Predictive biomarkers of pre-eclampsia and effectiveness of preventative interventions for the disease

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Introduction: Pre-eclampsia (PE) is one of the most common pregnancy complication characterized by placental and maternal vascular dysfunction. It affects about 3–8% of women during the second half of pregnancy and represents one of the major causes of neonatal morbidity and mortality. The etiology of PE largely remains unknown.

Areas covered: PE is considered a syndrome with multisystem involvement, so the ideal predictive test for it should utilize a combination of many predictors. Measurement in early pregnancy of a variety of biophysical and biochemical markers implicated in the pathophysiology of PE associated with clinical risk factors has been proposed to predict the development of the syndrome, thereby mitigating an adverse outcome.

Expert opinion: The identification of reliable indicators is a clinically relevant issue that could result in early therapeutic intervention and leading to the prevention of maternal and fetal injuries before the manifestation of clinical signs. Many factors complicate the prevention of PE cases. Most are attributed to unknown etiology, the low predictive value of current screening tests and the several presentations of the disease. Although preventative treatments have been studied extensively, an effective intervention to avoid the development of PE has not yet been discovered.

Keywords: predictive biomarkers, pre-eclampsia, preventative interventions

1. Introduction

Pre-eclampsia (PE) is a hypertensive multisystem disorder affecting about 3–8% of pregnancies. It is a leading cause of maternal and fetal morbidity and mortality, with an estimated 50,000–60,000 PE deaths/year worldwide [1,2]. The classification of hypertensive disorders in pregnancy was introduced in 1972 by the American College of Obstetricians and Gynecologists and revised in 2000 including four categories: PE/eclampsia, chronic hypertension, chronic hypertension with superimposed PE and gestational hypertension. PE is defined by hypertension as a persistent systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg after 20 weeks of gestation in a woman with previously normal blood pressure, dipstick of >1+ equivalent to 30 mg/dl in a single urine sample or >0.3 g in 24 h collection. PE can require delivery before 34 weeks (early forms), between 35 and 37 weeks (intermediate forms) or after 37 weeks (late forms): the early-onset form is associated with abnormal placentation and fetal growth restriction, while the intermediate and late-onset forms, the more frequent forms, are often associated with maternal metabolic disorders [3]. Pathogenetic mechanisms differentially
Pre-eclampsia (PE) is one of the most common pregnancy complications characterized by placental and maternal vascular dysfunction. The identification of reliable markers is a clinically relevant issue that could result in early therapeutic interventions, leading to the prevention of maternal and fetal injuries before the manifestation of clinical signs.

PE is considered a syndrome thus the ideal predictive test for PE should utilize a combination of maternal risk factors and biophysical and biochemical markers.

The administration of aspirin before 16 weeks of gestation as preventative treatment of PE seems to reduce the inflammation and to improve trophoblast invasion, thereby significantly reducing the development of PE.

Recently, several new targets have been proposed. However, treatments for placental disorders need to be extensively validated before their introduction in pregnancy management.

Prevention and prediction of PE are strictly connected: early prediction without the possibility of correcting the profile of a woman at risk does not have any benefits.

This box summarizes key points contained in the article.

2. Pre-eclampsia

In 2004, the World Health Organization compiled and published the criteria necessary to ensure that a screening test for PE is considered reliable [5]:

1) It should help to improve the prognosis and decrease the severity of pathology, creating an advantageous cost/benefit ratio.

2) It must be simple to perform, non-invasive, safe for the patient, accessible.

3) It must allow a clear classification between patients at risk and not at risk.

4) It must possess a high sensitivity, compared to a good specificity.

So far, a certain number of clinical risk factors (RFs), biophysical and biochemical markers have been associated with PE (Table 1), but not one has met the WHO 2004 criteria listed here.

2.1 Risk factors

The assessment of clinical RFs and medical history (Table 1) is a useful tool for the prediction of PE, but not sufficiently accurate, despite the incidence of PE in women at risk being high. In particular, in women with a history of previous PE, the risk of developing re-disease is 19%; in women with a family history of PE, it ranges from 15 to 35%; it is around 25% in women with chronic hypertension; 22% in women with diabetes; and 18% in women with nephropathy [6,7].

A recent study revealed low predictive values of clinical RFs in pregnancy, in particular in 37% of those who successively developed early-onset PE (onset before the 34th week of gestation) and in 29% of those who developed late-onset form, with false positive rate of 5% [8]. Ethnic differences are difficult to investigate because of confounding geographic, economical and cultural factors. Recent studies confirmed that the risk for PE is higher for women with Afro-Caribbean and South Asian racial origins, and this aspect must be taken into account when developing new models for PE prediction [9].

