestational weeks; PE, preeclampsia; PTB, preterm birth; SGA, small for gestational age.

In summary, amongst high-risk women the prevalence of adverse pregnancy outcome is more frequent compared to women without clinical risk factors. However, as approximately half of population is considered high risk, the PPV in the presence of a clinical risk factor for PE development remains low. The risk of developing PE is considerably increased for women with pre-pregnancy diabetes and/or PE/GH in previous pregnancy.

5.1.2 Biomarker based PE prediction

5.1.2.1 sFlt-1/PlGF test performance in PE prediction in the third trimester among asymptomatic women (Paper I)

The study to assess performance of sFlt-1/PlGF in the third trimester included 178 pregnancies, (**Tabel 5 and 8)** all normotensive at sampling. The study sample included 24 patients with subsequent PE, 12 with isolated GH and 142 normotensive controls **(Table 6).** In PE risk prediction by sFlt-1/PlGF ratio, 29 patients were classified as high-risk for PE development (sFlt-1/PlGF > 38) and 149 as low-risk (sFlt-1/PlGF ≤ 38) **(Table 11).**

Pregnancy outcome of women classified as high risk for PE by sFlt-1/PlGF ratio

The applied PE "diagnostic" cutoff of sFlt-1/PlGF ≥85 for <34 g.w. and sFlt-1/PlGF ≥110 for ≥34 g.w. was exceeded in 13 asymptomatic women out of 29. Within 30 days, nine of these women (69%; sFlt-1/PlGF ranging 85.3–571.3) developed clinical symptoms of PE and delivered by Caesarean section, preterm in seven cases. The remaining four patients with sFlt-1/PlGF "diagnostic" values had spontaneous preterm delivery, SGA newborn and/or operative delivery.

Among 16 high-risk (sFlt-1/PlGF >38) patients with the estimated sFlt-1/PlGF ratio lower than the "diagnostic cut-off", five cases (sFlt-1/PlGF = 40.5–58.3) were diagnosed with PE and delivered within 22–64 days after blood draw. Isolated GH was diagnosed in four cases (sFlt-1/PlGF between 39.3–64.4). Two patients remained normotensive, but experienced either a spontaneous preterm delivery (sFlt-1/PlGF 68.2) or the birth of SGA newborn (sFlt-1/PlGF 43.9). Third of 16 cases (n=5; 31%) with sFlt-1/PlGF ratio >38 (41.4–65.3), but not reaching the current "diagnostic cut-off" had an uneventful pregnancy course and delivered term neonates (28–47 d after sampling).

For the sFlt-1/PlGF measurements >38, the calculated AOR to develop PE was 15.9, (95% CI 5.4–46.7). The delivery due to PE within 30 days was estimated with AOR of 43.5, (95% CI 8.4–224.4).

Pregnancy outcome of women classified as low risk for PE by sFlt-1/PlGF ratio

Ten women (6.7%) out of 149 women from the low-risk group developed PE (sampling between 211–254 g.d.). Most of the cases (n=9; 90%) received PE diagnosis and delivered their newborn within 30–69 days after blood draw (**Table 11**).

Table 11. Maternal characteristics and pregnancy outcome stratified by sFlt-1/PlGF among asymptomatic women

Parameter	Ratio ≤ 38	Ratio > 38	*P* – value[b]
Study subjects (n)	149	29	
Gestational age at sampling (d)	227 (206–251)	230 (210–257)	0.2
Time from sampling to delivery (d)	50 (29–78)	29 (12–64)	**2.1×10^{-7}**
Gestational age at delivery (d)	282 (263–292)	265 (233–289)	**3.3×10^{-6}**
Preterm birth (n, %)	3 (2.0%)	10 (34.5%)	**7.8×10^{-10}**
Birthweight (grams)	3584 (2878–4328)	2782 (1510–3614)	**4.6×10^{-9}**
Birthweight centile	74.5 (18.7–97.9)	31.6 (1.0–85.3)	**4.1×10^{-7}**
Isolated SGA[a] (n, %)	2 (1.3%)	3 (10.3%)	7.0×10^{-3}
PE (n, %)	10 (6.7%)	14 (48.3%)	**2.0×10^{-9}**
delivery within 30 d after sampling	1	9	
delivery after 31 d after sampling	9	5	
GH (n, %)	8 (5.4%)	4 (13.8%)	1.8×10^{-2}
Delivery by Cesarean section	23 (15.4%)	20 (69%)	**7.2×10^{-10}**
GDM (n, %)	9 (6.0%)	2 (6.9%)	0.6

Data are given as median (5th–95th percentiles) or number (percentage) as appropriate

[a] Newborn was categorized as SGA in case the sex-and gestational age adjusted birth-weight was less than 10 centile. To convert the newborn birth weight into gestational age and sex-adjusted centiles the fetal growth calculator based on INTERGROWTH-21st Project was applied.(Villar et al., 2014)

[b] Groups were compared using Chi^2 test for categorical and Wilcoxon rank-sum test for non-categorical variables.

P value was adjusted according to Bonferron correction 0.05/11= 0.0045. Statistically significant nominal P-values are highlighted in bold.

d, days; GDM, gestational diabetes mellitus; GH, gestational hypertension; n, number of patients; PE, preeclampsia; PlGF, placental growth factors; sFlt-1, soluble fms-like tyrosine kinase; SGA, small for gestational age

Modified from Hanson et al., 2022b.

The overall detection rate (DR) to predict PE development was 58.3% with false positive rate (FPR) of ~ 10% **(Table 12).** The estimated DR was improved to 83.3%, (FPR of 3%). when the PE prediction period was constrained to 30 days. Most of the false positive cases (4 out of 5) represented pregnancies with SGA and/or preterm birth and gave birth via C-section within 30 days of sampling.

Timing of the sFlt-1/PlGF test for screening of PE in asymptomatic women in the third trimester

To evaluate the most optimal timeframe for the third trimester PE screening test based on sFlt-1/PlGF ratio among asymptomatic women, the outcomes of predicting PE were compared for sampling windows 210–224 g.d. (30–32 g.w.), 225–238 g.d. (32–34 g.w.) and 239–253 g.d. (34–36. g.w.) **(Table 12, Figure 6).**

Table 12. sFlt-1/PlGF test performance predicting development of preeclampsia within 30 days after sampling, until delivery and gestational age and time to delivery within different timeframes

	210–224 g.d	225–238 g.d	239–253 g.d	Within 30 days of sampling	Total PE
No of patients sFlt-1/PlGF >38/≤38	10/52	7/56	9/27	15/21	29/149
DR %	6/11 (54.5%)	4/6 (66.7%)	2/4 (50%)	10/12 (83.3%)	14/24 (58.3%)
FPR %	4/51 (7.8%)[a]	3/57 (5.3%)[b]	7/32 (21.9%)[ab]	5/166 (3.0%)	15/154 (9.7%)

Data are given as number (%). Groups were compared using Wilcoxon rank-sum test.
Detection rate (DR) was defined as the proportion of true positives among PE cases. False positive rate (FPR) was defined as ratio of false positives among controls.
GA, gestational age; g.d, gestational day PlGF, placental growth factor; sFlt-1, soluble fmf-like tyrosine kinase-1
[a]$P<0.05$ in comparison to time periods 210–224 gestational days and 239–253 gestational days
[b]$P<0.05$ in comparison to time periods 225–238 gestational days and 239–253 gestational days
Modified from Hanson et al 2022b.

No statistical difference was detected between the DRs between three timeframes (**Table 12).** However, sFlt-1/PlGF measurements after 239 g.d. exhibited higher FPR (22% *vs* 8% and 5%) compared to the earlier periods.

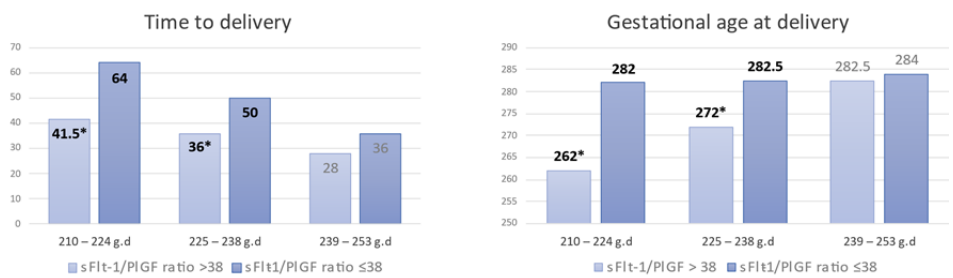

Figure 6. sFlt-1/PlGF test performance predicting time from sampling to delivery and gestational age at birth when blood is drawn within different timeframes.
Statistically significant differences are highlighted in bold and marked with asterix (*)

Among women sampled within 210–224 and 225–238 g.d., the individuals in the high-risk group delivered at earlier gestational age and sooner after the blood draw compared to low-risk cases. When sera had been sampled after 238 g.d., the sFlt-1/PlGF >38 did not predict gestational age and time of delivery **(Figure 6).**

To conclude**,** sFlt-1/PlGF ratio above "diagnostic cut-off" (≥ 85 for <34 g.w. and ≥110 for ≥34 g.w.) in asymptomatic women shows the high risk for subsequent PE, SGA newborn, preterm delivery, and operative delivery. Detection rate for PE is better within first 30 days after sampling. Sampling at later gestational age results in increased false positive rate.

5.1.2.2 Performance of sFlt-1/PlGF test among symptomatic women (unpublished)

To assess performance of the sFlt-1/PlGF ratio-based PE risk assessment during 2nd half of pregnancy among symptomatic women, 16 patients with hypertension or proteinuria at blood draw (between 209–257 g.d.) were tested. Within 28 days half of them were diagnosed with PE.

