Review

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Towards biomarker-based tests that can facilitate decisions about prevention and management of preeclampsia in low-resource settings

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Abstract: In recent years, an increasing amount of literature is emerging on candidate urine and blood-based biomarkers associated with incidence and severity of preeclampsia (PE) in pregnant women. While enthusiasm on the usefulness of several of these markers in predicting PE is evolving, essentially all work so far has focused on the needs of high-resource settings and high-income countries, resulting primarily in multi-parameter laboratory assays based on proteomic and metabolomics analysis techniques. These highly complex methods, however, require laboratory capabilities that are rarely available or affordable in low-resource settings (LRS). The importance of quantifying maternal and perinatal risks and identifying which pregnancies can be safely prolonged is also much greater in LRS, where intensive care facilities that can rapidly respond to PE-related health threats for women and infants are limited. For these reasons, simple, low cost, sensitive, and specific point-of-care (POC) tests are needed that can be performed by antenatal health care providers in LRS and that can facilitate decisions about detection and management of PE. Our study aims to provide a comprehensive systematic review of current and emerging blood and urine biomarkers for PE, not only on the basis of their clinical performance, but also of their suitability to be used in LRS-compatible test formats, such as lateral flow and other variants of POC rapid assays.

Keywords: biomarkers; low-resource settings; point-of-care; preeclampsia; rapid diagnostic test.

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Introduction

Preeclampsia (PE) is a pregnancy-specific disorder described by new-onset hypertension and proteinuria typically developing after 20 weeks of gestation. If left untreated, the condition progresses to eclampsia (E). Worldwide, PE complicates 2%–8% of all pregnancies and remains one of the leading causes of maternal and perinatal mortality and morbidity, particularly in developing countries [1]. The World Health Organization (WHO) estimates that PE/E accounts for at least 16% of maternal deaths in low-resource settings (LRS), which constitutes 63,000 deaths each year [2]. The increased burden of adverse outcomes in low- and middle-income countries is believed to be due primarily to the absence of trained health professionals, delays in triage, transport, and treatment [3].

There are many conditions and health risk behaviors that are thought to increase the risk of a woman developing PE during pregnancy. High-risk pregnancies include those of women with preexisting hypertension, chronic kidney disease, insulin-dependent diabetics, and women with previous PE [4, 5]. However, the use of so-called “risk factors” alone for prediction has a low positive and high false negative predictive value for PE [6, 7]. To date, no reliable test or complex of symptoms predicts the development of PE and providers continue to rely on measuring blood pressure (BP) and proteinuria during antenatal care (ANC) for early detection. Such clinical parameters are, however,

unreliable, non-specific, and subject to observer error and poor test accuracy for identifying women and infants at risk of adverse outcomes [8]. The 24-h urine protein excretion measurement remains the "gold standard" for quantifying urinary protein, but it requires hospitalization and is, therefore, an inconvenient and time consuming test. Alternatives to the 24-h collection include the urinary dipstick test, the urinary spot protein:creatinine ratio (Pr:Cr), and albuminuria to creatinine ratio (ACR). Of the three, the urinary dipstick test may be the quickest and simplest method, but its drawbacks are inconsistency and poor correlation with 24-h urine protein excretion level [9]. Alternatively, measurement of Pr:Cr in a spot urine sample accurately reflects the results of the 24-h urinary collection [10, 11]. Although the measurement of proteinuria is one of the formal diagnostic criteria for PE, 10% of women with clinical and/or histological manifestations of PE have been shown to have no proteinuria [12]. Furthermore, only 35% of women have been shown to have both proteinuria and hypertension before the development of E [13]. A need therefore exists for a diagnostic tool that can help to identify and monitor women at risk, but also to identify women with atypical PE (no proteinuria) in clinical practice.

New biomarkers could significantly aid the diagnosis of women suspected of having PE. Experts have long sought biomarkers for PE that are sensitive, specific, and reliable; that can be measured with precision; and that allow for an objective assessment of the disease. Furthermore, because the presence of increased urinary protein is usually a late manifestation of PE, experts have hoped that the use of biomarkers would enable earlier detection or even prediction of the disease [14].

