THE MOST RELIABLE CRITERION FOR DIAGNOSING SEVERE PRE-ECLAMPSIA IN PRETERM PREGNANCIES

A thesis submitted in partial fulfilment for the degree of Master of Medicine

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SUMMARY

Author name: Georgina Jacob

Title: The most reliable diagnostic criterion for diagnosing severe preeclampsia in preterm pregnancies.

Aim: The aim of this study is to find which factor is the most reliable for diagnosing severe preeclampsia in preterm pregnancies.

Objectives:
1. Which diagnostic criterion: blood pressure >160/100mmHg, proteinuria, liver enzymes, thrombocytes, headache is the most common when the diagnosis of severe preterm preeclampsia is made.
2. What is the impact of angiogenic factors (sFlt-1 and PlGF) for diagnosing severe preterm preeclampsia.
3. Which of the diagnostic criterions for preterm severe preeclampsia has the strongest correlation with sFlt-1/PlGF ratio.

Results:
From the entire sample, blood pressure ≥ 160/100 was the most common symptom with 75.7% of patients (n=56) suffering from it. Statistical analysis of the characteristics of patients with early preterm sPE and patients with late preterm sPE found that there is no significant difference in severity between the two subsets of preterm preeclampsia. Fetal weight and platelet count was found to have a significant correlation with sFlt-1/PlGF ratio. When trying to find the relation between severe preeclampsia diagnostic symptoms and adverse neonatal outcomes (fetal growth retardation, Apgar scores <7), correlation was found between blood pressure ≥160/100 and fetal growth retardation.

Conclusions:
1. The most common criterion for preterm severe preeclampsia was blood pressure ≥160/100mmHg.
2. Angiogenic factors (sFlt-1/PlGF ratio) is a reliable tool for diagnosing severe preterm preeclampsia.
3. The significant correlation with sFlt-1/PlGF ratio was found to be fetal weight and platelet count.
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CONFLICT OF INTEREST

The author reports no conflict of interest.
ETHICS COMMITTEE CLEARANCE

Ethics approval for this research was granted by Bioethics centre in the Lithuanian University of Health Sciences, on the 12th of December 2018. The ethics application number is BEC-MF-180.
DĖL PRITARIMO TYRIMUI

LSMU Bioetikos centras, įvertinęs Georginos Jacob pateiktus dokumentus, studentės tiriamajam darbui tema „The diagnosis of severe pre-eclampsia in preterm pregnancies“ pritaria.

[Signature]

dr. Eimantas Pečiulis

* Pastaba: šis pritarimas neatleidžia tiriamajį mokslinį darbą vykdantių asmenų nuo prievoles laikytis Bendrojo duomenų apsaugos reglamento nuostatų ir nuo atsakomybės gauti nacionalinio arba regioninio bioetikos komiteto leidimą, jei toks leidimas būtinas pagal LR Biomedicinių tyrimų etikos įstatyme numatytus reikalavimus.
ABBREVIATIONS LIST

ABG- arterial blood gas
ACOG- The American College of Obstetricians and Gynaecologists
AUC- Area under curve
BPD- Bronchopulmonary dysplasia
DBP- diastolic blood pressure
Eng-endoglin
eNOS-endothelial nitric oxide synthase
EO- early onset
Flt-1- fms-like tyrosine kinase 1
LDH-lactate dehydrogenase
LO- late onset
LUHS- Lithuanian University of Health Sciences
PE- Preeclampsia
PIGF- Placental growth factor
PLT- platelet
ROC- Receiver operating characteristic
SBP- systolic blood pressure
sEng- Soluble endoglin
sFlt-1- solouble fms-like tyrosine kinase 1
sPE- Severe Preeclampsia
STB- syncytiotrophoblast
Std. Deviation- Standard deviation
TGF-β- Transforming growth factor beta
VEGF- Vascular endothelial growth factor
VEGFR-2- Vascular endothelial growth factor receptor 2
VEGFR-1- Vascular endothelial growth factor receptor 1
1. INTRODUCTION

In Lithuania, 2% of pregnancies develop pre-eclampsia (1). It continues to affect 5–8% of pregnancies worldwide and is one of the leading causes of maternal and perinatal morbidity and mortality worldwide (2). Typically, preeclampsia (PE) manifests during the third trimester and usually resolves after the delivery of the fetus, and moreover, once the placenta has been removed. Some of the severe maternal complications that can occur are seizures, cerebral haemorrhage, hepatic rupture or insufficiency, renal failure, stroke and even death (3). Since preterm delivery is frequently induced, the most common fetal complications are growth restriction, hypoxia and intrauterine fetal death.

The etiology of preeclampsia is still relatively unknown. However, it is generally accepted that the placenta plays a pivotal role in the pathogenesis. It is believed that before the clinical signs of preeclampsia develop, there is incomplete trophoblast invasion in the uterine vasculature which results in the spiral arteries retaining their muscle elastic coating, and blood flow impedance persists (4). The inappropriate remodelling of the uterine spiral arteries results in limited supply of oxygen and nutrients to the placenta hence, placental hypoxia (5) which in turn is thought to lead to the secretion of soluble factors into the maternal bloodstream inducing a widespread endothelial dysfunction (6). The secreted factors that have a significant influence on the endothelial dysfunction are soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PIGF). sFlt-1 is a splice variant of VEGF (vascular endothelial growth factor) receptor Flt-1 that possesses antagonistic properties when binding to VEGF and PIGF. When sFlt-1 binds to VEGF and PIGF in circulation it prevents these growth, factors from interacting with their receptors Flt1 and Flk1 (7). As a result endothelial dysfunction ensues which results in hypertension, proteinuria and other systemic manifestations of the syndrome.

