

# Perspectives on the Use of Placental Growth Factor (PIGF) in the Prediction and Diagnosis of Pre-Eclampsia: Recent Insights and Future Steps

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**Abstract:** Pre-eclampsia (PE) is a complex multisystem disease of pregnancy that is becoming increasingly recognized as a state of angiogenic imbalance characterized by low concentrations of placental growth factor (PIGF) and elevated soluble fms-like tyrosine kinase (sFlt-1). PIGF is a protein highly expressed by the placenta with vasculogenic and angiogenic properties, which has a central role in spiral artery remodeling and the development of a low-resistance placental capillary network. PIGF concentrations are significantly lower in women with preterm PE, and these reduced levels have been shown to precede the clinical onset of disease. Subsequently, the clinical utility of maternal serum PIGF has been extensively studied in singleton gestations from as early as 11 to 13 weeks' gestation, utilizing a validated multimarker prediction model, which performs superiorly to the National Institute for Health and Care Excellence (NICE) and American College of Obstetricians and Gynecologists (ACOG) guidelines in the detection of preterm PE. There is extensive research highlighting the role of PIGF-based testing utilizing commercially available assays in accelerating the diagnosis of PE in symptomatic women over 20 weeks' gestation and predicting time-to-delivery, allowing individualized risk stratification and appropriate antenatal surveillance to be determined. "Real-world" data has shown that interpretation of PIGF-based test results can aid clinicians in improving maternal outcomes and a growing body of evidence has implied a role for sFlt-1/PIGF in the prognostication of adverse pregnancy and perinatal events. Subsequently, PIGF-based testing is increasingly being implemented into obstetric practice and is advocated by NICE. This literature review aims to provide healthcare professionals with an understanding of the role of angiogenic biomarkers in PE and discuss the evidence for PIGF-based screening and triage. Prospective studies are warranted to explore if its implementation significantly improves perinatal outcomes, explore the value of repeat PIGF testing, and its use in multiple pregnancies.

**Keywords:** pre-eclampsia, biomarkers, PIGF, sFlt-1, screening, aspirin, prevention, prediction, pregnancy complications

## Introduction

Pre-eclampsia (PE) is a complex multisystem disorder of pregnancy, characterized by the new onset or progression of hypertension with signs of maternal end-organ or fetoplacental dysfunction, at or after 20 weeks' gestation, and complicates 2–8% of pregnancies.<sup>1</sup> PE is an important cause of global maternal and perinatal morbidity and mortality, with an estimated 70,000 maternal deaths directly attributable to the condition each year.<sup>1,2</sup> PE is a disorder that encompasses a wide spectrum of disease, with variable onset and clinical course. Women who meet the diagnostic criteria for PE can range from asymptomatic at term with mildly elevated blood pressure and proteinuria, to patients at a periviable gestation with seizures and evidence of multi-organ failure. Whilst delivery of the fetus and placenta is the definitive treatment for PE, there is growing evidence of significant long-term morbidity for affected women, who remain

at greater risk of cardiovascular and cerebrovascular disease years after pregnancy.<sup>3–5</sup> It is uncertain presently if these long-term health consequences are due to underlying risk factors associated with PE, or the disease mechanism itself.

The definition of PE is constantly evolving; the International Society for the Study of Hypertension in Pregnancy (ISSHP) recommends that significant proteinuria is no longer essential for the diagnosis in the presence of neurological, biochemical, or hematological abnormalities or severe uteroplacental disease (Box 1). In comparison to the traditional description of PE, this broader definition encompassing evidence of uteroplacental dysfunction appears to better identify women and fetuses at risk of adverse outcomes including major maternal and perinatal morbidity,<sup>1,6</sup> but also captures women with milder disease phenotypes.<sup>7</sup>

Diagnosis based on clinical signs including hypertension and proteinuria is subject to observer error and poorly predicts adverse outcomes.<sup>8–10</sup> Many features of the condition are non-specific and thus it is estimated that approximately 30% of all pregnancies will undergo evaluation for PE.<sup>11</sup> This necessitates repeat hospital attendances and resource utilization for women with ambiguous presentations who require additional antenatal surveillance.<sup>12</sup>

PE is also the most common cause of iatrogenic prematurity and is implicated in over one-fifth of preterm births.<sup>13</sup> Morbidity and mortality in prematurity is inversely related to the gestational age at birth, with infants born extremely premature at greatest risk of severe complications including cerebral palsy, neurodevelopmental delay and bronchopulmonary dysplasia.<sup>14,15</sup> Early, effective identification of women at high risk for PE is a clinical priority, given the known benefits of aspirin prophylaxis, commenced prior to 16 weeks' gestation, in preventing preterm PE in this cohort.<sup>16</sup> Efforts should be concentrated on moving the focus of antenatal surveillance from reactive to predictive, thus inverting the pyramid of prenatal care.<sup>17</sup>

The exact etiology of PE is incompletely understood; however, it is largely accepted that malperfusion of the uteroplacental unit is central to its pathophysiology. The “two-stage model” has been postulated to explain the pathophysiology of preterm PE, occurring secondary to inadequate trophoblastic remodeling of the spiral arteries.<sup>18–20</sup> It is this defective placentation, and failure to establish a high volume, low-resistance uteroplacental blood supply, which triggers a resultant “maternal syndrome” of endothelial dysfunction and intravascular inflammation, altering the equilibrium of placental angiogenic factors, including PlGF and culminating in the clinical signs of PE including hypertension and proteinuria.<sup>21,22</sup> It has also been hypothesized that PE may not be a primary placental disorder, with uteroplacental hypoperfusion and endothelial dysfunction occurring secondary to suboptimal maternal cardiovascular adaptation.<sup>23</sup> This is biologically plausible, as both PE and cardiovascular disease share multiple risk factors including obesity, advanced maternal age, diabetes mellitus and chronic kidney disease,<sup>23</sup> and there is emerging evidence from clinical echocardiographic studies that maladaptation of the maternal cardiovascular system precedes the development of term PE.<sup>24</sup> Indeed, villous and vascular placental lesions, previously assumed to be characteristic of PE, have been shown to be neither sensitive nor specific markers for the disease and found in greater abundance in placentae from uncomplicated gestations.<sup>25</sup>

Studies have demonstrated that circulating angiogenic proteins have an important biological role in the pathophysiology of PE and may be utilized in the prediction, screening, diagnosis, and prognostication of adverse disease

#### Box 1 International Society for the Study of Hypertension in Pregnancy (ISSHP) Definition of Pre-Eclampsia (2018)

##### International Society for the Study of Hypertension in Pregnancy (ISSHP) Definition of Pre-Eclampsia (2018)

Pre-eclampsia is gestational hypertension accompanied by one or more of the following new-onset conditions at or after 20 weeks' gestation:

1. Proteinuria ( $\geq 300$  g in 24-hour collection or protein:creatinine ratio  $\geq 30$  mg/mmol).
2. Other maternal organ dysfunction, including
  - Acute Kidney Injury (AKI); creatinine  $\geq 90$   $\mu$ mol/L.
  - Liver involvement; elevated transaminases (ALT/AST  $>40$  IU/mL) with or without right upper quadrant or epigastric abdominal pain.
  - Neurological complications (eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, persistent visual scotomata).
3. Uteroplacental dysfunction (fetal growth restriction, abnormal umbilical artery Doppler, intrauterine fetal death, placental abruption, and angiogenic imbalance).

outcomes.<sup>26–28</sup> PIGF has been shown to have a particularly important role in first trimester prediction of PE and has a good diagnostic capacity in symptomatic women after 20 weeks' gestation.<sup>27,28</sup>

This literature review will appraise and evaluate the evidence for PIGF testing in the prediction and diagnosis of PE and discuss its potential future applications including serial biomarker testing and use in twin pregnancies.