To date, numerous candidate biomarkers have been proposed for the prediction and detection of PE, which are extensively described in recent reviews [15–18]. Candidate biomarkers for the early detection of PE are under investigation, of which biomarkers of angiogenesis are currently at the most advanced stage of development. These candidate biomarkers include soluble Fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) [19]. So far, most of the work on new novel PE biomarkers has focused on the needs of high-resource settings and high-income countries. Emerging methods, such as genomics-, proteomics-, and metabolomics-based research require laboratory capabilities that are not widely available in LRS. Also, the need for a sensitive and specific test to screen for and diagnose PE is much greater in LRS than in high-resource developed countries, as are the consequences of misdiagnosis due to a lack of emergency intervention capabilities.

In resource-limited environments, POC tests should be simple to operate, built from inexpensive components, and able to detect disease directly from biological fluids. Rapid diagnostic tests (RDTs), generally based on immunochromatographic strip (ICS) or lateral flow technologies, are one of the very few diagnostic technologies successfully used in the developing world [20, 21]. As RTDs rely on inexpensive, off-the-shelf components and reagents, they are affordable and cost less than US$1 to the end-user in many cases. Some ICS assays can achieve analytical sensitivities for proteins to the level of pg/mL, though most can detect only to the level of ng/mL. In addition, low-cost, instrument-free or minimally instrumented microfluidic enzyme-linked immunosorbent assay (ELISA) formats are becoming available [22]. These formats may prove especially useful for multiplexed PE biomarker assays that require somewhat higher analytical sensitivities in the low pg/mL range [23].

Research to uncover biomarkers for PE is largely targeted to the discovery of signature markers in blood or serum. In comparison to these markers, urinary biomarkers present an advantage because urine samples are more easily obtainable, pain free, available in sufficient volume, and neither infectious nor a biohazard in disposal. Also, urinary biomarkers may be present at higher concentration, suggesting that they could likely be detected with RDTs or dipsticks.

Our study aims to provide a comprehensive review of current and emerging biomarkers for PE, not only for their clinical performance, but also for their suitability to be used in LRS-compatible test formats. Such test formats include lateral flow and other variants of POC-compatible rapid assays that are based on parameters, such as clinical normal and predictive/diagnostic concentrations of the biomarkers, ease of detection, availability of antibodies, and likelihood of providing a clinically actionable result at the gestational age (GA) range at which women in LRS most likely present at ANC clinics.

Materials and methods

Literature search

We conducted a comprehensive literature review using the databases PubMed, Nexis, ClinicalTrials.gov, International Clinical Trial Registry, and Espacenet (European database of worldwide patents, including US patents). We also searched conference proceedings titles on the BME IDEA 2013 website, Global Health Innovation Conference 2013 website, and 1st International Federation of Gynecology and Obstetrics (FIGO) African Regional

Conference website. We tailored search strategies to each database. MeSH and text word terms were used in conjunction to increase sensitivity to potentially appropriate studies. The study protocol is provided in Supplementary Material that accompanies the article at http://www.degruyter.com/view/j/cclm.2015.54.issue-1/cclm-2015-0069/cclm-2015-0069.xml?format=INT. Our search covered a period of five years (1 January, 2008–1 October, 2013) except the search in Nexis (1 January, 2011–1 October, 2013) due to subscription terms.

Eligible studies

Studies were selected in a staged process. First, one reviewer screened titles and abstracts identified by the search (JG). Studies were excluded if they focused on the following: epidemiological studies, non-pregnancy related hypertension, renal disease, heart disease or diabetes. Second, two reviewers (TH and BHW) independently screened the selected abstracts from the first stage to determine if the study described any novel biomarker or new application of an existing biomarker. Third, full texts of relevant abstracts were then reviewed by one review author (NA) if they met the following main criteria: 1) the study describes a biomarker that is sufficiently sensitive and specific to PE; 2) the study shows that the biomarker detection level is sufficiently different from normal clinical level; and 3) the study shows that the biomarker is present at a sufficiently high concentration to be detectable in a rapid assay format (≥5–10 ng/mL levels).