Clinically, preeclampsia is identified when a pregnant woman after 20 weeks gestation has new onset elevated blood pressure ≥140/90 mm Hg, that has been found on at least 2 measurements taken at least four hours apart. In addition, the gestational women must have one or more of the following: proteinuria >0.3g/24 h; indications of further systemic involvement such as renal insufficiency (elevated creatinine), elevated transaminases and or right upper quadrant pain; neurological complications; haematological complications or fetal growth restriction (8). Preeclampsia can be subclassified according to gestational age for example preterm pre-eclampsia occurs before 37 weeks whereas term pre-eclampsia develops at or after 37 weeks. Patients that develop preterm pre-eclampsia are more likely to suffer from the severe form of pre-eclampsia (9). Severe pre-eclampsia (sPE) is pre-eclampsia with clinical features that increase the risk of mortality and morbidity. Patients are considered to have sPE when they present with one or more of the following: two recordings of blood pressure ≥160/110 mmHg with each reading taken six hours apart, proteinuria > 5g in 24 hours, oliguria < 500ml in 24 hours, thrombocytopenia, impaired liver function indicated by elevated levels of
liver enzymes, progressive renal insufficiency, pulmonary edema or new onset cerebral or visual disturbances(8)(10).

In order to avoid the severe complications associated with preterm pre-eclampsia, it is essential to make a timely diagnosis. A timely diagnosis would allow early management of the disease which in turn could reduce the development of severe complications. Furthermore, it is imperative that the diagnosis of sPE is as accurate as possible, because the only treatment we have as for today is removal of the placenta via delivery. This is very important when preterm sPE is diagnosed. Delivery of the preterm foetus, especially till 30th week of gestation is an action that can negatively affect the future life of the baby. Therefore, it is essential that we identify and diagnose patients who are at risk of developing sPE early. With the current diagnostic methods patients could have other ailments that are causing them to have blood pressure≥160/100 and proteinuria>5g/24hr, hence some patients could be wrongly diagnosed with sPE such an action can affect the prognosis of any patient. In this way, the current clinical criteria for diagnosing pre-eclampsia is inadequate and therefore additional diagnostic aids should be incorporated when diagnosing pre-eclampsia. This study aims to research which diagnostic criterion has the strongest relation with the diagnosis of sPE in preterm pregnancies.
2. AIMS AND OBJECTIVES

Aim: The aim of this study is to find which factor is the most reliable for diagnosing severe pre-eclampsia in preterm pregnancies.

Objectives:

1. Which diagnostic criterion: blood pressure >160/100mmHg, proteinuria, liver enzymes, thrombocytes, headache is the most common when the diagnosis of severe preterm preeclampsia is made.

2. What is the impact of angiogenic factors (sFlt-1 and PlGF) for diagnosing severe preterm preeclampsia.

3. Which of the diagnostic criterions for preterm severe preeclampsia has the strongest correlation with sFlt-1/PlGF ratio.
3. LITERATURE REVIEW

3.1 Severe preterm pre-eclampsia

Preterm pregnancy is defined as delivery before 37 weeks of gestation according to WHO(11). Preterm pregnancy is a rising issue, every year approximately 15 million babies are born preterm and, it is one of the major factors contributing to death in children under five years of age. For example, preterm pregnancy was responsible for the deaths of almost 1 million children under five in 2015. Research has shown that these deaths are avoidable with current cost-effective interventions.

Across the globe the rate of preterm births is 5-8%. Preterm delivery is a major cause of perinatal morbidity and mortality. Preterm birth can be further sub classified based on gestational age for example, extremely preterm is less than 28 weeks, very preterm is 28-32 weeks and moderate to late preterm is 32-37 weeks(11). Severe pre-eclampsia (sPE) also known as pre-eclampsia with severe features, increases the risk of preterm pregnancy by 80 fold(12). Preterm infants born to mothers suffering from pre-eclampsia with severe features are at an increased risk of developing intrauterine growth restriction(IUGR) when compared to term babies. Preterm pre-eclampsia with severe features is the interchangeable with preterm pregnancy with severe pre-eclampsia. Intrauterine growth restriction is a term that describes significantly reduced fetal weight for its gestational age. Newborns that suffer from IUGR are at greater risk of neonatal morbidity and mortality(13). Infants that are severely affected by IUGR can suffer from oxygen and nutrient deprivation, difficult cardiopulmonary transition with perinatal asphyxia, meconium aspiration or persistent pulmonary hypertension. The immediate complications IUGR neonates may suffer from include: hypothermia, hypoglycaemia, hyperglycaemia, polycythaemia, jaundice, feeding difficulties, feed intolerance, necrotizing enterocolitis, late onset sepsis and pulmonary haemorrhage to name a few.

Previous studies have shown that the clinical diagnostic criteria for pre-eclampsia is inadequate in sensitivity and specificity especially when identifying the development of complications. In 2013 the diagnostic criteria for PE was revised by ACOG to include women with end organ damage(renal, liver, brain or haematological) and exclude proteinuria as a component of the diagnostic criteria(14). All patients with PE with a gestational age>37 weeks are usually indicated for delivery. If a patient is suspected or diagnosed with PE in the preterm period it is essential that those at risk of developing sPE are recognised in order to commence management. The management of sPE involves deciding whether to admit the patient versus outpatient follow up, the use of medications such as antihypertensives and
magnesium sulfate which is administered for seizure prophylaxis, frequent lab draws, fetal monitoring and ultimately the delivery depends on sPE diagnosis.