2.2 Biophysical markers

2.2.1 Uterine artery Doppler

Uterine artery Doppler is one of the most investigated biophysical predictive markers of PE. In particular, in case of PE and intrauterine growth restriction (IUGR), the uteroplacental circulation is not transformed into a low resistance state, in contrast to what happens in normal pregnancy. This is thought to be the result of the failure of the trophoblast to invade the uterine arteries. The state of high resistance can be measured by ultrasound examination with velocimetry Doppler, which shows an increased impedance at the level of the blood flow of the arteries of the uterus in cases of PE and IUGR, anticipating the clinical manifestations.

A conspicuous number of studies showed that uterine artery Doppler provided a good early predictive marker of PE if carried out in the second trimester, particularly between weeks 22 and 24 of gestation in a population at risk [10,11]. In the last years, scientific interest moved to the first trimester, in order to improve early monitoring and start possible preventative treatments, such as aspirin. However, as stated by Cnossen et al. in a large metanalysis [10], the predictor ability of Doppler in the first trimester seems to be less accurate
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Table 1. Categories of possible predictive factors of PE.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Biophysical markers</th>
<th>Biochemical markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparity</td>
<td>Uterine artery Doppler</td>
<td>Placental proteins: PP13, PAPP-A</td>
</tr>
<tr>
<td>Previous PE pregnancy</td>
<td>Mean arterial blood pressure</td>
<td>Angiogenic and anti-angiogenic factors: sFlt-1, sEng, sFlt-1/PlGF ratio, PlGF, VEGF</td>
</tr>
<tr>
<td>Familial history of PE</td>
<td></td>
<td>Inflammatory proteins: IL, complement component, PTX3, chemokines</td>
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<tr>
<td>IVF</td>
<td></td>
<td>Hypoxia-induced factors: HIF1A, HbF and A1M</td>
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<tr>
<td>Chronic hypertension</td>
<td></td>
<td>Metabolomics</td>
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<tr>
<td>Renal disease</td>
<td></td>
<td>Cell-free fetal DNA and RNA</td>
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<tr>
<td>Diabetes mellitus type 1</td>
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<td>Obesity (BMI &gt; 30)</td>
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<tr>
<td>History of trombophilia</td>
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<td>LES</td>
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<tr>
<td>Multifetal pregnancy</td>
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<tr>
<td>Age &gt; 40</td>
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<tr>
<td>Ethnicity</td>
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</table>


than in the mid-trimester. Due to this, as reported in ‘Predictive multiple marker models’ session, during the first trimester it is worthy combining uterine artery Doppler with biochemical markers, avoiding its use alone for routine screening.

While it is widely accepted that uterine artery Doppler is a good predictor of PE in high-risk population, criticisms have been highlighted about the routine use of uterine artery Doppler in a low-risk population [12]. However, interesting results have recently been achieved in a population with low incidence of PE (2 - 3%) [13]. These contrasting results may be due to the heterogeneity of patient characteristics, timing of studies, different clinical definition used and method of recording, in particular during the first trimester. While there is agreement about the evaluation of uterine artery Doppler pulsatility index (PI) in the mid-trimester at the level of the apparent crossover with the external iliac artery, only recent studies have revealed that the correct site of uterine artery PI at 11 – 13 weeks is at the level of the internal cervical os [14]. This measurement seems to correlate better with the second trimester uterine artery PI.

Recent studies included in Pedrosa’s systematic review have focused attention on the contrast between the good sensitivity of an increased uterine artery Doppler as predictor of early-onset PE and the poor accuracy in detecting late-onset form [15]. At the beginning of year 2014, Verlohren et al. found that late-onset PE is associated with both small-for-gestational-age and an unexpected proportion of large-for-gestational-age fetuses, compared to controls [16]. The author explained this apparent paradox through the hypothesis that late-onset PE has a mixed etiology and, consequently, very different phenotypes. The heterogeneity of the late-onset PE form might be at the origin of the poor predictive accuracy of mid-trimester uterine artery Doppler for late-onset forms.

2.2.2 Mean arterial blood pressure

Mean arterial blood pressure was introduced by the Fetal Medicine Foundation during the Pregenesys study and it consists in twice the diastolic plus the systolic blood pressure, divided by 3. It appears to be better than considering only systolic or diastolic pressure and it does not show variability of prediction if considered in a low- or high-risk population [17].

2.3 Biochemical markers

2.3.1 Placental proteins: placental protein 13 and pregnancy-associated protein A

Placental protein 13 (PP13) is a member of the galectin family (a family of β-galactoside binding proteins) and it is produced exclusively by the syncytiotrophoblast of the placenta [18]. It has been extensively tested and analyzed as a marker of PE: it is abnormally low during the first trimester and it tends to rise and stay high until delivery in women who will later develop PE suggesting syncytiotrophoblast necrosis [19,20]. In a recent study Huppertz et al. made a meta-analysis on 19 studies on first trimester serum PP13 published between January 2006 and September 2012 [21]. In total 16,153 pregnant women were tested: 1,197 women subsequently developed PE of which 532 (45%) were early and intermediate PE cases combined and 224 (19%) were early PE cases. In all studies maternal blood PP13 level was lower in women who subsequently developed PE, although the range of differences was huge. The prediction by PP13 has a very broad range (17 - 91%). The mean detection rate for all PE cases was 47% (95% CI: 43 – 65) at a false positive rate of 10% [21].