Data extraction

For relevant articles, one reviewer (NA) extracted the following variables: 1) type of study; 2) target screening population (e.g., early- vs. late-onset PE and mild vs. severe PE); 3) study size; 4) type of patient sample; 5) collection period; 6) method of detection (e.g., ELISA); 7) whether a kit or commercial antibodies exist (and analytical performance if the test exists); 8) biomarker level; 9) biomarker clinical performance (sensitivity, specificity, positive predictive value, negative predictive value, and area under the receiver operating characteristic curve); and 10) use case.

The conditions for PE were adapted from the WHO criteria as follows: 1) mild PE: two readings of diastolic BP ≥90, but <110 mmHg 4 h apart after 20 weeks of gestation with proteinuria up to 2+ or >0.3 g/24 h urine collection; and 2) severe PE: diastolic BP ≥110 mmHg after 20 weeks of gestation with proteinuria ≥3+ or >5 g/24 h urine collection or any one of the signs/symptoms of severe PE (headache, blurred vision, oliguria, epigastric pain, right upper quadrant pain, and pulmonary edema). Early onset of PE was defined as occurring before 32–34 weeks of pregnancy [24].

Results

The study selection process is shown in Figure 1. The electronic database searches retrieved a total of 2783 citations comprising 1169 publications in PubMed; 971 in Nexis; 195 in ClinicalTrials.gov; 40 in International Clinical Trial Registry; 402 in Espacenet databases; six in conference proceedings between 2008 and 2013 of which a total of 135 unique blood and urine biomarkers and biomarker combinations associated with clinical PE presentation were identified. Key information about each biomarker is provided in Supplementary Table 1. Of the 135 biomarkers, 118 were blood biomarkers and 17 were urine biomarkers. Sixty-nine blood and eight urine biomarkers were subsequently excluded from further consideration on the basis of various criteria as illustrated in Figure 1. A total of 49 blood and nine urine biomarkers were selected for further analysis. Among the 49 blood biomarkers, 27 are single biomarkers and 22 are described for use in combination. Two of these 22 combination biomarkers are also used as single markers (CA-125 and NGAL).

Table 1 provides a summary list of the single blood and urine biomarker candidates and their relative biological functions. For example, these markers are involved in placental tissue damage, oxidative stress, renal function, or inflammation. While some of these biomarkers appear promising, most are investigational.

The 27 blood and nine urine single markers are referred to here as experimental markers. Four biomarkers known to be associated with PE – sFlt-1, vascular endothelial growth factor (VEGF), PlGF, and soluble endoglin (sEng) – were also included in our analysis and are referred to as established markers (Table 1). To our knowledge, no RDT uses any of these four well characterized biomarkers on the market or in development.

Among the 36 experimental biomarkers listed in Table 1, the following biomarkers have been shown to have some degree of clinical sensitivity and specificity to PE when compared to BP and/or proteinuria and could be of interest for future PE screening tests: 1) eight blood biomarkers consisting of CA-125 [32], C-terminal GRP78 [35], HSD17B1 [39], NGAL [55], plasma factor VII [50], serum uric acid (UA) [53], sFlt1-14 [56], and soluble ST2 [54]; and