Previously it was believed that PE was a short-term ailment that would only last during pregnancy and disappear after the delivery of the placenta however, various studies have proved this belief to be false. There is now increasing evidence suggesting that PE has significant long-term effects on the cardiovascular system, renal system and metabolic system. These effects can arise decades after PE(2)(8). Pregnant mothers with sPE are at a greater risk of developing these aforementioned complications. For example, a study conducted by Eriseida Ndoni et al (15) aimed to evaluate the maternal complications in sPE. The study found an association between maternal complications and sPE. The results from the research compared and contrasted the prevalence of complications between PE and sPE patients. Amongst patients with PE 0.5% developed stroke whereas, 1.9% of patients with sPE suffered from a stroke. From the investigation 1.3% of sPE mothers developed pulmonary edema and 2.6% suffered from renal failure. Thus, the study concluded that sPE was related to high rate of maternal severe morbidity(15). Information collected over 10 years, suggests that all women suffering from PE are at an increased risk of developing cardiovascular diseases later in life however, the risk for women who deliver before 37 weeks gestation are at an eight-nine fold increased risk. This risk has even been recognised by the American Heart association. The diseases that these mothers are more likely to suffer from are myocardial infarction, stroke and congestive heart failure. It is believed that PE doesn’t cause these cardiovascular diseases but rather that they share similar risk factors(8). The impact of PE on the future of kidney health has been largely speculated. Previously, certain studies showed that the average urinary albumin excretion rate was noticeably higher in women 3-5 years post PE than those in the normal control group. These findings were later confirmed in a systemic review however, serum creatinine and estimated glomerular filtration rates where not significantly different at the end of the 7-year follow up and therefore it was concluded that PE did not have lasting impact on kidney health. This conclusion, was contradicted by a large Norwegian registry database that showed the risk ratio of developing end stage renal disease in pregnancy was 4.7 however for women with PE the risk ratio was 15.5. These findings were further strengthened by a cohort study in Taiwan which found pre-eclamptic women had a hazard ratio of 14.0 to develop end stage renal disease when compared to women with no hypertensive diseases(2). Pre-eclampsia also has a significant effect on the metabolic and endocrine systems. For example, a registry cohort study conducted in Denmark found a 3.68-fold increase in diabetes mellitus in patients after suffering from severe pre-eclampsia. When compared to the control patients, patients with a history of PE had thyroid stimulating hormone levels 2.42 above the baseline while levels of free triiodothyronine were less than the controls.
Over the years more information had been obtained on how PE effects fetal complications. In addition to causing IUGR, pre-eclampsia can lead to the development of a condition called bronchopulmonary dysplasia. For healthy pulmonary vascular development proper angiogenic state is required therefore the anti-angiogenic state in PE can predispose infants to develop BPD. A study conducted by Hilal Ozkan et al (2) found 38.5% incidence of BPD in preterm infants born to pre-eclamptic mothers as opposed to 19.5% preterm infants with BPD born to normotensive women (2).

Preterm pre-eclampsia is believed to develop from abnormal trophoblast invasion of the spiral arteries which results in placenta malperfusion this then causes oxidative stress that triggers the release of cytokines and proinflammatory factors from the trophoblast (16). These anti-angiogenic factors cause widespread endothelial dysfunction which is responsible for the manifestations of pre-eclampsia.

The anti-angiogenic factors which are released from the placenta before the manifestation of symptoms are possible biomarkers for the early detection and diagnosis of preterm pre-eclampsia. Currently, there is extensive research being made on the use of these factors and other biophysical and sonographic findings to develop early diagnosis or screening of pre-eclampsia. Early diagnosis or screening of pre-eclampsia will allow risk stratification and the timely use of pharmacological interventions such as aspirin to reduce PE (16).

At present there are hardly any preventative methods and the only cure is delivery of the placenta. Women that present with preterm pre-eclampsia have iatrogenic delivery in order to avoid serious maternal or neonatal complications (16). If preterm pre-eclampsia could be diagnosed earlier the pregnancy could be prolonged for longer with intensive monitoring which would thus reduce maternal and fetal complications (17).

3.2 Angiogenic factors and pre-eclampsia

During angiogenesis, it is essential that there is a balance between pro- and anti angiogenic factors. One of the key pro-angiogenic factors is vascular endothelial growth factor, which also contributes towards maintaining endothelial and vascular health. These functions are accomplished through VEGFR-2, the receptor to VEGF. Another pro-angiogenic factor called placental growth factor, only binds to fms-like tyrosine kinase 1 also known as VEGFR-1 which is thought to trigger pathological angiogenesis under hypoxic or inflammatory environment. It also causes the recruitment and migration of other cells that support angiogenesis such as endothelial cells, macrophages, smooth muscle cells and pericytes. Soluble FLT-1 is formed when FLT-1 is shed from the cell surface and sFlt-1 binds to
VEGF, inhibiting it from direct angiogenesis. Thus, sFlt-1 is anti-angiogenic. Endoglin is a pro-angiogenic factor that binds to transforming growth factor beta and prevents apoptosis in hypoxic endothelial cells. An additional function of Eng is that it aids in the activation of eNOS which has a role in cardiovascular protection. Soluble Eng inhibits the function of Eng hence, it is an anti-angiogenic factor. Similar to sFlt-1, sEng is believed to be released from the syncytiotrophoblast during hypoxic condition. Alternatively, it had been speculated that sEng is released when oxysterol interacts with liver X receptors, expressed by trophoblasts(18).