Symptomatic patients with increased sFlt-1/PlGF ratio (>38) had shorter time to delivery after sampling (21 g.d. vs 31 g.d.), smaller median birthweight and birthweight centile of the newborn (2,968g vs 3,876g and 52.2 vs 87.3, respectively) and PE was diagnosed significantly more compared to low-risk group (n= 7 vs n=1; *P = 0.012*) (**Table 13**). In two cases where the sFlt-1/PlGF value remained below 38 (8.9 and 25.1) the patients delivered due to PE after 4 weeks of sampling (28 and 31 d).

Only one symptomatic patient without PE was classified as high risk by sFlt-1/PlGF value (sFlt-1/PlGF ratio 56.1). She gave birth 31 days after sampling an appropriate for gestational age new-born (3,548 g) and apart from gestational hypertension had uneventful pregnancy course.

Table 13. Maternal characteristics and pregnancy outcome stratified by sFlt-1/PlGF in symptomatic women

Parameter	sFlt-1/PlGF ≤ 38	sFlt-1/PlGF > 38	*P*-value
Number of patients	9	7	
Maternal age	33.5 (24.4–42.3)	29.5 (25.5–37)	0.6
BMI (kg/m^2)	31.2 (21.6–42.8)	29.7 (21.8–37.6)	0.6
Primiparity	4	6	0.4
Gestational age at sampling (d)	228.5 (209–257)	253 (206–256)	0.3
Preeclampsia	2	6	**0.012**
Gestational age at delivery (days)	274 (247–291)	267 (235–75)	0.2
Time sampling to delivery (d)	31 (18–82)	21 (1–32)	**0.049**
Newborn birthweight (grams)	3,876 (2,538–4,202)	2,968 (2,044–3,548)	**0.03**
Newborn birthweight centile	87.3 (44.5–98.5)	52.2 (7.2–77)	**0.02**
Cesarean section	4	2	0.5

Data are given as median (5th–95th percentiles). Groups were compared using Wilcoxon rank-sum test for non-categorical variables. Statistically significant nominal P-values are highlighted in bold.
BMI, mody mass index; d, days; PlGF, placental growth factor; s-Flt-1, soluble fms-like tyrosine kinase

To sum up, increased sFlt-1/PlGF ratio (>38) in symptomatic patients was able to predict development of PE, shorter time to delivery and newborn with smaller birthweight.

5.1.2.3 Performance of novel multimarker 6PLEX test in PE prediction in the third trimester (Paper II)

The study to develop test for PE prediction on the third trimester on the 6PLEX assay included 61 serum samples from Cohort II (Happy Pregnancy study) (**Table 5 and 8**).

The best biomarker-based PE prediction model included ADAM 12, sENG, PlGF, sFlt-1 with the DR of 96% and FPR 9.8%. Combining maternal characteristics (gestational age and maternal weight at the blood draw) with 6PLEX measurements of ADAM 12, sENG, PlGF, sFlt-1 and leptin the DR for subsequent PE was improved to 100.0% and FPR 3.5% **(Table 14).** Biomarker-based and combined prediction model had six and two false positive predictions, respectively. In three of those cases SGA was diagnosed (2 and 1, respectively). Rest of the false positive cases had uneventful pregnancy course. Biomarker-based model had also one false negative result, in this case the time from sampling from manifestation of PE was 62 days.

Table 14. Test performance of novel combined biomarker tests

	sFlt-1, PlGF, ADAM 12, s-Eng	sFlt-1, PlGF, sENG, ADAM 12, leptin, gestational age, maternal weight at the blood draw
True positives/cases	24/25	25/25
True negatives/healthy	26/32	30/32
False positive (n)	6	2
False negative (n)	1	0
FPR (%)	9.8%	3.5%
DR (%)	96%	100%

Detection rate (DR) was defined as the proportion of true positives among PE cases. False positive rate (FPR) was defined as ratio of false positives among controls
ADAM 12, a disintegrin and metalloproteinase-12; DR, detection rate; FPR, false positive rate; PlGF, placental growth factors; sENG, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase.

To conclude, PE prediction in the second half of pregnancy using combined model of several biomarkers and maternal characteristics allow to more accurate risk estimation compared to only biomarker or clinical assessment.

5.1.3 Challenges in applying biomarker tests in the III trimester in clinical decision-making

In several clinical cases the biomarker-based tests have a clear benefit in PE prediction or confirming/excluding the diagnosis (Cases A–B, **Table 15).** The challenge of interpreting the test results in clinical routine is to handle the false positive and false negative cases. False positive biomarker tests can refer to other complications most commonly SGA (Case C and E) but can also cause unreasonable anxiety and unnecessary visits in uncomplicated cases (Case F and G). Reason behind false negative sFlt-1/PlGF test result can be explained by long interval between sampling and PE manifestation (Case D).

Table 15. Example cases of sFlt-1/PlGF and 6PLEX tests performances in a clinical setting

At sampling						Pregnancy outcome				
Case	Time (g.d)	Sympt	sFlt-1/ PlGF	Risk	6PLEX	Delivery (g.d.)	Sampling to delivery time	BW (g)/ centile	Dgn	Comment
A	180	No	571.3	high	PE	199	19	814/7.9	ECL/SGA	Correct and useful prediction
B	251	HTN	14.3	low	No	285	34	4346/96	Transient HTN	Correct and useful prediction
C	228	No	148.9	high	PE	278	50	2,782/11	Small newborn	Wrong prediction of PE possibly due to small newborn
D	211	No	4.2	low	PE	275	64	2,622/7.2	PE/SGA	Correct prediction by 6PLEX, increased Flt-1/PlGF possibly due to long sampling to delivery time
E	223	No	240.3	high	No	235	12	1,510/7.4	SGA	Correct prediction by 6PLEX, high sFlt-1/PlGF possibly due to uteroplacental dysfunction
F	262	No	53.3	inter-mediate	No	283	21	3,512/52	none	Unreasonable anxiety to the patient by sFlt-1/PlGF
G	243	No	2.7	low	PE	283	40	3682/60	none	Unreasonable anxiety to the patient by 6PLEX

'High risk for PE' was determined as sFlt-1/PlGF >85 before 34 gestational weeks and sFlt-1/PlGF >110 after 34 gestational weeks; 'Low risk for PE' for 1 week as sFlt-1/PlGF ≤38; Intermediate risk for PE, the sFlt-1/PlGF value (38≤sFlt-1/PlGF≤85/110) (Stepan et al, 2015).
BMI, pre-pregnancy body mass index; BW, birthweight; Dgn, diagnosis; ECL, eclampsia; g, grams; g.d., gestational days; HTN, hypertension; PE, preeclampsia; SGA, small-for-gestational-age newborn; sympt, symptoms; yrs, years

5.2 Screening for gestational diabetes (Paper III)

5.2.1 The prevalence and predictive value of gestational diabetes and its risk factors

Between 2012 and 2018 the proportion of pregnant women presenting any of the GDM risk factors increased from 43.5% to 57.8%, and more referrals to OGTT were made (62.3% to 76.8%). Most frequently documented GDM risk factors were overweight (BMI 25–29.9 kg/m^2) followed by women with high fasting glycose level and "other" risk factors: glycosuria, excessive weight gain, suspicion of LGA foetus **(Table 16).**

Women with obesity and GDM or LGA in previous pregnancy were more likely to be subjected to correct GDM diagnostic algorithm. In addition to fasting glycose > 5.1 mmol/l, prior GDM and obesity were the most likely risk factors to result in GDM diagnosis, AOR 3.0 (95% CI 2.4–3.8), 6.7 (95% CI 3.8–11.9) and 2.5 (95% CI 1.9–3.2), respectively. Most common indication for referral to OGGT was "other" risk factors, however, resulted in a subsequent GDM diagnosis only in 13.9 % of cases **(Table 16 and 17).**

Table 16. Prevalence of GDM risk factors and pregnancy outcome in three cohorts[a]

Parameter	I (n=1,073)	II (n=2,176)	III (n=1,772)	*P* value[e]		
				I *vs.* II	I *vs.* III	II *vs.* III
GDM risk factors						
Individuals with any risk factor [b]	467 (43.5%)	1227 (56.4%)	1025 (57.8%)	5.1×10^{-12}	1.2×10^{-13}	n.s
Correctly tested among risk factor carriers	291 (62.3%)	702 (57.2%)	787 (76.8%)	n.s	1.5×10^{-4}	3.5×10^{-9}
BMI 25-30 kg/m^2 n (% of carriers)	198 (42.4%)	393 (32%)	316 (30.8%)	n.s	n.s	n.s
BMI >30 kg/m^2	92 (19.7%)	185 (15.1%)	210 (20.5%)	n.s	n.s	2.0×10^{-4}
Previous GDM	13 (2.8%)	26 (2.1%)	28 (2.7%)	n.s	n.s	n.s
Previous baby 4,500g	30 (6.4%)	36 (2.9%)	39 (3.8%)	n.s	n.s	n.s
DM among first degree relatives	78 (16.7%)	224 (18.3%)	161 (15.7%)	n.s	n.s	n.s
PCOS	24 (5.1%)	24 (2.0%)	9 (0.9%)	n.s	3.0×10^{-5}	n.s
Fasting glycose >5.1mmol/L	48 (10.3%)	471 (38.4%)	235 (22.9%)	3.3×10^{-36}	1.6×10^{-14}	6.0×10^{-10}
Polyhydramnion	19 (4.1%)	42 (3.4%)	40 (3.9%)	n.s	n.s	n.s
Other[c]	68 (14.6%)	116 (9.5%)	245 (23.9%)	n.s	6.1×10^{-10}	3.2×10^{-20}
Pregnancy outcome						
Gestational diabetes	56 (5.2%)	124 (5.7%)	243 (13.7%)	n.s	8.05×10^{-13}	6.4×10^{-18}
Preterm birth	50 (4.7%)	110 (5.1%)	87 (4.9%)	n.s	n.s	n.s
Gestational age at delivery (days)	280 (259–287)	281 (258–293)	279 (259–291)	1.38×10^{-17}	3.69×10^{-10}	n.s
Birthweight (grams)	3,596 (2,680–4,360)	3,569 (2,680–4,366)	3,590 (2,660–4,302)	n.s	n.s	n.s
Cesarean section	181 (16.9%)	363 (16.7%)	342 (19.3%)	n.s	n.s	n.s
LGA[d]	202 (18.8%)	341 (15.7%)	285 (16.1%)	n.s	n.s	n.s
LGA + GDM	23 (11.4%)	30 (8.8%)	57 (9.4%)			
SGA[d]	17 (1.6%)	52 (2.4%)	43 (2.4%)	n.s	n.s	n.s

Data are given as median (5th–95th percentiles) or number (percentage) when appropriate. Groups were compared using Chi^2 test for categorical and Wilcoxon rank-sum test for continuous variables.