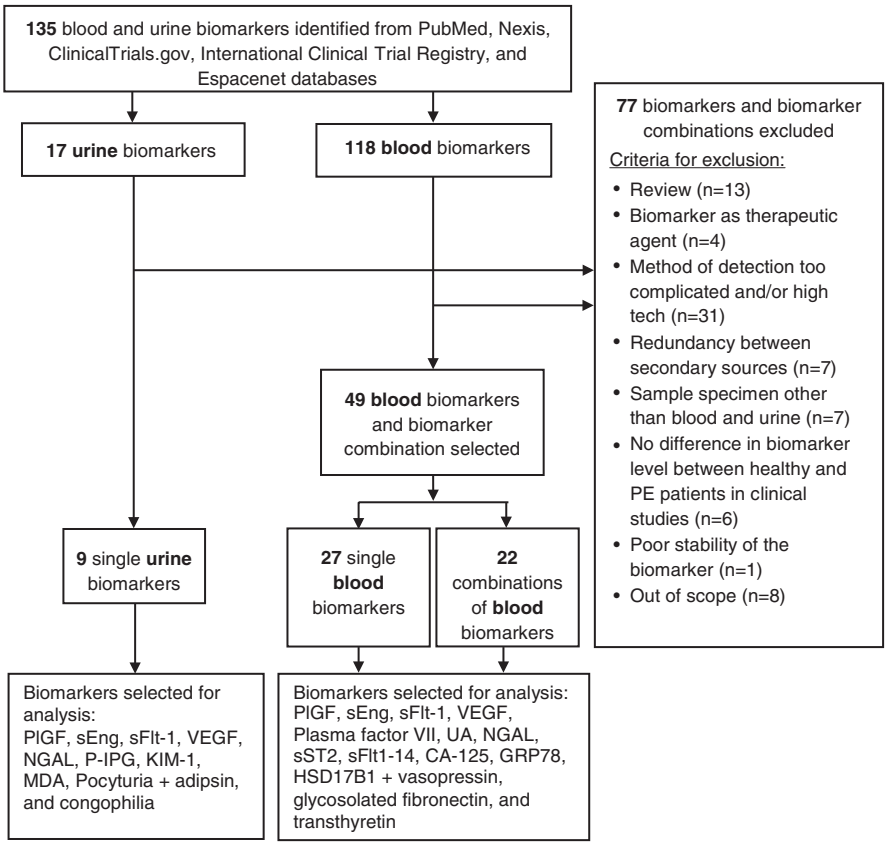

Figure 1: Flow chart summarizing the selection of blood and urine biomarkers.

2) five urine markers consisting of KIM-1 [43], NGAL [43], podocyturia [48], inositol [40], and MDA [44]. Another 10 blood experimental biomarkers (AT-1AA [31], calcyclin, copeptin [34], galectin-1 [36], Gas6 [37], HIF-1aOH [38], IGFALS [41], mammalian HtrA3 [45], NT-proBNP [47], and PTX3 [49]), and four urine ones (C5b-9 [33], nephrin [46], iodine [42], and prolactin [52]) had limited clinical evaluation information, which impeded the evaluation of their performance for the diagnosis of PE. No clinical data were found for the remaining nine blood markers, which consisted of adipsin, α enolase, ADMA, Ba, 2,3-BPGM, Fetal DNA, marinobufagenin, plasma UA [51], and soluble CD117. These latter markers have been described primarily in patents for which clinical and performance information is more limited than in the scientific literature.

As the number of studies describing novel candidate biomarkers predicting or diagnosing PE is continually growing, we performed a recent search in PubMed between 1 January 2014 and 31 December 2014. Three new blood biomarkers [glycosylated fibronectin (GlyFN) [57], transthyretin [58] and vasopressin [59]], and two new urine biomarkers (urine congophilia [60] and urinary adipsin [61]) were identified, which added to our preexisting list of experimental biomarkers (Figure 1). A total of 11 promising single experimental blood and seven urine biomarkers together with the four established markers were ranked according to the following criteria: 1) characteristics related to performance; and 2) other characteristics such as whether a kit or commercial antibodies exist (Table 2A and 2B). The total number of points a marker could receive was 12. If a marker scored from 1 to 4 points, it was ranked as poor; from 5 to 8 points, adequate; and from 9 to 12, good. The result of this scoring is that 5 of the 15 blood biomarkers were categorized as good, 5 as adequate, and 5 as poor for diagnosing/predicting PE as indicated in Table 2A. In contrast, Table 2B shows two of the 11 urine biomarkers categorized as good, six as adequate, and three as poor. This categorization had a few caveats since the studies describing these markers were not necessarily comparable (e.g., differences in sample size, GA, patient characteristics, and clinical definition of PE).