Recently there appeared a paper by Gonen et al. (March 2014) in the literature, wherein PP13 and RFs were evaluated as markers for predicting PE and the use of aspirin for PE
prevention was explained. First-trimester pregnancy screening was based on having PP13 level ≤ 0.4 multiple of the median (MoM) and/or at least one major RF for PE. The clinicians had adjusted the medical management of the patients based on the results of the prediction. Among the women that developed PE (n = 63, 7.7%) low PP13 was a better predictor for PE versus major RFs, particularly in young nulliparous women; however, combining low PP13 with RFs increased prediction accuracy. Moreover, they found that among patients identified as being at high risk, daily use of aspirin from the first trimester reduced the frequency of PE cases by 30% [22].

Pregnancy-associated protein A (PAPP-A) is a protein synthesized in the placenta and is known for the combination with β-human chorionic gonadotropin and molar trans- lency thickness to screen trisomies 21, 13 and 18. It has been evaluated as a predictive marker of PE but alone it does not have a good prediction rate (10 – 20%) [23]. Also in combined models, for example, in association with A-disintegrin metalloprotease 12 and uterine artery Doppler, it is not sufficiently predictive of PE (52% detection rate with a false positive rate of 10%) [24]. On the contrary, in 2011, Odibo et al. found a fair correlation between first trimester PAPP-A levels and pregnancies complicated by PE and IUGR. In particular, the surface areas of terminal and inter- mediate villi, and the volume of terminal villi were significantly smaller in placentas from pregnancies complicated by both of these pathologies; compared with the control group the mean PAPP-A (MoM) was lower in the pregnancies with abnormal placenta morphology, suggesting an important role of this marker in the prediction of PE combined with IUGR [25].

2.3.2 Angiogenic and anti-angiogenic factors

Soluble fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng) and placental growth factor (PIGF) appear to be the most promising predictive markers of PE, while a contradictory role is reserved to the VEGF [26]. We can identify two distinct groups on the basis of their angiogenic function: sFlt-1 and sEng are anti-angiogenic, whereas PIGF and VEGF are angiogenic. These biomarkers tend to vary from weeks to months before the clinical onset, so they have been introduced in lots of combined models. In particular, sFlt-1 is generally elevated 4 – 5 weeks before the onset, while PIGF starts to decrease between 9 and 11 weeks of gestation [27]. In 2012, Kleinrouxwer et al. investigated the accuracy of angio- genic predictive biomarkers by a metanalysis of 34 studies that included original publications testing PIGF, sFlt-1 and sEng in serum or plasma of pregnant women at < 30 weeks of gestation and before clinical onset of PE. They showed different concentrations in women who subsequently developed PE, but test accuracy was too poor to introduce them into clinical practice (PIGF, sFlt-1 and sEng sensitivities were respectively 32%, 26% and 18%, for a false positive rate of 5%) [28]. The same results were obtained by the authors of a recent study evaluating PIGF, sFlt-1 and sEng in 3,529 women between 14 and 16 weeks of gestation; the AUC was 0.84 using only PIGF and the clinical risk variables, and no further improvement was observed using uterine artery Doppler or the other two biomarkers. They stated that the analysis of plasma PIGF improved the identification of women at risk for preterm PE but the predictive performance of the test is not worthy of clinical use [29]. These last results may perhaps be due to the population enrolled: low-risk nulliparous women without recognized PE RFs [29].

In fact, in the same year, Maynard et al. compared angiogenic biomarker patterns in high-risk pregnancies and low-risk controls. In total 156 women at risk for PE and 59 low-risk controls were tested for sFlt-1, sEng and PIGF in three different temporal windows: 23 – 27, 28 – 31 and 32 – 35 weeks. The angiogenic profile of high-risk women was completely different from that of the controls, principally in women with prior PE and multiple gestations. In particular, women with prior PE had higher sFlt-1 and lower PIGF from 28 weeks until delivery [30]. However, there are two disadvantages in the use of these markers. First, the reliability of the assay: the biomarkers are present in the blood of pregnant women in very low concentrations [19]. Studies using circulating biomarkers of angiogenesis have produced conflicting and often confusing results and this may reflect either complex biology or lack of assay validation. This last point constitutes a critical component in biomarker research, and it is often the case that a biomarker proves to be not helpful in the clinic practice not because of the scientific rationale but rather from assay choice and lack of robust validation [31]. Second, they show similar patterns of appearance in cases of IUGR without PE, in particular sEng [32,33]; hence, they cannot be used for a specific prediction of PE.

