Diagnostic biomolecules and combination therapy for pre-eclampsia

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Abstract
Pre-eclampsia (PE), associated with placental malperfusion, is the primary reason for maternal and perinatal mortality and morbidity that can cause vascular endothelial injury and multi-organ injury. Despite considerable research efforts, no pharmaceutical has been shown to stop disease progression. If women precisely diagnosed with PE can achieve treatment at early gestation, the maternal and fetal outcomes can be maximally optimized by expectant management. Current diagnostic approaches applying maternal characteristics or biophysical markers, including blood test, urine analysis and biophysical profile, possess limitations in the precise diagnosis of PE. Biochemical factor research associated with PE development has generated ambitious diagnostic targets based on PE pathogenesis and dissecting molecular phenotypes. This review focuses on current developments in biochemical prediction of PE and the corresponding interventions to ameliorate disease progression, aiming to provide references for clinical diagnoses and treatments.

Keywords: Preeclampsia, Diagnosis, Biomarkers, Therapeutics

Background
Pre-eclampsia (PE) is a hypertensive complication during pregnancy that occurs at a rate of 3–5% worldwide [1], which has been subdivided into early-onset forms less than 34 weeks of gestation and late-onset forms of more than 34 weeks of gestation [2]. The pathophysiology of PE is still unclear. Increasing scientific evidence have suggested a series of stages of disease development (Fig. 1) [3–6]. Firstly, under the influence of genetic factors, environmental factors, and immunological factors, the placental insufficiency originates during the first and second trimesters of placentation [3, 7]. The genetic factors mainly include the imbalance of maternal and fetal soluble fms-like tyrosine kinase 1 (sFlt1) single nucleotide polymorphisms, decidual transcriptome, and heme oxygenase isofrom [8]. Meanwhile, diabetes mellitus, hyperglycemia, and chronic hypertension in pregnancy can greatly raise the morbidity of PE [9]. Additionally, immunological factors such as placental T helper 1 cell predominance, decidual natural killer cells and immunogenic human leukocyte antigen-C on trophoblasts will also affect the PE progress. Then, during the late second and third trimesters, the invasive trophoblast superficial invasion narrows maternal vessels, leading to placental ischemia [8]. The oxidative stress and persistent hypoxia of placenta will increase the levels of sFlt1 and soluble endoglin (sEng), syncytial decries, and pro-inflammatory cytokines in the maternal circulation [10]. High levels of sFlt1 and sEng can result in systemic vascular dysfunction including proteinuria, hypertension, eclampsia and HELLP syndrome (hemolysis, elevated liver enzymes and low platelets). Accordingly, PE patients have a larger probability to deliver small-for-gestational age infants.

Appropriate antenatal care, management and treatment are promising to reduce the risk of PE with early identification. Currently, the diagnosis of PE is...
Genetic factors

- SNPs in sFlt1
- Mutations in corin, TTN
- Transcriptionally inert
- L-3 hydroxyacyl-CoA dehydrogenase deficiency

Environmental factors

- Hyperglycemia/Diabetes
- Chronic Hypertension
- Obesity: BMI > 30
- Preexisting cardiovascular dysfunction

Immunological factors

- T helper cells shift to Th1 phenotype
- Inhibition of uterine NK cells
- HLA-C trophoblasts
- Elevated complement levels

Fig. 1 Pathophysiology of preeclampsia. Genetic factors, environmental factors, and immunological factors are contributing factors to preeclampsia (PE). Genetic factors mainly include single nucleotide polymorphisms in sFlt1, mutations in corin and Titin genes, transcriptionally inert and L-3 hydroxyacyl-CoA dehydrogenase deficiency. Also, maternal hyperglycemia, diabetes, chronic hypertension, obesity and pre-existing cardiovascular system may also induce the PE. Immunological factors mainly include the shift from T helper cells to Th1 phenotype, inhibition of uterine NK cells, increased HLA-C trophoblasts and elevated complement levels. These factors can lead to impaired trophoblast invasion and maternal vessel narrowing, causing placental ischemia and abnormal placentation. Then, the levels of circulating soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) will increase, that reduces angiogenesis and causes systemic vascular dysfunctions such as proteinuria, liver dysfunction, headache, stroke and hypertension. (Abbreviation: BMI, body mass index; sFlt1, soluble fms-like tyrosine kinase 1; sEng, soluble endoglin; SNP, single nucleotide polymorphism; TTN, Titin)

fundamentally based on the maternal characteristics including age, origin, body mass index (BMI), PE history, chronic hypertension, and conception method, followed by extrapolating and incorporating these characteristics into a designed mathematical formula to calculate the potential risk of PE occurrence [11]. By this traditional way, only 40% of all PE at false-positive rate (FPR) of 10% can be identified [12]. Besides, blood pressure (BP) is a routine test in antenatal care for PE assessment, and an exceeding threshold of 140/90 mmHg has been recognized as hypertension. Since the considerable variability among each individual hinders an accurate assessment of BP, the PE detection rates using BP ranges from 8 to 93% [13]. Based on these, the mean arterial pressure (MAP), showing higher predictability than systolic or diastolic BP, has been highly recommended by validated automated devices [14]. Patients who develop PE demonstrate the increase in MAP at 11–13 weeks of gestation, but the detection rate of using MAP in isolation was only 30% for early-onset PE at a 10% FPR, and the combined
MAP with maternal characteristics only raised the rate to 62.5% [13]. To sum up, novel biomarkers and diagnosis techniques are urgently needed to improve the efficiency of current routines for PE diagnosis.

Maternal uterine artery blood flow is essential for the intrauterine environment, placental function and fetal growth. During the development of PE, abnormal placental perfusion leads to trophoblast damage, primary trophoblast invasion defects, and ultimately inadequate transformation of the maternal uterine vascular system [15]. The blood flow in the maternal blood vessels can be monitored by the uterine artery Doppler ultrasound and recorded by pulsatility index (PI). Increased uterine artery PI induced by poor placental perfusion is a typical sign of PE development [16]. The detection rate of using PI in isolation is approximately 77.3% for early-onset PE and 26.8% for late-onset PE at a 10% FPR, while the combination of PI with maternal characteristics enhances the detection rate of early-onset PE to 100% with minor improvement to 46.5% for late-onset PE at the same FPR [17]. If PI and MAP are used synergistically with mathematically calculating PE risks using maternal characteristics, it will exhibit a high detection rate of 89% for early-onset PE, but still only 57% for late-onset PE at a FPR of 10% [18]. Since PE-induced pulmonary embolism commonly causes increased levels of mean platelet volume (MPV) and red cell distribution width (RDW), the blood tests detecting platelet count, mean platelet volume, and red cell distribution width have been raised as significant markers of PE [19, 20]. Although hemo-diagnosis possesses several advantages of fast and low-cost, their specificity and sensitivity are still unsatisfactory. That is mainly because multiple interferences in plasma may make accurate detection difficult, including the effects of detection time, physiologic and metabolic changes on the blood components [21, 22]. As the major cases of the PE occur in late-onset forms, alternative biomarkers remain a pressing need.