Taken together, these data suggest that GlyFN (Table 2A) and urinary adipsin (Table 2B), even though their role in PE is less documented, may have an advantage over the other "good" candidates because an RDT has been developed. GlyFN has the potential for high

Table 1: Summary of single blood and urine biomarker candidates and their physiological function.

Biomarker name	Also known as	Features/biological function	Specimen	References[a]
Established markers				
Placental growth factor	PlGF	Angiogenic factor	Blood/urine	[25]/[26]
Soluble endoglin	sEng	Antiangiogenic factor	Blood/urine	[27]/[28]
Soluble fms-like tyrosine kinase 1	sFlt-1	Antiangiogenic factor	Blood/urine	[28]/[29]
Vascular endothelial growth factor	VEGF	Angiogenic factor	Blood/urine	[26]/[30]
Experimental markers				
Adipsin	Factor D	Component of the alternative complement pathway	Blood	
α enolase	Enolase 1	Glycolytic enzyme	Blood	
Angiotensin II receptor type I autoimmune antibody	AT-1AA	Induce several signaling mechanisms	Blood	[31]
Asymmetric dimethylarginine	ADMA	Antiangiogenic factor	Blood	
Ba		Complement factor B cleavage product Ba	Blood	
Cancer antigen 125	CA-125, MUC16	Predicting ovarian cancer recurrence	Blood	[32]
Calcyclin	S100A6	Calcium-binding protein	Blood	
C5b-9		Complement factor 5b-9 complex	Urine	[33]
Copeptin	CT-proAVP	Surrogate marker for arginine vasopressin secretion	Blood	[34]
C-terminal glucose regulated protein 78 (GRP78)	BiP, HSPA5	Chaperone	Blood	[35]
Fetal 2,3 bisphosphoglycerate mutase	2,3-BPGM	Glycolytic enzyme	Blood	
Fetal DNA		Exogenous fetal material	Blood	
Galectin-1		β-galactoside binding protein	Blood	[36]
Growth arrest specific protein 6	Gas6	Antiangiogenic factor	Blood	[37]
Hydroxylated hypoxia Inducible factor 1-α	HIF-1aOH	Transcription factor	Blood	[38]
Hydroxysteroid (17-ß) dehydrogenase 1	HSD17B1	Alcohol oxidoreductases	Blood	[39]
Inositol phosphoglycan P-type	P-IPG	Putative second messengers of insulin	Urine	[40]
Insulin-like growth factor acid labile subunit	IGFALS	Binding protein	Blood	[41]
Iodine	I	Chemical element	Urine	[42]
Kidney injury molecule-1	KIM-1	Kidney injury molecule	Urine	[43]
Malondialdehyde	MDA	Marker for oxidative stress	Urine	[44]
Mammalian high temperature requirement A3	HtrA3	Important for placental development and function	Blood	[45]
Marinobufagenin	MBG	Steroid (called “cardiac glycoside”)	Blood	
Nephrin		Necessary for the renal filtration barrier	Urine	[46]
Neutrophil gelatinase-associated lipocalin	NGAL	Innate immunity (also used as a biomarker of kidney injury)	Blood/ Urine	[43]
N-terminal pro-brain natriuretic peptide	NT-proBNP	Natriuretic peptide	Blood	[47]
Podocyturia	Podocytes	Glomerular epithelial cells	Urine	[48]
Pentraxin 3	PTX3, TSG-14	Modulator of tumor-associated inflammation (also used as a biomarker of inflammation)	Blood	[49]
Plasma factor VII		Coagulation factor	Blood	[50]
Plasma uric acid		Product of the metabolic breakdown of purine nucleotides	Blood	[51]

(Table 1: Continued)

Biomarker name	Also known as	Features/biological function	Specimen	References[a]
Prolactin	PRL, luteotropic hormone		Urine	[52]
sFlt1-14		VEGF inhibitor	Blood	
Serum uric acid		Product of the metabolic breakdown of purine nucleotides	Blood	[53]
Soluble CD117	Proto-oncogene c-Kit	Cell surface marker	Blood	
Soluble ST2	ST2	Member of the IL-1 receptor family gene	Blood	[54]

[a]For studies identified from PubMed database.