2.3.3 Inflammatory proteins

Although inflammation has been associated with PE, its corre- lation and contribution to the pathogenesis remains unclear. Boij et al. analyzed a panel of 32 biomarkers in 114 women affected by PE, 31 with early-onset and 83 with late-onset form, and 100 controls. They found that PE was associated with decreased levels of IL-4, increased complement compo- nent C3a and pentraxin-3, more pronounced in the early- onset women. The Th1-associated chemokines CXCL10 and CXCL11 were significantly higher in PE women than in controls. This study showed that cytokines, chemokines and complement activation seem to be part of a Th1-like inflammatory response, more pronounced in PE, where chemokines might be more useful than cytokines as biomarkers [34].

2.3.4 Hypoxia-related factors

Recently, the role of hypoxia in determining PE has been reconsidered: oxidatively stressed syncytiotrophoblast over- secretes proteins that cause a cellular stress response. Irregular blood flow and intermittent oxygen supply are only two of the
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Multiple mechanisms involved in generating placental damage [35,36]. However, hypoxia is still to be considered an important pathogenic cause of PE, although it is not unique. Hypoxia-inducible factor 1-α subunit (HIF1A) is one of the most recent markers of PE investigated. HIF1A is oxygen sensitive, being rapidly inactivated and degraded in normoxia. Cell-free HIF1A mRNA in maternal plasma was reported to be increased in pregnant women with overt PE and IUGR [37]. There is some evidence for HIF1A being the molecular link between placental hypoxia and the downstream mediators of PE [38]. It was demonstrated that HIF1A is able to induce the anti-angiogenic factor sFlt-1 in placental explants [39]. In addition, endoglin, the membrane-bound protein that gives rise to its proteolytic product sEng, is upregulated by HIF1A [40], and it was recently clarified that sEng is induced by hypoxia in trophoblasts [41].

Recent reports are addressing attention to fetal hemoglobin (HbF) involvement in PE and the heme and radical scavenging protein α-1-microglobulin (A1M). The interest in this topic started in 2008 when a comparative study of gene and protein expression in placentas from patients with PE and normal pregnant women using microarray and immunohistochemistry was performed [42]. In total, 800 PE-associated genes were isolated and identified and 30 of them were significantly altered. On analyzing the pre- eclamptic placentas, an increased expression of HbF, both as mRNA and protein, was seen. The hypothesis was that HbF accumulation in the placenta caused injury to the barrier and, leaking into the maternal blood stream, caused endothelial damage. In order to evaluate the toxic effects of HbF, an ‘ex vivo’ placental perfusion model was used. The authors added HbF to the fetal circulation in the model and within 10 min PE manifested itself; after 1 h, signs of barrier damages were evidenced by the leakage of nutrient solutions from the fetal circulation to the maternal circulation [43]. To assess the potential anti-damage effect of A1M, a protein involved in antioxidant functions, mainly produced by the liver and responsible of binding free radicals and heme, the authors perfused the placental model with A1M; the HbF leakage from maternal blood to fetal circulation stopped and no structural damage was detected [43]. In the same period, Olsson and colleagues stated that the oxidative damage and leakage of the feto-maternal barrier results in increased maternal plasma concentration of HbF and induction of reactive oxygen species (ROS), hemoysis, and increased maternal plasma concentration of adult hemoglobin (HbA) in women collected just before delivery. HbA and ROS could induce an upregulation of A1M in placenta and maternal cells [44]. However, studies on the placenta or in term pregnancies can only speculate on the use of HbF and A1M as possible predictive markers of the disease, and studies on plasma women in the first trimester of pregnancy would serve to corroborate this hypothesis.

Recently, Anderson et al. followed a cohort of 96 pregnant women (60 with PE and 36 controls) analyzed between 10 and 16 weeks of gestation; they used a combined predictive model based on HbF/Hb ratio and A1M concentration [45]. These biomarkers were significantly elevated in serum from women with subsequent development of PE (p < 0.0001). This work mainly focused on late-onset form, as we can argue from the limited number of PE women (7 out of 20) that delivered before 34 weeks. Anyhow, early and late PE were not subjected to different analyses. The sensitivity in detecting women who later developed PE was 69% with 5% false positive rate, and AUC 0.89, while the optimal sensitivity chosen by authors from coordinate list of ROC curve representing as high sensitivity as possible for as low a screen positive rate as possible was 90% with 23% false positive rate. It is a natural consequence evaluating HbF and A1M as potential predictive markers of PE.

