SF1t1/PlGF ratio and risk of preeclampsia

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Abstract

Objective: To determine the diagnostic performance of sFlt1/PIGF ratio in predicting the risk of developing severe PE or associated complications in two different populations in late pregnancy: 1) low risk pregnant women past 20 weeks gestation without symptoms or signs of preeclampsia (PE), 2) pregnant women with subjective symptoms or signs associated with PE in the obstetric triage area.

Method: Two focused questions were formulated using the PIRO Acronym (population, index test, reference standard and outcome). Based on these a systematic literature search was conducted in the Pubmed and Embase databases. Data was collected based on pre-defined inclusion and exclusion criteria as well as an evaluation of the level of evidence in the article.

Results: Six articles were selected for evaluating the diagnostic performance of sFlt1/PIGF ratio in predicting the risk of developing severe PE or associated complications. PIRO 1, low risk women: Positive predictive value (PPV) was 4-8%, negative predictive value (NPV) was 99%. PIRO 2, women with symptoms or signs of PE: PPV was 71-86% whereas NPV was 87-96%

Conclusion: This literature study showed that the sFlt1/PIGF ratio has the potential to predict the risk of severe PE and complications in women with subjective symptoms or signs of PE. Among low risk pregnant women, the test has a limited utility to “rule-in”, but may be used to “rule-out” PE, reducing the apriori risk of approximately 5% to 1%. The use of the sFlt1/PIGF ratio could potentially reduce maternal and perinatal morbidity and mortality, and prevent over-diagnosis and over-treatment of women with suspected PE.
Danish summary

Formål: Formålet med dette litteratur studie er, at vurdere den diagnostiske værdi af sFlt1/PIGF i 2 forskellige populationer i graviditeten: 1) lavrisiko gravide efter 20. uge, uden tegn eller symptomer på PE, 2) Gravide med symptomer eller tegn på PE, der møder op til en obstetrisk vurdering.

Metode: Jeg formulerede to fokuserede spørgsmål efter PIRO akronym (population, index test, reference standard og outcome), og foretog derefter en systematisk litteratursøgning i Embase og Pubmed databaserne. Passende litteratur blev udvalgt på baggrund af forud definerede inklusions- og eksklusionskriterier samt ud fra en vurdering af evidensniveaet i artiklerne.

Resultat: Seks artikler blev udvalgt og gennemgået med henblik på sFlt1/PIGF ratioens evne til at forudsige, hvilke kvinder der var i høj risiko for at udvikle svær PE eller komplikationer associeret hertil. PIRO 1, lavrisiko gravide efter 20. uge: Positiv prædiktiv værdi (PPV) var 4-8%, negativ prædiktiv værdi (NPV) 99%. PIRO 2, kvinder med symptomer eller tegn på PE: PPV var 71-86% og NPV var 87-96%.

Konklusion: Dette litteraturstudie viste, at sFlt1/PIGF har potentialet til at forudsige om en gravid med symptomer eller tegn på PE er i høj eller lav risiko for at udvikle komplikationer til svær PE. Blandt lavrisiko kvinder uden tegn eller symptomer på PE, er ratioen kun brugbar til at udelukke svær PE, idet apriori risikoen kan nedsættes fra ca. 5% til 1% ved en negativ test. Brug af sFlt1/PIGF ratioen i praksis kan potentielt nedsætte maternel og neonatal morbiditet og mortalitet, samt nedbringe forekomsten af overdiagnosticerede og overbehandlede kvinder.
Aim of the study
The aim of this literature study is to evaluate the performance of the sFlt1/PlGF ratio to predict severe PE or the complications associated with PE, in two different populations in late pregnancy:

- Low risk pregnant women with no subjective symptoms or signs of PE
- Pregnant women presenting with unspecific subjective symptoms, isolated proteinuria or elevated blood pressure.

Introduction
Preeclampsia (PE) is a pregnancy-specific syndrome that affects about 2-7% of healthy nulliparous pregnant women(1). This multi-organ disease, commonly defined by the new onset of hypertension and proteinuria after 20 weeks gestation(2), is a leading cause of maternal mortality, accounting for 16-20% of maternal death in industrialized and developing countries respectively (3). In addition, PE is associated with a substantial maternal and fetal morbidity and represents a considerable healthcare resource burden (3).

The methods for diagnosing PE have not changed in the last 20-30 years (4). The diagnosis relies on the measurement of elevated blood pressure and proteinuria; however these methods have a limited value in predicting PE since they are neither particularly sensitive nor specific(5, 6). A number of studies have claimed that the sFlt1/PlGF ratio would be of great value in diagnosing and predicting the subsequent development of PE (4, 7, 8).

Pathophysiology of preeclampsia
The exact pathophysiology of preeclampsia remains unknown, but it is general opinion that it is caused by the placenta or the maternal response to placentation (1). Impaired cytotrophoblastic invasion of the spiral arteries and endothelial cell dysfunction are the two key features in the pathogenesis of PE (9). There are generally two theories dominating:

*Increased syncytiotrophoblast shedding*

Shedding of syncytiotrophoblasts is increased in PE compared to normal pregnancies(1). This is hypothesized to be a consequence of impaired trophoblastic invasion into the maternal decidua which, in part, leads to impaired transformation of the spiral arteries. The spiral arteries remain narrow affecting the placental perfusion and leads to periodically hypoxia, which triggers oxidative stress and apoptosis of the syncytiotrophoblast. As a consequence, the shedding of the microvillus particles of the syncytiotrophoblast is increased into the maternal circulation(4). This leads to a
raised concentration of free fetal DNA in the maternal blood(1), which is thought to trigger an exaggerated inflammatory response and systemic endothelial cell dysfunction(4).

**Immune maladaptation theory**

This theory states that an inappropriate maternal immunological response to the paternal antigen presented on the trophoblast, leads to impaired remodeling processes in the spiral arteries and endothelial dysfunction, caused by cytokines and other substances secreted by the immunological cells(1).

It is possible that the mechanisms might interact, and that the maternal-fetal immune maladaptation leads to superficial placentation with the subsequent increased shedding of syncytiotrophoblast initiating an exaggerated inflammatory response(1).

**Sflt1 and PlGF**

The angiogenic factors, Placental Growth Factor (PIGF) and Vascular Endothelium Growth Factor (VEGF) are important for the functioning of endothelial cells and the development of the placenta (10). They are released into the maternal blood stream by migrating trophoblasts and play a key role in the normal placental vascular development, regulation of normal trophoblast functioning, growth and differentiation and the stabilization of maternal endothelial cells (11). In addition, VEGF induces nitric oxide and vasodilatory prostacyclins in endothelial cells leading to a decrease in vascular resistance and blood pressure(11).