Current treatments for PE mainly include the prenatal aspirin for women at high risk, betamethasone for PE patients in early stage, intravenous magnesium sulfate and real-time postpartum BP monitoring, though timely delivery of the fetus remains the only ultimate treatment [23]. Currently, the American College of Obstetrics and Gynecology does not recommend medication for mild to moderate hypertension in PE, because it cannot reduce the risk of disease progression and may increase the risk of fetal restriction [24]. Treatment of PE patients with severe hypertension requires pharmacological therapy such as labetalol, nifedipine and methyldopa [24]. However, recent evidence from animal studies suggests that amlodipine may be superior to nifedipine due to its induction of Arrb1 and subsequent downregulation of the AT1-B2 receptor complex, but more clinical evidence is needed [25]. Although there exists some challenges in current management of PE, innovative medical therapies are emerging. In this review, we discuss promising PE diagnostic tools by focusing on the links among pathways associated with PE pathogenesis and propose the applications of cutting-edge findings on impeding PE development.

Methods
We studied the prediction, diagnosis and treatment of preeclampsia. A systematic search of PUBMED was performed to identify relevant studies by using the keywords of diagnosis or biomarkers or therapeutics of preeclampsia. Although we strived to include the current evidence on the topic, this is not a systemic review of the literature.

Novel biochemical markers with PE occurrence
To date, continuous understanding of pathogenic mechanisms, especially those underlying molecule changes in maternal blood, has inspired various targeted biochemical markers to identify PE disease process. Figure 2 shows promising predictive markers with their unique functions during PE pregnancy.

Placental associated proteins
Proteases play key roles in the placental remodeling process. The secretion of placental protein 13 (PP13), pregnancy-associated placental protein A (PAPP-A) and a disintegrin and metalloprotease 12 (ADAM12) positively correlates with the size of the placenta [26]. Therefore, reduction in these proteins can be recognized as the first trimester biomarkers of PE. PP13 from the galectin family produced by the syncytiotrophoblast of fetomaternal interference is reduced in PE patients even at 9–12 weeks of gestation [27]. The use of PP13 with PI leads to 90% detection rate in PE [28], and the combination of PP13 and PAPP-A reaches 91% of sensitivity and specificity at the first and second trimesters of PE [29]. Moreover, a significant decline in ADAM12 and PAPP-A levels at 11–14 weeks of gestation has been observed in PE patients compared with control group. In contrast, another study claimed that ADAM12 and PAPP-A in combination with maternal characteristics only identified 50% and 48% of patients who developed PE at a fixed FPR of 10%, respectively [30]. The prediction of PE has been analyzed by logistic regression using ADAM12 multiples of median (MoM), PAPP-A MoM, and uterine Artery Doppler pulse index MoM. To compare the screening efficiency of the nonparametric U statistic model, the sensitivity, specificity, and area under the subject operating characteristic curves were measured. Accordingly, combining the early pregnancy parameters or even the
Various circulating hormones, including human chorionic gonadotropin (hCG) [32, 33] and corticotropin-releasing hormone (CRH) [34], have been reported to elevate in PE cases at the second trimester, indicating their prognostic prediction capability in the late-onset PE. Nevertheless, no further clinical trial within diverse populations has proven their high sensitivity in PE detection. Moreover, the endothelial dysfunction is a key mediator during PE [35]. First, the placental selectin involved in leucocyte trafficking changes in PE progression. Increased E- and L-selectin but decreased P-selectin have been detected in the early-onset of PE [36]. Pentraxin 3 (PTX3), an inflammation biomarker, has been observed to elevate in the first trimester at 11–14 weeks in patients who subsequently developed PE [37, 38]. Similarly, asymmetric dimethylarginine (ADMA) also found to be increased in the first trimester at developing early-onset PE [39]. PTX3 and ADMA might possess mechanistic links between vascular effects in PE and later cardiovascular risk [40], and the abnormal elevations in both were able to segregate high risk PE patients at early stage [41].

Furthermore, renal blood flow and glomerular filtration rate (GFR) were commonly observed to be decreased in PE patients [42]. Proteinuria will be raised responding to the injury of the glomerular capillary. Currently, the gold standard for PE diagnosis is the 24-h urine collection that

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**Fig. 2** Current biochemical markers for early prediction of PE. A series of biochemical markers has been identified to predict the PE in the first and second trimesters, including exosomes, cell-free fetal DNA, cell-free maternal RNA, angiogenic markers and placental associated proteins. These markers possess specific detection time during the stages of pregnancy. (Abbreviations: ADMA, asymmetric dimethyl arginine; ADAM12, A disintegrin and metalloprotease12; CRH, corticotropin-releasing hormone; hCG, human chorionic gonadotropin; PTX, Pentraxin; PIGF, placental growth factor; PP-13, placental protein-13; PAPP-A, pregnancy-associated plasma protein A; sEng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase 1; VEGF, vascular endothelial growth factor; cffDNA, cell-free fetal DNA; miR, microRNA.)
assesses proteinuria. Moreover, the 12-h urine collection for proteinuria also shows high clinical efficiency (sensitivity, 92%; specificity, 99%) with convenient management [43]. However, the results may be unreliable, because the inaccurate collection may affect the results of PE diagnosis. The actual prevalence of significant proteinuria is varied by laboratory processing methods [44].