2.3.5 Metabolomics

There is a growing interest in the application of the metabolic profiles correlated to obstetric complications [46]. Metabolic profiles can reveal important information about the mechanisms that underlie PE, because every specific biochemical event causes the production of metabolites that are up- or downregulated even before the onset of the pathology. Bahado-Singh et al. in 2012 performed a case-control study of maternal plasma samples collected at 11 – 13 weeks of gestation comparing 30 women with early-onset PE and 60 unaffected controls. A nuclear magnetic resonance (NMR) spectroscopy analysis was used to identify and quantify metabolomic changes: between the two groups, 20 different metabolites were found. Four of this (citrate, glycerol, hydroxyisovalerate and methionine) seem to be highly predictive of early-onset PE especially if combined with uterine artery Doppler PI and fetal crown-rump length (detection rate of 82.6% at a false positive rate of 1.6%) [47]. In a subsequent study the same group performed an NMR-based metabolomics analysis on first trimester maternal serum in 30 cases of late-onset PE and 59 controls. Significant differences were seen between the two categories; combining these metabolites with maternal demographic characteristics, they obtained a model with 76.6% sensitivity at 100% specificity for late PE prediction, while by a simplified model using fewer predictors 60% sensitivity at 96.6% specificity [48]. Interestingly, two of the different metabolites presented strongly different profiles between PE women and controls: glycerol and carnitine, both relevant for lipid metabolism and mitochondrial energy productions based on lipids. Carnitine also has antioxidant functions. In the same study, the authors also compared 30 early-onset cases to the late-onset group. A relevant discrimination between the two categories was shown: glycerol, acetate, trimethylamine and succinate appeared to be the most significant metabolites to distinguish the two typologies of the disease.

Although metabolomics seems to be one of the most promising tools in studying PE, many environmental and lifestyle factors, such as diet, physical activity, smoking, time of day and so on, also contribute to changes in the metabolomic [48-50];
and need to be evaluated for the future use of this promising approach. Moreover, the current sample size is limited, and thus further studies involving a larger number of patients are mandatory to determine the variability introduced by demographic factors.

2.3.6 Cell-free fetal DNA and RNA

Fetal DNA and RNA released into maternal plasma is a well-known phenomenon that is being extensively studied in order to provide new tools for monitoring the pregnancy outcome. Quantitative aberrations in cell-free nucleic acids in maternal plasma have been shown to be associated with PE. Previous DNA-based assays through Y-chromosome specific sequence quantification (SRY or DYS14 loci) showed that PE substantially increases fetal DNA concentration in the plasma of male-bearing pregnant women [51-54]. Subsequently, the identification of universal DNA markers independent of fetal gender (the measurement of total cell-free DNA or the use of fetal epigenetic markers, such as DNA methylation) helps in overcoming this limitation. In particular, fetal and total DNA were analyzed based on methylation patterns in the Ras association (RalGDS/AF-6) domain family 1A (RASSF1A) promoter gene, methylated in placenta and unmethylated in maternal blood cells. Fetal sequences were quantified by quantitative real-time PCR after selectively removing maternal sequences by a methylation-sensitive restriction enzyme. The results obtained revealed that differentially methylated fetal and total DNA were elevated in the plasma of women with overt PE [55-57]. Recently, it has been reported that also in women that successively develop PE, elevated levels may be already seen in the first trimester as predictive marker of the disease [58]. Among specific mRNA sequences of fetal/placental origin, the transcript of the circulating corticotropin-releasing hormone (CRH) gene in maternal plasma was extensively studied. The levels of this hormone in maternal plasma rise exponentially throughout pregnancy and peak during labor till complete clearance after delivery [59]. It showed higher levels in PE than in uncomplicated pregnancies [55,59]. Moreover, Purwosunu et al. reported a correlation between the median values of CRH mRNA concentration and the severity of clinical symptoms in PE [60].

2.3.7 Predictive multiple marker models

Due to the fact that nowadays no single biomarker can predict PE, recent studies are concentrated on combining maternal anamnestic RFs, biophysical and biochemical markers in order to create a screening test with elevated predictive accuracy (Table 2) [29,61-67]. Since far, one of the best predictive accuracy obtained is still the model proposed by Poon et al. in 2009 [61]. They enrolled 7,797 women with a singleton pregnancy between 11 and 13 weeks, combining maternal characteristics, uterine artery Doppler PI, mean arterial blood pressure and two biochemical markers: PAPP-A and PIgf. The sensitivity for early-onset PE was 93.1 and 35.7% for late-onset forms, with a false positive rate of 5%. Another high-quality predictive model was proposed by Akolekar et al. in 2013: they found that screening by uterine artery PI, mean arterial blood pressure, PAPP-A and PIgf detected 96% of PE cases requiring delivery before 34 weeks and 54% of all cases of PE (early and late forms) at a fixed false positive rate of 10% [66]. These algorithms are very promising but need to be further tested in order to achieve a satisfactory predictive accuracy universally.