VEGF and PlGF exert their biological effects through high-affinity tyrosine kinase receptors (VEGFR-1 and 2). The former receptor has two isoforms, a transmembranous and a soluble form (soluble fms-like tyrosine kinase-1, sFlt1). The soluble receptor is a splice variant and lacks the signaling tyrosine kinase domain but contains the extracellular ligand-binding domain; thus sFlt1 is able to bind the VEGF and PlGF and prevent them from exerting their angiogenic effects (Figure 1)(10).

Numerous studies have demonstrated elevated levels of sFlt1 and decreased levels of PlGF in women with PE compared to normal pregnancies(4). In addition, it was found that an injection of sFlt1 to pregnant rats induced hypertension, proteinuria and glomerular endotheliosis, important characteristics of PE, and could be reversed by administering a VEGF isoform(12). These findings suggest that sFlt1 and PlGF may play a causative role in the endothelial dysfunction seen in PE.
In Preeclampsia the level of sFlt1 is elevated leading to a reduced level of free PlGF and VEGF. This reduces the activation of the transmembranous VEGF-receptor. Thus, sFlt1 antagonizes the biological effect of VEGF and PlGF.

**Figure 1**

<table>
<thead>
<tr>
<th>sFlt1</th>
<th>Free PlGF and VEGF</th>
<th>Receptor level (the bioactive part)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Stabilization of maternal endothelial cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Induction of NO and Vasodilatory prostacyclins in endothelial cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Normal placental development</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Regulation of normal trophoblast functioning, growth and differentiation</td>
</tr>
</tbody>
</table>

VEGFR = Vascular Endothelial Growth Factor Receptor; sFlt1 = soluble fms-like tyrosine kinase-1; PlGF = Placental Growth Factor; VEGF = Vascular Endothelial Growth Factor; NO = Nitric oxide

**Method**

**PIRO**

Based on the objective of this paper, two focused questions were formulated to specify the population, the diagnostic test and the outcome of interest before conducting the literature search. This was done using the Acronym PIRO (population, index test, reference standard, outcome) (13). The two focused questions are shown in table 1.
### Table 1 PIRO 1 and PIRO 2

<table>
<thead>
<tr>
<th>Focused question</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIRO 1: Can the measurement of sFlt1/PIGF ratio in low risk pregnant women after 20 weeks gestation predict the development of severe PE?</td>
<td>Low risk* pregnant women without symptoms or signs of PE **.</td>
</tr>
<tr>
<td>PIRO 2: Can the sFlt1/PIGF ratio predict adverse maternal or neonatal outcomes in women with unspecific subjective symptoms of PE or isolated proteinuria or elevated BP after 20 weeks gestation?</td>
<td>Women &gt;20 weeks gestation presenting to the obstetrical triage area with symptoms of PE*** or isolated proteinuria or BP &gt;140/90.</td>
</tr>
</tbody>
</table>

**Outcome**
1. Severe PE (according to DSOG)
2. Diagnostic performance of sFlt1/PIGF ratio

**Index test**
- sFlt1/PIGF ratio or PIGF/sFlt1 ratio

Differs between studies

**Reference standard**

PE = Preeclampsia; BP = Blood pressure; DSOG = Dansk Selskab for Obstetrik og Gynækologi  
*No major risk factors for PE (history of PE, multi-gestational pregnancy, essential hypertension, gestational diabetes mellitus, antiphospholipid antibodies (14)  
**No subjective symptoms (headache, epigastric pain, visual disturbances, nausea/vomiting), elevated blood pressure or proteinuria.  
***Headache, epigastric pain, nausea/vomiting, edema, vision changes  
Neonatal complications: Early preterm delivery (<34 weeks), fetal or neonatal death (15).

### Population

The population of interest in PIRO 1 is low risk pregnant women after 20 weeks gestation. The population in PIRO 2 are high risk women with subjective symptoms of PE, elevated blood pressure or proteinuria, but not yet diagnosed with PE.

### Index test

It is well established, that the sFlt1/PIGF ratio has the best diagnostic performance compared to sFlt1 or PIGF alone (4, 16-19). Thus sFlt1/PIGF (or PIGF/sFlt1) ratio is the index test of this study.
**Reference standard**

This represents the values of the sFlt1/PIGF ratio in the unaffected pregnancies. This differs between the various studies, since they use different cut-offs.

**Outcome**

The outcome of the questions has two dimensions: first, the outcome of the test, meaning severe PE and composite maternal and neonatal outcomes in PIRO 1 and 2 respectively. And second, the diagnostic performance of the test which, in this context, is considered the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Area under the ROC curve (AUC). A description of these metrics can be seen in the supporting data.

**Literature search**

To identify all articles reporting on sFlt1/PIGF ratio as a possible predictor of PE in late pregnancy, a systematic electronic search was performed on 5/8 2015. The search strategy was made with assistance from a search specialist (20).

Relevant studies were identified by searching PubMed and Embase databases. In PubMed both a Mesh-word search and a free-text search was conducted. The search strings are shown in the supporting data.

The quality of the information retrieval was tested by making sure relevant references found in reviews and core articles were included in the result of the electronic search.

**Inclusion- and exclusion of data**

In the first step of selecting relevant literature, initial inclusions and exclusions were made based on title and abstract. Inclusion criteria were: 1) Articles examining sFlt1 or sFlt1/PIGF ratio as predictor for PE in pregnant women. 2) Blood sample must have been taken after 20 weeks gestation. 3) The article must have severe PE or associated complications as endpoint. 4) The article must explain the definition of PE used in the study.

Exclusion criteria were: 1) Studies with a selected population based on a specific group of patients, e.g. patients with systemic lupus erythematosus (SLE), kidney disease or any other chronic disease. 2) Articles other than primary articles. 3) Articles without an abstract or full text available. 4) Articles written in a language other than English.
Repeated articles in the three search algorithms were excluded, so that one article was only included once.

**Final selection of literature**

After the initial inclusion, articles with the aim to determine an association between the biomarkers and PE, but with no metrics on diagnostic performance, were excluded.

Three of the fourteen remaining articles matched PIRO 1. All three were included. Five articles matched PIRO 2. Of these, three used a prospective cohort design, one used a retrospective cohort design and one used a case-control design. As pre-decided, no more than six articles would be included in this study, the five articles were narrowed down to three based on the level of evidence, and the two non-prospective studies were excluded by design.

**Definitions of Preeclampsia**

The common definition of preeclampsia is hypertension and proteinuria occurring after 20 weeks gestation in previously normotensive women (2). Hypertension is defined as a blood pressure ≥ 140 mmHg systolic or 90 mmHg diastolic and proteinuria is defined as an excretion of 0.3 g/24 hour or ≥ 1+ using a urine dipstick (2).