## Angiogenic Factors

PlGF is a member of the VEGF family and potentiates the angiogenic response of VEGF.<sup>29</sup> PlGF exists structurally as a glycosylated dimeric protein with both angiogenic and vasculogenic properties influencing trophoblastic growth, differentiation, and invasion into the maternal decidua.<sup>30–33</sup> The *plgf* gene, located on chromosome 14q.14, encodes four isoforms of PlGF, of which PlGF-1 and -2 are most abundant during pregnancy and are prominently expressed in villous trophoblast cells.<sup>34</sup> Whilst PlGF shows 53% homology in structure to VEGF-A, there are structural differences between the various isoforms with PlGF-2 and PlGF-4 possessing additional heparin-binding domains.<sup>34</sup>

sFlt-1, a splice variant of the VEGFR-1 receptor, is a circulating anti-angiogenic protein that acts as a potent VEGF and PlGF antagonist, inducing endothelial dysfunction.<sup>21</sup> The placenta is the major source of sFlt-1 in the maternal circulation with serum levels in PE up to five times greater than those in normotensive women.<sup>21</sup> Whilst this excess of sFlt-1 is mediated by the placenta in PE, exogenously administered sFlt-1 induces the clinical and pathological findings of PE in both pregnant and non-pregnant animal models, indicating the direct effect of sFlt-1 is on the endothelium irrespective of the presence of a placenta.<sup>21</sup>

Placental expression of PlGF increases in the second trimester corresponding to a “second wave” of spiral artery remodeling at 16 to 18 weeks' gestation.<sup>34</sup> From 25 weeks' PlGF is believed to contribute to a change in branching to non-branching angiogenesis, controlling the expansion of a low-resistance placental capillary network.<sup>35–37</sup>

PlGF concentrations reach a peak between 29 and 32 weeks, then fall progressively until term.<sup>38,39</sup> Whilst PlGF expression is upregulated in response to pathological injury in non-trophoblastic cells, the expression of trophoblastic PlGF is specifically inhibited in response to placental hypoxia, a consequence of uteroplacental ischemia.<sup>34</sup>

Accurate and early detection of high-risk patients provides a significant opportunity for clinicians to implement an effective screen-and-prevent protocol in contrast to reacting to the clinical signs of PE, which are late features of the disease. Reduced levels of PlGF are evident from as early as 11–13 weeks' gestation which provides an opportunity for screening at the time of the first trimester booking appointment.<sup>39–41</sup>

## First Trimester Prediction of PE Utilizing PlGF

Serum concentrations of PlGF vary considerably with maternal characteristics and comorbidities; levels are greater in parous women, cigarette smokers, and in individuals of Afro-Caribbean, South Asian, and East Asian origin, and are lower in women with obesity or insulin-dependent diabetes mellitus.<sup>42–44</sup> Consequently, it is necessary to adjust for these variables and other variations in biomarker testing and assay analysis to standardize results and enable comparisons between research studies.<sup>40</sup> To achieve standardization of results, PlGF values can be expressed in multiples of the expected median (MoM). This is an essential step in applying a competing risks model to prediction of PE in the first trimester utilizing Bayes' theorem, which necessitates the combination of the a priori risk from the maternal history with measurements of the uterine artery Doppler pulsatility index (UtPI), mean arterial blood pressure (MAP) and the PlGF MoM result to calculate a patient-specific risk for PE.<sup>45,46</sup> The prior risk estimated from maternal characteristics and history is then modified with the incorporation of these biomarkers to produce an adjusted estimate of the probability of delivery with PE. This survival time approach assumes that if pregnancy was to continue indefinitely, all women would develop PE, and the experience of this outcome before a specified gestation is dependent on competition between delivery before or after the development of PE.<sup>45,46</sup> The incorporation of individual maternal characteristics and biomarker levels (MAP, UtPI and PlGF) modifies the mean distribution of gestational age at delivery with PE. In low-risk pregnancies, the gestational age distribution is shifted to the right which implies that delivery is likely to occur prior to a diagnosis of PE. In high-risk women, the gestational age distribution is shifted to the left indicating that delivery is likely to occur after the development of PE.<sup>45,46</sup> This allows for individualized risk calculation, incorporating not only

maternal risk factors but protective factors, such as a previous normal pregnancy.<sup>45</sup> Another advantage of this approach is that partial combinations can be used to calculate patient-specific risks when not all biomarkers are available.

Studies have consistently shown that women who develop preterm PE have significantly lower PIGF levels in the first trimester than those with normal pregnancies (Table 1). Furthermore, a significant association is seen between serum PIGF concentrations and the severity of PE, defined by both the gestation at the time of iatrogenic delivery and the neonatal birthweight percentile.<sup>40</sup> PIGF as an independent biomarker has detection rates (DR) of 55% and 33% for the identification of early- and late-onset PE, respectively (at a fixed false-positive rate (FPR) of 10%).<sup>40</sup> However, there is now significant evidence that the DR of preterm PE can be improved using the aforementioned competing risks model, known as the Fetal Medicine Foundation (FMF) triple test between 11 and 13 weeks' gestation.<sup>28</sup>

The model was first developed from a study of 58,884 women which showed its accuracy in the detection of preterm PE (DR 77.3% at a fixed FPR of 10%).<sup>46</sup> This algorithm encompassed maternal factors, MAP, UtPI and PIGF and additionally included serum pregnancy-associated plasma protein A (PAPP-A) levels, which are also known to be decreased in the first trimester in pregnancies later affected by PE.<sup>28</sup> Including PAPP-A yielded a similar DR of 76.6%.<sup>46</sup> Prospective data from first trimester screening of 35,948 women was used to update the original algorithm and assess its performance in the detection of preterm and term PE, which was 75% and 47%, respectively, with an FPR of 10%.<sup>28</sup> The inclusion of PAPP-A to this combined model including PIGF did not improve the screening performance, a finding which has been replicated by Tan et al.<sup>47</sup> A subsequent systematic review and meta-analysis has also demonstrated that PIGF is superior to PAPP-A in the first trimester prediction of PE.<sup>48</sup> Whilst PAPP-A may be utilized in the FMF model if PIGF is unavailable, it results in reduced detection of preterm PE with DRs reported to be up to 7.1% (95% CI, 3.8–10.6%) lower with PAPP-A compared to using PIGF.<sup>49,50</sup>

**Table 1** Studies Comparing First Trimester Serum PIGF Levels in Women Who Develop Pre-Eclampsia and Normotensive Controls