[a] Cohort I represented women recruited for antenatal care in 2012; Cohort II in 2013–2015 and Cohort III in 2018.

[b] Individual with any risk factor exhibited one or more of the following risk factors: pre-pregnancy overweight/obesity (BMI 25–29.9 />30 kg/m^2), GDM and/or birth of a newborn >4500g during any of the previous pregnancies, DM among first-degree relatives, PCOS, fasting glucose >5.1 mmol/L, “other“ risk factors.

[c] “Other” risk factors were classified as glycosuria, excessive weight gain (more than 3 kg in 4 weeks) or suspicion of LGA fetus in index pregnancy

[d] For the assignment of large or small-for-gestational-age (LGA or SGA, respectively) diagnosis, the fetal growth calculator based on INTERGROWTH-21st Project (Villar et al., 2014)was applied to convert the newborn birthweight into gestational age and sex-adjusted centiles. Newborn was categorized as LGA in case the sex-and gestational age adjusted birth centile was more than 95 and SGA in case the sex-and gestational age adjusted birth centile was less than 10. centiles

[e] *P* value was adjusted according to Bonferron correction for 22 tests and 3 subgroups $0.05/3*21 < 7.9 \times 10^{-4}$

DM, diabetes mellitus; GDM, gestational diabetes mellitus;LGA, large for gestational age; NA, not available; n.s, non- significant; PCOS, polycystic ovary syndrome; SGA, small for gestational age

Modified from Hanson et al 2022a.

Table 17. Odds to receive GDM diagnosis after testing

Risk factor	Individuals with the risk factor [a] n=2,719	Tested [b] n=1,780	GDM [c] n=423	AOR [d] (95% CI)
BMI 25–29.9 kg/m^2	907	500 (55%)	113 (22.6%)	0.9 (0.7–1.2)
BMI ≥ 30.0 kg/m^2	482	371 (77%)	142 (38.3%)	**2.5 (1.9–3.2)**
Previous GDM	67	63 (94%)	38 (60.3%)	**6.7 (3.8–11.9)**
DM in a relative	463	321 (69%)	78 (24.3%)	1.0 (0.8–1.3)
PCOS	57	44 (77%)	14 (31.8%)	1.9 (0.9–3.7)
Previous LGA	105	92 (88%)	32 (34.8%)	1.5 (0.9–2.4)
Fasting glycose >5.1 mol/l	754	443 (59%)	176 (39.8%)	**3.0 (2.4–3.8)**
Polyhydramnion	101	63 (62.4%)	16 (25.4%)	1.2 (0.6–2.1)
Other [e]	429	416 (97%)	58 (13.9%)	0.4 (0.3–0.5)

[a] Individual with any risk factor exhibited one or more of the following risk factors: pre-pregnancy overweight/obesity (BMI 25–29.9 />30 kg/m^2), GDM and/or birth of a newborn >4500g during any of the previous pregnancies, DM among first-degree relatives, PCOS, fasting glucose >5.1 mmol/L, "other" risk factors.
[b] Percentage of tested individuals is shown
[c] Percentage of tested individuals receiving GDM diagnosis is shown
[d] Adjusted for cohort, age. AOR is calculated from tested.
[e] "Other"risk factors were classified as glycosuria, excessive weight gain (more than 3 kg in 4 weeks) or suspicion of LGA fetus in index pregnancy
AOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; GDM, gestational diabetes mellitus; LGA, large for gestational age; PCOS, polycystic ovary syndrome.
Adapted from Hanson et al 2022a.

In conclusion, with growing number of GDM risk factor carriers the number of women being allocated and referred to OGTT is increasing. Fasting glycose >5.1 mmol/l, obesity and GDM in previous pregnancy most frequently result in GDM diagnosis.

5.2.2 Pregnancy outcome in different GDM risk groups

The women were divided into four subgroups according to presence of GDM risk factors and outcome of the OGTT test and the pregnancy outcome was assessed. Compared to other subgroups, the lowest gestational age at delivery was in group 4 (GDM). They were also more likely to deliver an LGA baby and via C-section **(Table 18).** The neonates with highest median birthweight were born to the mothers in group 3 (GDM risk factors, but normal OGTT). Irrespective of the OGTT result, women presenting risk factors to GDM and receiving OGTT (groups 3 and 4) delivered newborns with higher birthweight centiles (~82 percentile) compared to other groups (groups 1 and 2) **(Table 18).**

Table 18. Pregnancy outcome in women allocated into four subgroups according to GDM risk factors and OGTT result

Outcome	Low risk Pregnancies[d] (group 1)	High risk pregnancies [e] No OGTT (group 2)	OGTT normal (group 3)	GDM (group 4)
Number of women	2,302	939	1,357	423
GA at delivery (days)	280 [i] (259–292)	280 [k] (255–292)	280 [l] (260–293)	276 [i,k,l] (252–289)
Birthweight (grams)	3502 [g,h,i] (2644–4233)	3576 [g,j,] (2642–4320)	3705 [h,j] (2808–4468)	3635 [i] (2695–4430)
Birthweight centile	70.7 [g,h,i] (13.7–97.3)	75.7 [g,j,k] (18.4–98.2)	82.2 [h,j] (25.0–99.3)	82.6 [i,k] (26.5–99.3)
LGA [a]	243 (10.5%) [g,h,i,]	160 (17.1%) [g,j,k]	315 (23.2%) [h,j]	110 (26.0%) [i,k]
SGA [a]	70 (3.0%)	15 (1.6%)	23 (1.7%)	4 (0.95%)
Cesarean section	322 (14.0%) [h,i]	175 (18.7%) [k]	274 (20.2%) [h]	114 (27.0%) [i,k]
Preterm delivery	104 (4.5%)	55 (5.9%)	58 (4.3%)	27 (6.4%)
Shoulder dystocia[b,c]	6/1,468 (0.4%)	3/627 (0.5%)	6/886 (0.7%)	1/266 (0.4%)
Perineal rupture ≥3 grade [b,c]	14/1,468 (1.0%)	3/627 (0.5%)	9/883 (1.0%)	2/266 (0.8%)
Preeclampsia [c]	23/1699 (1.4%)	2 /760 (0.3%)	9/1122 (0.8%)	6/367 (1.6%)
Gestational hypertension [c]	20/1699 (1.2%) [i]	26 (3.4%)	50 (4.5%)	25 (6.8%) [i]

Data are given as median (5th–95th percentiles) or number (percentage) when appropriate.

[a] For the assignment of large or small-for-gestational-age (LGA or SGA, respectively) diagnosis, the fetal growth calculator based on INTERGROWTH-21st Project was applied to convert the newborn birthweight into gestational age and sex-adjusted centiles (Villar et al., 2014). Newborn was categorized as LGA in case the sex-and gestational age adjusted birth centile was more than 95 and SGA in case the sex-and gestational age adjusted birth centile was less than 10 centiles.

[b] Percentage is calculated from vaginal deliveries only

[c] Data available for 2013–2015 and 2018 cohorts.

[d] Low risk pregnancies for GDM were defined as absence of GDM risk factors (see below), for those induviduals OGTT is not indicated.

[e] High-risk pregnancies for GDM were defined as presence of any of the following risk factors: BMI > 25 kg/m^2, GDM or LGA in previous pregnancy, fasting glycose >5,1 mmol/l, PCOS, polyhydramnion, DM in family history, "other" risk factors (glycosuria, excessive weight gain (more than 3 kg in 4 weeks) suspicion of LGA fetus in index pregnancy). The presence of any of the risk factors is an indication for OGTT.

[f] Wilcoxon rank-sum test was used for continuous variables and Chi^2 test for categorical variables, statistical significance level adjusted according to Bonferron correction for 11 parametes and 4 groups $0.05 / 66 < 7.6 \times 10^{-4}$

Pairwise comprisonis between groups: [g] Group I vs group II; [h] group I vs group III; [i] group I vs group IV; [j] group II vs group III; [k] group II vs group IV; [l] group III vs group IV.

DM, diabetes mellitus; GA, gestational age; GDM, gestational diabetes mellitus; LGA, large for gestational age; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome, SGA, small for gestational age
Modified from Hanson et al 2022a.

Compared to the GDM cases (group 4), the low-risk women (group 1) had lower C-section rate, smaller newborn birthweight and less LGA babies (**Table 18**).

5.2.3 Comparison of high-risk women with normal and no OGTT result

There was no difference in maternal age and pre-pregnancy BMI between the two groups of high-risk women with normal or no OGTT result (groups 3 and 2) (Table 19). While fasting glycose level >5.1 mmol/l accounted for a third of no OGTT group (33.1% vs 19.7%), the proportion of women having "other" risk factors was considerably higher in normal OGTT group (26.4% vs 3.6%).