This definition of PE is often suggested as a “research definition” as it is more specific at recruiting true preeclamptics into scientific studies (21). But a less restrictive definition is often used in the clinical context, with de novo hypertension after 20 weeks gestation and one or more findings associated with PE (Proteinuria, renal insufficiency, liver disease, neurological problems, hematological disturbances or fetal growth restriction) (21). This means that diagnosis of PE practically does not require the presence of high levels of protein in the urine, which leads to a higher sensitivity. This wider definition is recently adopted by the American College of Obstetricians and Gynecologists (ACOG)(22) and the Society of Obstetricians and Gynecologists of Canada (SOGC)(23).

PE can be defined as severe if one or more severe complications or findings occur. Definitions according to different associations are shown in table 2.

Despite some differences, the definition of severe PE is quite similar in the three different obstetrical associations. This means that comparisons can be made between studies that refer to different definitions.
In addition to the subdivision of PE in regards to severity, PE can also be classified according to when clinical signs or symptoms appear: “early onset” is defined as before 34 weeks gestation and “late onset” as 34-42 weeks gestation(4).

Table 2 Definitions of preeclampsia according to different associations.

<table>
<thead>
<tr>
<th>Definition of mild PE</th>
<th>ACOG (22)</th>
<th>SOGC(23)</th>
<th>DSOG(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP ≥140/90 after 20 weeks gestation AND</td>
<td>BP ≥140/90 after 20 weeks gestation AND</td>
<td>BP ≥140/90 after 20 weeks gestation AND proteinuria</td>
<td></td>
</tr>
<tr>
<td>- Proteinuria OR - Any features below</td>
<td>- Proteinuria OR - Any features below</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Definition of severe PE</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Any of the following:</td>
<td>BP ≥140/90 AND one or more of the following:</td>
<td>Any of the following:</td>
</tr>
<tr>
<td>BP ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic</td>
<td>BP ≥160mmHg systolic or ≥ 110 mmHg diastolic Proteinuria &gt; 3 g/day</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia (&lt;100,000/l)</td>
<td>Thrombocytopenia</td>
<td>Thrombocytopenia (&lt;100,000/l),</td>
</tr>
<tr>
<td>Elevated transaminases</td>
<td>Elevated transaminases Elevated transaminases or bilirubin</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine &gt; 1.1 mg/dl or doubling of serum creatinine</td>
<td>Elevated serum creatinine or uric acid Oliguria (&lt;400 ml/day), Urate &gt;45 mmol/l, Creatinine &gt;110 mmol/l,</td>
<td></td>
</tr>
<tr>
<td>Elevated WBC, elevated INR or aPTT Low plasma albumin</td>
<td>DIC Hemolysis or HELLP syndrome</td>
<td></td>
</tr>
<tr>
<td>Severe persistent RUQ or epigastric pain</td>
<td>RUQ or epigastric pain Epigastric pain,</td>
<td></td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>Vomiting</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>Headache</td>
<td></td>
</tr>
<tr>
<td>New onset visual disturbances</td>
<td>Visual symptoms</td>
<td>Vision changes</td>
</tr>
<tr>
<td>New onset cerebral disturbances</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>Chest pain/dyspnea SAT &lt;97% Dyspnea/ chest tightness</td>
<td></td>
</tr>
<tr>
<td>Abnormal FHR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IUGR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal Doppler</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PE = Preeclampsia; ACOG = American College of Obstetricians and Gynecologists; SOGC = Society of Obstetricians and Gynaecologists of Canada; DSOG = Dansk Selskab for Obstetrik og Gynækologi; SAT = saturation; WBC = white blood count; RUQ = right upper quadrant; FHR = fetal heart rate; IUGR = intrauterine growth retardation; DIC = Disseminated intravascular coagulation

Results

Figure 2 illustrates the identification of studies included. Six articles were selected, three for each PIRO.
**Figure 2** Flowchart illustrating the search process

**Pubmed, Mesh search**
- 136 articles
- 102 excluded based on inclusion/exclusion criteria*
- 34 articles
- 16 excluded (early pregnancy, reviews)
- 18 articles
- 13 excluded (No focus on clinical utility)
- 5 articles

**Pubmed, free-text search**
- 204 articles
- 172 excluded based on inclusion/exclusion criteria**
- 36 articles
- 16 articles repeated from Mesh search
- 20 articles
- 16 excluded (early pregnancy, reviews)
- 8 articles
- 12 excluded (early pregnancy, reviews)
- 5 articles
- 5 excluded (No focus of clinical utility)
- 3 articles

**Embase search**
- 331 articles
- 295 excluded based on inclusion/exclusion criteria***
- 36 articles
- 17 articles repeated from Pubmed Mesh-free-text search
- 19 articles
- 6 excluded (early pregnancy, reviews)
- 13 articles
- 7 excluded (No focus on clinical utility)
- 6 articles

---

**First exclusion:**

- *Mesh search, Pubmed:*
  - 20 Non-relevant outcome
  - 9 Non-relevant exposure
  - 9 Wrong biomarker
  - 11 Specific population with a certain illness (SLE, etc.)
  - 10 Only high risk population
  - 19 Biological mechanisms
  - 2 biomarker not measured in peripheral blood
  - 5 Analysis procedure
  - 16 Measurement of biomarker after diagnosis
  - 1 No abstract available

- **Free-text search, Pubmed**
  - 32 Non-relevant outcome
  - 21 Non-relevant exposure
  - 24 Wrong biomarker
  - 16 Specific population with a certain illness
  - 14 High risk only
  - 40 Biological mechanisms
  - 8 Biomarker not measured in peripheral blood
  - 4 Analysis procedure
  - 9 Measurement of biomarker after diagnosis
  - 2 Case reports
  - 2 No abstract available

- ***Embase search:***
  - 55 Non-relevant outcome
  - 30 Non-relevant exposure
  - 49 Wrong biomarker
  - 19 Specific population with a certain illness
  - 14 High risk only
  - 94 Biological mechanisms
  - 19 Biomarker not measured in peripheral blood
  - 4 Treatment of PE
  - 9 Analysis procedure, or cost benefit
sFlt1/PIGF predicting severe preeclampsia in low risk pregnant women, PIRO 1
Definitions of study population, index test and outcome in the three studies reporting on the performance of sFlt1/PIGF ratio to predict severe PE in low risk women past 20 weeks gestation, are shown in table 3.