Author	Gestation (weeks)	Pre-Eclampsia		Controls		P
		N	PIGF (pg/mL)	n	PIGF (pg/mL)	
Tidwell et al, 2001 <sup>41</sup>	5–15	14	20.1	25	58.5	<0.0001
Ong et al, 2001 <sup>111</sup>	11–14	131	1.09 (MoM)	400	0.98 (MoM)	0.07
Thadhani et al, 2004 <sup>112</sup>	11–14	40	23	80	63	<0.01
Erez et al, 2008 <sup>113</sup>	9–14	17* 39^	20.3* 26.2^	201 201	35.4 35.4	0.002 0.003
Akolekar et al, 2008 <sup>40</sup>	11–13	29* 98**	0.611 MoM* 0.822 MoM**	609	0.991 MoM	<0.0001
Wortelboer et al, 2010 <sup>114</sup>	8–13	88	0.73 MoM	480	1.00 MoM	<0.0001
Poon et al, 2010 <sup>115</sup>	11–13	37* 128**	23.0 (0.59 MoM)* 30.1 (0.85 MoM)**	8061	34.2 (0.96 MoM)	<0.0001
Akolekar et al, 2011 <sup>116</sup>	11–13	56* 104** 186^	0.64 MoM* 0.72 MoM ** 0.85 MoM ^	2143	0.99 MoM	<0.0001
Kuc et al, 2013 <sup>117</sup>	8–13	59* 91**	0.94 MoM* 0.90 MoM**	482	1.00 MoM	<0.016*
Crovetto et al, 2015 <sup>118</sup>	11–13	57* 256**	21.9* 27.5**	9159	33.4	<0.05
Diguisto et al, 2017 <sup>119</sup>	11–13	219	37.1	197	57.7	<0.001

**Notes:** \*Early PE<34 weeks; \*\*Intermediate to late PE ≥34 weeks ^Late PE>37 weeks.

**Abbreviation:** MoM, multiples of the median.

The FMF prediction models have been prospectively evaluated in multiple studies worldwide which have reported comparable predictive performances. O’Gorman et al performed a prospective, multicenter study to validate the FMF algorithm in 8775 first trimester singleton pregnancies including 239 women (2.7%) with PE. This study reported DRs of 100%, 75% and 43% for very early (<32 weeks’), preterm and term PE with a 10% FPR, in keeping with the results of the preceding studies.<sup>51</sup> The algorithm was also subsequently employed in the multicenter, double-blind, placebo-controlled Aspirin for Evidence-Based Preeclampsia Prevention (ASPREE) trial which reported DRs of preterm and term PE of 77% and 43% respectively with a 9.2% FPR.<sup>52</sup>

The largest study assessing the performance of the FMF triple test in the first trimester included 61,174 pregnant women and 1770 cases of PE (2.9%), and aimed to identify differences in the detection of PE between women of Caucasian and Afro-Caribbean racial origins.<sup>48</sup> Previous evidence has demonstrated a significant association between maternal ethnicity and the risk of PE, which is highest amongst Afro-Caribbean and South Asian women, and lowest in the East Asian population.<sup>53</sup> Women of Afro-Caribbean racial origin constituted 16.5% of the study population but contributed 48.3%, 37.1% and 30.2% of the cases of early, preterm and term PE, respectively, in keeping with the known increased risk of PE in this population. The DRs for early, preterm and term PE were also higher in this group (100%, 92% and 75%, respectively). Comparatively in Caucasian women, the DRs for early, preterm and term PE were 88%, 69% and 40% of cases, respectively.<sup>48</sup>

The performance of the FMF algorithm has also been evaluated in a prospective multicenter study in Asia, achieving DRs of 64.0% and 71.8% for preterm PE at 10% and 15% FPRs, respectively, which is comparable to previously published data in this population, but inferior to those achieved in Caucasian and Afro-Caribbean women.<sup>28,54,55</sup> These findings have validated the FMF algorithm amongst populations that are vastly different from the original British and mixed-European cohorts. It must, however, be noted that the risk cut-off and screen positive rate to achieve a given fixed DR of preterm PE may vary according to the race of the study population and whether PIGF or PAPP-A is used in the FMF model.<sup>49</sup>

Subsequently, the International Federation of Gynecology and Obstetrics (FIGO)<sup>56</sup> and the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG)<sup>57</sup> guidelines recommend the use of the multimarker FMF model to screen for PE in the first trimester. However, despite the volume of high-quality evidence for the FMF algorithm, the UK National Institute for Health and Clinical Excellence (NICE) and the American College of Obstetricians and Gynecologists (ACOG) currently endorse screening for PE by the identification of maternal risk factors from the woman’s demographic characteristics and medical history.<sup>58,59</sup> Whilst ISSHP supports the use of the FMF algorithm for first trimester PE prediction when available, it otherwise also recommends the use of clinical risk factors to identify high-risk women.<sup>1</sup> All pregnancies deemed to be at high risk of PE, defined by one high-risk factor or two moderate-risk factors, are recommended to be offered low-dose aspirin prophylaxis until delivery (Table 2).

These approaches treat each risk factor as a separate screening test with additive DR and screen positive rate.<sup>51</sup> Whilst the listed risk factors have statistically significant associations with PE, their individual likelihood ratios (LR) are low and thus the utilization of this checklist method performs poorly.<sup>60</sup> O’Gorman et al demonstrated that two-thirds of women classify as high-risk for PE when following the ACOG checklist with a 64.2% FPR.<sup>51</sup> The DR of preterm PE was also shown to be low at only 39% when utilizing NICE guidance.<sup>51</sup> The SPREE study also reported a similarly low DR of 40.8% preterm PE using the NICE checklist, in comparison to using the FMF method (DR 81.7% <37 weeks, 42.6% >37 weeks).<sup>54</sup>

These findings indicate that the FMF algorithm is superior to the method advocated by NICE and ACOG. Indeed, the use of risk factors, determined by their historic association with PE as opposed to a strong prospective evidence base, poorly discriminates between women who develop PE and those who do not. This has implications for patient compliance with aspirin administration, which is lower when recommended for a larger, less targeted population in comparison to a carefully selected group of women who receive specific counseling on their individual risk of PE.<sup>61</sup> The SPREE study demonstrated this by reporting a 23% rate of compliance with aspirin prophylaxis when it was recommended by the NICE guidance.<sup>54</sup> This is significant given the abundance of robust evidence that treatment of high-risk women with aspirin reduces the risk of preterm PE if the prescribed dose is greater than 100 mg and the treatment is commenced prior to 16 weeks.<sup>16,28,52,54</sup> In the ASPREE study, participants identified as high risk by the FMF algorithm

**Table 2** Maternal Factors Categorized by Moderate or High Risk according to recommendations of the National Institute for Health and Clinical Excellence (NICE), the American College of Obstetricians and Gynecologists (ACOG) and the International Society for the Study of Hypertension in Pregnancy (ISSHP)<sup>1,58,59</sup>

	High Risk	Moderate Risk
<b>NICE (2019)</b>	<ul style="list-style-type: none"> <li>• History of hypertensive disease in pregnancy</li> <li>• Chronic hypertension</li> <li>• Type 1/Type 2 diabetes</li> <li>• Chronic kidney disease</li> <li>• Autoimmune disease – systemic lupus erythematosus or antiphospholipid syndrome</li> </ul>	<ul style="list-style-type: none"> <li>• Nulliparity</li> <li>• BMI &gt;35 kg/m<sup>2</sup></li> <li>• First-degree relative with history of PE</li> <li>• Age &gt;40 years</li> <li>• Multifetal pregnancy</li> <li>• &gt;10-year pregnancy interval</li> </ul>
<b>ACOG (2021)</b>	<ul style="list-style-type: none"> <li>• History of PE</li> <li>• Multifetal pregnancy</li> <li>• Chronic hypertension</li> <li>• Type 1/Type 2 diabetes</li> <li>• Chronic kidney disease</li> <li>• Autoimmune disease – systemic lupus erythematosus or antiphospholipid syndrome</li> </ul>	<ul style="list-style-type: none"> <li>• Nulliparity</li> <li>• BMI &gt;30 kg/m<sup>2</sup></li> <li>• First-degree relative with history of PE</li> <li>• Age &gt;35 years</li> <li>• Black race</li> <li>• Lower income</li> <li>• IVF</li> <li>• &gt;10-year pregnancy interval</li> <li>• Previous small for gestational age infant</li> </ul>
<b>ISSHP (2018)</b>	<ul style="list-style-type: none"> <li>• History of PE</li> <li>• Chronic hypertension</li> <li>• Type 1/Type 2 diabetes</li> <li>• Chronic kidney disease</li> <li>• BMI &gt;30 kg/m<sup>2</sup></li> <li>• Antiphospholipid syndrome</li> <li>• Assisted reproductive technology</li> </ul>	<ul style="list-style-type: none"> <li>• Nulliparity</li> <li>• First-degree relative with history of PE</li> <li>• Age &gt;35 years</li> <li>• Short duration of sexual relationship prior to pregnancy (&lt;6 months)</li> <li>• &gt;5-year pregnancy interval</li> <li>• Primipaternity</li> <li>• Connective tissue disorders</li> </ul>