However, women with normal OGTT were characterized by increased gestational weight gain and increased proportion of LGA newborns, compared to no OGTT group **(Table 19).**

Table 19. Maternal characteristics and pregnancy course among high risk pregnant women without GDM diagnosis

	OGTT normal N=1,357	No OGTT N=939	*P*-value [e]
Basic characteristics			
Age (years)	28 (20–38)	29 (21–39)	n.s
BMI (kg/m^2)	24.4 (18.7–34.6)	25.3 (18.6–32.9)	n.s
Multiparous [a]	48.5%	49.3%	n.s
Risk factors (n (% of carriers))			
Previous LGA	60 (4.4%)	13 (1.4%)	1.2×10^{-4}
Prior GDM	25 (1.8%)	4 (0.4%)	n.s
DM among first degree relatives	243 (17.9%)	142 (15.1%)	n.s
PCOS	30 (2.2%)	13 (1.4%)	n.s
Fasting glycose >5.1mmol/L	267 (19.7%)	311 (33.1%)	7.0×10^{-14}
Polyhydramnion	47 (3.5%)	38 (4.0%)	n.s
Other [b]	358 (26.4%)	35 (3.7%)	1.3×10^{-57}
Pregnancy course and outcome			
Weight gain (0–23 g.w) (kg)	7 (0–16)	6 (-1–14)	2.2×10^{-6}
Weight gain (24–42 g.w) (kg)	11 (3.6–22)	10 (2.9–17)	2.0×10^{-5}
Total weight gain (kg)	17.7 (4–36.5)	15.8 (3–29.6)	3.6×10^{-5}
Excessive weight gain [c]	62.4%	53.9%	2.9×10^{-4}
Male newborn	52.5%	50.0%	n.s
Cesarean section	274 (20.2%)	175 (18.7%)	n.s
If LGA [d] (% of Cesarean sections)	87 (31%)	32 (18%)	2.0×10^{-3}

Data are given as median (5th–95th percentiles) or number (percentage) when appropriate. Groups were compared using Chi^2 test for categorical and Wilcoxon rank-sum test for continuous variables.

[a] Data not available for 2012 cohort

[b] "other" risk factors were considered as glycosuria, excessive weight gain (more than 3 kg in 4 weeks) or suspicion of LGA fetus in index pregnancy.

[c] Total weight gain during the pregnancy was considered excessive when it exceeded recommendations by Rasmussen (Rasmussen et al., 2010).

[d] For the assignment of large for-gestational-age (LGA) diagnosis, the fetal growth calculator based on INTERGROWTH-21st Project was applied to convert the newborn birthweight into gestational age and sex-adjusted centiles (20). Newborn was categorized as LGA in case the sex-and gestational age adjusted birth centile was more than 95 centile

[e] *P* value was adjusted for multiple testing according to Bonferroni correction for 2 groups $0.05 / 17 < 2.9 \times 10^{-3}$

BMI, body mass index; DM, diabetes mellitus; GA, gestational age; g.w, gestational weeks; GDM, gestational diabetes mellitus; LGA, large for gestational age; n.s, non-significant; OGTT, oral glycose tolerance test; PCOS, polycystic ovary syndrome

Adapted from Hanson et al 2022a.

The difference in gestational weight gain was observed especially after 24 g.w. when the referral to OGTT is usually made. Women with normal OGTT (group 3), the weight gain during pregnancy was median 17.7 kg, of which. 11 kg after 24 g.w., compared to 15.8 kg and 10 kg in no OGTT group (group 2), respectively **(Table 19)**. In comparison, the total weight gain in women with GDM was 11.7 kg, and after 24 g.w. 8 kg.

Additionally, both birthweight and centile of newborns were significantly higher in group 3 in contrast to group 2 **(Table 18)**. Although in both groups approximately every fifth woman delivered by C-section, 31% of operative deliveries among women with normal OGTT (group 3) resulted in the birth of an LGA baby compared to 18% in no OGTT subgroup (group 2) **(Table 19)**.

In participants having GDM risk factors but OGTT result was negative, the odds to deliver an LGA baby was nearly as high as in the GDM diagnosis group (AOR 2.3 vs 2.4) **(Table 20)**.

Table 20. Crude and adjusted odds ratios for selected pregnancy outcomes between groups devided according to GDM risk factors and OGTT result

Outcome	Group	Number of women	OR (95% CI)	AOR (95% CI)
LGA newborn [a,b]				
	Low risk	243	1	
	No OGTT	160	1.8 (1.4–2.2)	1.6 (1.2–2.2)
	Normal OGTT	315	2.6 (2.1–3.1)	2.3 (1.8–3.0)
	GDM	110	3.0 (2.3–3.9)	2.4 (1.7–3.4)
SGA [b,c]				
	Low risk	70	1	
	No OGTT	15	0.5 (0.3–0.9)	0.6 (0.3–1.1)
	Normal OGTT	23	0.5 (0.3–0.9)	0.6 (0.4–1.0)
	GDM	4	0.3 (0.1–0.8)	0.3 (0.1–0.9)
GH [d,e]				
	Low risk	20	1	
	No OGTT	26	2.7 (1.9–4.9)	1.3 (0.7–2.5)
	Normal OGTT	50	3.7 (2.2–6.2)	1.7 (1.0–3.1)
	GDM	25	5.0 (2.7–9.2)	2.0 (1.0–4.0)
Cesarean section [f]				
	Low risk	322	1	
	No OGTT	175	1.4 (1.2–1.7)	1.2 (0.9–1.5)
	Normal OGTT	274	1.6 (1.3–1.9)	1.3 (1.1–1.7)
	GDM	114	2.3 (1.8–2.9)	1.5 (1.1–2.1)

[a] Adjusted to previous parities, previous LGA baby, BMI, age, cohort and gestational age at delivery

[b] For the assignment of large or small-for-gestational-age (LGA or SGA, respectively) diagnosis, the fetal growth calculator based on INTERGROWTH-21st Project was applied to convert the newborn birthweight into gestational age and sex-adjusted centiles (Villar et al., 2014). Newborn was categorized as LGA in case the sex-and gestational age adjusted birth centile was more than 95 and SGA in case the sex-and gestational age adjusted birth centile was less than 10 centile.

[c] Adjusted to cohort, previous parities and GA at delivery

[d] Adjusted to BMI and cohort

[e] Missing detailed data for 2012 cohort

[f] Adjusted to previous parities, LGA baby, BMI, age, cohort and gestational age at delivery

AOR, adjusted odds ratio; CI, confidence intervall; GDM, gestational diabetes mellitus; GH, gestational hypertension; LGA, large for gestational age; OGTT, oral glyose tolerance test; OR, odds ratio; SGA, small for gestational age.

Adapted from Hanson et al 2022a.

The risk factors mostly contributed to the birth of LGA baby without GDM were prior LGA or GDM (AOR 5.2 and 2.0, respectively) **(Table 21).**

Table 21. Maternal characteristics and prevalence of risk factors in women with or without LGA neonate

Risk factors	No LGA n=4,193	LGA [g] n=828	*P*=value [h]	AOR (95% CI)
Maternal age	28 (20–38)	29 (21–38)	**9.1×10^{-7}**	
	1,863	441	**2.3×10^{-11}**	
Multiparity	(44.4%)	(53.3%)		
	22.3	23.4	**2.3×10^{-15}**	
BMI	(18.1–32.0)	(19.2–33.9)		
	280	280	0.005	
GA at birth	(256–292)	(266–293)		
Excessive weight gain [a]	158 (3.8%)	629 (76%)	**7.7×10^{-8}**	**1.8 (1.5–2.2)**
	2,121	478		
Newborn male sex [b]	(50.6%)	(57.7%)	**1.0×10^{-4}**	**1.5 (1.2–1.7)**
DM in a relative [a]	377 (9%)	86 (10.4%)	0.2	1.1 (0.8–1.5)
PCOS [a]	48 (1.1%)	9 (1.1%)	0.9	0.7 (0.3–2.1)
Previous LGA [c]	47 (1.1%)	58 (7%)	**2.1×10^{-24}**	**5.2 (3.3–8.4)**
BMI 25–29.9 kg/m^2 [d]	728 (17.4%)	179 (21.6%)	**0.003**	1.2 (0.9–1.5)
BMI ≥ 30.0 kg/m^2 [d]	366 (8.7%)	116 (14%)	**2.2×10^{-6}**	**1.7 (1.2–1.9)**
Fasting glycose				
>5.1 mmol/l [c]	594 (14.2%)	160 (19.3%)	**1.6×10^{-4}**	**1.4 (1.2–1.8)**
Prior GDM [e]	43 (1%)	24 (2.9%)	**0.001**	**2.0 (1.2–3.3)**
Polyhydramnion [f]	64 (1.5%)	37 (4.5%)	**3.6×10^{-8}**	**3.3 (2.1–5.3)**
GDM[c]	313 (7.5%)	110 (13.3%)	**3.6×10^{-8}**	**1.4 (1.1–1.8)**

Data are given as median (5th–95th percentiles) or numbers (percentage). Groups were compared using Chi^2 test for categorical and Wilcoxon rank-sum test for continuous variables.

[a] Adjusted for parity, age, BMI, GDM

[b] Adjusted for parity, BMI, GDM

[c] Adjusted for BMI, parity

[d] Adjusted for age, parity, GDM

[e] Adjusted for BMI

[f] Adjusted for BMI, GDM, parity

[g] For the assignment of large or small-for-gestational-age (LGA or SGA, respectively) diagnosis, the fetal growth calculator based on INTERGROWTH-21st Project was applied to convert the newborn birthweight into gestational age and sex-adjusted centiles (Villar et al., 2014). Newborn was categorized as LGA in case the sex-and gestational age adjusted birth centile was more than 95 and SGA in case the sex-and gestational age adjusted birth centile was less than 10 centile.