Evidence suggests that placental malperfusion produces oxidative stress, placental debris, innate immune activation, cytokines, eicosanoids and angiogenic imbalances. The imbalance between the pro and antiangiogenic factors is believed to be associated with endothelial dysfunction(19). The most influential antiangiogenic factors in PE soluble fms-like tyrosine kinase 1 and soluble endoglin. The proangiogenic factors that have a significant role in placental angiogenesis are placental growth factor and vascular endothelial growth factor, both are believed to be secreted from trophoblast cells (20). The antagonising function of sFlt-1 contributes to vasoconstriction and endothelial dysfunction(20). A study conducted by Fangxian et al(20) injected sFlt-1 carrying adenovirus into pregnant mice. The increase in circulating sFlt-1 levels resulted in hypertension in the mice. In addition, the pregnant mice displayed signs of decreased fetal, placental weight, hepatic, renal and haematological findings consistent with pre-eclampsia(20). Another study performed on primates found that when the uteroplacental perfusion was reduced the levels of sFlt-1 increased. Therefore, increasing levels of sFlt-1 links placental dysfunction with maternal endothelial dysfunction. Similarly, soluble endoglin (sEng) released by the placenta circulates in the blood and binds to TGF-β and inhibits its proangiogenic function. Research on pregnant rats found that elevated levels of sEng induced signs of severe pre-eclampsia(21). Investigations by Chappell et al (16) and colleagues studied the diagnostic accuracy of low plasma PIGF concentration in suspected pre-eclamptic women between 20 and 35 weeks gestation. They found that the low levels of PIGF had a 98% negative predictive value of predicting the development of pre-eclampsia that needed delivery within 14 days. A prospective, multicentre observational study carried out by Zeisler et al (22) discovered that sFlt-1:PIGF ratio cutoff of 38 or lower has a negative predictive value of 99.3%. Therefore, a sFlt-1:PIGF ratio of 38 or lower can be used to predict the short term absence of pre-eclampsia in women in whom the syndrome is suspected clinically.
3.3 The role of sFlt-1 in the diagnosis of severe pre-eclampsia in preterm pregnancies

Soluble fms-like tyrosine kinase-1, a splice variant of angiogenic factor VEGF receptor Flt-1, is believed to be released into the maternal circulation when the placenta becomes hypoxic due to abnormal trophoblast invasion. When sFlt-1 is released into the bloodstream it binds and inhibits PIGF and VEGF. It prevents VEGF signalling in target organs such as the kidneys, liver and brain which are key proangiogenic factors for normal growth of blood vessels. Therefore, the maternal endothelium becomes dysfunctional which leads to the clinical manifestations of PE. A study led by Karumanchi et al(7) found that circulating levels of sFlt-1 was elevated in pregnancy but significantly higher in pre-eclamptic women. In addition, they found that over expressing this receptor in rats caused a pre-eclampsia like syndrome to develop. Furthermore, they discovered that sFlt-1 levels were elevated weeks before PE became evident (7). Multiple studies have shown there is a positive correlation between levels of sFlt-1 and PE. The first published evidence for an imbalance of angiogenic factors in PE was by Maynard et al (23). The study found that not only were sFlt-1 levels increased in PE patient but that sFlt-1 levels were almost five times higher in severe PE cases (18)(23). Additionally, they found that the patients displayed a decrease in VEGF and PIGF proportionate to the increase in sFlt-1(18)(23). Findings from a prospective cohort study carried out by Ahmed M. Abbas et al(24) in Assuit University further corroborated the results of Maynard et al(23). The study was used to investigate the levels of sFlt-1 in PE patients and the prognostic ability of sFlt-1 in detecting sPE(24). The patients involved in the study were divided into groups: sPE (group 1) and PE (group II) and women with the same age, parity and gestational age were placed in a control group (group III). On the day of admission and two days after, the following measurements were taken: serum sFlt-1, complete blood count, coagulation profile, liver function, kidney functions, serum uric acid, urine analysis and random blood sugar. Blood pressure was also recorded every four hours after admission until 24hours after delivery. Measurements of sFlt-1 was taken by sandwich ELISA technique. The patients were also followed up after the delivery for maternal outcomes; admission to ICU, duration of hospital stay and route of delivery. Fetal outcomes were also monitored such as: fetal status, fetal weight, Apgar score and referral to paediatric care unit. The results of the investigation found a strong association between sFlt-1 values and the severity of PE(24).

Multiple studies have indicated the link between sFlt-1 levels and the symptoms of PE hence, one of the solutions that was offered was the possibility to remove sFlt-1 extracorporeally via therapeutic apheresis and this would theoretically reduce the maternal symptoms of PE and allow the pregnancy to be prolonged. An investigation conducted by Ravi Thadhani et al(25) aimed to extracorporeally
separate sFlt-1 from maternal circulation and to see whether this would reduce the clinical symptoms of PE and thus allow the pregnancy to be prolonged instead of delivering the placenta. The study used dextran sulfate cellulose apharesis columns to separate the sFlt-1 proteins from the mother's blood they found that depleted levels of sFlt-1 prolonged pregnancy in women with very preterm PE and the dextran was well tolerated by both mother and baby(25).

Another study published in an international journal of women's cardiovascular health 2018 edition investigated serum concentration of sFlt-1, sEng and PIGF in normotensive, preterm and term pre-eclamptic pregnancies. These biomarkers were assessed on their clinical utility in monitoring PE severity and intrauterine growth retardation. The participants of the study were divided into four groups: preterm PE, preterm control, term PE and term control. The preterm category was stratified into with and without severe features. Results of the investigation found concentrations of sFlt-1 were significantly higher in preterm and term pre-eclampsia than the corresponding control. In addition, the serum sFlt-1 levels were also associated with the severity of preterm pre-eclampsia. However, the research showed that the ratio of sFlt-1/PIGF had a more positive correlation with the severity of preterm PE(26). The positive association between sFlt-1 levels and severe PE in preterm pregnancies makes it a possible biomarker that can be used for early diagnosis of sPE in preterm pregnancy.