To combat this, more convenient and accurate diagnostic tests that are capable of prognosticating kidney injury of PE patients have been developed recently. Valdes et al. [45] reported that the protein/creatinine ratio (PCR) yielded a high positive predictive value of 96.4% at FPR 4.4% (AUC, 0.802) which could substitute the 24-h urine collection. Unfortunately, creatinine is not sensitive to the real GFR decline, as it could be largely affected by the secretion from renal tubules, muscle mass, protein uptake, and physical activity [46]. Instead, Cystatin C filtered by glomerulus provides potential advantages for accurate PE prediction. Patients with high Cystatin C concentration have been widely reported to have a risk for subsequent PE development [47–49]. To conclude, more accurate studies focusing on the efficiency of placental associated protease are needed.

**Angiogenic markers**

Angiogenesis in the placental vascularization refers to the remodeling of new blood capillaries from the pre-existing vasculature mediated by the synergistic actions of pro- and anti-angiogenic factors [50]. Both endothelial cell proliferation and migration are activated by vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) that restore the vascular integrity [51]. Soluble Feline McDonough Sarcoma-like tyrosine kinase 1 (sFlt-1), a decoy receptor, sequesters VEGF and PlGF interaction with the VEGF-1 receptors on endothelial cells to impair the endothelial cell–cell communication and cause vascular dysfunction and hypertension [52]. As a result, PE patients normally show increased circulating sFlt1 concentrations but decreased levels of VEGF and PIGF. Single PIGF determination has been reported to predict PE with a sensitivity of 100% at a concentration threshold of 120.16 pg/mL and a diagnosis accuracy of 70.8%, while sFlt-1 only exhibited the diagnostic accuracy of 76.9%, sensitivity of 73.1% and specificity of 80.8% [53]. Hence, Zeisler et al. [54] suggests that a sFlt-1/PIGF ratio of less than 38 can discover the short-term absence of PE in women with suspicion of PE. A recent study demonstrated that PE diagnostic performance using the sFlt-1/PIGF ratio can achieve 95% sensitivity and 91% specificity which is superior to VEGF, PIGF or sFlt-1 [55]. Lately, several studies have attempted to use the VEGF genetic polymorphisms for early prediction of PE but there remains a lack of clinical competence [56, 57].

Chronic placental hypoxia exposure to the pro-inflammatory cytokines gradually increases the secretion of transforming growth factor-β (TGF-β) superfamily including inhibin-A, activin-A and soluble endoglin (sEng), which can be considered as endocrine markers for disorder prediction [58]. However, sEng-based diagnostic accuracy in maternal serum was only shown with 62.1% sensitivity and 56.8% specificity [53]. Even the combinations of sEng and PP13, PAPP-A, ADAM12, inhibin A or activin A only achieved 60%-80% sensitivity and approximately 80% specificity in the first or early second trimester [59], which causes poorer results than that of the sFlt-1/PIGF.

Overall, the angiogenic markers may be useful as a screening test, but no solid evidence performs to date documenting clinical implementation. The clinical studies are limited by single national region, small scale of participant population and lacking convenience sampling.

**Cell-free fetal DNA**

Maternal blood containing the placenta-derived cell-free fetal DNA (cfDNA) have provided a new way for non-invasive prenatal testing (Table 1). CfDNA plasma level increases during PE pregnancy at an early gestational age.

| Marker gene to quantify | Gestational age (in weeks) | Results in PE prediction |
|-------------------------|----------------------------|-------------------------|
| Hypermethylated RASSF1A | 8–17                      | cfDNA concentration at cutoff value of 7.49 GE/ml showing 100% sensitivity and 50% specificity [60] |
|                         | 11–13                     | cfDNA concentration at cutoff value of 512 GE/ml showing 100% sensitivity and 50% specificity [60] |
|                         | 15–28                     | cfDNA concentration increased 3.3-fold [59] |
|                         | 21–40                     | The positive correlations of hypermethylated RASSF1A with PAPP-A, PP-13 and urine protein in PE [59] |
| DSCR3, RASSF1A, HYP2    | 6–14                      | The combination of DSCR3, RASSF1A, HYP2 and PAPP-A with 66.9% of detection rate at 10% of FPR [58] |
| Y chromosome specific gene | 15–20                   | cfDNA concentration at cutoff value of 2.62 GE/ml showing 90% sensitivity and 85% specificity for early-onset PE [64] |
and can reflect the placental apoptosis and necrosis [60]. The principle of cffDNA analysis is based on the promoter, RASSF1A gene (Ras association domain family 1 isoform A), which was hypermethylated in placenta but hypomethylated in maternal blood cells [61, 62]. Saraswathy et al. [63] observed hypermethylated RASSF1A concentrations elevated 3.3-fold in PE before the occurrence of clinical symptoms. At gestational age of 8–17 weeks, the cffDNA concentration turned positive before the PE onset, with 100% sensitivity and 50% specificity [64]. This is consistent with a recent study, in which maternal blood at 11–14 weeks of pregnancy estimating a cut-off value of cffDNA concentration at 22.54 GE/ml could predict the PE with 85.0% sensitivity and 81.8% specificity [65]. Except for RASSF1A, other gene makers have been demonstrated to identify the cffDNA. For instance, the Sex-degenerming region Y [66–68], β-globin [68] and DYS [60] have shown great potential for PE prediction. As shown in Table 1, the quantity of cffDNA is a promising marker [69]. Moreover, further studies are necessary to minimize the heterogeneity of population scale, result assessments and evaluation methods.

Cell-free RNA
Plasma cell-free RNA (cfRNA), a non-invasive manner, possesses a marked and stable level in the early gestation of PE patients (Table 2). Thus, cfRNA can reveal the pregnancy progression and determine the PE risk months before clinical symptoms present. The changes of cfRNA are enriched for genes specific to the neuromuscular, endothelial and immune cells and tissues, reflecting the correlation between PE progression and maternal organ health [70–73]. According to Mira et al., the identification and independent validation of a panel of 18 genes measured between 5 and 16 weeks of gestation can form the basis of a liquid biopsy test, shown in the Table 2 [74]. The cfRNA extracted from a single blood draw can track the pregnancy progression at the placental, maternal and fetal levels and reliably predict PE with a sensitivity of 75% and a positive predictive value of 32.3% [75]. Notably, the cfRNA characteristics are independent of various clinical factors including the maternal age, body mass index, and race, cumulatively accounting for less than 1% of the model variance. cfRNA also provides molecular evidence for the pathogenesis of PE caused by early placental abnormalities and systemic endothelial dysfunction [75]. Regardless of the type or severity of onset, placental signaling in PE is weakened, and the platelets and endothelial cells drive changes in cfRNA in PE patients, especially before the 20-week gestation. The increase in cell type-specific cfRNA may occur in part through the cellular signal transduction and secretion. Congenital and adaptive immune systems are also responsible for changes in cfRNA associated with significant metastases in the bone marrow, T cells, B cells, granulocytes, and neutrophils, consistent with previous studies on maternal placental interfaces and PE [76–78]. Generally, cfRNA has been considered as a novel, efficient and non-invasive method to predict PE in early stage and discover the underlying signaling pathways of PE pathology.