**Notes:** Women are at increased risk for pre-eclampsia if one or more high risk factors or two or more moderate risk factors are identified. For women at increased risk, NICE recommends aspirin 75–150 mg daily from 12 weeks', ACOG recommends aspirin 81 mg daily from 12 to 28 weeks' and ISSHP recommends commencing 100–150 mg of aspirin before 16 weeks' gestation.

had 79.9% compliance to at least 85% of the prescribed 150 mg aspirin tablets.<sup>62</sup> Rates of preterm PE were significantly lower in the aspirin group when compared to the placebo group (1.6% vs 4.3%, adjusted odds ratio (OR) 0.38; 95% CI 0.20–0.74,  $p=0.004$ ), with no significant reduction in term PE.<sup>51</sup> A secondary analysis of the ASPRE study specifically stressed the importance of compliance to aspirin prophylaxis in the successful prevention of PE, as results showed that the reduction in the incidence of preterm PE is reliant on good adherence to treatment; a 75% reduction was seen in those with compliance  $\geq 90\%$  compared to only a 40% reduction in those with compliance  $< 90\%$ .<sup>63</sup> Comparatively, women with lower adherence to prophylaxis ( $< 90\%$ ) appear to have a greater rate of PE (OR 2.3; 95% CI 1.2–11.6,  $p=0.03$ ), fetal growth restriction (FGR) (OR 5.8; 95% CI 1.2–8.3,  $p=0.001$ ) and preterm birth (OR 5.2; 95% CI 1.5–8.7,  $p=0.008$ ).<sup>64</sup> Guy et al have shown that use of the FMF algorithm also results in a statistically significant increase in targeted aspirin use by clinicians (99% vs 28.9%, OR 241.6; 95% CI 89.6–652) with a subsequent reduction in the rate of overall PE compared to those screened by the NICE guidelines (2.8% vs 3.6%  $p=0.016$ ).<sup>60</sup> Notably, the implementation of a first trimester screening program for the detection of preterm PE and targeted aspirin prescription in women identified as high risk has the potential for substantial cost savings to healthcare systems. A secondary analysis of the ASPRE study reported a statistically significant 68% reduction in the length of neonatal unit stay in the aspirin group compared to the placebo group (11.1 vs 31.4 days  $p=0.008$ ).<sup>65</sup> The cost savings from implementing screening were estimated to be in excess of \$5.6 million due to shorter neonatal stays.<sup>65</sup> A Canadian cost-effectiveness analysis further estimated that national integration of the FMF model into routine antenatal could save an estimated C\$140 million over a 10-year period.<sup>66</sup>

Similarly to ASPRE, Guy et al's study did not identify any significant reduction in the incidence of term PE, and it is notable from the above evidence that the first trimester FMF model is poor in predicting term PE compared to preterm PE. These findings support the possibility that there are varying pathophysiological mechanisms contributing to the PE syndrome. The influence of different contributors in both early onset and late onset PE has been debated and, consequently, it has been theorized that term PE is not an isolated placental-mediated condition, instead developing secondary to underlying maternal cardiovascular and metabolic disease, thus explaining why it is largely unaffected by aspirin prophylaxis.<sup>23,67,68</sup> An alternative hypothesis centers on preterm PE and term PE being graduations of the same disease process, with aspirin administration delaying the clinical presentation of PE to a later gestational age.<sup>69</sup> As term PE constitutes over 80% of PE cases, it has a large impact on healthcare systems given that maternal morbidity remains significantly increased in this group compared to women without PE.<sup>68,70</sup> Thus, there remains a substantial need for effective predictive strategies to identify and treat women at risk of term PE.

## Second and Third Trimester Prediction of PE

Screening for PE in the second and third trimesters aims to estimate the patient-specific risk, determining the frequency and content of an individual's future antenatal surveillance. It is not primarily directed towards prevention of PE, given the diminished window of opportunity for current prophylactic intervention.<sup>16</sup> Combining maternal risk factors, mean UtPI, MAP and PIGF has a similar, if not superior, predictive value to first trimester screening for early and preterm PE. Studies have demonstrated that this combined screening between 19 and 24 weeks' gestation predicts 99% of early PE, 85% of preterm PE and 46% of term PE with a 10% FPR, outperforming the DRs achieved by maternal factors alone (52%, 47% and 37%, respectively).<sup>71</sup>

Screening in the third trimester between 30 and 34 weeks can also predict 98% of preterm PE but still fails to detect over half of term PE cases.<sup>72</sup> The best DR of term PE by combined testing is achieved at 35 to 37 weeks' (70%) and includes sFlt-1 levels in addition to MAP, PIGF and maternal factors, as the addition of UtPI does not improve detection at this gestation.<sup>73</sup> To facilitate the clinical risk assessment of asymptomatic women in the first, second and third trimesters, the FMF has developed a free online PE risk calculator which is accessible on their website (<https://fetalmedicine.org/research/assess/preeclampsia>). Whilst first trimester screening by the FMF model has been established to be cost-effective in high resource settings, it is unclear presently if there are economic benefits with second and trimester screening for PE in asymptomatic women.

## Screening for PE in Low- and Middle-Income Settings

Pregnant women in low- and middle-income countries (LMIC) are disproportionately affected by serious maternal morbidity and mortality, thus any meaningful strategies to reduce the global burden of PE must also be applicable to these settings.<sup>74</sup> However, there are significant barriers to implementing screening for PE utilizing the FMF algorithm as these models rely on dependent access to ultrasound and laboratory testing, and additionally skilled clinicians who are competent in performing, and interpreting these results. This is a significant challenge given that most women in these low-income settings are not supervised by medical experts at any stage of their pregnancy.<sup>75</sup> Therefore, there are strategies of equal importance that should be implemented, including workforce and provider education, accurate blood pressure measurement, the availability of essential drugs for prophylaxis and treatment of PE, and financing of healthcare provision in LMIC.<sup>56</sup> FIGO recommends that in LMIC, when resources are limited, the FMF algorithm can be considered combining maternal characteristics and medical history with MAP.<sup>56</sup> Whilst the DR for preterm PE will be reduced, the risk calculation can still be performed, allowing targeted aspirin prescription.<sup>56</sup> Validation of this prediction model in LMIC is paramount and may be limited by populations with low health literacy.<sup>75</sup>

## Serum PIGF and sFlt-1 in the Screening and Diagnosis of PE in Women with Suspected Disease

There are four commercially available PIGF-based assays recommended by NICE to rule-in or out PE between 20 and 36 +6 weeks' gestation.<sup>76</sup> These tests may measure PIGF alone or additionally quantify sFlt-1 levels providing an sFlt-1/

PIGF ratio. As the assays have different affinities for each PIGF isomer, the thresholds for diagnosing PE vary between each test. Similarly, the manufacturers indicate different gestational ranges for testing and thus each assay should be used per its product guidance. Conversion of second trimester raw data values to MoM may also be performed, eliminating significant variations amongst different commercial testing platforms and allowing direct comparison of inter-assay results.<sup>77</sup> More recently, the ISSHP, NICE and German (AWMF) guidelines have included angiogenic testing as part of PE diagnosis.