3.4 The role of PIGF in the diagnosis of severe pre-eclampsia in preterm pregnancies

Placental growth factor (PIGF) is an angiogenic factor that belongs to the VEGF sub-family. The source of PIGF is believed to be the syncytiotrophoblast, serum levels of PIGF begin to rise with gestation peaking at 26-30 weeks and reducing towards term. However, subjects with PE have elevated levels of sFlt-1 which binds to PIGF hence, women with PE would display an abnormally reduced level of PIGF (27). PIGF encourages the growth of new blood vessels which is essential for a healthy placenta(28).

The early diagnosis of sPE in preterm pregnancies will help start early management and hence reduce the chances of fetal and maternal complications. Angiogenic markers such as PIGF have been researched over the years for their clinical utility. Currently, the diagnosis of PE is made from maternal blood pressure and proteinuria. These two factors are imprecise and have low predictive value for disease progression and associated complications(29). A study carried out by Chappell et al(27) found PIGF to be of significant use when diagnosing pre-eclampsia in women <35 weeks gestation. The
investigation showed that PlGF had 0.96 sensitivity and 0.98 negative predictive value. Another study conducted by Chappell et al (27) aimed to find if incorporating PlGF testing in a clinical management algorithm for women with suspected preterm PE would save the use of unnecessary resources and expenses. The findings of the study indicated that using PlGF in the management of women with PE does in fact reduces cost and save resources (27).

A study conducted by H. Stepan et al (29) investigated the diagnostic ability of PlGF and sFlt-1/PlGF ratio in diagnosing pre-eclampsia. The objective of the study was to compare the diagnostic utility of PlGF and sFlt-1/PlGF ratio. Levels of sFlt-1/PlGF ratio was measured using Elecsys immunoassay and PlGF serum concentration was recorded using Triage PlGF assay. The results from the investigation indicated that Elecsys immunoassay sFlt-1/PlGF ratio used in conjunction with gestational age related cut-off values, was better at identifying individuals with PE than PlGF measurements alone (29).

Further studies have evaluated the use of PlGF alone to detect and diagnose PE. For example, a systematic review carried out by Frampton GK et al (28) on PlGF being used alone or in combination with sFlt-1 for the assessment of suspected PE in women. The review evaluated the results of Triage PlGF test in the PETRA and PELICAN study. Meanwhile, the results of Elecsys sFlt-1/PlGF ratio from the PROGNOSIS and Álvarez-Fernández et al (28). study was reviewed. After analysing the published evidence from the various studies the review concluded that the Triage PlGF test had high prognostic sensitivity for predicting PE that needs delivery within two weeks of testing whereas Elecsys sFlt-1/PlGF ratio has high diagnostic sensitivity to rule out PE within 1 week of testing and good specificity to rule in PE after four weeks of testing even though it had a high rate of false positives. The PELICAN investigation found that for Triage PlGF testing with cut offs <100pg/ml and <5th percentile of PlGF concentration there was high sensitivity of 96% in recognising women likely to develop PE requiring delivery within 14 days. On the other hand, the PROGNOSIS studies results suggest that Elecsys sFlt-1/PlGF ratio can successfully rule out PE within a week of testing in 99% of the patients. However, the review does state that there would be clinical benefit in using either PlGF or sFlt-1/PlGF ratio in addition to current clinical assessment for women between 20-37 weeks of gestation suspected to have PE.

3.5 sFlt-1/PLGF

It is believed, that patients with PE have a high sFlt-1/PlGF ratio which can be detected before the onset of the disease (10). Levine et al (30) found the serum concentration of sFlt-1 to be elevated five
weeks before the development of clinical symptoms of PE. Meanwhile, the PlGF concentrations were reduced thus the ratio of sFlt-1/PlGF ratio was increased. Therefore, an elevated level of sFlt-1/PlGF ratio can diagnosis PE while, a low sFlt-1/PlGF ratio can be used to rule out PE, within a week (30).

Cut off values for sFlt-1/PlGF ratio can indicate which patients will develop PE, and which will not. It is important to establish appropriate cut-off values which can either diagnose PE or rule it out. A review conducted by Herraiz et al (31) summarised the current evidence surrounding sFlt-1/PlGF ratio cut-off values. After analysing the PROGNOSIS study Herraiz and colleagues concluded that using a sFlt-1/PlGF cut off value of ≤ 38 between 24-36 weeks gestation, to rule out PE within 1 week had a negative predictive value of 99% and a value >38 to predict PE developing 4 weeks from the assessment had a negative predictive value of 95%. Herraiz and his colleagues also found that using a cut-off >85 for early onset PE and >110 for late onset PE has a high specificity of 99.5% and 95.5% respectively (31). On the other hand, a study carried out by Theng Theng Chuah et al (32) found the ideal cut off ratio for early onset PE was 32. This cut off value increased sensitivity to 95.8% and specificity to 100%. In contrast, this cut off value limited the diagnostic ability of sFlt-1/PlGF for late onset PE. The higher the sensitivity and specificity of sFlt-1/PlGF ratio the better it will diagnose PE (32). Research conducted by Stefan Verlohren et al (33) found that the cut-off value of 85 for sFlt-1/PlGF ratio for early onset PE had a sensitivity of 89% and specificity of 97%. However, when the same cut-off value of 85 was used for late onset PE the sensitivity was just 74% while the specificity was 89%. Hence, the cut-off values should change according to the subset of PE that is to be diagnosed (33).