2.4 Conclusion

Only diseases with a preclinical phase, during which the disease can be detected by a marker, are amenable to screening. Based on our current knowledge, we can state that PE fulfills the prerequisites for a disease for which screening by multiple markers may be appropriate. An early identification of pregnant women at risk of developing PE is of primary importance in order to start early possible preventative treatments and to allow a better allocation of economic resources.

3. Expert opinion

Until now all the major screening studies for PE prediction through biochemical and biophysical markers were performed in the context of clinical trials and not in the more realistic context of clinical routine and pregnancy management. Although these results are encouraging, the 2013 ACOG task force focused attention on the fact that no evidence has been located to support the hypothesis that predicting PE, in particular early-onset PE, can be followed by therapeutic interventions or close follow up that might improve maternal or fetal outcomes [2]. Repke reported recently that it is useless to implement a PE screening test until we have a proven intervention to prevent PE in women that are diagnosed as at risk for developing the disease [4]. We will illustrate the more trialed preventative treatments, often showing contrasting results.

3.1 Antiplatelets and antithrombotic agents

Since it was hypothesized that aspirin during pregnancy could improve trophoblast invasion and reduce inflammation, several studies concentrated on the possible use of this drug as a preventive treatment of PE. In the last years contradictory results were achieved: an initial small trial suggested positive effects for high-risk pregnant women [68]. This findings were not confirmed by a successive large controlled trial conducted in 1994 by the CLASP group (Collaborative Low dose Aspirin Study in pregnancy) [69]. In 2007, a Cochrane meta-analysis marked the turning point about the ‘Aspirin dilemma’: 59 trials with > 37,000 pregnant women found a reduction of 17% of PE development, particularly evident in high-risk women [70]. Moreover, a recent metaanalysis including 27,222 women showed that starting low-dose aspirin treatment before or at 16 weeks of gestation can reduce perinatal death, PE, severe PE and preterm birth more significantly than starting the treatment after 16 weeks [71].
Concerning antithrombotic agents, the use of heparin alone as a preventative treatment for PE in women without a diagnosis of thrombophilia has not been evidenced yet [72]. Furthermore, information about serious adverse infant and long-term childhood outcomes are still lacking [73].

### 3.2 Calcium supplementation

Calcium supplementation as a preventative treatment for hypertensive disorders in pregnancy has been studied for a long time; in particular, it has been observed that women with high levels of calcium intake, such as Guatemala Indians and Ethiopians have a low incidence of PE [74]. Recently, it has been clarified that its role is relevant only in a subgroup of women from South America and other countries, where nutrition is characterized by low calcium intake [75].

In particular, in 1997, Levine found that calcium supplementation in a large cohort of nulliparous US women without obstetrical conditions or preexisting diseases did not reduce PE development [76]. In 2010, a Cochrane metanalysis of 15,730 women involved in 13 trials compared at least 1 g/day of calcium supplementation to placebo or no intervention. The results showed a significant reduction in PE development in women from South America and other countries with low calcium intake, but no significant effect in other regions [75].

### Table 2. Combined models for PE prediction in chronological order.

<table>
<thead>
<tr>
<th>Author</th>
<th>Population</th>
<th>Markers</th>
<th>Gestational age at testing</th>
<th>Detection rate</th>
<th>False positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright et al. 2012 [63]</td>
<td>58,884 women with singleton pregnancy: 1,426 PE and 57,458 controls</td>
<td>Maternal history Doppler ut PI MAP</td>
<td>11 – 13 weeks</td>
<td>90% early-onset</td>
<td>10</td>
</tr>
<tr>
<td>Myatt et al. 2012 [64]</td>
<td>683 women with singleton pregnancy: 174 PE and 509 controls</td>
<td>Maternal history ADAM12 PAPP-A PP13 PlGF sFlt-1 sEng</td>
<td>9 + 0 – 12 + 6 weeks</td>
<td>46% overall PE</td>
<td></td>
</tr>
<tr>
<td>Myers et al. 2013 [29]</td>
<td>3,529 women with singleton pregnancy: 187 PE and 3,342 controls</td>
<td>Maternal history PlGF sEng sFlt-1</td>
<td>14 – 16 weeks</td>
<td>45% overall PE</td>
<td>5</td>
</tr>
<tr>
<td>Scazzocchio et al. 2013 [65]</td>
<td>5,170 women with singleton pregnancy: 136 PE and 5,034 controls</td>
<td>Maternal history Doppler ut PI MAP PAPP-A fβ-hCG</td>
<td>11 – 13 + 6 weeks</td>
<td>69.2% early-onset</td>
<td>5</td>
</tr>
<tr>
<td>Park et al. 2014 [67]</td>
<td>262 women with singleton pregnancy: 8 PE and 254 controls</td>
<td>PAPP-A βFlt-1/PlGF ratio</td>
<td>11 – 13 weeks</td>
<td>87.5% overall PE</td>
<td>5</td>
</tr>
</tbody>
</table>

of calcium versus placebo and found a reduction of the average risk of PE, more pronounced in high-risk women and in those with a low calcium intake (< 600 mg/day). Low calcium intake is not frequent in developed countries, thus calcium supplementation could be a possible preventative treatment in low and middle income countries [77,78].