### Elecsys<sup>®</sup> Immunoassay sFlt-1/PIGF Ratio (Roche Diagnostics, Mannheim, Germany)

The Elecsys<sup>®</sup> sFlt-1/PIGF ratio is formed by combining the results from two electrochemiluminescence immunoassays. It is not available as a point-of-care test and analysis requires the use of a large-scale automated laboratory analyzer. The measurement ranges have been defined as 10–85,000 pg/mL and 3–10,000 pg/mL for sFlt-1 and PIGF, respectively.<sup>78</sup> The sFlt-1/PIGF ratio is recommended for use in conjunction with clinical information in triaging (rule-out and rule-in) women suspected to have PE (Table 3).

Rana et al first identified that using the Elecsys<sup>®</sup> sFlt-1/PIGF ratio was superior in the prediction of adverse maternal outcomes than traditional late clinical parameters of PE, including systolic blood pressure, proteinuria, deranged liver transaminases and elevated serum creatinine measurements with an area under the receiver-operating characteristics (ROC) curve (AUC) of 0.899.<sup>79</sup> This study established a threshold of 85 for predicting adverse outcomes in women with early-onset PE (sensitivity 72.9%, specificity 94%) and identified that the sFlt-1/PIGF ratio was inversely correlated with time-to-delivery.<sup>79</sup> Dröge et al have also shown that hypertension and proteinuria are not sufficiently predictive of PE-related adverse outcomes unless integrated into a multimarker regression model with the sFlt-1/PIGF ratio (AUC 0.69 vs 0.887) and that patients with a ratio >85 have a significantly shorter time-to-delivery than patients with values lower than 85 (median 4 vs 29 days,  $p < 0.001$ ).<sup>26</sup>

The PROGNOSIS prospective, multicenter, observational study reported that a ratio <38 accurately rules out the diagnosis of PE for 1 week (negative predictive value (NPV) 99.3%).<sup>80</sup> An exploratory post-hoc analysis of data collected from the PROGNOSIS study has subsequently identified that an sFlt-1/PIGF ratio of  $\leq 38$  can rule-out the onset of PE for up to 4 weeks (NPV 94.3%), thus providing both clinicians and symptomatic women reassurance about the appropriateness of outpatient management.<sup>80,81</sup> The INSPIRE prospective, randomized interventional trial investigated the impact of revealed sFlt-1/PIGF testing on inpatient hospitalization. Whilst the number of admissions did not differ between groups, a “revealed” ratio of >38 correctly identified all women that developed PE within 7 days demonstrating 100% sensitivity and 100% NPV, in comparison to a sensitivity of 83.3% and NPV of 97.8% in the concealed cohort.<sup>82</sup> Stumpf et al have since demonstrated that routine clinical use of sFlt-1/PIGF testing can improve prognostic accuracy for women with PE which results in shorter hospital stays with potential economic benefits for healthcare systems (median 1 vs 4 days,  $p = 0.009$ ).<sup>83</sup>

However, the positive predictive value (PPV) for ruling in a diagnosis of PE within 4 weeks when utilizing an sFlt-1/PIGF ratio >38 is low (36.7%).<sup>80</sup> Subsequently, higher thresholds for ruling in a diagnosis of suspected PE have been

**Table 3** Recommended sFlt-1/PIGF Ratio Cut-Offs Utilizing the Elecsys<sup>®</sup> Immunoassay, data from the NICE Guidance<sup>76</sup>

Intention	Gestation	Decision Rule	sFlt-1/PIGF Ratio
Diagnosis of PE	20–33+6 weeks	Rule-out cut-off	33
Diagnosis of PE	20–33+6 weeks	Rule-in cut-off	85
Diagnosis of PE	34 weeks–birth	Rule-out cut-off	33
Diagnosis of PE	34 weeks–birth	Rule-in cut-off	110
Short-term prediction of PE	24–36+6 weeks	Rule-out PE for 1 week	38 or less
Short-term prediction of PE	24–36+6 weeks	Rule-in PE within 4 weeks	Over 38



established dependent on gestation: ratios  $\geq 85$  between 20 and 33+6 weeks' gestation and  $>110$  between 34 weeks and birth.<sup>76</sup>

Multiple studies utilizing the Elecsys<sup>®</sup> immunoassays have shown that the sFlt-1/PIGF threshold of 85 reflects a state of severe angiogenic imbalance, detecting preterm PE with high sensitivity and specificity and significantly predicting adverse maternal and perinatal outcomes.<sup>24,79,84–86</sup> The performance of an sFlt-1/PIGF ratio of  $\geq 85$  was analyzed as a secondary endpoint of the INSPIRE study which noted a significantly higher PPV of 71.7% to rule-in preterm PE within the following 4 weeks.<sup>87</sup> Similarly, the ROPE study recorded a PPV of 74.4% for the presence of severe PE amongst symptomatic patients  $<34$  weeks with a ratio  $>85$  (NPV=91.0%).<sup>88</sup>

A higher cut-off of 110 at later gestations improves the specificity and the LR of the sFlt-1/PIGF ratio in the diagnosis of PE in these patients (specificity 95.5% and positive LR 13; 95% CI 7.3–23.0).<sup>85</sup> Whilst many studies have utilized a ratio threshold of 38, NICE have employed a cut-off of 33 for diagnosing PE as Verlohren et al demonstrated that this result was highly sensitive and specific for PE in both early and late gestations and had the lowest negative likelihood ratio in women with PE (sensitivity and specificity of 95% and 94%, respectively, between 20 and 33+6 weeks, and 89.6% and 73.1%, respectively, after 34 weeks; negative LR 0.05, 95% CI 0.02–0.13).<sup>85</sup>

### Triage<sup>®</sup> PIGF Test (Quidel Cardiovascular Inc., San Diego, CA)

The Triage<sup>®</sup> PIGF test is a single-use fluorescence immunoassay recommended by NICE to either rule-in or rule-out the diagnosis of PE in symptomatic women between 20 and 35 weeks' gestation.<sup>76</sup> The analyzer can be installed as a point-of-care test in a clinical area or in a laboratory, producing a test result in 15 minutes. The assay quantifies PIGF concentrations in the range of 12–3000 pg/mL with a result  $<100$  pg/mL used as the threshold for a positive diagnosis.<sup>76,89</sup> Angiogenic biomarkers appear to be less useful in discriminating the need for delivery in later PE given that most women in this cohort will have abnormal PIGF and sFlt-1 levels.<sup>90</sup> The PEACOCK study assessed their diagnostic performance in determining the need for delivery within 7 days in women with late-onset preterm PE (34–36+6 weeks'). Whilst PIGF levels  $<100$  pg/mL have high sensitivity for PE (97.9%), testing at this gestation poorly discriminates which individuals are at greatest risk (specificity 8.4%).<sup>90</sup> The trial subsequently reported AUCs of less than 0.70 for PIGF, hence the NICE recommendation to screen until 35 weeks' gestation.<sup>76,90</sup>