Research suggests that sFlt-1/PlGF ratio is a better diagnostic marker than sFlt-1 and PlGF individually. Data collected by Stefan Verlohren et al (33) during a study investigating the use of Elecsys in measuring sFlt-1/PlGF ratio showed that sFlt-1/PlGF ratio is better at diagnosing early onset PE, late PE and all PE cases. By calculating the area under the receiver operating characteristic curve, the study compared the diagnostic capability of sFlt-1, PlGF and sFlt-1/PlGF ratio. The sFlt-1/PlGF ratio had the highest AUC when used to diagnose all patient sub groups. However, the most sensitive and specific diagnosis was for patients in the early onset PE subgroup with an AUC of 0.97 (33).

A meta-analysis conducted by Y.Liu et al (34) reviewed various studies that investigated the diagnostic accuracy of sFlt-1/PlGF ratio. The review also concluded that sFlt-1/PlGF ratio has a higher diagnostic efficiency in early onset PE than with normal PE or late onset PE. With a positive likelihood ratio of 15.00 compared to the desirable, which is 10.0 and, a negative likelihood ratio of 0.06 where the standard is 0.1 (34).
It is evident, that sFlt-1/PlGF ratio is a useful diagnostic tool for diagnosing PE. It would be even more beneficial if sFlt-1/PlGF could indicate whether women were going to develop severe PE. Some research has been done to investigate whether sFlt-1/PlGF can be used to diagnose sPE. A study conducted by Rana et al (14) evaluated if sFlt-1/PlGF can accurately diagnose sPE, also known as PE with adverse outcomes, in gestational women. The results of the research found a sFlt-1/PlGF ratio > 85 was associated with sPE that required imminent delivery within two weeks of presentation. The data from the study also showed that sFlt-1/PlGF ratio had the highest predictive value over all the other biomarkers for women less than 34 weeks gestation (14).
4. RESEARCH METHODOLOGY AND METHODS

The research was conducted in the Obstetrics and Gynaecology department in the Lithuanian Health Sciences University and is a retrospective cohort study.

Before collecting and recording the data, ethical approval was granted by the Bioethics department in the Lithuanian University of Health Sciences. Following the approval from the bioethics department we searched in Lithuanian University of Health Sciences hospital archives, for patients that were diagnosed with severe preterm pre-eclampsia in the Obstetrics and Gynaecology department, from 2017 till 2018. The patients were diagnosed with severe preterm pre-eclampsia on the basis of any of the following: blood pressure ≥160/100 mm Hg, elevated liver enzymes (AST, ALT, ALP), low platelet count, elevated lactate dehydrogenase and proteinuria>5g. We excluded cases that had fetal death, twin pregnancy or immediate delivery (no 24-hour urine protein test). From the search 55 cases matched the criteria required however, only 47 cases were available in archives. These cases were matched with 26 cases of severe pre-eclampsia which was diagnosed in 2010-2013 and had serum fms-like tyrosine kinase 1 and placental growth factor values recorded. The 26 cases with serum fms-like tyrosine kinase 1 and placental growth factor values were collected prospectively by Dr. V. Tarasevičienė and where then analysed retrospectively.

From the patient’s cases data regarding diagnosis, complications, blood pressure levels, laboratory findings, ultrasonographic findings delivery and neonatal outcomes was obtained. From the patient’s history values for certain variables were acquired: the actual fetal weight; the neonates APGAR score; blood pressure. In addition, from the history gravidity, weeks of gestation upon delivery were found and recorded. Data from the blood tests (full blood count, liver enzymes, haptoglobin levels, LDH level, PH) were found in the cases and noted. Biochemical tests such as, 24-hour urine protein test showed values of proteinuria in each patient and was recorded. Symptoms such as: headache, vomiting, nausea, visual disturbances, pain in right costal region were found in the patient’s complaints and recorded.

From the 26 cases that were collected from October 2010-January 2013, we recorded the values of the same variables we assessed in the 47 cases. In addition, we recorded the sFlt-1 and PIGF levels from the 26 cases.

Once the data was collected, all the recorded values were presented in a table on Microsoft Excel. For all of the patient cases, the calculations were performed using IBM® SPSS 25.0 and significance when p<0.05 was used.
Shapiro-Wilk test was used to find the distribution of the data. We suspected the non-normal distribution may have been due to outliers. In order to confirm the cause of the non-normal distribution in the data we analysed the data for outliers using histograms and boxplot graphs. We found outlier for: age, average DBP, PLT count, AST levels, ALT levels, LDH levels, proteinuria, fetal weight and PH. Hence, we concluded the non-normal distribution of the data is due to outliers. Therefore, we decided to use non parametric tests for our investigation, since they can be used for data that is normally distributed and data that is non normally distributed.

For the characteristics of the patients and laboratory findings the median and the range was used.

The non-parametric Mann-Whitney U test was used to find if there was any significant difference between the characteristics of the early preterm sPE patients and the late preterm sPE patients.

To perform the inferential statistics, the non-parametric nominal by interval Eta correlation was used on all of the cases in the study. Spearman’s correlation coefficient was used to find the association between sFlt-1/PlGF and all the variables except for blood pressure ≥160/100 mm Hg and fetal growth retardation. Nominal by interval Eta value was used to find the association between sFlt-1/PlGF and blood pressure ≥160/100 mm Hg and fetal growth retardation.