**Table 3** Definition of population, index test and outcome in studies selected for PIRO 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Index test</th>
<th>Reference standard</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaiworapongsa et al. 2013 (24) (prospective cohort study)</td>
<td>Singleton pregnant women, who delivered &gt; 34 weeks gestation. No signs or symptoms of PE.</td>
<td>PlGF/sFlt1 &lt;0.3MoM</td>
<td>PlGF/sFlt1 &gt;0.3 MoM</td>
<td>Severe PE (definition according to ACOG)</td>
</tr>
<tr>
<td></td>
<td><strong>Exclusion:</strong> Preterm labor, PPROM, PE or impaired fetal growth at time of blood sample, known major fetal anomaly, fetal death, active vaginal bleeding, renal insufficiency, congestive heart disease, chronic respiratory insufficiency or active hepatitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Time of blood sample:</strong> 30-34 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engels et al. 2013(25) (Case-control study)</td>
<td>Singleton pregnant women &gt; 24 weeks gestation. No signs or symptoms of PE</td>
<td>sFlt1/PIGF ≥ 70</td>
<td>sFlt1/PIGF &lt;70</td>
<td>Severe PE (definition according to ACOG)</td>
</tr>
<tr>
<td></td>
<td><strong>Exclusion:</strong> APS, SLE or any other autoimmune disease, HIV, chronic hepatitis infection, chronic corticosteroid or NSAID use.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Time of blood sample:</strong> Median = 35 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest et al. 2013(26) (Nested case-control study)</td>
<td>Pregnant women &gt; 20 weeks gestation. No signs or symptoms of PE.</td>
<td>sFlt1/PIGF (no given cutoff)</td>
<td></td>
<td>Severe PE (definition according to SOGC)</td>
</tr>
<tr>
<td></td>
<td><strong>Exclusion:</strong> &lt;18 years old, chronic hepatic or renal disease, major fetal abnormalities, pregnancies ending in termination, miscarriage or fetal death before 24 weeks gestation. No information on whether multiple gestations were included in the study.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Time of blood sample:</strong> 20-32 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PE = Preeclampsia; PPROM = preterm prelabor rupture of membranes; APS = Antiphospholipid syndrome; SLE = systemic lupus erythematosus; ACOG = American College of Obstetricians and Gynecologists; SOGC = Society of Obstetricians and Gynaecologists of Canada
None of the three studies excluded women with history of PE, essential hypertension or gestational diabetes mellitus in their study population; however, these were accepted as low risk since they were not high risk populations and did not have symptoms or signs of PE.

Two of the studies (24, 25) used the diagnosis of severe PE according to ACOG. The last study (26) used the definition according to SOGC. These are in consistency with the definition used in Danish guidelines apart from a few differences (table 2).

**Findings**

The inclusion of low risk women in the three studies results in a low prevalence of severe PE ranging from 0.9-1.8% (24, 26). One study did not provide a true prevalence since this was a case-control study which made it artificially high (25).

The overall ability of the sFlt1/PlGF (or PlGF/sFlt1) ratio to discriminate who would develop severe PE and who would not among low risk women past 20 weeks gestation was measured by the area under the ROC curve in all three studies. AUC ranged from 0.75 in the study by Forest et al. (26), 0.84 in the one by Chaiworapongsya (24), to 0.99 in the study by Engels (25).

Specific cut-offs of the ratio were evaluated. The cut-offs used were different in the three studies. Chaiworapongsya et al used PlGF/sFlt1 <0.03 MoM which led to a sensitivity to predict severe PE of 74% and a specificity of 84% (24). Engels et al. used sFlt1/PlGF > 70 (raw value) which had a sensitivity of 100% and a specificity of 94% (25). Forest et al found, that at a fixed false positive rate (FPR) of 10%, equivalent to a specificity of 90%, the test had a sensitivity of 44% (26); however they did not specify which cut-off this was related to.

Since positive- and negative predictive values depend on the prevalence of the disease in the population, these were only calculated in two of the studies: In the prospective cohort study and

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1 MoM = multiple of the median, is a measure of an individual test compared to a median value obtained from unaffected pregnancies. This is used in prenatal screening when the tested analyte changes according to gestational age. Using values in MoM makes it comparable among groups without adjustment for gestational age at sampling [27].

the nested case-control study (24, 26).\(^2\) For PlGF/sFlt1 <0.03 MoM the PPV was 8% and NPV was 99% (24). PPV and NPV matching a cut-off with a FPR of 10% was 4% and 99.5% respectively (26).

Descriptions of the studies included for PIRO 1, including strengths and limitations, can be found in the supporting data.

The diagnostic performance of the sFlt1/PIGF (PIGF/sFlt1) ratio in PIRO 1 is summarized in table 4.

<table>
<thead>
<tr>
<th></th>
<th>Chaiworapongsa et al. (24)</th>
<th>Engels et al. (25)</th>
<th>Forest et al. (26)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size of population</strong></td>
<td>1269 women</td>
<td>296 women</td>
<td>449 women</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>Severe PE</td>
<td>Severe PE or HELLP</td>
<td>Severe PE</td>
</tr>
<tr>
<td><strong>Cut-off</strong></td>
<td>PIGF/sFlt1 &lt;0.3 MoM</td>
<td>sFlt1/PIGF ≥70</td>
<td>-</td>
</tr>
<tr>
<td><strong>Outcome prevalence</strong></td>
<td>1.8%</td>
<td>-</td>
<td>0.9%</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>74% (52-90)</td>
<td>100%</td>
<td>44% (32-56)</td>
</tr>
<tr>
<td><strong>specificity</strong></td>
<td>84% (81-86)</td>
<td>94%</td>
<td>90% (FPR at 10%)</td>
</tr>
<tr>
<td><strong>Positive predictive value</strong></td>
<td>8% (5-12)</td>
<td>-</td>
<td>4% (3-5)</td>
</tr>
<tr>
<td><strong>Negative predictive value</strong></td>
<td>99% (99-100)</td>
<td>-</td>
<td>99.5% (99.2-99.6)</td>
</tr>
<tr>
<td><strong>AUC (Area under the ROC curve)</strong></td>
<td>0.84</td>
<td>0.99</td>
<td>0.75 (0.66-0.83)</td>
</tr>
</tbody>
</table>

sFlt1/PIGF predicting adverse outcomes in women with suspected preeclampsia, PIRO2

The three studies reporting on the performance of sFlt1/PIGF ratio to predict adverse outcomes in women with signs or symptoms of PE are shown in table 5.

Both study populations and outcomes were relatively similar in the three studies.