Exosomes
Exosomes secreted by placenta increase in the PE maternal circulation, and their abilities for PE pathophysiology evaluation and disease prediction have been investigated [79–81]. Plasmatic exosomes from PE patient deliver abundant sFlt-1 and sEng to endothelial cells, causing vascular dysfunctions [82]. As mentioned before, a candidate marker PlGF also presented in exosomes, achieving 100% sensitivity and 78.6% specificity of PE prediction [83]. As PE can disrupt the immune balance, the quantification of exosomal Th1/Th2 cytokines including increased IL-2 and TNF-α with decreased IL-10 shows clinical significance in PE diagnosis [84]. In addition to the effect on protein, the placental-derived exosomes can also alter the RNA cargo [85, 86]. A study mapping the microRNA profiles in plasma exosomes extracted from maternal plasma of PE patients compared with those from normal pregnancies showed significantly different expression levels of miR-134, miR-376c, miR-486-3p and miR-590-5p in the first trimester (2–12 weeks) [87]. Moreover, novel miR-483, miR-424, miR-101, miR-203a-3p and miR-548c-5p were reported to be downregulated [88–92], while miR-431, miR-15a-5p, miR-125a-5p, miR-486–1-5p and miR-486–2–5p was aberrantly upregulated in the exosomes of PE patients [93–96]. Based on these identifications, exosomal miRNAs as a critical factor significantly expands the prospects for PE prediction. New detection methods such as microarrays chip, molecular beacons, and electrochemical sensors show the sensitivity and specificity for evaluating exosomes with heterogeneous subtypes [97], but systemic high-throughput analysis of exosomal RNA is still challenging as it translates from bench to bedside.

Single-cell transcriptomes
The single-cell RNA sequencing offer unique opportunity for identifying the abnormal gene expression with high resolution in tissues and organs including the trophoblast-decidual interactions [98]. The Illumina Hiseq and Genome Analyzer II systems are popular tools in the placental research and have been extensively used thanks to the low-cost. The first single-cell atlas of the maternal–fetal interface was reported in 2018, revealing the landscape of early pregnancy. An array of unique
Table 2  Preeclampsia prediction relies on 8 increased cfRNA genes and 10 decreased cfRNA genes (Summarized from Moufarrej et al., 2022 [70])

| Gene trends | Marker gene to quantify | Biological process | Molecular function |
|-------------|-------------------------|--------------------|-------------------|
| Enriched in PE DERA (Deoxyribose-phosphate aldolase) | - Pentose-phosphate shunt | - Deoxyribose-phosphate aldolase activity | |
| KIAA 1109 | - Synaptic vesicle endocytosis | - Protein binding | |
| NMRK1 (Nicotinamide riboside kinase 1) | - NAD metabolic process | - Ribosynicotinamide kinase activity | |
| PI4KA (Phosphatidylinositol 4 kinase alpha) | - Multi-organism membrane organization | - Kinase activity | |
| PRTFDC1 (Phosphoribosyl transferase domain containing 1) | - Guanine salvage | - Protein homodimerization activity | |
| Y_RNA | - GMP catabolic process | - Hypoxanthines phosphoribosyltransferase activity | |
| Y_RNA | - Purine ribonucleoside salvage | - Protein homodimerization activity | |
| YWHAQPS (YWHAQ pseudogene 5) | - Guanine salvage | | |

Decreased in PE CAMK2G (Calcium/ calmodulin dependent protein kinase II gamma) | - Nervous system development | - Calcium-dependent protein serine/ threonine phosphatase activity | |
| FAM46A (Terminal nucleotidytransferase 5A) | - Protein phosphorylation | - Identical protein binding | |
| LRRCS8 (Leucine rich repeat containing 58) | - Regulation of neuron projective development | - Protein homodimerization activity | |
| MYLIP (Myosin regulatory light chain interacting protein) | - Regulation of skeletal muscle adaptation | | |
| NDUVF3 (NADH: ubiquinone oxidoreductase subunit V3) | - Nervous system development | - Ubiquitin protein ligase activity | |
| PYGO2 (Pygopus family PHD finger 2) | - Protein phosphorylation | - Cytoskeletal protein binding | |
| RNF149 (Ring finger protein 149) | - Regulation of low-density lipoprotein particle receptor catabolic process | - Metal ion binding | |
| RNF149 | - Mitochondrial electron transport | - NADH dehydrogenase activity | |
| TFPI11 (Tuftelin interacting protein 11) | - NADH to ubiquinone | - Protein binding | |
| TRIM21 (Tripartite motif containing 21) | - Positive regulation of chromatin binding | - RNA binding | |
| USB1 (U6 snRNA biogenesis phosphodiesterase 1) | - Ubiquitin-dependent protein catabolic process | - RNA binding | |

Abbreviation NR Not reported
fetal cell types derived from the early embryo, including villous cytotrophoblast cells, syncytiotrophoblast cells, extravillous trophoblast cells and maternal immune cells, have been detected [98]. Taking advantage of this discovery, Guo et al. [99] further compared both placental and peripheral blood transcriptomes to distinguish the fetal and maternal differences between women diagnosed with early- and late-onset PE. Interestingly, classical biomarkers such as sFlt-1, sEng and PAPP-A were only upregulated in the early-onset PE while the maternal blood-derived factors EBI3, IGF2, ORMDL3, GATA2 and KIR2DL4 were identified as new biomarkers for the late-onset PE. In the future, increasing PE diagnostic studies using single-cell transcriptomes will undoubtedly help to drive the field forward.

