Original Article

Strategy for Standardization of Preeclampsia Research
Study Design
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Abstract—Preeclampsia remains a major problem worldwide for mothers and babies. Despite intensive study, we have not been able to improve the management or early recognition of preeclampsia. At least part of this is because of failure to standardize the approach to studying this complex syndrome. It is possible that within the syndrome there may be different phenotypes with pathogenic pathways that differ between the subtypes. The capacity to recognize and to exploit different subtypes is of obvious importance for prediction, prevention, and treatment. We present a strategy for research to study preeclampsia, which will allow discrimination of such possible subtypes and also allow comparison and perhaps combinations of findings in different studies by standardized data and biosample collection. To make studies relevant to current clinical practice, the definition of preeclampsia can be that currently used and accepted. However, more importantly, sufficient data should be collected to allow other diagnostic criteria to be used and applied retrospectively. To that end, we present what we consider to be the minimum requirements for a data set in a study of preeclampsia that will facilitate comparisons. We also present a comprehensive or optimal data set for in-depth investigation of pathophysiology. As we approach the definition of phenotypes of preeclampsia by clinical and biochemical criteria, adherence to standardized protocols will hasten our understanding of the causes of preeclampsia and development of targeted treatment strategies. (Hypertension. 2014;63:00-00.) ● Online Data Supplement

Key Words: hypertension ▪ placenta ▪ pre-eclampsia ▪ pregnancy ▪ proteinuria

Preeclampsia remains a major problem worldwide for mothers and babies. It is estimated that yearly 50,000 women die in developing countries from preeclampsia.1 Careful maternal observation for the signs of preeclampsia and delivery of women with increasingly severe preeclampsia is the cornerstone of management (as it has been for the past 100 years). Maternal mortality is, therefore, much less in developed countries with the capacity for careful perinatal observation, but morbidity is considerable and remains the leading cause of admissions to intensive care for pregnant women.2 Also, the appropriate delivery of women who develop increasingly severe preeclampsia early in gestation accounts for 8% of all preterm births.3

Why No Advances in Clinical Management?
During the past 20 years, there has been an explosion in our knowledge of preeclampsia. The recognition of inflammation, including endothelial dysfunction as potential unifying pathophysiological concepts and the appreciation of the multisystemic nature of preeclampsia, has directed attention away from blood pressure as the sole or even most important pathophysiological issue of preeclampsia.4 This concept has resulted in recognition of other origins of organ dysfunction. Despite this, we have not managed to affect the management or early recognition of preeclampsia with this information. Large, well-designed multicenter, clinical intervention trials have, at best, demonstrated a minimal effect on outcome except in perhaps the highest risk cases. Attempts to use factors implicated in the pathophysiology of the disorder to predict preeclampsia have also not as yet provided adequate sensitivity and specificity to be adopted for use in routine clinical practice.5

Is There >1 Subtype of Preeclampsia?
Why is this? A recurring theme is success in small studies of prediction, prevention, or treatment of preeclampsia, and failure in larger adequately powered multicenter trials. This...
is often interpreted as a result of publication bias. However, an alternative explanation is that the important difference between small and large studies is that small studies are usually within homogeneous populations, whereas large multicenter studies include a much more heterogeneous group of women. Furthermore, another explanation for the poor predictive power of studies guided by proposed pathogenic factors is that none of these factors can be demonstrated in all women with preeclampsia (Figure S1 in the online-only Data Supplement). These findings lead to the hypothesis that not all preeclampsia is the same, that subtypes may be present.

This is supported by clinical and epidemiological data. Most preeclampsia occurs in the last month of pregnancy; however, the 10% of earlier cases are strikingly different than those occurring at term. The excess of small for gestational age deliveries that occurs in preeclampsia is associated with disease presenting before 37 weeks of gestation when preeclampsia tends to be more severe. Epidemiological data indicate major differences in the risk of later life cardiovascular disease with the risk with earlier onset preeclampsia 8- to 10-fold versus a doubling when preeclampsia occurs close to term. A similar increased cardiovascular risk is present with recurrent preeclampsia. Clinically, preeclamptic women at any gestational age may present with fulminant preeclampsia that goes from recognition to life-threatening disease over hours to days or the syndrome may be indolent with little progression in the same time frame.