Levels  $<100$  pg/mL have been shown to have high sensitivity and NPV for diagnosing PE necessitating delivery within 14 days (Table 4).<sup>26,91,92</sup> The PELICAN study showed that the AUC for low PIGF  $<5$ th percentile was superior to all other standard tests for PE requiring delivery within 14 days including blood pressure, proteinuria, uric acid and alanine transaminase measurements (AUC 0.87).<sup>27</sup> Chappell et al additionally demonstrated in this study that very low PIGF results  $<12$  pg/mL have a much higher specificity for PE requiring delivery within 14 days compared to levels  $<100$  pg/mL (90%, 95% CI 85%–94%).<sup>27</sup>

PIGF levels of normal ( $>100$  pg/mL), low (12–100 pg/mL) and very low ( $<12$  pg/mL) concentrations were shown in the PETRA study to independently predict time-to-delivery with median values of 45, 10 and 2 days, respectively.<sup>92</sup>

“Real-world” PIGF testing was implemented in a multicenter stepped-wedge cluster-randomized cluster trial (PARROT) to investigate the clinical effects of revealed PIGF testing on time-to-diagnosis and both maternal and perinatal outcomes.<sup>91</sup> The median time to PE diagnosis was significantly reduced in the revealed testing cohort (4.1 vs

**Table 4** A Summary of the Results from Prospective Clinical Studies Utilizing the Quidel Triage<sup>®</sup> PIGF Assay and a Threshold of  $<100$  Pg/ML in PE Requiring Delivery within 14 Days

Author	Gestation (weeks)	PLGF $<100$ in the Prediction of PE Requiring Delivery within 14 Days					
		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Chappell et al, 2013 <sup>27</sup>	20–35	96 (89–99)	56 (49–63)	44 (36–52)	98 (93–99.5)	2.2 (1.9–2.6)	0.07 (0.02–0.22)
Duhig et al, 2019 <sup>91</sup>	20–35	94.9 (82.7–99.4)	52.7 (45.9–59.3)	25.7 (18.8–33.6)	98.3 (94.2–99.8)	2.00 (1.71–2.34)	0.10 (0.03–0.38)
Barton et al, 2019 <sup>92</sup>	20–35	92.5	63.8	69.8	90.3	–	–

1.9 days,  $p=0.027$ ) and there was also significantly less maternal adverse outcomes (as defined by the fullPIERS consensus) in this group (5% vs 4%, adjusted OR 0.32; 95% CI 0.11–0.96,  $p=0.043$ ) with no evidence of increased adverse perinatal outcomes.<sup>91</sup> This specifically highlights the potential benefits of real-time PIGF testing as a diagnostic adjunct when women present to hospital with suspected PE.

### DELFLIA Xpress® PLGF 1-2-3 (PerkinElmer Life and Analytical Sciences, Waltham, USA)

The DELFLIA Xpress® PIGF 1-2-3 assay is a solid phase, two-site fluoroimmuno-metric sandwich assay, which quantifies serum levels of PIGF isoforms 1–3 between 1.9 and 4000 pg/mL. Prior to the 2022 update, the NICE guidelines on PIGF testing did not recommend the adoption of the DELFLIA Xpress® PIGF 1-2-3 test into clinical practice for diagnosing or predicting time-to-delivery in PE, given that research remained limited and primarily focused on its use in first trimester prediction.<sup>76</sup> DELFLIA® PIGF cut-offs have subsequently been validated to rule-in and rule-out the diagnosis and aid short-term prediction of PE<sup>93–95</sup> and are now advocated by NICE for this purpose from 20 weeks' gestation.<sup>76</sup>

Whilst the different assays perform similarly,<sup>77</sup> the rule-out threshold of 150 pg/mL was derived following the results of the COMPARE study which aimed to compare the performances of the commercially available PIGF-based tests in the prediction of time-to-delivery in women with PE prior to 35 weeks.<sup>94</sup> DELFLIA Xpress® PIGF 1-2-3 concentrations <150 pg/mL were noted to identify the same proportion of positive test results as the Triage® test and thus this cut-off was used for comparisons between assays. All three assays provided similar test performances in the prediction of time-to-delivery in women with PE prior to 35 weeks' gestation (DELFLIA® AUC 0.846, Quidel Triage® AUC 0.847, Elecsys® AUC 0.863) with no significant differences observed in AUCs ( $p=0.795$ ), sensitivities ( $p=0.249$ ), positive predictive values ( $p=0.765$ ) or negative predictive values ( $p=0.92$ ).<sup>94</sup>

Giblin et al also conducted a further secondary analysis of the COMPARE study demonstrating the optimal rule-in threshold of 50 pg/mL for the DELFLIA Xpress® PIGF 1-2-3 assay.<sup>93</sup> The specificities for the established Triage® PIGF and Elecsys® sFit-1/PIGF rule-in thresholds (<12 and >85, respectively) were utilized to calculate the corresponding rule-in threshold for PE requiring delivery within 14 days using the DELFLIA® test with an equivalent specificity (Table 5). The cut-off of 50 pg/mL for the DELFLIA Xpress® test demonstrated a positive LR of over 10 suggesting good predictive ability for the population and endpoint studied, with a specificity of 95% indicating its suitability as a rule-in threshold.<sup>93</sup> Despite variation in the quantitative thresholds for all of the commercially available assays, their individual clinical performances in the prediction of delivery in women with suspected PE have thus been demonstrated to be equivalent.<sup>76,93</sup> Bremner et al have provided new prospective evidence of the diagnostic accuracy of the DELFLIA Xpress® PIGF 1-2-3 test to rule-in and rule-out PE at the previously established thresholds (Table 6).<sup>95</sup> Their findings show that between 20 and 33+6 weeks' gestation, a PIGF level of >150 pg/mL is highly predictive in ruling out PE for up to four weeks, which is a significant advantage of the DELFLIA® test as other studies have not investigated the use of PIGF alone to predict PE at 28 days.<sup>95</sup>

**Table 5** The Performance of PIGF-Based Testing Assays in Predicting PE Requiring Delivery within 14 Days in 305 Women Presenting Prior to 35 Weeks' Gestation<sup>93</sup>

Test	DELFLIA Xpress® PIGF 1-2-3	Quidel Triage® PIGF	Roche Elecsys® sFit-1/PIGF
<b>Rule-in Threshold</b>	<50 pg/mL	<12 pg/mL	>85
Sensitivity (%)	53.8	53.8	65.4
Specificity (%)	95.0	95.3	95.3
PPV (%)	50.0	51.9	56.7
NPV (%)	95.7	95.7	96.7
Positive LR	10.7	11.6	14.0
Negative LR	0.5	0.5	0.4

Notes: Data from Giblin et al.<sup>93</sup>

**Table 6** Absolute Sensitivity and Specificity Values for PerkinElmer DELFIA Xpress PIGF 1-2-3 Levels and sFlt-1/PIGF Ratios, Showing High Specificity for Rule-in Thresholds of <50 Pg/ml and sFlt-1/PIGF  $\geq 70$ , and High Sensitivity for Rule-Out Thresholds of  $\geq 150$  Pg/ml and sFlt-1/PIGF  $\geq 90$ . Areas Under the Receiver-Operating Characteristics Curves Are Also Shown to Compare the Test Performances<sup>95</sup>

	PE within 7 Days			PE within 28 Days		
	Sensitivity (%)	Specificity (%)	AUROC	Sensitivity (%)	Specificity (%)	AUROC
<b>20–33+6 weeks (n=136)</b>						
DELFLIA <50 pg/mL	38.9	90.7	0.85	47.2	99.0	0.90
DELFLIA $\geq 150$ pg/mL	72.2	78.0	N/A	80.6	90.0	N/A
DELFLIA ratio <50	50.0	89.0	N/A	55.6	98.0	N/A
DELFLIA ratio $\geq 70$	38.9	89.8	0.81	50.0	99.0	0.88
<b>&gt;34 weeks (n=128)</b>						
DELFLIA <50 pg/mL	26.3	86.2	0.73	26.7	87.8	0.70
DELFLIA $\geq 150$ pg/mL	84.2	34.9	N/A	83.3	36.7	N/A
DELFLIA ratio <50	52.6	78.9	N/A	50.0	81.6	N/A
DELFLIA ratio $\geq 90$	21.1	93.6	0.75	23.3	95.9	0.59

Note: Data from Bremner et al.<sup>95</sup>

Abbreviations: AUROC, area under the receiver-operating characteristics curve; N/A, Not available.