Diagnostic recommendation

As shown above, while individual biomarkers demonstrate the connections between PE progression and secreted factor imbalance, none of them have demonstrated sensitivity and to be used as a test in isolation. Multiparametric biomarker testing may offer a path to improve the PE diagnosis. Early-onset PE appears less frequently (0.4–1%), but causes more significant disease burden due to prematurity, fetal growth restriction, and increased long-term maternal cardiovascular morbidity, while late-onset PE is regarded as secondary to maternal cardiovascular and metabolic predisposition for endothelial dysfunction [100]. In this case, splitting PE screening into two arms is recommended, including an early integrated test at 11–13 weeks and a second screen at 30–33 weeks for evaluating cases with late-onset PE [101].

Placental proteins, cffDNA and exosomes exhibit positive correlations with PE development, but considerable heterogeneity exists in the diagnostic accuracy according to current research. Hence, for the screening of low-risk population, biochemical biomarkers and Doppler ultrasonography are not recommended [102]. Currently, several organizations suggest screening for early-onset PE by obtaining BP measurements incorporating the maternal characteristics and urinalysis for proteinuria at each antenatal visit as adequate [103]. Subsequently, the late-onset PE assessment would depend on the changes in angiogenic factors (PIGF/sFlt-1), indicating an ideal predictive rate for estimating the onset of PE post 34 weeks of gestation.

Clinical management of PE

To avoid neonatal respiratory distress syndrome in fetus born with preterm birth, delivering the placenta is the only cure for PE. Besides, PE has long-term implications for women. For instance, the occurrence of high blood pressure within 2 years of birth in women with PE history is sixfold higher than in women with no history of PE [104]. Therefore, effective prevention and treatment for this condition are sorely needed. Firstly, PE women should practice good health habits in the antenatal period, including sleeping adequate amounts, consuming a high-protein diet, ceasing smoking, exercising mildly to moderately, as well as maintaining a healthy weight. Additionally, salt restriction and bed rest are not recommended in women at risk of PE, and folic acid together with multi-vitamins are preferred to prevent spina bifida and help normal brain development.

Though detailed expectant managements of PE in the international clinical practice guidelines are still inconsistent, their principles primary rely on two characteristics: the gestational age and the disease severity. We encourage a delivery for women with confirmed PE at 37 weeks of gestation. Elective delivery beyond 34 weeks should be considered. In cases of severe PE with IUGR below 26 weeks, the evidence suggests that termination of pregnancy might be a necessary form of management to prevent maternal morbidity [105, 106]. Regarding to maternal-neonatal outcomes, clinicians need to fully compare the risk of elective delivery against expectant management of severe PE less than 34 weeks of gestation to achieve optimal maternal and child outcomes. We do not encourage elective delivery for PE before 34 weeks of gestation unless failure to correct severe maternal hypertension or maternal complications (e.g. eclampsia, liver failure, kidney failure, disseminated intravascular coagulation, placental abruption, HELLP syndrome and acute pulmonary edema), non-reassuring fetal status or unavailable monitoring. Prior to 34 weeks of gestation, expectant management for severe PE patients should be the first priority at facilities with high volume maternal and neonatal intensive care resources.

Magnesium sulfate has been considered as the first-line therapy for the prophylaxis against maternal eclampsia. Magnesium sulfate has the effects on lowering maternal BP as well as fetal neuroprotection [107], demonstrating superiority to other anticonvulsants in the prevention of maternal eclampsia. The common dose protocol for magnesium sulfate in PE is a 4 to 6 g intravenous (IV) loading dose with a continuous 1 g/h IV maintenance dose. Mild side effects of magnesium sulfate mainly include fever, flushing, nausea, vomiting, muscle weakness, dizziness and irritation at the injection site [108]. In randomized trials, the reported rates of these side effects range from 15 to 67% [109–111]. In addition, serious side effects such as respiratory depression and postpartum bleeding require monitoring of the correct dose. Maternal deaths from the overdose of magnesium have been reported in previous clinical trials [112]. As for women with
gestational hypertension (Systolic BP ≥ 140 mmHg and/or Diastolic BP ≥ 90 mmHg), antihypertensive agents are recommended to prevent maternal morbidity and fetus injury. For instance, Labetalol, a beta-blocker, is the first-line treatment for hypertension in pregnancy. Nifedipine, a calcium channel blocker, is an alternative if labetalol is not suitable.

As for severe PE patients at 26–34 weeks gestation, antenatal corticosteroid therapy for acceleration of fetal lung maturity is recommended. Glucocorticoids can trigger the synthesis of ribonucel acid that codes for proteins involved in the phospholipid biosynthesis or glycogen breakdown [113]. Epidemiological evidence and animal studies suggest that the prenatal exposure to corticosteroids may lead to adverse long-term consequences [114]. Some animal studies have demonstrated impaired glucose tolerance and elevated blood pressure in adult animals following prenatal exposure to corticosteroids [115–117]. In addition, premature and full-term infants exposed to a single course of corticosteroids can cause reduced brain growth [118, 119]. Thus, the side effects of corticosteroid therapy will be monitored carefully.

During pregnancy, physical monitoring and assessments are required to detect the PE progression. The blood pressure, respiratory rate, and continuous cardiotocography are needed to be considered. Additionally, the maternal assessments mainly include urea and electrolytes, full blood count and platelets, liver function test/ lactate dehydrogenase, coagulation screen, group and hold serum.

**Novel PE treatments**

PE, characterized by the release of anti-angiogenic factors and increased oxidative and inflammatory stress, jointly causes endothelial dysfunction, systemic maternal vascular disorder and hypertension [120]. Based on this, current treatments of PE targeting anti-angiogenic factors, inflammation, oxidative stress, endothelial injury and dysfunction have been researched (Fig. 3).

**Pravastatin** shows potential effects to prevent and treat PE through promoting the angiogenic signaling, endothelial colony-forming cell function and protein expression [120, 121]. They also play a role in reducing blood lipid, improving the vascular endothelial cell function, reducing the oxidative stress and inflammatory injury of systemic vascular systemic [122]. Though various animal experiments have achieved great progress of the application of pravastatin, its clinical use has been delayed, mainly because statins are classified by the food and drug administration (FDA) as class X [123, 124]. That means that the pravastatin may lead to fetal abnormalities.