This hypothesis predicts that no 1 test will predict and no 1 treatment will prevent preeclampsia. However, to offer encouragement, this also means that if we could identify subtypes of preeclampsia, appropriate predictors could more successfully predict, and the appropriate treatment more effectively prevents the different subtypes of preeclampsia.

**What Should We Do Differently?**

Study designs that aggregate what might be different forms of preeclampsia, resulting from different pathophysiological pathways, are part of the problem. Amalgamation of the less obvious heterogeneous phenotypes is compounded by studies that combine obviously dissimilar subsets. Considering all causes of increased risk of preeclampsia as resulting in a group of homogeneous high-risk subjects is one such obvious error. Should it be surprising that a preeclamptic woman with a large placenta, as present with multiple gestations or diabetes mellitus, would not respond to the same preventive therapy as a woman with chronic hypertension or previous preeclampsia?

Also important differences between recurrent and first pregnancy preeclampsia are often ignored and early and late onset preeclampsia are usually combined for analysis. Another discrimination, the difference between preeclampsia with proteinuria and gestational hypertension with no obvious systemic changes is often not made. It is likely that some cases of gestational hypertension are early preeclampsia. It is also possible that others reflect chronic hypertension, masked in early pregnancy by the physiological decrease in blood pressure that occurs at that time. Gestational hypertension without systemic involvement could also be a distinct and unrelated phenotype. In most settings, the findings with gestational hypertension are intermediate between normal pregnancy and preeclampsia. However, the increasing certainty that gestational hypertension is not a manifestation of a multisystemic syndrome (absence of hyperuricemia and markers of endothelial dysfunction) suggests that it generally is of more benign origin with outcomes for mother and baby not different than in normal pregnancy. There may, therefore, be a form of new onset hypertension in pregnancy, which has minimal effect on mother or baby.

The capacity to recognize and to exploit different subtypes is of obvious importance for prediction, prevention, and treatment. As an analogy, our progress in the successful management of diabetes mellitus would have been far less if all patients with carbohydrate intolerance were thought to be insulinopenic.

**What Might Be the Subtypes of Preeclampsia?**

Obvious subtypes of preeclampsia are early and late onset, recurrent and nonrecurrent, and preeclampsia with the different types of high-risk pregnancies. Clinically it is also possible that severe and mild preeclampsia and preeclampsia with and without intrauterine growth restriction could be different (see online-only Data Supplement). In addition, preeclampsia seems to have a different target in different cases. The primary organ involvement may be hepatic, renal, cardiovascular, or placental. Do these define different subtypes in which prediction and prevention may require different strategies?

Another interesting subclassification could exploit the differences in pathophysiological biomarkers associated with preeclampsia. In this regard, the most valuable findings would be those present before clinically evident disease. Once disease is established, the biochemical consequences of multiple organ involvement will mask causal pathways. However, we know that not all subjects manifest the same early markers, so should we begin to redefine preeclampsia on the basis of, for example, inflammatory, angiogenic, oxidative stress, or endoplasmic stress-mediated subtypes? Some caution is required here, however, because some biochemical clusters may reflect different steps in a common pathway. Nonetheless, the common strategy of amalgamating all is increasingly undermined by the current evidence base. To achieve progress in prediction and prevention inevitably demands recognition of subtypes (see online-only Data Supplement). Although the hypothesis of several subtypes of preeclampsia to explain the discrepant findings and outcomes in preeclampsia is attractive, it has also recently been proposed that true preeclampsia is only present when excess angiogenic or deficient angiogenic factors are present. Without these findings, preeclampsia is a misdiagnosis. The argument is that the most dangerous features of new onset gestational hypertension with proteinuria or other organ involvement are much more common when angiogenic imbalance is evident from laboratory findings. These abnormal angiogenic findings are also more prevalent in early onset, the most serious form of this disorder. To a certain extent this concept is not without potential risk if applied to current clinical practice. Most deaths from preeclampsia are in developing countries with late onset preeclampsia, which is less likely to be accompanied by these laboratory findings. Nonetheless the understanding of preeclampsia regardless of semantics will be aided by more standardized definitions and data and biological sample collection.