Table 2A: Overall performance of 15 blood biomarker candidates of preeclampsia (PE).

Ranking category[a]	**Good**				
Biomarker	GlynFN	sEng	sFlt-1[h]	PlGF[i]	Serum UA
Detection level[b]	√	√	√		√
Sensitivity to PE[c]	√	√	√	√	√
Specificity to PE[c]	√	√	√	√	√
Clinically relevant[d]	√	√	√	√	√
RDT[e]	√				
Antibodies[f]	√	√	√	√	
Assay[g]	√	√	√	√	√
Use in first trimester	√			√	
Ranking category	**Adequate**				
Biomarker	Vasopressin	NGAL	Plasma factor VII	Soluble ST2	Transthyretin
Detection level		√	√	√	√
Sensitivity to PE	√		√		√
Specificity to PE		√			√
Clinically relevant	√	√	√	√	
RDT					
Antibodies	√	√	√	√	√
Assay	√	√	√	√	√
Use in first trimester	√				
Ranking category	**Poor**				
Biomarker	sFlt1-14	CA-125	GRP78	HSD17B1	VEGF
Detection level				√	
Sensitivity to PE	√	√	√		
Specificity to PE	√				
Clinically relevant					
RDT					
Antibodies	√	√	√	√	√
Assay	√	√			
Use in first trimester			√		

[a]Performance of the biomarker in predicting PE; [b]≥5 ng/mL; [c]≥85%; [d]Significant difference of biomarker level between control group and PE patients; [e]Rapid strip assay prototype has been developed; [f]Antibodies commercially available; [g]Assay commercially available; [h]Elecsys® sFlt-1Roche; [i]Alere Triage® PlGF; [i]Antibodies developed but not commercially available.

sensitivity (92%) and specificity (91%) to PE. Its concentration remains constant in controls throughout pregnancy in non-PE patients; whereas, its levels start at a higher value in PE patients in the first trimester and increase throughout pregnancy – suggesting that GlyFN could be used as a predictive screening or diagnostic tool [57]. In contrast, urinary adipsin generally has a lower specificity (70%) than GlyFN, but the specificity is 100% when combined with an increased diastolic BP reading [61].

Another interesting and promising candidate is urine congophilia or Congo Red dot test (CRD), which is a rapid, inexpensive, and usable diagnostic method in

Table 2B: Overall performance of 11 urine biomarker candidates of preeclampsia (PE).

Ranking category[a]	**Good**					
Biomarker	Urine congophilia (Congo Red dot test)				Adipsin	
Detection level[b]	√				√	
Sensitivity to PE[c]	√				√	
Specificity to PE[c]	√				√	
Clinically relevant[d]	√				√	
RDT[e]	√				√	
Antibodies[f]	√				√	
Assay[g]	√				√	
Use in first trimester						
Ranking category	**Adequate**					
Biomarker	Podocyturia	NGAL	P-lPG	sEng	PlGF	KIM-1
Detection level		√				√
Sensitivity to PE	√		√		√	
Specificity to PE	√	√		√		
Clinically relevant RDT	√	√	√	√	√	√
Antibodies	√	√	√	√	√	√
Assay		√	√	√	√	√
Use in first trimester			√			
Ranking category	**Poor**					
Biomarker	MDA		sFLT-1		VEGF	
Detection level	√					
Sensitivity to PE						
Specificity to PE						
Clinically relevant RDT	√		√			
Antibodies			√		√	
Assay			√		√	
Use in first trimester						

[a]Performance of the biomarker in predicting PE; [b]≥5 ng/mL; [c]≥85%; [d] Significant difference of biomarker level between control group and PE patients; [e]Rapid strip assay prototype has been developed; [f]Antibodies commercially available; [g]Immunoassay has been developed.