In the past decade, pravastatin has shown its effect on preventing PE in two pregnancies, and it did not show teratogenic effects on pregnant women. In a cohort study of 890,000 people in the United States, approximately 1200 pregnant women who took statins in the first trimester of pregnancy experienced no increased incidence of congenital malformations [125]. Other organizations have reported similar epidemiological results [126, 127]. Although pravastatin has been used in a small number of high-risk pregnant women for several years, randomized controlled trials have not been reported. Recent studies have shown that pravastatin has favorable pharmacokinetic characteristics during pregnancy, and its safety has been confirmed by accumulating case studies [128]. In the future, it is vital to figure out the targets and duration of use. Additionally, large-scale clinical studies are needed to validate whether pravastatin may be an effective drug for PE prevention and treatment, improving outcomes for pregnant women and newborns at risk for PE.

**Metformin** has been reported to reduce the secretion of sFlt-1 from placenta as one of the markers of endothelial dysfunction. It can promote angiogenesis through inhibiting the mitochondrial electron transport chain, thus implying its potential to treat PE [129]. Metformin, with a molecular weight of 129 Dalton, can be directly diffused across the placenta without affecting the transfer of glucose. Double infusion of human placental lobules in vitro showed a lag time of rapid transfer of metformin from maternal to fetal circulation of 1.7 ± 0.28 min. During a randomized controlled clinical trial, nondiabetic or obese pregnant women were randomly assigned to receive metformin at 12–16 weeks of gestation [130]. The starting dose of 500 mg/day was increased to a maximum tolerable dose not exceeding 2500 mg. Accordingly, metformin decreased the frequency of PE, which is consistent with the results of a previous Meta-analysis [131].

Additionally, metformin had no significant effect on the birthweight, maternal weight, or combined adverse pregnancy outcomes. An in vivo study reported that metformin was detected in the neonatal cord blood of women with polycystic ovary syndrome treated with metformin during pregnancy, while such concentration of metformin in fetal umbilical arteries and umbilical veins are negligible [132]. Several meta-analyses have demonstrated that metformin has no effect on embryonic development including congenital malformations, and metformin is currently classified as class B in the United States and class C in Australia [133–135]. In pregnant women, adverse effects have been reported mainly in the gastrointestinal tract including nausea and diarrhea [135]. In addition, long-term use may produce some side effects, such as mild erythema and reduced absorption of vitamin B12 [136]. Therefore, although metformin can
reduce the incidence of PE, the dose and duration of use need to be properly controlled on a case-by-case basis.

Aspirin has been initially used for PE prevention via the inhibition of platelet aggregation. Patients with high PE risk should be treated with antiplatelet therapy, regardless of gestation stage [137]. Aspirin acts on a variety of immune cells and exert anti-inflammatory and immunomodulatory effects. First of all, Aspirin inhibits the natural killer cell activity induced by melanoma [138], and inhibits the secretion of macrophage derived cytokines including lipopolysaccharide induced NF-κB and TNF-α [139]. Aspirin also regulates the maturation and differentiation of dendritic cells, thus affecting their functions such as activating downstream T cell proliferation [140]. Besides, Aspirin increases the number of Treg, and indirectly enhances the function of Treg by inducing the decrease of costimulatory factor expression on immune tolerant dendritic cells and the upregulation of costimulatory factor expression required for Treg activation [140, 141]. The use of Aspirin reduces the risk of proteinuric PE by 18%, preterm birth less than 37 weeks by 9%, fetal deaths, neonatal deaths or death before hospital discharge by 14% as well as small gestational age and serious adverse pregnancy outcomes [142]. Most clinical practice guidelines for pregnancy hypertension recommend a low-dose Aspirin therapy for the prevention of PE in high-risk women [143]. Currently, four randomized clinical trials have shown that low-dose Aspirin can significantly minimize the incidence of PE [144, 145]. The most recent trial applying 1776 subjects found that
Aspirin apparently reduced the incidence of PE from 11 or 14 weeks to 36 weeks of pregnancy, suggesting an early supplement from 3 months or 6 months of pregnancy. Evidence also showed that Aspirin prophylaxis significantly reduces the early PE onset as well as preterm birth [146]. In our center, a daily dosage of 50–150 mg Aspirin taken at bedtime is highly recommended to patients with a PE history, initiating from 12 or 16 weeks until 35 weeks of gestation. To sum up, systematic studies on its regulatory effect on maternal fetal immunity in PE will be continued.

**Calcium** supplementation (≥ 1 g/day) during pregnancy may significantly decrease the risk of PE and preterm birth, especially in patients with a calcium diet deficiency (<600 mg/d) [147]. Furthermore, its efficiency is highly correlated with the time of supplementation, as only taking calcium at early pregnancy stage may play a role in decreasing PE occurrence and pregnancy loss [148]. Recently, a systemic Meta-analysis of 27 randomized trials involving 28,492 pregnant women showed that high doses (1.2 to 2 g/day), medium doses (0.6 to 1.2 g/day) and low doses (<0.6 g/day) of calcium supplementation were associated with a reduced risk of PE [149]. However, further studies of direct concentration comparisons are needed to determine the ideal calcium dose for PE prevention.

**Melatonin** is an endogenous antioxidant that can improve PE maternal condition. In primary trophoblast cells, melatonin increases the release of antioxidant enzyme TXN and decreases the sFlt release [150]. In placental explant models with melatonin treatment, lower oxidative stress (8-isoprene) and higher antioxidant markers (Nrf2 transposition, HO-1) were observed, but the secretion of anti-angiogenic factors (sFlt, sEng and activin A) remained steady. Similarly, melatonin reduces the expression of vascular cell adhesion molecules induced by TNF-α and upregulates both antioxidant TXN and GCLC expression in human umbilical vein endothelial cells (HUVECs) without altering the secretion of sFlt or sEng. Melatonin has shown its safety and efficacy for both mothers and fetuses in a phase I clinical trial, leading to extended delivery interval by 6 ± 2.3 days, and the less requirement of antihypertensive medication on days 3–4 decreased from 71 to 13%, days 6–7 dropped from 51 to 8%, and at delivery reduced from 75 to 26% [151]. Hence, melatonin can be a possible adjuvant therapy to prolong the pregnancy time and improve the clinical results of PE [151].