---

\(^2\) Nested case-control study: Meaning that the case-control study is nested in a bigger, well-defined cohort (28. Juul Sf. Epidemiolog og evidens. 2. udgave ed. Kbh.: Munksgaard; 2012. 293 p.). In the nested case-control study by Forest et al (26), PPV and NPV were based on an adjustment of the prevalence of severe PE in the entire cohort.
Table 5: Definition of population, index test and outcome in studies selected for PIRO 2

<table>
<thead>
<tr>
<th>PIRO 2: Can the Sflt1/PIGF ratio predict adverse maternal or neonatal outcomes in women with unspecific subjective symptoms of PE or isolated proteinuria or elevated blood pressure after 20 weeks gestation?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Chaiworapongsa et al. 2014 (29)</strong> (Prospective cohort study)</td>
</tr>
<tr>
<td><strong>Exclusion:</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Rana et al. 2012 (19)</strong> (Prospective cohort study)</td>
</tr>
<tr>
<td><strong>Exclusion:</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Moore et al. 2013 (8)</strong> (Prospective cohort study)</td>
</tr>
<tr>
<td><strong>Exclusion:</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

GA = Gestational age; ICU = intensive care unit; RDS = respiratory distress syndrome; BPD = bronchopulmonary dysplasia; DIC = Disseminated intravascular coagulation; UADV = Umbilical artery Doppler velocity; *All 3 studies defined symptoms of PE as headache, vision changes, nausea/vomiting or epigastric/right upper quadrant pain. **Common definition of complications between all 3 studies (any of the following): Elevated transaminases, renal insufficiency, pulmonary edema, thrombocytopenia (<100,000/l), abruptio placentae, Seizure/Eclampsia, fetal/neonatal death.
Findings
The women in these populations were at high risk of developing complications associated to severe PE, which is illustrated by the prevalence of 30-45% of maternal or fatal/neonatal complications in the three studies\(^8\), \(^{19}\), \(^{29}\).

The sFlt1/PIGF ratio was significantly associated with the development of adverse outcomes in women with signs or symptoms of PE \(^8\), \(^{19}\), \(^{29}\). In two of the three studies \(^{19}\), \(^{29}\), the analysis was restricted to women presenting before 34 weeks, since this subgroup showed the strongest association between the angiogenic markers and development of complications. In the study performed by Moore et al, they did not find a significant association after 37 weeks gestation between the sFlt1/PIGF ratio and development of complications in women presenting with signs or symptoms of PE \(^8\). Therefor they limited their analysis to women presenting at or before 37 weeks gestation.

In the prospective cohort study by Rana et al.\(^{19}\) the overall ability of the sFlt1/PIGF to predict complications of PE in women with signs or symptoms of PE was measured as an AUC of 0.89. In the study by Moore\(^8\), the AUC of sFlt1/PIGF alone was 0.76, whereas a multivariable model composed of maternal characteristics, sFlt1 and PlGF had an AUC of 0.91.

Rana et al. determined a cut-off of sFlt1/PIGF ≥85, which yielded a sensitivity and specificity of 73% and 94% respectively\(^{19}\). Chaiworapongsar used PlGF/sFlt1 ≤ 0.035 MoM as cut-off which had a sensitivity of 92% and a specificity of 83\%\(^{29}\). Moore et al. did not specify a cut-off\(^8\).

PPV ranged from 71% to 86% and the NPV ranged from 96% to 87% \(^{19}\), \(^{29}\). Description of the studies included for PIRO 2, including strengths and limitations, can be found in the supporting data.

The diagnostic performance of sFlt1/PIGF (PIGF/sFlt1) ratio in PIRO 2 is summarized in table 6.
**Table 6** Diagnostic performance of sFlt1/PIGF (or PlGF/sFlt1) ratio to predict adverse outcomes, in women with signs or symptoms of PE.

<table>
<thead>
<tr>
<th></th>
<th>Chaiworapongsa et al. (29)</th>
<th>Rana et al. (19)</th>
<th>Moore et al. (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Size of population</strong></td>
<td>85 women</td>
<td>616 women</td>
<td>276 women</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>Maternal or neonatal complications within 2 weeks (&lt;34 weeks gestation)</td>
<td>Maternal or neonatal complications (&lt;34 weeks gestation)</td>
<td>Maternal or neonatal complications (&lt;37 weeks gestation)</td>
</tr>
<tr>
<td><strong>Cut-off</strong></td>
<td>PIGF/sFlt1 ≤ 0.035 MoM</td>
<td>sFlt1/PIGF ≥ 85</td>
<td>No specific cut-off</td>
</tr>
<tr>
<td><strong>Outcome prevalence</strong></td>
<td>30%</td>
<td>34%</td>
<td>45%</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>92%</td>
<td>73%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>83%</td>
<td>94%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Positive predictive value</strong></td>
<td>71%</td>
<td>86%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Negative predictive value</strong></td>
<td>96%</td>
<td>87%</td>
<td>-</td>
</tr>
<tr>
<td><strong>AUC</strong></td>
<td>-</td>
<td>0.89</td>
<td>sFlt1/PIGF alone: 0.76 (0.66-0.85) Clinical multivariate model including sFlt1/PIGF: 0.91 (0.85-0.97)</td>
</tr>
</tbody>
</table>

**Discussion**

**Main findings**

In a low risk population of pregnant women after 20 weeks gestation, the sFlt1/PIGF ratio was associated with the development of severe PE. The PPV of the test was 4-8%, whereas NPV was 99%.

The sFlt1/PIGF ratio also showed a significant association with the development of complications of PE in pregnant women with unspecific subjective symptoms or clinical signs of PE. The PPV in this population was 71-85%. The NPV was 87-96%.

**Strengths and limitations**

The strengths of this literature study are the systematically performed information retrieval and that the PIRO questions were formulated before the literature search was conducted. The studies had low risk of bias in general and the results were quite homogenous. This consistency indicates a true applicability of this test to predict severe PE and associated complications.
The limitations of this study include, that only three articles for each focused questions were evaluated. At the end of the literature retrieval, two articles were excluded because it was pre-decided that no more than six articles would be included. However these were excluded based on an assumption that they had a lower level of evidence than the rest, since they had a retrospective and case-control design. Another limitation is that the studies did not use the same cut-off, and the ratios were expressed in either raw values or MoM, making the results difficult to compare, and no cut-off is repeatedly evaluated better than another.

**Possible implementation of the sFlt1/PIGF ratio?**

The sFlt1/PIGF ratio could principally be used in two ways: To “rule-in” or “rule-out” PE. The low PPV (4-8%) found in this study limits the utility of the test to “rule-in” PE in low risk pregnant women. In patients presenting to the obstetrical triage area with symptoms or signs of PE, the test showed better PPV (71-85%), and has the potential to be an additional diagnostic tool in determining who is in high risk of developing severe complications of PE. However as this was only based on women before 34/37 weeks gestation, it is uncertain whether this can be applied to women after 37 weeks gestation.

An even more promising role in the obstetrical clinic is for the sFlt1/PIGF ratio to “rule-out” PE. In both high and low risk populations, the test showed a very high NPV (99% and 87-96% respectively) and would, therefore, lead to a low rate of false negatives.