[h] Statistically significant nominal P-values are highlighted in bold. *P* value was adjusted for multiple testing according to Bonferroni correction for 2 groups $0.05 / 15 < 3.3 \times 10^{-3}$

AOR, adjusted odds ratio; BMI, body mass index; GDM, gestational diabetes mellitus; PCOS, polycystic ovary syndrome.

In conclusion, women with risk factors and negative OGTT have increased total weight gain and they give birth to larger new-borns, compared to women with GDM risk factors but no OGTT.

6. DISCUSSION

6.1 Inverse trends of the prevalence of preeclampsia and gestational diabetes

Between 2013 and 2018, the prevalence of PE decreased and GDM, in contrary, increased in our study population (**Figure 1**). The same trend was seen in proportion of pregnant women with one or more PE or GDM risk factors for. The shift from nulliparous to multiparous women promotes rather GDM than PE (Robillard et al., 2022; Sweeting et al, 2022). However, proportion of women with obesity, well-known risk factor for both pregnancy complications, is increasing (Robillard et al., 2020; Sweeting et al, 2022).

According to Estonian Medical Birth Registry data similar decline in the prevalence of PE was seen in Estonia, from 2.7% in study population in the Cohort II (2013–2015) to 1.6% in the Cohort III (2018) compared to 2.1% in Estonia in 2013 to 1.7% in 2018 (tai.ee). GDM prevalence went through a more notable rise in our study population compared to Estonian Medical Birth Registry data, from 5.7% in Cohort II to 13.7% in Cohort III compared to 4.3% in 2013 in Estonia to 9.8% in 2018, possibly due to active referral to OGTT (tai.ee).

In concordance with the trend in Estonia, the prevalence of PE is decreasing in other developed countries. A population-based study from Norway Medical Birth Registry included data from 1999 to 2018 to estimate the risk of PE. They concluded that despite the increasing number of risk factor carriers the prevalence of PE was reduced, possibly due to ASA prophylaxis and increased incidence of labor induction (Sole et al., 2022).

In Estonia, ASA is available without a prescription, thus information about ASA consumption is limited. We have detailed data about medication usage from patient questionnaires for Cohort II (2013–2015) and only handful of women used ASA during the pregnancy. The respective information for the Cohort III (2018) is missing. However, as the routine first trimester screening was not introduced in Tartu since before 2020, the ASA prophylaxis was not common in clinical practice.

The higher prevalence of GDM in our study population compared to country's general might be explained by the fact that GDM is laboratory diagnosis and highly dependent on testing activity. With the changing guidelines for testing and increasing trend of obesity, the prevalence of GDM is rising worldwide (Behboudi-Gandevani et al., 2019). Study from Finland including data from Finnish Medical Birth Registry and Hospital Discharge Register demonstrated increased prevalence of GDM between two timeframes from 7.2% in 2006–2008 to 11.3% in 2010–2012. They explained the finding with the change of risk assessment policy from high-risk to universal screening and demonstrated increased prevalence of GDM among obese women (Ellenberg et al., 2017).

6.2 Hunt for the ideal test for preeclampsia prediction is still in progress

The aim for PE screening is to prevent the development of disease. On the second half of pregnancy when women present PE symptoms, the maternal condition can quickly deteriorate, and the only treatment is delivery. Screening for PE at the beginning of pregnancy facilitates sifting out patients for prevention, so as few as possible would develop PE and its complications. As PE is a heterogenous disease, the screening of all forms (especially term PE) remains a challenge.

The most available tool for risk assessment for PE is medical history. This method has been proposed as a screening test in several guidelines (Espinoza et al., 2020; NICE guideline, 2019; WHO 2013). In our study, more than half of the population included had at least one risk factor for PE. Pre-pregnancy diabetes, prior PE, nulliparity and BMI $\geq$ 30kg/m^2 were the risk factors most likely related to subsequent PE. Neither maternal age nor multiple pregnancy increased the risk of PE in our population. Surprisingly, chronic hypertension was not related to increased risk of PE in our study. This might be due to the small number of included patients with this disease, but still, both patients with chronic hypertension developed early (<34 g.w) PE. Study by Villa et al. from Finland had similar results. Strong risk factors related to severe and preterm PE were prior PE, diabetes, and chronic hypertension. Obesity increased the risk of term and severe PE (Villa et al., 2017). Villa et al. also pointed out different risk factor profiles for preterm and term PE (Villa et al., 2017). However, in our population we did not find significant differences related to early or late onset PE.

Although easily applicable, the value of screening by maternal history alone remains poor. Several studies have shown low DR by screening for PE according to risk factors recommended by NICE guidelines (39% for preterm PE and 34% for term PE) (O'Gorman et al., 2017; Tan et al., 2018). Superiority of combined screening of PE involving biochemical and biophysical markers in addition to maternal history has been demonstrated repeatedly, reaching DR´s of the delivery with PE <37 and ≥37 weeks of gestation 75% and 47%, respectively, (O'Gorman et al., 2016; 2017; Tan et al., 2018).

Despite of combined screening tests detecting most of the patients with preterm PE, the predictive performance for term PE which accounts for most of the PE cases remains low. Adding more biomarkers indicating the different abnormal functions, cell-free DNA, or assessment of cardiovascular indices might help to improve the prediction (Garcia-Gonzalez et al., 2020).

6.3 Biomarker tests in the aid of early PE detection in the third trimester

The screening of PE in the third trimester is based on assessment of symptoms characteristic to PE, measurement of blood pressure and protein in urine. However, not every new onset of hypertension in the second half of pregnancy will result in PE. To distinguish PE from gestational or transient hypertension, sFlt-1/PlGF ratio test has been validated for the prediction of short-term absence or presence of PE (Zeisler et al., 2016). These biomarkers also represent a promising test for asymptomatic women as the ratio starts to increase already five weeks before the onset of symptoms (Levine et al., 2004). Our findings indicate that asymptomatic women with sFlt-1/PlGF ratio >38 are more likely to deliver preterm due to PE. Nevertheless, the overall DR for PE remains moderate (58.3%). Sovio et al. showed similar results. At 28 g.w. sFlt-1/PlGF >38 among unselected nulliparous women without PE risk factors was useful for predicting preterm PE (32% risk of delivery prematurely due to PE), however with a low sensitivity of 23%. At 36 g.w. roughly 5% of women were considered as a risk group either based on increased sFlt-1/PlGF ratio >110 or >38 plus risk factors and less than half of them (43%) developed PE. They concluded that sFlt-1/PlGF ratio measurement is a useful clinical tool for PE risk prediction at term in nulliparous women (Sovio et al., 2017). Another study, including 12,035 unselected pregnancies found DR of 76.6% for subsequent PE for following 4 weeks with sFlt-1/PlGF >38, but reported steep fall in DR to 20.7% later than 28 days of testing. Whether all the patients in this study were asymptomatic was not stated (Dragan et al., 2017).

Several studies, including our data show that sFlt-1/PlGF has a limited prediction window for PE (< 28 days) (Dragan et al., 2017; Zeisler et al., 2019). In our population, the DR for 30 days after blood draw was remarkably higher compared to PE prediction for the remaining pregnancy duration generally, 83.3% vs 58.3%, respectively. Another limitation to be aware of while using the test in clinical practice is the physiologic rise of sFlt-1/PlGF with advancing gestational age (Dragan et al., 2017; Levine et al., 2004). According to our data, the DR is dependent rather on the closeness of the PE development than the gestational age at sampling, the FPR, however, increases closer to term. Thus, for PE prediction in asymptomatic women, the sFlt-1/PlGF test is more useful before 34 g.w., when women with sFlt-1/PlGF ratio >38 would benefit from more frequent antenatal visits and once the early signs of PE appear, the antenatal corticosteroids for lung maturation and magnesium sulphate for fetal neuroprotection.

The DR can be improved by addition of biomarkers and maternal characteristics. We were able to develop single-tube multimarker 6PLEX test combining sFlt-1, PlGF, sENG, leptin, and ADAM 12 with gestational age and maternal weight achieving DR of 100% in a small sample set (Ratnik et al., 2020). The performance in a larger population is a subject of a future studies.

As most biomarker tests on the third trimester are based on placenta derived markers, they bare the same limitations. Both commercial sFlt-1/PlGF ratio test and developed 6PLEX multimarker test rather refer to placental insufficiency and pick up the women with placental related problems such as fetal growth retardation, preterm birth, and/or fetal distress.

To improve screening particularly for term PE inclusion of markers of maternal cardiovascular system maladaptation to pregnancy might be useful. A study by Garcia-Gonzalez et al demonstrated that increased chamber filling pressure (E/e´ ratio) and left ventricular mass indexed to body surface area (LVMI) referred to high risk for the development of term PE despite of low PE risk score (combination of maternal characteristics sFlt-1/PlGF ratio and mean arterial pressure) (Garcia-Gonzalez et al., 2020; Melchiorre et al., 2022). However, the screening group who would benefit from the additional cardiac assessment remains to be determined.

6.4 The search for most optimal screening strategy for gestational diabetes

For GDM the purpose of screening is to achieve diagnoses as early as possible reducing the risk of complications with lifestyle changes, diet, and treatment. The GDM diagnosis is based on the abnormal results of OGTT. However, the referral to OGTT is dependent on the subjective assessment of risk factors by midwives or obstetricians, as well as the patient's consent and understanding of the necessity of the test. Benhalima et al. studied selective screening for GDM in European countries and showed that more than a third of GDM cases are missed by using the risk factors-based selection for OGTT referral compared to universal screening (Benhalima et al., 2019). In our study population, during different observed periods roughly half of the pregnant women had at least one risk factor but less than three out of four (57–76%) were referred to OGTT, as suggested by the guidelines (Vaas et al., 2018). Our finding is in line with other studies from Nordic countries showing poor adherence to risk-based screening with less than a half (31%–49%) of high-risk population being screened for GDM (Persson et al., 2009; Grønvall et al., 2022).