It is with this goal of translating current and emerging understanding to define, treat, and prevent disease that we make the following proposal for the investigation of preeclampsia.
Proposal

Appreciation of Preeclampsia as a Syndrome

The current definition of preeclampsia requires renal (proteinuria) and cardiovascular (blood pressure) dysfunction. These were established historically as the first signs preceding what at the time was considered a pregnancy-specific seizure disorder, eclampsia. They were not selected as sensitive or specific indicators of maternal or fetal morbidity. However, in combination they predict increased risk for mother and baby and indicate that preeclampsia affects many organ systems. This is confirmed by the increased risk associated with gestational hypertension when accompanied by other systemic involvement, even without proteinuria. Thus, a key feature in studying preeclampsia is recognizing the fact that it is a syndrome and that it can occur in the absence of proteinuria.

Identification of Preeclampsia Subtypes

Identifying possible preeclampsia subtypes is clearly an important goal in translating findings of preeclampsia research into effective modifications of clinical care. We have presented obvious candidates. How do we modify current research strategies to address this goal? Either we must examine homogenous groups of women—only nulliparas, only obese women, only women with previous preeclampsia, only early onset preeclampsia, etc—or the study population should be of an adequate size to enable separate study of these obviously different groups. There should, at the very least, be an effort to look at results in relation to these different possible subtypes (and allow readers to also make these comparisons), given the problems of inadequate power in smaller studies. The solution to this quandary is big science that is the merging of data and biological samples from several centers. This is a major goal of the Global Pregnancy CoLaboratory, which has authored this article because data and biosample sharing can only be successful with standardized data and sample collection.

We present a strategy for research to study preeclampsia and suggest that further large multicenter trials be deferred until rigorous exploration for subtypes of preeclampsia has been attempted.

Approach

Comparisons and interpretation of the data generated in the many studies of preeclampsia remain complicated because of differences in study sizes, study designs, definition of patient groups, and outcomes measured. There is a need for standardization of study design, including patient selection, data collection, and definition of outcome, to allow comparable studies and trials to be performed and allow comparison of data sets and integration for meta-analyses. To facilitate comparison of studies or trials, at a minimum, the patient groups selected, information collected, and definitions used need to be similar. Critical components of this approach are unambiguous and unbiased definitions. With this in mind, we recommend collecting the clinical and laboratory information necessary to make the diagnosis that is then examined retrospectively in a blinded manner by impartial observers rather than relying on clinical diagnoses made by care providers.

We offer here an outline that can be used for study design and clinical trials. We also present what we think are the minimum requirements for a data set in a study of preeclampsia that will facilitate comparisons (Table 1). Subsequently, we define a comprehensive or optimal data set (Table 2) together with recommendations for collection of biological materials (Table 3). This, we consider, would provide all that is needed for in-depth investigation of pathophysiology.

Key Definitions

For studies to be relevant to current clinical practice, the definition of preeclampsia can be that currently used and accepted. However, diagnostic criteria change. Thus, sufficient data should be collected (Tables 1 and 2) to allow retrospective analysis not only to satisfy new diagnostic recommendations but also to facilitate the development of novel and improved methods of diagnosis (see online-only Data Supplement). Defining the syndrome by only traditional criteria is too limited and does not facilitate progress.

Gestational Age

Gestational age should be determined using information from the last menstrual period, if known, and first or second trimester ultrasounds with standardized criteria for resolving discrepancies between menstrual history and ultrasound findings or, if last menstrual period is not known, preferably by first trimester ultrasound (Table S1, online-only data supplement). Gestational age should be recorded by completed weeks and days.

Fetal Variables

A proportion of pregnancies complicated by preeclampsia is also associated with intrauterine growth restriction. In all cases, birth weight and gestational age data should be recorded to determine whether the fetus is small for gestational age. Population-specific birth weight centiles adjusted for gestational age, ethnicity, and sex should be calculated.

Control Subjects

Mechanistic Studies

Parity, age, race, ethnicity, smoking, and body mass index are all recognized to influence the incidence of preeclampsia and patients in case control studies should, therefore, be carefully matched for each of these factors. Also, in these studies, it is appropriate to compare women with preeclampsia with women with normal outcomes to identify the specific pathophysiology of preeclampsia. In case control studies, case and controls should be matched for gestational age and parity.