No exact similar studies as those presented in this paper are made for blood pressure or hematological test, which are commonly used as predictors of adverse outcomes in the clinical setting. However one study (30) examined commonly used clinical variables in the prediction of maternal or neonatal complications associated with PE within 48 hours after admission to the obstetrical unit due to PE or suspicion of PE. AUC for systolic/diastolic blood pressure were 0.65/0.63, and AUC for platelet count and elevated alanine transaminase was 0.69 and 0.72 respectively (30). With AUC of the sFlt1/PIGF ratio of 0.89 in one study and 0.76 alone and 0.91 in combination with maternal characteristics in another (8, 19), the diagnostic performance of sFlt1/PIGF seems to be at least as good, if not better, at predicting adverse outcomes, than the variables currently used.
With the development of the automated method for the measurement of sFlt1 and PlGF and a total duration of the assay of only 18 minutes (18), it is now practicable to use these markers in the clinical context and not for experimental use only. The approximate cost of a test result is estimated to 500 Danish Kroner (31).

**Perspectives**

The sFlt1/PIGF ratio has the potential to help identify (rule-in) those in high risk of developing severe complications, among women with signs or symptoms of PE. However this might be of limited benefit. As there is no intervention in late pregnancy known to prevent PE and no good treatment but to deliver the placenta, earlier identification of a high risk of severe PE does not seem particularly beneficial. Nevertheless, early detection of severe PE leads to the possibility of more intensive surveillance in specialized hospitals, which could possibly allow fast prophylactic intervention against complications, with steroids for lung maturity, seizure prophylaxis and antihypertensive therapy (32). In addition, many studies are currently ongoing to find a cure or preventive therapy for PE. This includes extra-corporal removal of sFlt1 by apheresis (33) and administration of Pravastatin (34, 35). Both are hypothesized to reverse the angiogenic imbalance associated with preeclampsia and restore the endothelial functioning.

Finding a treatment or preventive therapy for PE, would make a predictive test even more beneficial.

Until a cure or preventive therapy is found, the sFlt1/PIGF ratio was shown to have a more promising role to “rule-out” severe PE. A test that can accurately rule-out PE is desirable especially in a high risk setting, as this has the potential to prevent over-diagnosis and over-treatment of women with suspected preeclampsia. A simple blood sample would be less invasive in the woman’s life, than hospitalization, frequent blood testing and evaluation of fetal wellbeing, which is often required in women with unspecific symptoms. In addition, it may allow more efficient allocation of the resources according to patient’s risk (3). In a cost analysis on the implementation of the plasma sFlt1/PIGF ratio in an obstetrical triage setting, Schnettler and colleagues (36) found a potential reduction of the average per-patient cost of 1,215 US Dollars.

It is unclear whether the sFlt1/PIGF ratio could have a role in ruling out the risk of severe PE in low risk women in late pregnancy. Some have stated that even though the sFlt1/PIGF ratio shows a
high NPV, this would not be useful, since even in the absence of any testing, the probability of a woman being healthy is very high (37). However, in a population with prevalence of PE of 5%, the apriori risk would be reduced from 5% to 1% if the test is negative.

One approach of using the sFlt1/PIGF ratio in the clinical context is suggested by Verlohren et al. (6). By using two gestational specific cut-offs, one with high sensitivity and the other with high specificity, the women can be stratified into three risk-zones according to their sFlt1/PIGF ratio: low risk, intermediate risk and high risk. This results in a very high diagnostic accuracy in zone one and three. In the study approximately 7% was placed in the intermediate zone, which would require retesting and monitoring (6). This approach takes into account that even though a cut-off is used to dichotomize (disease/no disease) a test with a numerical outcome, values just above or just below the threshold are more unreliable and should be observed carefully.

**Conclusion**

The sFlt1/PIGF ratio is associated with development of severe PE in low risk pregnant women after 20 weeks gestation, and with development of maternal or neonatal complications in women with signs or symptoms of PE. It has the potential to identify symptomatic women in high risk of developing severe complications, which could potentially reduce maternal and neonatal morbidity and mortality.

In both low risk pregnant women and in women with signs or symptoms of PE the sFlt1/PIGF ratio is able to accurately rule-out severe PE or associated complications. This could potentially reduce healthcare costs and prevent over-diagnosis and over-treatment of women with suspected PE.

Larger multicenter studies are needed to determine the best cut-offs before implementation to the clinical setting.
References


Niels Tørring. Department of Biochemistry. Aarhus University Hospital, Skejby


Supporting data

Contents

1. Performance of a diagnostic test
2. Search algorithms
3. Description of studies included for PIRO 1
4. Description of studies included for PIRO 2

1. Performance of a diagnostic test

The evaluation of a diagnostic performance of a test is commonly based on sensitivity, specificity, positive predictive value and negative predictive value. The Sensitivity refers to the test's ability to detect patients who has the disease, meaning the proportion with the disease that was correctly identified by the test. Specificity is the proportion of healthy individuals, who are correctly identified as such by the test (28).

Positive predictive value (PPV) and Negative predictive value (NPV) are of special interest in the clinical setting, compared to sensitivity and specificity. PPV is the probability of disease if the test is positive, and NPV is the probability of no disease if the test is negative (28). It is important to remember that PPV and NPV depend on the prevalence of the disease. Thus the same test will have different predictive values in high-risk and low risk populations, which should be kept in mind when applying the test to a certain population.

A common used statistic tool is the receiving operator characteristics (ROC) curve. This is a plot of sensitivity against 1-specificity for different numerical values of the test (38). This can be used to determine cut-offs, meaning turning a numerical variable into a binary classification (38), by choosing the cut-off with the best combination of sensitivity and specificity.

Area under the ROC curve (AUC) is also used to evaluate how well a continuous measure is able to discriminate between who will get the disease and who will not (38). Since the ROC curve is a plot of sensitivity against 1-specificity, an ideal test with 100% discrimination would have an area under the ROC curve of 1, meaning 100% sensitivity and 100% specificity. A test that is not able to discriminate at all would have an AUC of 0.5. Thus, the closer the AUC gets to 1 the better ability of the test to discriminate between who will get the outcome and who will not.
Figure S1 Test result and disease

<table>
<thead>
<tr>
<th>Test result</th>
<th>Disease</th>
<th>No disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test +</td>
<td>True positives (TP)</td>
<td>False positives (FP)</td>
</tr>
<tr>
<td>Test -</td>
<td>False negatives (FN)</td>
<td>True negatives (TN)</td>
</tr>
</tbody>
</table>

Definitions (28)

Sensitivity = \( \frac{TP}{TP+FN} \)

Specificity = \( \frac{TN}{TN+FP} \)

Positive predictive value (PPV) = \( \frac{TP}{TP+FP} \)

Negative predictive value (NPV) = \( \frac{TN}{TN+FN} \)

False positive rate (FPR) = \( \frac{FP}{FP+TN} \)

2. Search algorithms

**Pubmed, Mesh search**


Following filters were used: English language, full text available, only humans.