In order to find out, which variable out of all the cases had the strongest association with adverse fetal outcome, Spearman’s correlation coefficient and nominal by interval Eta correlation coefficient was used.
5. Results

5.1 Characteristics of patients

73 cases of preterm severe preeclampsia were analysed. The characteristics of the study population is shown in table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median(Range) (n=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(yrs)</td>
<td>29(19-44)</td>
</tr>
<tr>
<td>Gestational age upon delivery(wks)</td>
<td>33(25-36)</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1(1-10)</td>
</tr>
<tr>
<td>DBP(mmhg)</td>
<td>100(70.5-150.0)</td>
</tr>
<tr>
<td>SBP(mmhg)</td>
<td>160(130.5-250.0)</td>
</tr>
<tr>
<td>PLT</td>
<td>186(92-700)</td>
</tr>
<tr>
<td>AST</td>
<td>29(12-242)</td>
</tr>
<tr>
<td>ALT</td>
<td>25(2-181)</td>
</tr>
<tr>
<td>ALP</td>
<td>102(16-221)</td>
</tr>
<tr>
<td>LDH</td>
<td>255(4.1-514.)</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>593(46-2905)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>2.91(0.1-14.94)</td>
</tr>
<tr>
<td>Actual fetal weight(g)</td>
<td>1770(495-3630)</td>
</tr>
</tbody>
</table>

From the sample, 50% of the mothers (n=37) were primiparous. In the study group 75.7% of patients (n=56) had blood pressure $\geq 160/100$mmHg. 24 hour proteinuria of 5g and more appeared in 29.7% (n=22). There were 17 cases of elevated ALT (23.3%), 22 cases of elevated AST (30.1%) and 36 cases of elevated ALP (49.3%). When considering other symptoms of severe preeclampsia, 36.99% (n=27) patients had headache, 5.48% (n=4) had nausea and the same percentage vomited, 5.48% (n=4). The proportion of patients that complained to have suffered visual disturbances was 2.74% (n=2), 1.37% (n=1) reported to have suffered from pain in right costal region and less urination, respectively. The rate of patients that had primary hypertension was 19.2% (n=14) while, 80.8% (n=59) did not have primary hypertension. 32.4% (n=24) of patients had a PLT count less than 150 (thrombocytopenia).
Out of the sample 72.5% (n=53) were late preterm pregnancies (31 to 36 weeks of gestation) the other 27.5% (n=20) were early preterm pregnancies (25-30 weeks of gestation).

### Table 1.2: Characteristics for early and late preterm patients.

<table>
<thead>
<tr>
<th></th>
<th>Early preterm sPE (25-30wks of gestation) N=20</th>
<th>Late preterm sPE (31-36 wks of gestation) N=53</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>28.5(24-44)</td>
<td>30(19-41)</td>
<td>0.906</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>162(142-200)</td>
<td>160(130.5-250)</td>
<td>0.317</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>100(85-130)</td>
<td>100(70.5-150)</td>
<td>0.78</td>
</tr>
<tr>
<td>Thrombocytes (nx10^9/l)</td>
<td>186(94-402)</td>
<td>185(92-700)</td>
<td>0.833</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>26.5(13-94)</td>
<td>29(12-242)</td>
<td>0.569</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>23.5(5-168)</td>
<td>26(2-181)</td>
<td>0.678</td>
</tr>
<tr>
<td>LDH</td>
<td>255.5(132-514)</td>
<td>255(4.1-514)</td>
<td>0.706</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>524(134-1368)</td>
<td>620(46-2905)</td>
<td>0.485</td>
</tr>
<tr>
<td>24 h proteinuria</td>
<td>1.65(0.14-13.46)</td>
<td>3(0.1-14.94)</td>
<td>0.383</td>
</tr>
<tr>
<td>Actual fetal weight(g)</td>
<td>1060.5(495-1770)</td>
<td>1920(1160-3630)</td>
<td>0.00</td>
</tr>
</tbody>
</table>
5.2 Characteristics of the patients in the group where sFlt-1 and PlGF were collected vs other group

Table 2.1: Characteristics of patients with sFlt-1/PlGF measurements (26) and without (47)

<table>
<thead>
<tr>
<th></th>
<th>26 cases</th>
<th>47 cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>25-30 weeks</td>
<td>6</td>
<td>22.2</td>
</tr>
<tr>
<td>31-36 weeks</td>
<td>20</td>
<td>74</td>
</tr>
<tr>
<td>BP≥ 160/100</td>
<td>22</td>
<td>81.5</td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>5</td>
<td>18.5</td>
</tr>
<tr>
<td>Elevated AST</td>
<td>9</td>
<td>33.30</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>9</td>
<td>33.3</td>
</tr>
<tr>
<td>Proteinuria&gt;5g(24hrs)</td>
<td>4</td>
<td>14.8</td>
</tr>
<tr>
<td>Headache</td>
<td>11</td>
<td>40.7</td>
</tr>
</tbody>
</table>

Table 2.1 displays the difference in characteristics between the 26 cases with recorded measurements of sFlt-1 and PlGF and the 47 cases that lacked values of sFlt-1 and PlGF.

Out of 26 cases with sFlt-1 and PlGF 88,5% (n=23) had sFlt-1/PlGF ratio more than 85, which according to the literature, confirms severe preeclampsia diagnosis. The mean of sFlt-1 was found to be 15827.46 while the range was from 6598 till 34746. For PlGF the mean was 61.88 and the range was from 15.94 till 294.8. Meanwhile, for sFlt-1/PIGF the mean was 375.34 and the range was from 59.9 till 1387.61.

We checked the correlation of sFlt-1/PlGF ratio with these variables: average SBP, average DBP, PLT count, proteinuria, AST, ALT, ALP, actual fetal weight, fetal growth retardation and blood pressure ≥160/100. We found that only PLT count and actual fetal weight had statistically significant correlation to sFlt-1/PlGF ratio, with significance (p) of 0.049 and 0.03 respectively.

When trying to find out whether any of the symptoms of severe preeclampsia (average SBP, average DBP, AST, ALT, proteinuria, PLT count and blood pressure ≥160/100 mm Hg) could have relationship with adverse neonatal effects (fetal growth retardation, Apgar score less than 7 at 1 and 5 minutes), we found that only blood pressure ≥160/100 mmHg was significantly associated with fetal growth restriction. None of other symptoms and laboratory tests correlated with fetal hypoxia.