Prediction Studies

For studies of predictors, it is not appropriate to compare women with preeclampsia with women with normal outcomes. This will inevitably falsely enhance the predictive capability. When a predictive test is used in the real world, it will attempt to identify women with preeclampsia as distinct from all other outcomes, both normal and abnormal. Therefore, the use of the test in this scenario should always be evaluated. Any information not known at the times of testing (eg, eventual pregnancy outcome) must not influence the selection of controls. It is equally inappropriate to combine high-and low-risk women in a prediction study; populations...
should be predefined according to the risk status. Eventually, after such trials of defined risk subjects, clinical data and biomarkers can be combined for prediction.

**Clinical Trials**

**Low-Risk Subjects**

Standardization of studies of low-risk subjects should use the following exclusion criteria at recruitment:

1. Two or more blood pressures with systolic pressure $\geq 135$ mm Hg or diastolic blood pressure $\geq 85$ mm Hg during this pregnancy. If the screening blood pressure is the only blood pressure that exceeds this cutoff, a repeat blood pressure should be taken within 30 to 60 minutes. If this second blood pressure remains $\geq 135$ mm Hg systolic or $\geq 85$ mm Hg diastolic, the patient is excluded.
### Table 2. Optimal Data Set for Studies on Preeclampsia

<table>
<thead>
<tr>
<th>Maternal data (data from minimal data set plus the following)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical history</td>
</tr>
<tr>
<td>Gestational age at start of documented maternity care</td>
</tr>
<tr>
<td>Number of prenatal visits at doctor/midwife/hospital in present pregnancy</td>
</tr>
<tr>
<td>Blood transfusions</td>
</tr>
<tr>
<td>in life-time</td>
</tr>
<tr>
<td>In present pregnancy</td>
</tr>
<tr>
<td>Fertility history</td>
</tr>
<tr>
<td>Assisted reproductive technology</td>
</tr>
<tr>
<td>present pregnancy</td>
</tr>
<tr>
<td>any previous attempted pregnancy</td>
</tr>
<tr>
<td>IVF</td>
</tr>
<tr>
<td>ICSI</td>
</tr>
<tr>
<td>Artificial insemination</td>
</tr>
<tr>
<td>Partner/donor sperm</td>
</tr>
<tr>
<td>Egg recipient</td>
</tr>
<tr>
<td>Embryo recipient</td>
</tr>
<tr>
<td>Age at menarche</td>
</tr>
<tr>
<td>Birthweight of the pregnant woman</td>
</tr>
<tr>
<td>Duration of preconception sexual intercourse with biological father of child (months [list as zero if donor semen])</td>
</tr>
<tr>
<td>Previous pregnancy outcomes (indicate numbers, and if with same partner or a previous partner and gestational age at occurrence)</td>
</tr>
<tr>
<td>Miscarriage</td>
</tr>
<tr>
<td>Stillbirth</td>
</tr>
<tr>
<td>Induced abortion</td>
</tr>
<tr>
<td>Recurrent spontaneous pregnancy loss</td>
</tr>
<tr>
<td>Gestational hypertension</td>
</tr>
<tr>
<td>Preeclampsia</td>
</tr>
<tr>
<td>Eclampsia</td>
</tr>
<tr>
<td>HELLP</td>
</tr>
<tr>
<td>SGA and IUGR</td>
</tr>
<tr>
<td>Gestational diabetes mellitus requiring treatment with insulin or oral hypoglycemic agents</td>
</tr>
<tr>
<td>Preterm delivery (&lt;37 wk)</td>
</tr>
<tr>
<td>Neonatal death</td>
</tr>
<tr>
<td>Relevant maternal family history</td>
</tr>
<tr>
<td>Mother, sister, or cousin with preeclampsia</td>
</tr>
<tr>
<td>Validated or self-reported</td>
</tr>
<tr>
<td>Family history (siblings, parents, and grandparents) of cardiovascular disease (none, hypertension, CHD, stroke, and actual age [y] at occurrence)</td>
</tr>
<tr>
<td>Family history (siblings, parents, and grandparents) of diabetes mellitus</td>
</tr>
<tr>
<td>Relevant paternal family history</td>
</tr>
<tr>
<td>Has he fathered a preeclamptic pregnancy? (this mother/other mother)</td>
</tr>
<tr>
<td>Mother, sister, or cousin with preeclampsia</td>
</tr>
<tr>
<td>Validated or self-reported</td>
</tr>
<tr>
<td>Family history (siblings, parents, and grandparents) of cardiovascular disease (none, hypertension, CHD, stroke, and actual age [y] at occurrence)</td>
</tr>
<tr>
<td>Nicotine history</td>
</tr>
<tr>
<td>(Continued)</td>
</tr>
</tbody>
</table>