**Pubmed, Free-text search**

The search algorithm is composed of 3 pillars:
This was made up to the following search algorithm:

```
(((((((("Pre-Eclampsia"[Mesh]) OR pre-eclampsia) OR preeclampsia) OR "toxemia of pregnancy") OR "Pregnancy toxemia") OR "EPH Complex") AND (((((((("Vascular Endothelial Growth Factor Receptor-1"[Mesh]) OR "soluble fms-like tyrosine kinase 1") OR "soluble fms-like tyrosine kinase one") OR "s-flt-1") OR "sFlt1") OR "sFlt-1") OR "sVEGFR-1") OR "sVEGFR1") OR "soluble vascular endothelial growth factor receptor 1") OR "soluble vascular endothelial growth factor receptor one") AND ((((("Risk Assessment"[Mesh]) OR risk assessment) OR "risk assessment") OR "risk assessments") OR "Risk"[Mesh]) OR "Risk") OR "Risks") OR predictors) OR predicting) OR predictor)
```

Following filters were used: English language, full text available, only humans.

**Embase search**

#4: #3 AND ('clinical article'/de OR 'controlled study'/de OR 'human'/de OR 'major clinical study'/de) AND ('article'/it OR 'review'/it) AND ('endothelial dysfunction'/de OR 'hellp syndrome'/de OR 'hypertension'/de OR 'hypoxia'/de OR 'inflammation'/de OR 'maternal hypertension'/de OR 'maternal morbidity'/de OR 'placenta disorder'/de OR 'preeclampsia'/de OR 'pregnancy complication'/de OR 'proteinuria'/de) AND ([article]/lim OR [review]/lim) AND [female]/lim AND [humans]/lim AND [english]/lim AND [abstracts]/lim

#3: #1 AND #2

#2: ‘Vaculotropin receptor 1’/exp

#1: ‘Preeclampsia’/exp

### 3. Description of studies included for PIRO 1

**Chaiworapongsa et al. (24)**

In the prospective cohort study performed by Chaiworapongsa (24), 1269 singleton pregnant women had blood samples taken at 30-34 weeks gestation, and PI GF and sFlt1 ratio was measured by an ELISA immunoassay. A median PI GF/sFlt1 ratio concentration in relation to gestational age were measured among uncomplicated pregnancies (n= 886) and used to generate MoMs for each patient. The prevalence of severe PE in the study population was 23/1269 = 1.8%. A ROC curve was calculated, and used to determine a cutoff of PI GF/sFlt1 ratio < 0.3 MoM. The study found,
that women with plasma PI GF/sFlt1 ratio < 0.3 MoM were significantly more likely to experience severe PE than those with MoMs at or above the threshold (Odds ratio = 12.2 (95%CI 4.6-32.0)). The sensitivity of the test was found to be 74% and the specificity 84%. Positive predictive value was estimated to 8%, whereas negative predictive value was estimated to 99%.

Strengths and limitations

The strengths of this study are that a relatively large number of women are included, and that a prospective cohort study was used as design. In addition, MoM values are made which makes it comparable regardless of gestational age at the time of sampling. The laboratory personnel were blinded to clinical data, which leads to a low risk of bias in the interpretation of the test.

The Limitation of this study is few. Since this study wishes to compare the performance of PI GF/sFlt1 ratio in third trimester to that of the ratio and uterine artery Doppler Velocity (UADV) in first and second trimester, 1285 women were excluded based on missing plasma samples and UADV in the first two trimesters (the study consisted of several arms). This could potentially result in a selection bias. However the distribution of baseline characteristics did not differ significantly between included and excluded women, which reduce the risk of bias.

Two other limitations are present in this study as well as the rest of the studies presented, except for one (29). The cut-off is based on the study data, and therefore not replicated on an independent population. In addition blood samples were stored, which is not comparable with a clinical diagnostic setting. However sFlt1 and PI GF are said to be quite stable when stored at this low temperature ((29)), so this is not thought to affect the applicability.

Engels et al. (25)

Engels performs a case-control study with 64 cases of PE, of which 26 cases were severe PE or HELLP syndrome, in comparison to 232 healthy controls. All pregnancies included were after 24 weeks gestation. sFlt1 and PI GF were measured in serum samples on an automated immunoassay3. Blood samples were taken at the time of admission to the labor and delivery unit or at routine visits in the outpatients unit. This study found a significantly higher sFlt1/PI GF ratio in

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3 The automated assay is based on electrochemiluminescence technology, and the total duration of the assay is about 20 minutes, making it more applicable to the clinical setting, than the ELISA method (18. Verlohren S, Galindo A, Schlembach D, Zeisler H, Herraiz I, Moertl MG, et al. An automated method for the determination of the sFlt-1/PI GF ratio in the assessment of preeclampsia. American journal of obstetrics and gynecology. 2010;202(2):161.e1-.e11.)
severe PE than the controls (P<0.001). A ROC analysis was made and a sFlt1/PlGF cutoff of 70 was found to have the best diagnostic performance. AUC for sFlt1/PlGF was 0.99 for severe PE and 0.99 for HELLP syndrome. Sensitivity and specificity of sFlt1/PlGF ≥70 to diagnose severe PE/HELLP was 100% and 94% respectively. No confidence interval was specified. Since the study design was case-control, no positive- or negative predictive value was given, since these are dependent on the prevalence of the disease in the population, which is artificially high in a case-control study.

Strengths and limitations
This study uses the automated assay for analysis of sFlt1 and PlGF which is more applicable to the clinical setting than the regular ELISA method. In addition all cases of PE and healthy pregnancies, fulfilling the inclusion and exclusion criteria, were included in the study. Since no inappropriate exclusions were made there is a low risk of bias based on the selection of patients.

The limitations of this study were the case-control design, and that no information was given on whether laboratory personnel were blinded to clinical the status. However because of the character of the test, the risk of information bias caused by staffs being influenced on clinical status is not high in this case.

*Forest et al. (26)*
A nested case-control study based on a prospective cohort of 7929 pregnant women was performed by Forest et al. Of the 7929 women, 111 cases and 338 controls were matched by maternal age, gestational age, parity, smoking status and duration of sample storage. Blood samples were taken between 20-32 weeks gestation, and serum sFlt1 was measured by ELISA technique, while serum PlGF was measured on an automated immunoassay. Medians at different gestational ages were obtained from 50 randomly selected women with normal pregnancy outcome and measurements of sFlt1/PlGF were converted into MoM values. Based on a ROC analysis, the sensitivity of the ratio to predict severe PE at a fixed false positive rate of 10%, was found to be 44%. As the false positive rate is 1-specificity (38), the specificity was 90%. Positive and negative predictive values were estimated to 4% and 99.5% respectively, based on an adjustment of the prevalence of severe PE in the entire cohort.
Strengths and limitations

The strengths of this study are the use of MoM values, and that cases and controls were matched, which reduces the risk of selection bias. In addition, laboratory personnel were blinded to case-control status and clinical date, eliminating risk of bias in interpretation of the test.