6 Discussion

Preeclampsia is still a very miscellaneous disease. There are many different criterions for the diagnosis of PE, due to this many different laboratory tests have to be performed. As our patient case histories showed, other than blood pressure measurements, proteinuria during 24 hours, liver enzymes, CBC, haptoglobin and LDH should be investigated. Performing multiple investigations like this is not economically feasible and also costs time and resources. In our study, we found that the most common criterion for the diagnosis of severe preeclampsia in preterm pregnancies, was blood pressure > 160/100mmHg, which 75.7 % of patients suffered from. However, if we only paid attention to the blood pressure, 24.3% of severe preeclampsia cases, would have been missed. Proteinuria of 5g and more appeared in only 29.7% of cases. According to literature, up to 10% of preeclampsia cases and 20% of eclampsia cases can have no proteinuria at all (35). Similarly, patients could have other diseases, such as primary hypertension, which could result in them having elevated blood pressure of more than 160/100mmHg. In such cases, it is even more difficult to diagnose severe preeclampsia. In sPE, we know that the treatment is delivery of the fetus and placenta. In cases of preterm pregnancies, the induction of labour would significantly influence the future life of the baby, and this is a decision that should be made very carefully.

Furthermore, when a patient has kidney disease or systemic disease, proteinuria can be present beside gestational hypertension which could also distort the results and thus, patients could end up being wrongly diagnosed with sPE. Some studies have shown, that the amount of proteinuria when severe preeclampsia is present, is correlated with adverse fetal outcomes (35).

In our study we tried to compare early preterm (26-30wks of gestation) severe preeclampsia group with late preterm (31-36wks of gestation) severe preeclampsia group in order to see, if there were any differences of blood pressures, liver enzymes, haptoglobin, LDH, platelets, 24 hour proteinuria and fetal weight between the groups. Since, it is known, that the earlier preeclampsia starts, the more severe it is. But we found no significant differences for the parameters we calculated, except for the weight of the newborns, which is expected because the groups were divided by gestational age. These findings resonate with a study that was conducted by Johannes Stubert et al (36). Stubert and his colleagues performed a study, to find clinical differences between early onset and late onset sPE. When they compared values of blood pressure, proteinuria and liver enzymes between early onset and late onset sPE patients, they found no difference in the values (36). But the difference in neonatal outcomes were significant. According to the literature, current assessment of preeclampsia with blood
pressure and proteinuria had reported a positive predictive value of only 20% in detecting preeclampsia related adverse neonatal outcomes (37).

During the last decade, the role of angiogenic factors, such as sFlt-1 and PI GF has been widely discussed and in many hospitals across the world they are implemented in clinical practice. It is known that a sFlt-1/PI GF ratio of more than 85 can diagnose preterm preeclampsia with the specificity of 99.5% (31). A study conducted by Duckworth et al (27) found using PI GF testing as part of the diagnosis of sPE made a saving of £592 per women tested and 1000 women were tested in the study (27). In this way using angiogenic tests to diagnose sPE can save money, resources and time.

Unfortunately, we only had this ratio for 26 cases. A small number of cases is the limitation of this study. We found the ratio of sFlt-1/PI GF less than 85 only in 3 cases. For these 3 cases the diagnosis of preeclampsia was established without considering the values of angiogenic factors. It is possible that in these 3 cases the high blood pressure (>160/100) was due to primary hypertension rather than severe preeclampsia.

Since sFlt-1/PI GF ratio is still not implemented in clinical practice in LUHS, we wanted to find out which criterion of preeclampsia had the strongest correlation with sFlt-1/PI GF ratio. The strongest correlation to sFlt-1/PI GF happened to have fetal weight. Platelet count had a weak correlation. As the number of study patients was rather small, it could happen that if we tried to reproduce this correlation with a bigger group of severe preterm preeclampsia patients, the significance of correlation could disappear. Furthermore, when we investigated which variable out of: actual fetal weight, fetal growth retardation, Apgar score<7 at 1 and 5 minutes had the strongest association with the same parameters we tested on sFlt-1/PI GF ratio, we found that the strongest relation was between fetal growth retardation and blood pressure ≥160/100. Therefore, these findings suggest that the higher the blood pressure, the bigger chance to have a growth restriction of the fetus and all complications that are associated with this condition. From our data we can make a conclusion, that estimated fetal weight is not always the same as actual weight of the newborn. So as fetal growth restriction is associated with high blood pressure, and as actual fetal weight has correlation with sFlt-1/PI GF ratio, it would be important to have the values of the angiogenic factors mentioned above in cases of severe preterm preeclampsia for better decision making of the timing of delivery.
7. CONCLUSIONS

The results from our study showed that:

1. The most common criterion for preterm severe preeclampsia was blood pressure >160/100mmHg.
2. Angiogenic factors (sFlt-1/PIGF ratio) are reliable tools for diagnosing severe preterm preeclampsia.
3. The significant correlation with sFlt-1/PIGF ratio was found to be fetal weight and platelet count.
8. PRACTICAL RECOMMENDATIONS

As it is known that preeclampsia is a miscellaneous disease, sFlt-1/PIGF ratio could help diagnosing severe forms of the disease and therefore we suggest implementing it in daily clinical practice.
LITERATURE LIST


32. Chuah TT, Shi TW, Jack NM et al. Serum sFlt-1/PIGF ratio has better diagnostic ability in early- compared to late-onset pre-eclampsia. J Perinat Med. 2018;47:35.