Reaching GDM diagnosis is important because those with undiagnosed GDM are prone to GDM-related complications, including stillbirth (Muin et al., 2022). A study in Finland found that even mild untreated hyperglycemia resulted in an increased Cesarean section rate and larger birthweight (Koivunen et al., 2020). A more stringent screening strategy might be beneficial, for example adding additional OGTT after 30 g.w. to determine late onset GDM related to increased risk of operative delivery (Mohr Sasson et al., 2019). Mohr Sasson et al. demonstrated higher Cesarean section rates after pathological OGTT at term due to the suspicion of LGA (Mohr Sasson et al., 2019).

To improve testing by risk factors, Benhalima et al suggested all women at age 30 or more and/or BMI $\geq$ 25 kg/m2 should be assessed for GDM. This

would result in 70% of pregnant women needing screening and only 18.6% cases of GDM would be missed (Benhalima et al., 2019).

To ensure that every woman would be at least offered a screening, universal testing is an option resulting in maximum number of GDM cases. Offering OGTT to every pregnant woman as part of antenatal care would inevitably lead to increased workload of medical personnel and healthcare costs, however, seems more likely to be cost effective than targeted screening. (Mo et al., 2021).

Benefit of detecting all women with GDM, is the option to reduce the risk of complications by careful monitoring and timely interventions and medication when necessary. Studies have shown the positive effect of GDM treatment on maternal gestational weight gain, perinatal outcome, and the possible long-term effects on lifestyle changes (Rasmussen et al., 2020).

However, the opposite results have been demonstrated. Ellenberg et al recognized improved detection of GDM after broadening the screening in Finland but did not detect improvement in pregnancy or neonatal outcomes. The reason behind this could be increased number of diagnosis of GDM among younger women with normal BMI who delivered newborns with normal birthweight. In addition, similarly to our results, increasing birthweight during the study period was noted among severely obese women without GDM diagnoses (Ellenberg et al., 2017).

6.5 Benefits of risk factors assessment

For preeclampsia, as a spectrum of disease, many risk factors are identified and therefore high DR is accompanied by high FPR. Risk factors carriers can be classified as low risk after testing by more accurate combined screening tests. However, receiving negative result from screening does not equal to low-risk pregnancy. A thorough assessment of maternal history can help to sift out a high-risk pregnancy prone to adverse pregnancy outcome. Women with risk factors in our study were in addition to PE more likely to develop isolated SGA, GH, or have a spontaneous preterm birth.

Furthermore, assessment of GDM risk factors can give additional information about pregnancy risks apart from GDM. Our data showed a comparable number of LGA neonates between the GDM group and women with risk factors but normal OGTT result. This could be explained by the fact that GDM is not the only factor resulting in fetal macrosomia; other known risk factors are multiparity, older age, previous LGA and a male newborn. Also, pregnancy weight gain and pre-pregnancy BMI have been shown in previous studies to be related to GDM but also to isolated LGA newborns (Lin et al., 2022; Usta et al., 2017; Zhao et al., 2018).

While it is possible to screen for several pregnancy complications in the beginning of pregnancy, successful antenatal follow-up and counselling does not end there. Some risk factors might develop later or could be modifiable during pregnancy due to early detection.

Increased gestational weight has been shown to be related to several pregnancy complications. In our study population, women with GDM risk factors and a normal OGTT result had substantially higher gestational weight gain, especially on the second half of pregnancy, and more frequent need for operative delivery due to LGA compared to women of high risk but not referred to OGTT. We may assume that weight gain was the reason for the referral to OGTT. But we can also speculate that by associating LGA newborns only to GDM, the normal OGTT result could offer false reassurance of a normal pregnancy course and less motivation for weight management after testing. Therefore, women with high risk of GDM despite of normal OGTT results should be closely monitored and counselled as they are at increased risk of gestational weight gain and a LGA newborn. As a large proportion of these women are overweight, focusing on a healthy diet and exercises have been shown to significantly reduce gestational weight gain (Peaceman et al., 2018).

Similar applies to PE. A study from Sweden demonstrated strong association with high pregnancy weight gain in nulliparous women and term PE. Surprisingly, this association was more pronounced in women with lower BMI before pregnancy (Hutcheon et al., 2018). In contrary, optimization of gestational weight gain is beneficial of reducing late onset PE in women with overweight and obesity for 30–40% (Robillard et al., 2020)

6.6 Strengths and limitations

The strengths of the study:

a) inclusion of a large unselected heterogeneous cohort to assess the prevalence of risk factors and pregnancy outcome throughout timespan of seven years;
b) the availability of detailed medical information collected during routine antenatal follow-up and self-reported questionnaires;
c) access to pregnancy data gave the opportunity to retrospectively assess changes in biomarker levels in relation to developing symptoms (asymptomatic/ symptomatic);
d) valuable information of applicability of the sFlt-1/PlGF test for screening purposes before the onset of symptoms was reported. As the test is becoming more widely available, it is important to be aware of its performance and limits when used as a screening test in asymptomatic women.

The limitations of the study:

a) the properties of the sFlt-1/PlGF test and multimarker test in differentiating subsequent development of PE or GH could not be properly addressed due to small sample size.
b) effects of ASA to the development of PE could not be assessed due to missing data about medication use for cohort III (2018).

6.7 Future research

The results of the studies raise several thoughts for future research.

1. PE is a multisystem disorder. The currently used biomarkers are focusing on placental insufficiency but changes in these biochemical markers refer to placental syndromes rather than PE specifically. Addition of markers reflecting maternal adaption to pregnancy could be useful.
2. Multimarker PE prediction models incorporating several biomarkers and clinical data need validation in larger cohorts and in different subgroups (high risk, low risk, multiple pregnancy etc).
3. More targeted and prospective studies are needed to assess if diet and additional OGTT testing in the III trimester and active management (timed delivery) could help to reduce the number of LGA newborns among women with GDM risk factors but negative OGTT result.

7. CONCLUSIONS

1. The number of risk factors carriers and prevalence of PE has decreased in time (2013–2015 compared to 2018). Although the strongest risk factors were pre-pregnancy diabetes and prior PE, the PPV of risk factors in the prediction of PE in high-risk population is low. The screening for PE can be improved by tests including maternal characteristics and combination of biomarkers.
2. In the third trimester, the highest DR for sFlt-1/PlGF ratio test for prediction of PE among asymptomatic women is within first 30 days of sampling. Testing at later gestational age results in increased false positive rate due to physiological increase of sFlt-1/PlGF ratio. In symptomatic women increased sFlt-1/PlGF ratio (>38) can predict development of PE, shorter time to delivery and newborn with smaller birthweight. The most false positive cases in asymptomatic women using placenta derived biomarker-based tests in the prediction of PE in the third trimester are related to other placental problems such as fetal growth retardation, preterm birth, GH and/or fetal distress. Additional biomarkers (leptin, ADAM 12, sENG) can improve PE prediction.
3. Number of risk factors carriers and prevalence of GDM is increasing. The increase was confirmed in over 7 years observational period following the worldwide trend. The strongest risk factors and most likely to result in diagnosis of GDM are obesity and GDM in previous pregnancy.
4. Women high risk for GDM but negative OGTT results are prone to increased total gestational weight gain and birth of a larger newborns. Risk factors related to LGA without GDM are LGA or GDM during previous pregnancy and polyhydramnios.

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SUMMARY IN ESTONIAN

Rasedustüsistuste riski hindamise ja varase diagnoosimise kliinilised ja biokeemilised markerid

Enamus rasedustest kulgeb probleemideta ja sünnib terve laps. Siiski võivad ka raseduse ajal tekkida tüsistused, mis ähvardavad ema ja loote tervist raseduse ja sünnituse ajal ning mõjutavad pikaajalist elukvaliteeti.

Üheks maailmas enim emade tervist kahjustavaks ja suremust põhjustavaks tüsistuseks on preeklampsia (PE). Seda iseloomustab raseduse teises pooles esmakordselt tekkiv vererõhu tõus, millele lisanduvad teiste organite kahjustuse sümptomid näiteks valk uriinis, maksaensüümide tõus, neuroloogilised või hematoloogilised komplikatsioonid ja/või loote kasvupeetus (Magee et al., 2022).

PE tekkepõhjuseks peetakse viljastunud munaraku pesastumise häiret, mille tagajärjel platsenta areng jääb puudulikuks. Raseduse teises pooles, kui loode hakkab kiiresti kasvama, ei suuda puudulikult arenenud platsenta loote vajadusi tagada. Ülekoormatud platsenta paiskab ema vereringesse põletikku soodustavaid ja angiogeneesiga seotud valke, mis kahjustavad ema endoteeli, tagajärjeks on vasokonstriktsioon ning vererõhu tõus (Staff, 2019).

PE ainsaks põhjuslikuks raviks on sünnitus. Kuigi raseduse ajal tekkivad sümptomid kaovad, ei ole PE ajal tekkinud kardiovaskulaarsüsteemi kahjustused sageli pöörduvad ja hilisemas elus on PE põdenud naistel suurem risk haigestuda kroonilisse hüpertooniasse ning II tüübi diabeeti (Melchiorre et al., 2022; Weissgerber et al, 2015).

Rasedusaegne diabeet (GDM) on teine sageli esinev rasedustüsistus. Seoses elanikkonna rasvumisega on selle esinemissagedus tõusutendentsiga (Sweeting et al., 2022).