LRS. Buhimschi et al. recently demonstrated that CRD had better predictive value for PE and clinical outcomes than Pr:Cr ratio, sFlt-1, and PlGF, as well as their ratio – implying that CRD carries diagnostic and prognostic potential for PE [60]. It should be noted, however, that the kidney is frequently affected in amyloidosis, suggesting that CRD could have cross-sensitivity with other kidney dysfunctions that are not due to PE.

The following blood and urine biomarkers sEng, sFlt-1, serum UA, and PlGF are promising candidates as well. Although two automated assays exist for PlGF and sFtl-1 (Triage® PlGF, Alere; Elecsys® sFlt-1, Roche), they are not suitable and affordable for use in LRS in their current format, mainly because of their high complexity requiring well equipped laboratory with constant availability of electricity and highly trained technicians. The existing test kit to measure serum UA level requires a mix of enzymes. This means that the kit would need to be kept at a low temperature, which is rather difficult in LRS environments. Vasopressin, though not a candidate meeting "good" criteria, is still worth mentioning. Santillan et al. showed that maternal plasma vasopressin concentration is predictive of the development of PE in all three trimesters with clinically significant sensitivities ranging from 78% to 88% and specificities ranging from 71% to 84% [59].

Discussion

Diagnosing PE in remote and/or poor settings is difficult without access to well equipped clinical laboratories and trained medical personnel. Consequently, developing diagnostics for PE that are affordable and compatible with the needs and conditions of LRS remain an important goal in global health.

Despite extensive research to date, a reliable screening test with high sensitivity, specificity, and predictive value has yet to be developed. Although numerous clinical and laboratory studies have examined biomarkers listed in Table 1, many of the studies have not reported sensitivity and specificity. Some studies have reported promising positive or negative predictive values but with large confidence intervals due to the low number of patients. Other studies have compared assay performance in women with established disease or have tested at a fixed time point rather than at presentation. Overall, the existing data are in many cases contradictory and confusing.

Despite the lack of definitive data on biomarkers some emerging assay development activities look promising. Two automated immunoassays specific for sFtl-1 and PlGF, (Elecsys® sFlt-1/PlGF from Roche and the Alere Triage® PlGF test) have become available for clinical use to diagnose PE. Although these two tests have not yet been approved by the US Food and Drug Administration for commercial use in the US, a direct comparison between Triage® PlGF and Elecsys® sFlt-1/PlGF found a clinical sensitivity and specificity of 100% and 96% for Triage® and 64% and 100% for Elecsys® in diagnosing early-onset PE [19]. Although these tests offer hope for improved PE screening, they are not suitable and affordable for use in LRS in their current form. Both sFtl-1 and PlGF, however, could be used as the basis for simpler rapid assays, although probably with less sensitivity and accuracy.

Using an arbitrary scoring method, we found five blood and two urine biomarker candidates that are promising and warrant further investigation and validation

Additionally, of these seven biomarkers, a POC prototype test has been described for the analysis of GlyFN, urine congophilia, and urinary adipsin levels in blood and urine, respectively (Table 2A and 2B). The ability to use this biomarker tests in a POC format could quickly determine risk for PE in pregnant women and support their utility in diverse settings, including LRS.

While further clinical studies are needed, PE-specific blood and/or urine assays could potentially be developed by creating a lateral flow-assay based on one or several of the "good" candidates listed above. Subsequently other biomarkers (e.g., vasopressin) might join the "good" group. Assay development for urine- and blood-based biomarkers would be part of a future research and development (R&D) effort that would ultimately lead towards product development. From a programmatic perspective, a strategic plan could be outlined detailing not only the R&D pathway but also corroborating pathways (e.g., clinical validation, commercial development, advocacy, implementation, and monitoring and evaluation) that must be undertaken as parallel efforts to lead to viable prototypes and an eventual commercialized product.

Ultimately, the test for a biomarker for PE would need to be integrated into the current model of interventions during ANC with minimal disruption to increase the likelihood that the test will actually be ordered and that the results will influence the individual woman's care plan.