3.3 Vitamins C and E
In July 2010, Roberts and his collaborators published results of a large, multicenter, randomized, double-blind trial on the use of vitamins C and E to prevent complications related to hypertensive disorders in pregnancy [79]. The hypothesis was that the antioxidant effect of vitamins administered between 9 and 16 weeks of gestation in a nulliparous, low-risk population had to improve maternal and fetal prognosis. The study did not reveal a significant advantage resulting from the supplementation of vitamins; thus it has been suggested that vitamins prophylactic treatment alone is not sufficient to improve the profile risk of hypertensive disorders of a low-risk population.

3.4 Dark chocolate
In December 2013, Mogollon et al. published a review with the aim of analyzing the association between chocolate consumption and prevention of PE [80]. They hypothesized that flavanol-rich chocolate consumption reduces the risk of PE via improvement of endothelial function in pregnant women. Theobromine, a major constituent of dark chocolate, could account for, or contribute to, enhancing the effect of flavonoids, antioxidants capable of inducing NO-dependent vasodilatation. Few observational and experimental studies have been designed to evaluate the associations between chocolate consumption and/or theobromine with PE; moreover, the results were conflicting [81-83]. Additional experimental trials are necessary to better evaluate the benefits of chocolate consumption on the risk of PE.

3.5 Lifestyle modification
Obesity is a recognized RF for PE. Obese women have a cardiovascular profile that predispose them to hypertension disorders during pregnancy [84]. Alimentary restrictions in normal-weight women, in particular sodium restriction, and bed rest do not prevent PE development. On the other hand, there is no evidence of PE prevention through physical exercise. A recent study found a correlation between physical activity in early pregnancy and a worse placental development, evaluated in terms of lower PAPP-A and PI GF levels and lower placental volume in 94 nulliparous women [85]. Furthermore, a randomized controlled trial involving 116 women at high risk for PE development (for chronic hypertension or prior PE) compared two groups: 58 women performing physical exercise with a stationary bike at controlled intensity once a week and 58 not engaged in any activity. There were no differences between groups in terms of delivery and maternal and fetal outcomes [86]. Larger controlled trials are needed to establish the utility of performing physical exercise in the prevention of PE.

3.6 New targets
In physiological pregnancy, NO contributes to vasodilatation, decreased responsiveness to vasopressors and increased uteroplacental blood flow. In PE, availability of NO is reduced, but it is unclear whether there is reduced production or increased degradation [87]. There are contradictory results among therapeutic agents that increase NO levels, or NO donors and precursors. Some studies have demonstrated that administration of NO donors is associated with a reduction in uterine artery resistance in women with PE suggesting that NO may have a role in prevention and treatment of PE. However, in 2007, a Cochrane review that included six trials for a total of 310 women enrolled established that there was insufficient evidence to draw reliable conclusions about whether NO donors and precursors prevent PE or its complications [88]. A second topic proposed as a possible preventative measurement for PE was the sFlt-1 apheresis treatment. It has been suggested that excess placental derived sFlt-1 mediates the signs and symptoms of PE and elevated circulating levels are associated with clinical PE. A pilot study of extracorporeal removal of sFlt1 in very preterm PE was performed by Thadhani et al. to test the potential of apheresis treatments in prolonging pregnancy [89]. They demonstrated that apheresis with dextran sulfate cellulose columns reduces circulating sFlt-1 in a dose-dependent fashion in women with PE. In 3 patients with very preterm PE (gestational age at admission 28, 30 and 27 + 4 weeks), repeated apheresis treatments reduced circulating levels of sFlt-1 and proteinuria, stabilized blood pressure and potentially prolonged pregnancy without apparent adverse events occurring to either mother or fetus. In particular pregnancy lasted for 15 and 19 days in women treated twice and 23 days in a woman treated four times [89]. The authors treated only a limited number of patients, and as they themselves suggested, only randomized trials with additional patients will allow definitive conclusions on how apheresis could really prolong pregnancy in PE patients. A complementory approach to subtract sFlt-1 from maternal circulation include administration of VEGF (its natural ligand). A recent animal PE model on pregnant rats evidenced a decrease of sFlt-1, whose expression was induced by administration of recombinant VEGF through adenovirus injection, with subsequent decrease of hypertension and proteinuria. This could be a future preventative treatment, assuming that women with high sFlt-1 levels are at higher risk of developing PE [90].