GDM diagnoositakse, kui rutiinse rasedusaegse testimise käigus tuvastatakse emal hüperglükeemia (Hod et al., 2015). Selle teket põhjustab pankrease β-rakkude funktsiooni häire, mille tõttu ei suudeta tagada piisava hulga insuliini tootmist, mis on vajalik raseduse ajal tekkiva insuliiniresistentsuse tõttu (Sweeting et al., 2022).

Raseduse ajal soodustab GDM teiste rasedustüsistuste (PE, isoleeritud rasedusaegne hüpertensioon) teket ja loote liigkasv hüperglükeemia tõttu tõstab sünnitusel tekkivate tüsistuste riski (erakorraline keisrilõige, õlgade düstookia, perineumi ruptuur) (Anderberg et al., 2010). GDM põdemine raseduse ajal mõjutab naise tervist ka hilisemas elus, suurendades II tüübi diabeeti haigestumise riski 10-korda. (Vounzoulaki et al., 2020).

Rasedustüsistustega kaasnevaid ohte aitab vähendada riskirasedate tuvastamine ning õigeaegne profülaktika või ravi.

Traditsiooniliselt on hinnatud PE riski anamneesi alusel. Kõrgele riskile viitavad PE varasema raseduse ajal, krooniline hüpertensioon, diabeet, kehaväline viljastamine (*in vitro* fertilization, IVF), mitmikrasedus, esimene oodatav sünnitus, ema vanus üle 40. eluaasta ning rasvumine (kehamassi indeks (KMI)

üle 30 kg/m^2). Selle meetodi probleemideks on aga madal ennustusväärtus (DR (detection rate) <40%, FPR (false positive rate) 10%, NICE) või kõrge valepositiivsete hulk (DR >90%, FPR 64%, ACOG) (O'Gorman et al., 2017). Efektiivseimaks PE riskihinnangu meetodiks peetakse biomarkerite (PAPP-A, PlGF), biofüüsikaliste markerite (vererõhu mõõtmine, ultraheliuuring) ja anamneesi kombineerimist (Mosimann et al., 2020).

Kolmandal rasedustrimestril on kasutusel angiogeneesiga (sFlt-1 ja PlGF) seotud markerite suhte määramine. Kui testi väärtus on ≤38, siis on ühe nädala vältel PE kujunemine vähetõenäoline (Harald Zeisler et al., 2016). Haigusele võiksid viitata sFlt-1/PlGF suhe üle 85 enne 34. või üle 110 pärast 34. rasedusnädalat (Verlohren et al., 2014). Kuna muutused sFlt-1 ja PlGF väärtustes tekivad juba viis nädalat enne sümptomite teket, võiks see olla ka paljulubav test PE skriinimiseks asümptomaatilistel naistel.

GDM korral on oluline veresuhkru taseme alandamine dieedi ja kehalise aktiivsuse abil, seega on vajalik varane diagnoos. GDM on laboratoorne diagnoos, kuna puuduvad haigusele viitavad sümptomid. Samas ei ole rahvusvahelist konsensust nn „kuldse standardi" testi osas. Enim levinud on suukaudne 75 g glükoosi tolerantsus test (GTT), kus diagnoosi kinnitab üks või enam väärtustest üle kokkulepitud normi. Vastavalt Eestis kehtivale raseduse jälgimise juhendile suunatakse GTT-le patsient, kellel on üks või enam järgnevatest riskiteguritest: GDM anamneesis, varasem suurekaaluline vaststsündinu >4500 g, polütsüstiliste munasarjade sündroom (PCOS), diabeet I astme sugulasel, liigne kaaluiive raseduse ajal (>3 kg nelja nädala jooksul), loote makrosoomia või polühüdramnioni kahtlus käesoleva raseduse ajal, paastu veresuhkur >5,1 mmol/L, raseduseelne ülekaal või rasvumus (KMI 25–29.9/≥30 kg/m^2) (Vaas et al., 2018).

Kuna GDM osas skriinitakse valikuliselt st. ainult riskiteguritega naisi, siis diagnostika sõltub paljuski testimise aktiivsusest, ämmaemandate ja arstide hinnangust, keda lisauuringule saata. Seetõttu rasedad, kelle riskitegurid on jäänud tähelepanuta ja potentsiaalselt GDM diagnoosimata, võivad olla ohustatud GDM-ga kaasnevatest tüsistustest. Raseduse tulem GDM riskiteguritega rasedatel, kellel on GTT normaalse tulemusega või tegemata, vajab rohkem tähelepanu.

Uurimistöö eesmärgid

Uuringu eesmärk oli hinnata sagedamini esinevate rasedustüsistuste, preeklampsia ja gestatsioondiabeedi esinemissagedust ning erinevaid võimalusi tüsistuste riski hindamiseks kliiniliste ja biokeemiliste markerite abil.

1. Hinnata PE riskitegurite esinemissagedust ja ennustusväärtust.
2. Selgitada välja biomarkeritel põhinevate testide efektiivsus PE riskihinnangul.
3. Hinnata GDM riskitegurite esinemissagedust ja ennustusväärtust.
4. Leida seoseid glükoosi tolerantsustesti (GTT) tulemuste ja loote makrosoomia vahel.

Patsiendid ja metoodika

Uuringusse kaasati 5735 SA Tartu Ülikooli Kliinikumi naistekliinikus rasedusega jälgimisel olevat naist aastatel 2012 (kohort I, n=1373), 2013–2015 (kohort II, n=2334) ja 2018 (kohort III, n=2028).

Andmed raseduse riskitegurite, kulu ja tulemi kohta saadi haigla elektroonilisest andmebaasist või koguti ämmaemandate poolt.

Analüüsid uuringu jaoks võeti samal ajal raseduse jälgimise jaoks ettenähtud vereanalüüsidega. Uuringust väljaarvamise kriteeriumiteks olid raseduse katkemine/katkestamine enne 22. rasedusnädalat ja andmete puudumine raseduse kulu või tulemi kohta. Üks uuritav jäeti II kohordist välja vastsündinul diagnoositud Edwards´i sündroomi tõttu.

Uuringud on heakskiitnud Tartu Ülikooli inimuuringute eetika komitee.

PE riskitegurite hinnang

PE riskitegureid hinnati II ja III kohordi andmete põhjal, kummastki vastavalt 2334 ja 2028 rasedat.

PE riskigruppi liigitati naine, kellel esines üks või enam järgnevatest riskiteguritest: PE või rasedusaegne hüpertensioon eelneva raseduse ajal, krooniline hüpertensioon, raseduseelne diabeet, rasvumine (KMI üle 30 kg/m^2), IVF, esmassünnitaja, ema vanus üle 40 aasta.

PE diagnoositi vastavalt ISSHP 2014 juhtnööridele: esmane vererõhu tõus (≥140/≥90 mmHg) pärast 20. rasedusnädalat (r.n), millele on lisandunud viited teiste organsüsteemide kahjustusele (valk uriinis, trombotsütopeenia, maksaensüümide tõus, hemolüüs, neuroloogilised sümptomid) (Tranquilli et al., 2014).

Vastsündinu hinnati väikesekaaluliseks, kui tema sünnikaal oli alla 10. tsentiili. Makrosoomia diagnoositi, kui sünnikaal oli üle 95. tsentiili. Sünnikaal arvutati ümber raseduse kestuse ja sooga kohandatud tsentiilideks kasutades INTERGROWTH-21st kalkulaatorit (Villar et al., 2014).

PE biomarkerite uuringud

SFlt-1/PlGF testi jaoks kaasati II kohordist 178 uuritavat: PE oli diagnoositud neist 24-l ja isoleeritud rasedushüpertensioon (GH) 12-l. Vereanalüüsi võtmise hetkel (180 kuni 259 raseduspäeva) olid kõik patsiendi ilma sümptomideta. Kontrollgruppi (n=142) valiti rasedad, kes sarnanesid juhtudega vanuse ja raseduse kestuse poolest vereanalüüsi võtmisel.

Lisaks hinnati 16 naist, kellel olid PE-le viitavad sümptomid (vererõhu tõus või valk uriinis) verevõtul raseduse kestuses 209 kuni 257 päeva.

sFlt-1 ja PlGF tase mõõdeti ka Synlabi laboris Saksamaal kasutades BRAHMS-i sFlt-1 Kryptor/BRAHMS PlGF plus Kryptor PE testi (Thermo Fisher Scientific, Henningdorf, Saksamaa).

Kui sFlt-1/PlGF oli >38, hinnati risk PE tekkeks kõrgeks ning vastupidiselt, kui sFlt/PlGF oli ≤38, siis madalaks (Harald Zeisler et al., 2016). PE diag-

noosile viitavaks väärtuseks loeti sFlt-1/PlGF ≥85 raseduse kestuses 24+0 kuni 33+6 või ≥110 alates 34+0. r.n kuni sünnituseni (Stepan, et al., 2015).

Esimese trimestri multimarker testi väljatöötamiseks kasutati 34 seerumit (võetud raseduse kestuses 70 kuni 98 päeva; PE n=14 ja kontroll n=20) 31-lt rasedalt ning III trimestri testi jaoks uuringu jaoks 61 seerumit (võetud raseduse kestus 180 kuni 275 päeva, PE n=29 ja kontroll n=32) 53-lt rasedalt.

Multimarker testi väljatöötamise jaoks kasutati Luminex® xMAP-i platvormi, kus määrati samaaegselt seerumist ADAM 12, sENG, leptin, PlGF, sFlt-1 ja PTX3.

GDM uuring

GDM uuringusse kaasati 5021 rasedat kõigist kolmest kohordist (vastavalt 1073, 2176 ja 1772 naist).

Mitmikrasedusega või varasemalt diagnoositud diabeediga naised jäeti uuritavate hulgast välja.