**Mesenchymal stem cells** (MSCs) have received increasing attention for PE treatment due to their low immunogenicity, powerful immunomodulation, angiogenesis and regenerative therapy, as shown in Fig. 4 [152]. Bone marrow is the main source of MSCs, but the extraction process is invasive, and the number of MSCs decreases with age, which limits the clinical application of MSCs [153]. Human cord blood has been found to be rich in MSCs, whose characteristics are similar to those of bone marrow MSCs [154]. Therefore, the MSCs derived from placenta or umbilical cord may have the potential to treat endotoxin-induced hypertension in patients with PE. Studies have shown that placental derived MSCs have great therapeutic potential in PE ischemia [155, 156]. In vitro culture, MSCs have successfully differentiated into polysyncytic trophoblasts and HLA-G+ extra-villous trophoblasts [157]. During PE development, inflammatory Th1 cells would exacerbate immune imbalances and reduce the number of regulatory T cells and anti-inflammatory cytokines, leading to chronic inflammation and associated oxidative stress. In this case, MSCs can significantly improve the symptoms of Th1 cells of PE mouse model, implying that MSCs may play a significant role in maternal interface immunity [158]. Consistent with these, another study demonstrated that MSCs could improve the functions of mouse trophoblasts and endothelial cells to promote angiogenesis through attenuating the hypoxia-induced mitochondria damage [159]. As for the underlying mechanisms, there is increasing evidence that the beneficial effects of MSCs are exerted through the secretory bodies and reducing SFLT-1 to restore angiogenesis. Additionally, human placental mesenchymal-like adherent stromal cells have been found to secrete IL-6 and VEGF to confer neuroprotection to nerve growth factor-differentiated PC12 cells exposed to ischemia [160–162]. Recently, Todd et al. demonstrated the mechanisms of FKBPL in MSC-mediated angiogenic and anti-inflammatory effects [163]. MSCs enhanced trophoblast migration and endothelial tubule formation under hypoxia and normoxia, which associated with decreased levels of anti-angiogenic protein FKBPL and increased expression of pro-angiogenic CD44 mRNA. Besides inhibiting angiogenesis, FKBPL is involved in the regulation of inflammatory pathways, such as STAT3 [164], which are associated with trophoblast functions [165, 166]. Thus, MSCs may improve the vascular response and symptoms of PE by restoring normal levels of FKBPL. Despite such promising results, a series of questions of MSC therapy in PE, including cellular quality and dosage control, need to be addressed prior to a clinical setting.

The MSC-derived exosomes have raised great attention due to its ability to transport angiogenesis factors, lipids and microRNAs. As the regulatory factors transfer biologically active membrane and cytosolic components to target cells, the dysfunctional intercellular communication can be improved. In PE, the application of MSC-derived exosomes alleviates degenerative angiogenesis
through regulating the levels of ERK, AKT, angiopo- 
etin, PKA, VEGFR-2, VEGF and β-catenin. MicroRNAs (miRNAs) are small (21–23 nucleotides) single-stranded RNA molecules that play an important role in the post-transcriptional regulation of gene expression by promoting RNA instability or translation inhibition [167, 168]. The miRNAs in exosomes have been considered to char-acterize the disease progression, and thus can be used as novel biomarkers to predict PE [169, 170]. In addition, exosomes of human umbilical cord mesenchymal stem cells (uc-MSCs) have been reported to protect ovarian granulosa cells from cisplatin-induced damage and chemotherapy-induced apoptosis in vitro [171]. MiR-133b, a member of big miRNA family, is downregulated in PE though targeting serum and glucocorticoid-regulated kinase 1 (SGK1) [172, 173]. SGK1 is a serine/threonine kinase that plays a restrictive role in regulating cell survival, proliferation and differentiation. Kong et al. applied the trophoblast cell HPT-8 and HTR8-S/Vneo co-cultured with human uc-MSC-derived exosomes that had been transfected with miR-133b plasmids [174]. Accordingly, miR-133b is down-regulated and SGK1 is up-regulated in...
PE groups, and miR-133b derived from exosomes in uCMSCs facilitates trophoblast cell proliferation, migration and invasion in PE via constraining SGK1. Two recent studies have shown that the up-regulation of placental miR-210 is a hallmark of severe PE [175, 176]. MiR-210 is a primary miRNA of hypoxic response and a common component of tumor microenvironment, that can be induced in a hypoxia-inducible factor (HIF)-1α dependent manner [177]. Immunoprecipitation analysis showed that HIF-1α regulates miR-210 expression in a variety of tumors. Lee et al. suggested that the iron-sulfur cluster scaffold homologue down-regulated by miR-210 perturbing trophoblast iron metabolism is associated with defective placentation of PE [177], indicating that miR-210 is promising to treat PE.

Currently, the oncogenicity of MSC therapy has turned researchers’ attention to the MSC-derived exosome treatment. The exosome, a subtype of extracellular vesicle, possesses lower susceptibility to damage by hostile injury microenvironment (e.g. high concentrations of cytokines and hypoxia) than that of MSCs. Additionally, the exosomes can retain their efficacy after freezing, and thus the costly stem cell facilities are no need for MSC-exosome production. Nevertheless, in contrast to MSCs, the nonrenewable exosome may be used up in vivo. In clinical applications, the repeated injection line and procedure of MSC-exosome in specific phases may be considered.

**Discussion**

PE is a syndrome with various phenotypes, with long-term effects on the fetus and mother including the an increased possibility of adult disease in fetus and a larger risk of cardiovascular disease in mother after pregnancy. There is an urgent need to identify PE in early stage in order to achieve their specific treatment. Such studies require large-scale samples, which can be achieved by setting rules of standardized and coordinated collection of clinical data and biological specimens. Future research should include samples scaled globally, and cutting-edge methods such as machine learning and artificial intelligence can be considered to collect and analyze data. The objective is to determine the precise early PE prediction and the implementation of phenotypic specific therapies.