### Table 2. Continued

<p>| Cigarette/cigar smoker                                       |
| Snuff user                                                   |
| Chews tobacco/takes nicotine                                 |
| None of above used ever                                      |
| Used irregularly/regularly only before pregnancy             |
| Continues (no. of cigarettes/d: 1–10, 11–20, &gt;20 per day, no. of cigars 1, 2–5, &gt;5 per day) |
| In third trimester (28–36 wk), Stopped since early pregnancy, restarted since early/before pregnancy, continues to smoke [no.] |
| Alcohol use                                                 |
| At baseline (never/gave up before pregnancy/gave up during pregnancy/this pregnancy) number of units/wk |
| In third trimester (stopped since early pregnancy, restarted since early/before pregnancy, continues to drink) number of units/wk |
| Recreational drugs/drug abuse (yes/no)                      |
| Cannabis (yes/no)                                            |
| Cocaine (yes/no)                                             |
| Opiates (heroin/morphine/codeine/methadone; yes/no)          |
| Methamphetamine                                             |
| Ecstasy/other central stimulating drugs? (specify)           |
| At baseline (never/gave up before pregnancy/gave up during pregnancy/this pregnancy) |
| In third trimester (stopped since early pregnancy, restarted since early/before pregnancy, continues to use) |
| Clinical data                                               |
| Blood pressures                                             |
| First blood pressure (and gestational age)                   |
| Two highest systolic and diastolic blood pressures at each visit (can be at different times) or each week if visit lasts &gt;1 wk |
| At diagnosis of preeclampsia                                 |
| Two highest systolic blood pressures within 2 wk of delivery |
| Two highest diastolic blood pressures within 2 wk of delivery |
| Urine protein values (at each visit)                        |
| First urinalysis (and gestational age)                       |
| 24 h or timed collections                                    |
| Protein/creatinine ratio                                     |
| Weight gain during pregnancy                                 |
| Weight gain since last delivery                              |
| Growth by ultrasound                                         |
| Constant (above, below, or on curve)                        |
| Falling off with increasing gestation                        |
| Macrosomia                                                   |
| Uteroplacental blood flow indices at mid gestation (16–25 wk), performed/not performed |
| Notching (yes/no)                                            |
| Unilateral (yes/no)                                          |
| Bilateral (yes/no)                                           |
| Pulsatility index (mean of bilateral measurements)           |
| Umbilical blood flow indices if clinical suspicion of FGR or documented FGR (done/not done) |
| Gestational age at which performed                           |
| Pulsatility index (value) and resistance index (value)        |</p>
<table>
<thead>
<tr>
<th>Table 2. Continued</th>
</tr>
</thead>
</table>
| Absent end diastolic flow (yes/no)  
Reversed end diastolic flow (yes/no)  
Fetal growth ultrasound  
12 wk  
18–20 wk  
28 wk  
36 wk  
And if clinical indication of FGR or documented FGR |
| Labor (active phase, yes/no; labor defined as uterine contractions which result in cervical dilatation and effacement)  
Spontaneous (yes/no)  
Induced (yes/no)  
Induction indicated for hypertensive disorder (yes/no)  
Cesarean section (yes/no)  
C section indicated for hypertensive disorder (yes/no)  
Medical conditions before pregnancy (in addition to those in minimal data set)  
Select either  
In pregnancy alone  
Before pregnancy  
Before and continuing during pregnancy  
Other endocrine disease  
Thyroid disease  
Adrenal disease  
Liver disease  
Hematologic disorder, including alloimmune or isoimmune  
Epilepsy or seizure disorder  
Heart disease  
Cancer  
Metabolic syndrome (any 3 of the 5 criteria described in Alberti et al21 are present before pregnancy)  
PCOS (≥2 of the following 3 features are present)  
oligo- and anovulation  
clinical and biochemical signs of hyperandrogenism  
polycystic ovaries and exclusion of other pathogeneses (congenital adrenal hyperplasia, androgen-secreting tumors, Gushing syndrome)  
Infectious disease  
Malaria  
Placental yes/no, laboratory diagnosis yes/no  
HIV  
CD4 count  
TB  
Active or inactive  
Schistosomiasis  
Hepatitis B  
STD  
Gonorrhea  
Syphilis  
Chlamydia  
Herpes  
Trichomoniasis |
| Table 2. Continued |
| Genital warts  
Other  
Urinary tract infection  
Antibiotics (yes/no)  
Other infectious disease  
Medications before and during pregnancy  
Select either  
In pregnancy alone (Which week started?)  
Before pregnancy  
Before and continuing during pregnancy  
Vitamins  
Vit C  
Vit D  
Vit E  
Other  
Multivitamins  
Folate  
Fortified foods available in country of residence (yes/no)  
List additives used for fortification  
Aspirin  
Platelet active drugs  
Antioxidants  
High dosages of vit C (>500 mg)  
High dosages of vit E (>400 IU)  
β-carotene  
Resveratrol  
Selenium  
Coenzyme Q10  
Other (specify)  
Fish oil  
Calcium (specify amount)  
Iron supplements (specify)  
Diuretics (specify)  
Antihypertensive agents (specify)  
Antibiotics (specify)  
Anticoagulants (specify)  
Anticonvulsants  
MgSO4  
Other (specify)  
Antidepressants (SSRIs; specify)  
Antiglycemic agents  
Insulin  
Metformin  
Other (specify)  
Long-term immunosuppressants  
Thyroid supplements  
Antithyroid treatment for thyrotoxicosis  
Other (specify)  
Postnatal maternal care  
Length of stay in hospital, d |
| (Continued) |
| (Continued) |
Table 2. Continued