GDM riskigruppi klassifitseeriti rasedad, kellel olid järgmised riskitegurid: raseduseelne ülekaal või rasvumus (KMI 25-29.9/≥30 kg/m^2), GDM või makrosoomne vastsündinu (>4500 g) anamneesis, diabeet esimese astme sugulasel, polütsüstiliste munasarjade sündroom, glükosuuria, suur kaaluiive raseduse ajal, loote makrosoomia kahtlus või polühüdramnion, paastusuhkur >5,1 mmol/l (Vaas et al., 2018).

GDM diagnoositi vastavalt GTT tulemustele, kui tühja kõhu veresuhkru tase oli ≥5.1 mmol/l ja/või pärast 75 g glükoosi suukaudset manustamist 1h ja 2h pärast vastavalt ≥10.0 mmol/l ja ≥8.5 mmol/l (Vaas et al., 2018).

Uuritavad jagati GDM riskitegurite olemasolu ja GTT tulemuste järgi nelja gruppi: 1) grupp I/madal risk: rasedad, kelle GDM riskitegureid ei esine ja seega puudub näidustus GTT-ks (n=2,302, 46%); 2) grupp II/GTT tegemata: naised, kellel olid GDM riskitegurid, aga GTT ei ole tehtud või on dokumenteeritud ainult üks patoloogiata GTT enne 20. r.n. (n=939, 19%); 3) grupp III/OGTT patoloogiata: rasedad, kellel on GDM riskitegurid, OGTT tehtud pärast 20. r.n. ja tulemus normis.(n=1,357, 27%); 4) grupp IV/GDM: naised, kellel patoloogilise GTT tõttu on diagnoositud GDM (n=423, 8%).

Rasedusaegne kaaluiive hinnati liigseks, kui see ületas rahvusvaheliselt tunnustatud soovitusi: >9.0 kg rasvunud naisel (raseduseelne KMI ≥30.0 kg/m^2); >11.5 kg ülekaalulistel (25.0–29.9 kg/m^2), >16.0 kg normaalkaalulistel (18.5–24.9 kg/m^2) and >18.0 kg alakaalulistel rasedatel (< 18.5 kg/m^2) (Rasmussen et al., 2010).

Peamised tulemused

PE riskitegurite hinnang

PE riskitegurite hindamiseks moodustatud valim koosnes 4056-st rasedast II ja III kohordist (vastavalt 2254 ja 1802 naist). PE esinemissagedus selles popu-

latsioonis oli 2,2% (n=90). Kahe perioodi võrdluses on riskiteguritega rasedate hulk kerges langustrendis (56.3% vs 52%, $P = 0{,}006$)

Riskiteguritega uuritavatel (n=2,206; 54,4%) oli 7,8 korda suurem šanss PE tekkeks kui madala riskiga naistel. Samuti suurem tõenäosus loote hüpotroofia, spontaanse enneaegse sünnituse või isoleeritud rasedushüpertensiooni tekkeks. Raseduseelne diabeet ja PE eelneva raseduse ajal on seotud suurema riskiga PE tekkeks ka käesoleva raseduse ajal, šansi suhe vastavalt 14,5 (95% CI 5,2 – 40,6) ja 9,6 (95% CI 3,8 – 24,5). Kuigi 90%-l PE diagnoosi saanud naistel oli vähemalt üks riskitegur, on riskiteguri olemasolu PE tekkeks siiski madala positiivse ennustava väärtusega (3,7%).

PE biomarkerite uuringud

Parima tulemuse (ennustusväärtus, DR, 100%) PE riski hindamiseks I trimestril andis multimarker testi mudel, kus on määratud PTX3, sFlt-1 ja ADAM12 väärtused ja kombineeritud kliiniliste andmetega (eelnevad sünnitused ja raseduse kestus verevõtul). Test tuvastas kõik rasedad, kellel hiljem diagnoositi PE. Saadi ka neli valepositiivset analüüsi vastust, kus kahel juhul sündis makrosoomne laps, ühel rasedal diagnoositi rasedusaegne hüpertensioon ja ühel juhul kulges rasedus probleemideta.

sFlt-1/PlGF testi kasutusvõimaluste hindamiseks III trimestril, uuriti 178 asümptomaatilist rasedat II kohordist. Rasedad, kes olid klassifitseeritud kõrge riski gruppi (n=29) vastavalt sFlt-1/PlGF testi tulemusele (sFlt-1/PlGF > 38) sünnitasid võrreldes madala riskiga rasedatega varem (raseduse kestus 282 *vs* 265 päeva) ja väikesema sünnikaaluga vastsündinud (2782 g *vs* 3584 g).

Patsientidest, kelle sFlt-1/PlGF tase ületas diagnostilise väärtuse (≥85 enne 34 r.n. ja ≥110 pärast 34 r.n; n=13) diagnoositi üheksal juhul PE (69%; sFlt-1/PlGF 85.3–571.3), kõik sünnitasid keisrilõike teel ning seitsmel juhul enneaegselt. Ka ülejäänud neljal patsiendil oli patoloogiline rasedustulem: spontaanne enneaegne sünnitus, väikesekaalulise lapse sünd või sünnitus keisrilõike teel.

Madala riskiga (sFlt-1/PlGF ≤ 38; n=149) naistest kümnel (6,7%) diagnoositi PE, neist enamusel (n=9; 90%) rohkem kui 30 päeva (30–69 päeva) pärast vereanalüüsi. Üldiselt esines selles grupis võrreldes kõrge riski grupiga oluliselt vähem patoloogilist rasedustulemit (nt enneaegne sünnitus, väikesekaalulise lapse sünd).

PE kujunemise ennustusväärtus oli 58,3% ja valepositiivsuse määr (false positiive rate, FPR) ~ 10%. DR on oluliselt parem esimese 30 päeva jooksul pärast vereanalüüsi (83,3%, FPR 3%). Pärast 34. rasedusnädalat võetud testide hulgas oli rohkem valepositiivseid tulemusi.

Sümptomaatilistel rasedatel, kellel sFlt-1/PlGF tase oli üle 38, diagnoositi sagedamini PE (n= 7 vs n=1; $P = 0.012$) ja sündis väiksemakaaluline vastsündinu (2968g vs 3876g) võrreldes madala riskiga (sFlt-1/PlGF ≤ 38) naistega.

Parima ennustusväärtusega (100%) multimarker test PE riski hindamiseks kolmandal trimestril hõlmas ADAM12, sENG, PlGF, sFlt-1, leptini väärtuste kombineerimist ema kaalu ning raseduse kestusega verevõtul. Sellisel juhul oli

FPR 3,5%. Valepositiivsetel juhtudel sünnitasid naised väikesekaalulise vastsündinu. Ühel valenegatiivsel naisel diagnoositi PE, kuid alles 62 päeva pärast vereanalüüsi.

GDM uuring

Ajavahemikul 2012 kuni 2018 on GDM riskitegurite kandjate hulk suurenenud (43,5%-lt 57,8%-ni). Sagedamini esinevad riskitegurid on ülekaalulisus (KMI 25-29.9 kg/m^2) ja „muu“ (glükosuuria, liigne kaaluiive, loote makrosoomia kahtlus). GTT-le suunati kõige sagedamini rasvunud (KMI ≥ 30 kg/m^2) rasedaid ja neid, kellel anamneesis oli GDM.

Vastavalt GDM riskitegurite olemasolule ja GTT tulemustele jagati uuritavad nelja gruppi. Võrreldes teiste gruppidega, sünnitasid GDM diagnoosi saanud rasedad (grupp IV) varem, suurema tõenäosusega makrosoomse lapse ja keisrilõike teel. Naistel, kes olid liigitatud gruppi III (GDM riskitegurid, aga GTT normis) sündisid suurema sünnikaalu lapsed kui teistes gruppides (3705g vs < 3635g).

Normaalse GTT tulemusega naisi iseloomustas võrreldes II grupiga (GDM risk, GTT tegemata) suurem rasedusaegne kaaluiive (grupp 3: median 11 kg vs grupp 2: median 10 kg) ning vastsündinu sünnikaal (3705g vs 3576g).

Järeldused

1. PE riskiteguritega rasedate arv on ajavahemikus 2013 kuni 2018 langenud. Olulisimad riskitegurid olid raseduseelne diabeet ning PE varasema raseduse jooksul. Siiski on riskiteguritel põhinev PE riskihinnangu positiivne ennustav väärtus madal. Ennustusväärtust parandab kombineeritud riskihinnang, mis sisaldab lisaks ema riskiteguritele ka biomarkerite väärtusi.
2. Asümptomaatiliselt rasedatel on sFlt-1/PlGF test parima ennustusväärtusega 30 päeva jooksul pärast vereanalüüsi. Pärast 34. r.n esineb rohkem valepositiivseid testi tulemusi. Naistel, kellel on PE-le viitavaid sümptome, viitab sFlt-1/PlGF tõus üle 38 suuremale tõenäosusele PE tekkeks, lühemale ajale sünnituseni ja väiksema sünnikaaluga lapsele. Enamus valepositiivsetest juhtudest asümptomaatilistel naistel, kasutades platsentast pärit biomarkereid, on seostatavad teiste platsentaarsete patoloogiatega nagu loote kasvupeetus, spontaanne enneaegse sünnitus ja/või GH.
3. GDM esinemissagedus ja riskiteguritega rasedate hulk on tõusuteel. Olulisemateks riskiteguriteks, millega sageli kaasneb ka GDM diagnoos, on rasvumus ja GDM eelneva raseduse ajal.
4. Rasedaid, kellel on GDM riskitegurid, aga GTT tulemus on normaalne, iseloomustab suurem rasedusaegne kaaluiive ja suuremakaaluliste vastsündinute sünd. Loote makrosoomia riskiteguriteks on suurekaaluline vastsündinu või GDM eelneva raseduse ajal ning polühüdramnion.

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Industrial property

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Tööstusomand

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