Currently, the only effective treatment for PE is childbirth, but new treatments are being developed to reduce complications and prolong pregnancy. Aspirin has been recommended as a preventive therapy for PE in preterm labor, and statins are being explored as another potential intervention. In the future, it may be possible to target PE therapy by using pro-angiogenic factors or removing anti-angiogenic factors to restore angiogenic balance. MSCs provide a promising tool for future therapeutic applications because of their anti-inflammatory, pro-angiogenic, antioxidant and immunomodulatory potentials, as well as their easy isolation, great self-renewal ability and lack of immunogenicity. MSCs can be obtained from different tissue sources and vary in differentiation stage, proteome, and genome profile, achieving different functions. Although several molecular pathways have been identified, the intact mechanisms by which MSCs play a therapeutic role in PE are still not fully understood [152]. According to Zhang et al., direct cell-to-cell contact was insignificant during MSC treatment [178]. Given the pathogenesis of PE, each secreted molecule from MSCs plays a partial role in restoring normal functions. In the future, the exact molecules can be identified by inhibiting or knocking them out individually or simultaneously, and their therapeutic potential need to be investigated. There are some issues that need to be addressed in bringing MSC therapy from in vivo PE studies into clinical trials. Currently, no clinical trial of long-term exposure to MSCs has been reported and there is a lack of complete examination of the effects of MSCs on fetal and maternal health [179]. Due to their regenerative and angiogenic effects, MSCs may promote malignancy which needs to be fully studied prior to treatment [152]. In general, standardized procedures of isolation and propagation need to be developed, which will maximize the therapeutic potential and minimize the side effects of MSC therapy.

While various drugs show great potential in preclinical practice, the off-target effects remain of concern when deliver to a pregnant woman, as they can harm a developing fetus [180]. Nanoparticles, a targeted delivery system, can prevent the off-target effects by delivering therapeutic drugs to specific cells, tissues or organs. Nanoparticles are similar in size to proteins, usually less than 100 nm [181], and can facilitate their functions by leveraging existing cellular mechanisms [182]. Nanoparticles can be targeted to epidermal growth factor receptor, which is expressed about 3000 times more in placenta than in other tissues [183]. Nanoparticles can be designed to cross the placenta easily into the fetal chamber, or modified to deliberately prevent them from crossing the placenta, or synthesized so that the placenta is the site of action [184]. Nanoparticles tend to have longer half-lives and thus, the effective doses are commonly lower than therapies used alone.

An array of types of nanoparticles are promising for PE treatment, including liposomes, polymers, dendritic molecules and inorganic nanoparticles. Liposomes are vesicular bimolecular layers synthesized from phospholipids, which can increase the solubility of drugs [185], but their low loading capacity may lead to rapid drug release and unstable storage [182].
can be synthesized from natural or biodegradable synthetic materials and can change their physical or chemical properties in response to environmental signals [182]. Dendrite molecules are larger synthetic nanoparticles, usually with a dendritic structure, strong loading capacity, drug sitting on the external branch, and predictable pharmacokinetics [186]. Inorganic nanoparticles, such as gold and iron oxide, have a large load capacity and low toxicity [187], but they have not yet been explored for use in pregnancy due to their unclear safety profile. Emerging treatments have used target delivery therapy. For instance, bacteria-derived organic nanoparticles have been used to deliver doxorubicin into placental tissue as a non-invasive treatment for ectopic pregnancy [188]. Magnetic iron oxide nanoparticles have been used in a similar approach using magnetic targeting instead of EGFR targeting [189]. Generally, combining drugs used to treat PE with novel nanoparticle targeted delivery systems may further improve drug efficacy and reduce off-target effects.

One of the challenges of PE research is their reproducibility in animal models, because spontaneous PE is exclusive to human pregnancy, making it difficult to replicate in animal models accurately. At present, cutting the abdominal aorta and uterine aorta in pregnant rats is one of the most common models, appearing the symptoms including systolic hypertension, increased renal endothelial cells and proteinuria [190]. Besides, a transgenic blastocyst with a trophoblast ectoderm expressing human sFlt1 via lentiviral vectors has been implanted [191]. During the pregnancy progress, human sFlt1 level increases in maternal serum, with the corresponding development of PE features. However, these rodents failed to develop all severe PE features as humans do. In addition, genetic rodent models have been developed. For instance, the BPH/5 model showed elevated MAP, proteinuria, progressive glomerular damage, and a significant decrease in birth rate during the third trimester of pregnancy [192]. COMT knockout mouse models showed similar systolic hypertension, increased sFlt1 level, and decreased proteinuria after delivery, but there was no evidence of systemic vascular injury or fetal growth restriction, suggesting that the model closed to gestational hypertension or mild PE [193]. Based on the observation that human placenta chemically induces systemic hypertension, an old rodent model has been applied to simulate gestational hypertension by manipulating the renin-angiotensin system [194]. Although in human, PE is characterized by inhibition of renin and angiotensin II, this animal model can mimic the molecular environment of uteroplacental hypoperfusion and various cytokines and proteins released into the maternal circulation. Following these, animal models can extend the relevant data observed in human to causal relationships and can be a key tool for assessing toxicity and efficacy of new therapies. Of course, animal models for this human-specific disease are imperfect, as they cannot prove thrombocytopenia, HELLP syndrome, eclampsia and other signs and symptoms that define the severe features of PE in human [23]. The main drivers of PE, names inadequate trophoblast infiltration and failure of spiral artery remodeling, have not been accurately modeled [195]. Since the gestation period of humans is significantly longer than that of rodents, animal models need to be exposed to higher doses of circulating toxins. Interestingly, studies have found that rodents did develop severe PE characteristics when the levels of sFlt1 and sEng were increased [196–198]. With the development of tissue engineering, 3-dimensional in vitro models have attracted various researches due to the lack of reliable animal models. To ensure a good resemble of complex in vivo microenvironment, scaffold materials, seeding cells, and growth factors are significant for cell–cell interactions and cell–matrix interactions [199]. Haider et al. constructed a long-term expanding organoid from purified first-trimester cytotrophoblasts embedded in Matrigel containing a mixture of growth factors and signaling inhibitors including epidermal growth factor, Noggin and R-spondin [200]. To further research the underlying mechanisms and appropriate managements of PE, innovative experimental models are needed.

Conclusion
The emergence of multiple biomarkers with accurate test outcomes provides multi-dimensional targeting to complete the antenatal and postnatal care of PE, aiming towards ultra-rapid bedside prediction of women with the suspicion of PE. Various biochemical factors combined with improved therapeutic approaches, which have been displayed to be valuable and expandable into widespread clinical practice, will be integrated into the management standard of patients with suspected or confirmed PE over the coming years.

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JQ, BW and XC wrote the manuscript. XY and WW designed and revised the manuscript. All authors have approved this version of the article.

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Declarations

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Not applicable.

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Competing interests
The authors declare that they have no conflicts of interest to this work.

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