### DELFLIA Xpress<sup>®</sup> sFlt-1/PIGF 1-2-3 Ratio (PerkinElmer Life and Analytical Sciences, Waltham, USA)

The DELFLIA Xpress PIGF 1-2-3<sup>®</sup> test can be used in combination with the PerkinElmer DELFLIA Xpress sFlt-1<sup>®</sup> kit to determine the sFlt-1/PIGF ratio for both the diagnosis and short-term prediction of suspected PE. Bremner et al determined that an sFlt-1/PIGF ratio of <50 most accurately rules out a PE diagnosis within seven and 28 days, whilst the best performing cut-offs for ruling in a diagnosis are  $\geq 70$  between 20 and 33+6 weeks' and  $\geq 90$  over 34 weeks' gestation (Table 6).<sup>95</sup>

Notably, the performance of biomarker testing does not appear to be improved by the additional measurement of sFlt-1 levels with the DELFLIA Xpress sFlt-1<sup>®</sup> kit.<sup>95</sup> Thus, using the DELFLIA Xpress PIGF 1-2-3<sup>®</sup> assay alone to determine PIGF levels may provide a competitive and cheaper alternative to other market competitors with similar clinical accuracy. However, NICE have concluded that compared with standard assessment, the DELFLIA Xpress<sup>®</sup> sFlt-1/PLGF 1-2-3 ratio is a cost-effective measure and have subsequently recommended its use in clinical practice based upon locally available resources.<sup>76</sup>

### Use of PIGF-Based Testing to Predict Adverse Maternal and Perinatal Outcomes

“Real-world” data from the PARROT trial demonstrated significant reductions in serious adverse maternal outcomes with revealed PIGF testing, with clear clinical benefits in utilizing angiogenic biomarkers as diagnostic adjuncts to target increased antenatal surveillance to high-risk women.<sup>91</sup> Recent evidence has confirmed that compared to women who experience PE without severe features, individuals who experience the potentially life-threatening complications of hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, disseminated intravascular coagulation, severe renal involvement or eclampsia, have the most significant alterations in serum sFlt-1 and PIGF levels. Women with HELLP syndrome appear to acquire the greatest derangement in angiogenic factor levels with a 74% reduction in PIGF levels and a ten-fold increase in the sFlt-1/PIGF ratio, followed by those who experience eclampsia with 57% lower PIGF levels and a four-fold increase in the sFlt-1/PIGF ratio.<sup>96</sup> The number of maternal complications also appears to correlate to the extent of angiogenic markers derangement, as when compared to women without severe features, those with PE and  $\geq 2$  adverse outcomes including severe hypertension, left ventricular failure, stroke and coma in addition to those detailed above, have significantly elevated sFlt-1/PIGF levels (5.70-fold change; 95% CI: 3.37–9.63,  $p < 0.001$ ).<sup>96</sup>

Altered levels of sFlt-1 and PIGF have also been associated with fetal complications including SGA, preterm birth and intrauterine death.<sup>79,92,97–99</sup> Low maternal PIGF concentrations appear to have an excellent predictive performance for the detection of SGA fetuses with a sensitivity of 93.2% and NPV of 89.7% in women presenting with suspected PE, outperforming ultrasound assessment alone (sensitivity 71.2%, NPV 78.5%) and suggesting a role for PIGF in the detection of presumed growth restriction.<sup>99</sup> PIGF has further been suggested as a potential discriminator between placentally mediated FGR and constitutionally small fetuses as a significant independent relationship has been demonstrated between PIGF levels <5th percentile and histological placental features of malperfusion.<sup>95</sup> In a multicenter study, low PIGF levels outperformed gestational age, abdominal circumference measurement and umbilical artery resistance index in predicting FGR with an AUC of 0.96, NPV of 99.2%, 98.2% sensitivity, and 75.1% specificity.<sup>98</sup> PIGF levels <5th percentile also demonstrated a significantly higher proportion of intrauterine deaths compared with normal levels ( $p < 0.05$ ).<sup>98</sup> A systematic review by Sherell et al investigating maternal PIGF levels as a predictor of adverse intrapartum and perinatal outcomes further demonstrated that lower concentrations of PIGF are consistently associated with fetal compromise necessitating Caesarean delivery, neonatal intensive care admission, and stillbirth.<sup>97</sup>

Elevated sFlt-1/PIGF ratios have also been reported in pregnancies complicated by early-onset FGR and appear to correlate well with ultrasound Doppler findings of absent or reversed end-diastolic flow in the umbilical arteries.<sup>100</sup> Garcia-Manau et al identified statistically significant differences in Elecsys<sup>®</sup> sFlt-1/PIGF levels with increasing FGR severity, defined by the extent of resistance in the umbilical artery Doppler ( $p < 0.05$ ).<sup>100</sup> Optimal sFlt-1/PIGF thresholds were calculated to identify the severity of early-onset FGR, with a value  $< 97.46$  (AUC 0.852, 95% CI 0.772–0.932) ruling out absent or reversed end-diastolic flow with a sensitivity and specificity of 78.3% and 97.1% respectively, and a ratio  $\geq 523.78$  (AUC 0.751; 95% CI 0.578–0.924) identifying reversed end-diastolic flow with a sensitivity of 70.6% and specificity of 87.2%.<sup>100</sup> Significant correlations were also found between higher sFlt-1/PIGF values and adverse outcomes including preterm birth ( $p < 0.001$ ), time-to-delivery ( $p < 0.001$ ), lower birthweight ( $p = 0.026$ ), and neonatal intensive care unit stay ( $p = 0.006$ ).<sup>100</sup> Associations between low serum levels of PIGF and elevated sFlt-1 levels have also been observed in small studies investigating late-onset FGR;<sup>101</sup> however, there are no proposed sFlt-1/PIGF cut-offs. Currently, biomarker testing as an adjunct to ultrasound for detecting FGR is not recommended outside of clinical research, given the paucity of prospective, interventional trial data and larger multicenter studies are recommended to investigate their role in fetal growth surveillance.<sup>102</sup>

The association of elevated sFlt-1/PIGF values with adverse perinatal events has been echoed by other studies, including a retrospective, “real world” study of 1117 women  $< 36 + 6$  weeks’ gestation, which reported that integrating sFlt-1/PIGF with maternal information aids the prediction of unfavorable outcomes including preterm birth, respiratory distress syndrome and fetal death with an AUC of 0.887.<sup>26</sup> Significantly, the sFlt-1/PIGF ratio in suspected or confirmed PE has been shown to perform well in predicting a composite of other adverse perinatal outcomes including intraventricular hemorrhage, hypoxic-ischemic encephalopathy, necrotizing enterocolitis, and retinopathy of prematurity (AUC 0.87, 95% CI 0.81–0.93).<sup>103</sup>

Recognition that low PIGF levels and elevated sFlt-1/PIGF ratio correlate with poor perinatal outcomes is significant as this is arguably more clinically relevant than prediction of time-to-delivery, which may be influenced by a different threshold to deliver with advancing gestational age.