Limitations were the use of a case-control design. However since it was a nested case-control study, meaning that it is based on a larger cohort, the PPV and NPV was still estimated by adjustment of the prevalence in the total cohort. No specific cut-off value correlated to the metrics on the diagnostic performance was given.

4. Description of studies included for PIRO 2

Chaiworapongsa et al (29)

Based on a previous retrospective cohort study, which found prognostic value of PlGF/sFlt1 in patients with suspected PE, Chaiworapongsa et al (29) performed a prospective cohort study to investigate whether this could be replicated on an independent population. They use the same cut-off as found earlier, PlGF/sFlt1 ≤ 0.05 MoM for the entire population and ≤0.035 for women presenting before 34 weeks gestation. 85 singleton pregnant women between 20-36 gestational weeks, presenting with subjective symptoms, elevated blood pressure or proteinuria, were included in the study. Maternal and neonatal complications within 2 weeks of presentation were recorded. Blood samples were collected on presentation to the triage unit and stored at -70°C until analyzed on an ELISA immunoassay. The concentrations were then turned into MoM based on previously determined gestational age-dependent expected medians.

Plasma concentrations of PlGF/sFlt1 ≤0.05 MoM were not significantly associated with composite maternal and neonatal morbidity in all women presenting to triage unit, when adjusted for potential confounders (OR = 2.8 (95% CI 0.9-8.8)). Restricting the test to women presenting before 34 weeks gestation, a PlGF/sFlt1 ratio ≤0.035 were significantly associated with composite maternal and neonatal complications within 2 weeks of presentation (OR = 19.5 (95% CI 2.5-150.1)).

The article does not present sensitivity, specificity or positive- or negative predictive values. But based on data in the article, it is possible to calculate these metrics. 43 women of the cohort presented to the hospital before 34 weeks gestation, and of these 13 developed maternal and/or
neonatal complications. 12 out of the 13 had a PI GF/sFlt1 ratio ≤0.035 MoM, which gives a sensitivity of the test of 92%. Of the remaining 30 women, 25 had a ratio >0.035 MoM, leading to a specificity of 83%. Negative- and positive predictive value are 71% and 96% respectively.

Calculations:

**Table S3** Classification by PI GF/sFlt1 ratio and development of complications

<table>
<thead>
<tr>
<th>Complications</th>
<th>No complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test positive</td>
<td>12</td>
</tr>
<tr>
<td>Test negative</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

Prevalence of composite maternal and neonatal complications = 13/43 = 30.23%

Sensitivity = 12/13 = 92.3%

Specificity = 25/30 = 83.3%

PPV = 12/17 = 70.6%

NPV = 25/26 = 96.2%

**Strengths and limitations**

The strengths of this study are the prospective design and the independent replication of previously findings, which suggests a high level of evidence. In addition, the use of MoM values makes the test result comparable among different gestational ages. Physicians were blinded to the test result and could not be influenced by this in the clinical decision making process.

The main limitation of the study is the small size of the population.

**Rana et al. (19)**

Rana et al performed a prospective cohort study with 616 women with singleton pregnancies, who presented to the obstetrical triage unit with symptoms of PE, elevated blood pressure or proteinuria. Blood samples were taken at presentation and stored at -80°C until analysis by an automated assay. Information on maternal or neonatal complications within 2 weeks since presentation, were then registered. They do not specify a lower limit for the gestational age to be included in the study. But since PE by definition does not occur before 20 weeks gestation, it is
reasonable to think that the women presenting to the triage unit with suspected PE, were beyond 20 weeks gestation.

The sFlt1/PlGF ratio at presentation was associated with development of adverse outcomes (P<0.001). When patients were divided into tertiles based on sFlt1/PlGF level (tertile 1: sFlt1/PlGF ≤9.7, tertile 2: sFlt1/PlGF >9.7 to <39.2, tertile 3: sFlt1/PlGF ≥39.2), the risk of adverse outcomes were elevated in women in the third tertile compared to those in the first tertile (OR = 9.5 (95 % CI 6.1-15.0)). In women presenting <34 weeks gestation, the association were even stronger with OR of 47.8 (95% CI 16.6-156.6).

In women presenting <34 weeks gestation, the sFlt1/PlGF ratio had superior performance of predicting subsequent adverse outcomes to other parameters measured at presentation, including blood pressure (p<0.01), defined by a greater AUC.

Based on ROC curves a cut-off of sFlt1/PlGF ≥ 85 was chosen. Metrics on the predictive performance was only specified for women presenting before 34 weeks gestation. Sensitivity and specificity was 73% and 94% respectively (no confidens intervals were given). Positive predictive value was 86% whereas negative predictive value was 87%.

Strengths and limitations
The prospective design strengthens the level of evidence in the results of this study. In addition, the automated assay was used, and the assay operators as well as the physicians, were blinded to clinical data and test result respectively. The size of the population was large, compared to the other two studies on the subject.

A limitation of this study is that a sFlt1/PlGF cut-off is only evaluated on patients presenting before 34 weeks gestation.

Moore et al.(8)
In the prospective cohort study performed by Moore et al. (8), 276 women with singleton pregnancies and symptoms, elevated blood pressure or proteinuria were followed until delivery in order to register any complications. Blood samples were taken at presentation and stored at -80°C until analyzed on immunoassays (ELISA).
In all women presenting to the triage unit sFlt1 were significantly higher (P=0.015) and PI GF were significantly lower (P<0.0001) in women who subsequently developed complications, than those who did not. When a subgroup analysis was made on women who presented after 37 weeks gestation, the biomarker levels were not significantly different in those who developed complications compared to those who did not. Based on this, the predictive analysis was restricted to women, who presented before 37 weeks gestation. In this subgroup of women, the odds ratio for complications were 2.37 (95% CI 1.45-3.87) per 100 unit increase in sFlt1/PI GF ratio. A ROC analysis was made and AUC was calculated. The sFlt1/PI GF ratio had an AUC of 0.76 (95% CI 0.66-0.85) and was not significantly different from that of clinical diagnosis of PE or gestational age at presentation. But when using a multivariate model consisting of maternal clinical variables available at the time of presentation and Sflt1/PI GF ratio, this was more predictive of complications, than any single model predictor, with AUC of 0.91 (95% CI 0.85-0.97).

Strengths and limitations
The strengths of this study are the prospective design and the low risk of bias in the interpretation of the test, since the assay was handled by a single investigator blinded to clinical information.

The limitation of this study is the lack of information on sensitivity, specificity, PPV and NPV. In addition the size of the study population is relatively small.

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4 The clinical multivariate model included: Race, chronic hypertension, history of renal disease, gravidity (primi- vs multipara), PE history, maternal age, smoking status, obesity (BMI>30 kg/m^2), pregestational diabetes mellitus, clinical diagnosis of PE and gestational age at presentation (reference Moore)