At this point, three scenarios for using a PE biomarker could be envisaged. First, such a biomarker could be predictive in the first trimester of pregnancy. Ideally, a predictive screening test for PE could be performed early in ANC, preferably at 8–12 weeks of gestation. All pregnant women would be screened at their first ANC visit, implying that the test is available at all POCs where ANC is offered. As the biomarker in this scenario is being used to predict PE, proteinuria would continue to be an important diagnostic criterion for PE.

It was demonstrated that calcium supplementation reduces the incidence of PE during pregnancy, especially in populations with low baseline calcium intake [62]. Recently, Gizzo and colleagues [63] proposed a biochemical screening test of maternal calcium metabolism pattern during first trimester of pregnancy (12th gestational week) using calcium serum levels as a biomarker. As calcium levels in serum can be determined electrochemically [64] or via optochemical sensing using calcium-specific fluorescent dyes [65, 66], it may be possible to design a screening test for the measurement of calcium in the blood of pregnant women at a relatively low-cost.

Second, the biomarker could be used to confirm/rule out diagnosis of PE. The biomarker will ultimately replace proteinuria in the definition of PE. Pr:Cr tests, however, will continue to be beneficial for use in testing for kidney disease and urinary tract infection. The potential advantages of this approach include improvement in the sensitivity and specificity of the diagnostic criteria for PE/E, the ability to differentiate between PE and other disease entities that present with proteinuria and/or hypertension, and the ability to predict adverse outcomes.

Third, the biomarker would be used as a prognostic tool to assist with decisions about timing of childbirth in women diagnosed with PE/E. To improve maternal and perinatal outcomes from PE, having a biomarker that could quantify maternal and perinatal risks and aid to stratify care would be beneficial. Recently, Chappell et al. reported that low PlGF concentration (<5th centile or ≤100 pg/mL) has high sensitivity (0.96%) and negative predictive value (0.98%) in determining which women presenting with suspected disease at <35 weeks of gestation are likely to need delivery for PE within 14 days [67].

The concentrations of most biomarkers will likely either significantly increase or decrease as pregnancy progresses. An assay that is able to determine this increase or decrease most accurately (i.e., expensive and complex ELISAs and proteomics-based assay panels under development for high-resource settings) would potentially become a predictive assay. A less accurate and sensitive test might only be able to determine biomarker level changes once they become significant enough – later in pregnancy – and thus become a useful diagnostic tool. Novel, sensitive, but still relatively inexpensive emerging platforms, such as microfluidics-based ELISAs, could achieve sensitivity and accuracy similar to the more complex assays but at a lower cost.

Ultimately, the question of which combination of biomarker(s), assay format, and use algorithm is the most appropriate way of balancing clinical predictive and diagnostic performance with cost, ease of use, and ease of access in LRS needs to be answered. Given the physiological complexity of PE/E, a large, collaborative multisite study along the lines of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study [68] would be ideally suited to answer this question. Such a study would ultimately result in consensus regarding a screening and diagnostic algorithm. At the end of 2012, IMPROvED (Improved Pregnancy Outcomes via Early Detection), a consortium comprising of European enterprises and academic institutions launched a Phase 2a prognostic multicenter hospital-based clinical study [69] using biomarkers previously identified through metabolomic and proteomic platforms for PE prediction [41, 70]. This ongoing study will allow assessment of the predictive performance of the

proteomic and metabolomics tests throughout pregnancy, and offers mothers accurate risk assessment for PE, thus impacting the provision of ANC care in high-resource countries.

In conclusion, we believe that the implementation of a biomarker-based screening and/or diagnostic test is vital because it offers the potential to have substantially higher sensitivity and specificity than current clinical methods. It could also predict adverse outcomes, reducing maternal mortality and morbidity in low-resource countries. For use in LRS, an ideal biomarker-based test requires only limited technical expertise, is low cost, and has a high degree of accuracy – enabling quick screening for and/or diagnosis of PE.

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