## Repeat PIGF Testing

NICE do not currently recommend repeat PIGF testing for women at risk of or suspected to have PE.<sup>76</sup> However, they have explicitly recommended further research to investigate if serial testing offers additional clinical and economic benefits compared to performing a single PIGF measurement. Notably, 10.9% of the women with normal PIGF levels in the PARROT study ( $> 100$  pg/mL) subsequently received a PE diagnosis later in pregnancy.<sup>104</sup> Indeed, a multicenter case-series of 289 women undergoing repeated PIGF measurements identified that nearly one-third of women with suspected PE and an initial normal PIGF level  $> 100$  pg/mL (Triage<sup>®</sup>) have an abnormal result on repeat testing, indicating the dynamic changes in PIGF levels at the onset of clinical disease.<sup>104</sup> The diagnostic accuracy of repeat PIGF testing was high and performed similarly to the cohort’s first test showing high sensitivity and NPV in predicting PE requiring delivery within 14 days (AUC 0.78, 95% CI 0.72–0.84 vs 0.80, 95% CI 0.68–0.89, respectively).<sup>104</sup> Zeisler et al’s post-

hoc analysis of the PROGNOSIS study additionally demonstrated that women who develop PE and/or adverse perinatal outcomes have significantly higher median increases in interval sFlt-1/PIGF ratio results when repeated after 2 and 3 weeks, compared to those who do not ( $p < 0.001$ ).<sup>81</sup> However, this study was limited by its retrospective design, a lack of validation for the optimal interval between tests and the absence of performance statistics for repeat tests which were indeterminable due to the small sample size. Thus, serial testing may have a role in the risk stratification of women with ongoing clinical suspicion of PE despite an initial normal PIGF result, but prospective studies are needed to determine the benefit of repeat PIGF testing.

The PARROT-2 study aims to address this research gap and is recruiting participants to a prospective multicenter randomized controlled trial to investigate whether knowledge of serial repeat PIGF results (using either Elecsys<sup>®</sup> sFlt-1/PIGF or Triage<sup>®</sup> quantitative PIGF) in symptomatic women decreases the incidence of severe perinatal and maternal adverse outcomes. The investigators additionally aim to perform a cost-effectiveness analysis of repeat testing, including additional attendances, mode of delivery and neonatal care in addition to the price of PIGF tests.<sup>105</sup>

## Use of PIGF-Based Testing in Twin Pregnancies

Twin pregnancies are at increased risk of gestational hypertension (relative risk (RR) 2.04, 95% CI 1.6–2.59) and PE (RR 2.62; 95% CI 2.03–3.38) compared to singleton gestations.<sup>106</sup> Previous research has primarily addressed the prognostic value of angiogenic biomarkers in predicting and diagnosing PE in singleton pregnancies, excluding multiple gestations. Women with normal twin pregnancies appear to have at least double the levels of sFlt-1 compared to singleton pregnancies.<sup>107,108</sup> This is theorized to be a result of the enlarged placental mass in twin gestations, which subsequently increases the placental expression of sFlt-1 out of proportion of the circulating PIGF levels, thus potentiating the characteristic maternal endothelial dysfunction associated with PE.<sup>20,107</sup>

In keeping with singletons, women with twin pregnancies and PE have significantly lower PIGF levels and greater sFlt-1 levels when compared to gestational age-matched cohorts with uneventful twin pregnancies ( $p < 0.001$ ).<sup>108</sup> Thus, median sFlt-1/PIGF ratios are also significantly higher in these patients ( $p < 0.001$ ).<sup>108</sup> Dröge et al determined an optimal sFlt-1/PLGF threshold of 53 for ruling in PE in twin pregnancies in a European multicenter study utilizing the Elecsys<sup>®</sup> assay (sensitivity 94.4%, specificity 74.2%).<sup>108</sup> Use of the NICE recommended singleton lower cut-off of 33, initially established by Verlohren et al,<sup>85</sup> resulted in a sensitivity of 100% but lower specificity (67.7%) in the twin cohort in comparison with singleton pregnancies <34 weeks (95% sensitivity, 94% specificity) suggesting a need to establish reference ranges for PIGF testing for twin pregnancies.<sup>108</sup> De La Calle et al subsequently published an analysis which reported comparable sFlt-1/PIGF ratios between singletons and twins until 29 weeks' when levels rise substantially more in twin gestations.<sup>109</sup> In this study, an exploratory analysis of the sFlt-1/PIGF ratio threshold of  $\leq 38$  had a NPV of 91.9% and 83.8% to rule-out PE within a week and 4 weeks respectively.<sup>109</sup> Binder et al also demonstrated that a cut-off of 38 can rule-out PE requiring delivery due to maternal complications of PE within one and 2 weeks with NPVs of 98.8% and 96.4%, respectively.<sup>110</sup> Indeed, sFlt-1/PIGF performed better than PIGF alone for predicting delivery due to maternal complications within 2 weeks (AUC 0.88 vs 0.80,  $p = 0.001$ ) and had a stronger linear relationship with the sampling-delivery interval compared with PIGF.<sup>110</sup>

However, PPVs using this threshold appear to be much lower; 20% at 1 week and 44% at 4 weeks, which may be explained by the rising sFlt-1/PIGF ratios seen in normal twin pregnancies after 29 weeks' which often surpasses the cut-off of 38 even in the absence of PE.<sup>108</sup> Higher PPVs are reported when using cut-offs of 80 and 67 to predict delivery within one and 2 weeks, respectively (54.5% and 61.0%).<sup>110</sup> Thus, an sFlt-1/PIGF cut-off of 38 is not clinically useful to accurately rule-in or sufficiently screen for PE but appears promising to rule-out a diagnosis of PE in twin pregnancies and identify women eligible for expectant management. Further analysis and particularly prospective studies are required to understand the differences in angiogenic marker levels in multiple gestations and to validate sFlt-1/PIGF cut-offs before implementation into clinical practice.

## Conclusion

A state of angiogenic imbalance is central to the pathogenesis of PE and this knowledge is increasingly informing the use of angiogenic biomarkers to improve the prediction, diagnosis and management of the disease. The use of PIGF testing,

combined with maternal characteristics, medical history and other biomarkers, is superior to current national guidance in first trimester risk prediction performance, allowing individualized antenatal care from the first hospital visit. This ensures targeted aspirin use by clinicians and patient understanding of the purpose of pharmacological prophylaxis, with resultant positive effects on treatment adherence and prevention of preterm PE. The value of PIGF and sFlt-1/PIGF as rule-out tests in the diagnosis and short-term prediction of PE has been clearly established in singleton pregnancies, outperforming traditional clinical parameters in determining time-to-delivery and predicting adverse perinatal outcomes.

Whilst the use of biomarkers in delivery planning of women with PE has not yet been adequately investigated and is not recommended, there is evidence that knowledge of PIGF levels can reduce serious maternal morbidity. Further research is required to ascertain the benefits of elective early delivery on perinatal outcomes when utilizing PIGF-based testing as more than just an adjunct to other clinical information in decision-making. There is emerging evidence of the potential advantages of angiogenic biomarkers to diagnose and predict other complications including fetal growth restriction and stillbirth, and this is a research priority given the association of these complications with pre-eclampsia.

## Disclosure

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