A new pilot study involving a different animal model and an alternative PE preventative intervention showed that, in gravid rats, PP13 can significantly improve placentation, in terms of trophoblast invasion [91]. Replenishing PP13 in women with low serum levels early in pregnancy may help to prepare their vasculature for pregnancy. In vivo PP13 function is still unknown, especially when circulating
in the maternal bloodstream, but Huppertz et al. suggest PP13 replenishing in women with a first trimester low dosage as a possible future pharmacological approach to PE [20].

In 2013, Hansson et al. suggested a different potential therapeutic way: the use of A1M to prevent oxidative damage [92] based on the experiment proposed by May et al. [43]. Placentas were perfused with free Hb in the fetal circulation while A1M was added to the maternal circulation to simulate potential future intravenous therapy. The effects of A1M were clear: the Hb leakage from the fetal to the maternal circulation ceased completely, and structural damage was not detected after A1M treatment. Moreover A1M treatment led to upregulation of genes coding for the structural components of the extracellular matrix, which indicates that a healing process is initiated by A1M.

Finally, whilst our understanding of the end-stage pathophysiology of PE has come a long way in the past decade, there is still a huge amount we do not understand about the root cause of these disorders, and how stem cells (either trophoblast stem cell or mesenchymal stem cell [MSC]) could contribute to normal and abnormal placentation [93]. MSCs seem to be particular promising in the treatment of tissue injury and degenerative diseases. In all pre-clinical and clinical studies using MSCs, migration into the damaged tissue is a crucial process for the efficacy of treatment. Therefore, understanding the tissue and cell specific factors that regulate MSC trafficking, both from the site of administration and to/from their stem cell niche within the placenta will be crucial for effective administration of MSCs. The ability of MSCs to be delivered through an arterial route suggests that they could be administered to the placenta in utero via the umbilical artery, a delivery method previously established for fetal transfusions in rhesus disease [94]. Allogenic transplantation of stem cells is likely to be necessary to treat placental disorders. Fortunately due to their unique immunophenotype, allogenically transplanted MSC are well tolerated. When considering the situation in utero it is unlikely the primary host, the fetus itself, would reject the transplanted cells, as its immune system is relatively immature and still learning to recognize self from non-self antigens; thus, the ability of allogenically transplanted placental stem cells to successfully hide from the maternal immune system is imperative to the success of such treatments. If allogenic transplantation does not prove to be therapeutically viable, an alternative approach could be to stimulate stem cell function pharmacologically using peptides or nanoparticles specific to placental stem cell populations [93]. The major question that must be addressed in considering stem cell based therapies for the treatment of placental disorders is the potential risks involved. Treatments for placenta disorders would clearly need to be extensively validated, first in in vitro models to understand how stem cells injected into the placenta directly or via the umbilical circulation may migrate and function within the tissue, and then in hemochorial animal models in order to evaluate not only their safety and efficacy, but the potential for fetal chimerism as a result of cellular engraftment outside the placenta, and the long-term effects that this may have.

Although preventive treatments have been studied extensively, an effective intervention to prevent the development of PE has not yet been discovered. Prevention and prediction of PE are strictly connected: early prediction without the possibility of correcting the profile of a woman at risk does not make sense. In order to help pre-eclamptic women manage clinical information, the focus needs to be on PE prevention and on better assessing the level of cardiovascular risks that such women may face later on in life [95].

### Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.
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Papers of special note have been highlighted as either of interest (●) or of considerable interest (★★) to readers.

5. Discusses the concept that new insights in pathogenesis and prediction of PE could help in discovering new targets for pharmacological intervention.
7. Discusses the requested criteria for a PE screening test in order to be reliable.
13. It is a very important systematic review that highlights the fact that uterine artery Doppler ultrasonography provides a more accurate prediction of PE when performed in the second trimester than in the first trimester.
19. It is a systematic review that focuses on the contrast between the good sensitivity of an increased uterine artery Doppler as predictor of early-onset PE and the poor accuracy in detecting late-onset form.
22. Critically discusses current knowledge regarding early markers of preeclampsia to identify priorities and opportunities for future research.
23. Consider issues that may need to be addressed in future recommendations and highlight key issues in cost effectiveness and national policies concerning prediction and early screening for the risk of developing pre-eclampsia.
Predictive biomarkers of PE and effectiveness of preventative interventions for the disease


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• Represents one of the best predictive multiple marker models that combine maternal anamnestic risk factors, biophysical and biochemical markers, reaching a very high accuracy in PE prediction.


• Compares early versus late administration of low-dose aspirin and highlights how low-dose aspirin initiated at ≤ 16 weeks of gestation is associated with a greater reduction of perinatal death and other adverse perinatal outcomes than when initiated at > 16 weeks.


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