<table>
<thead>
<tr>
<th>Infant data (data from the minimal data set plus the following)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
</tr>
<tr>
<td>APGAR scores (1, 5, and 10 min if recorded)</td>
</tr>
<tr>
<td>Umbilical cord gases</td>
</tr>
<tr>
<td>Admitted to NICU (yes/no)</td>
</tr>
<tr>
<td>Length of stay in NICU, d</td>
</tr>
<tr>
<td>Outcome at discharge from NICU</td>
</tr>
<tr>
<td>IVH</td>
</tr>
<tr>
<td>BPD</td>
</tr>
<tr>
<td>RDS</td>
</tr>
<tr>
<td>NEC</td>
</tr>
<tr>
<td>Hypoxic ischemic encephalopathy</td>
</tr>
<tr>
<td>Convulsions</td>
</tr>
</tbody>
</table>

Placenta data

<table>
<thead>
<tr>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
</tr>
<tr>
<td>Pathology report (if sent for pathology)</td>
</tr>
<tr>
<td>Photograph against a scale bar</td>
</tr>
</tbody>
</table>

Appendix for other important information

BPD indicates bronchopulmonary dysplasia; CD4, cluster of differentiation 4; CHD, coronary heart disease; FGR, fetal growth restriction; HELLP, hemolysis elevated liver enzymes low platelets; ICSI, intra-cytoplasmic sperm injection; IUGR, intrauterine growth restriction; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; PDA, polycystic ovary syndrome; RDS, respiratory distress syndrome; SGA, small for gestational age; STD, sexually transmitted disease; SSRI, selective serotonin reuptake inhibitor; Vit, vitamin; and TB, tuberculosis.

Table 3. Collection of Biological Materials

<table>
<thead>
<tr>
<th>Timing of samples (preferably coordinate with clinical examination/visit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8–10 wk</td>
</tr>
<tr>
<td>16–20 wk (fasting)</td>
</tr>
<tr>
<td>28 wk</td>
</tr>
<tr>
<td>36 wk</td>
</tr>
<tr>
<td>At delivery before labor</td>
</tr>
<tr>
<td>At discharge from hospital (list time after delivery)</td>
</tr>
<tr>
<td>6–24 mo postpartum (maternal sample)</td>
</tr>
</tbody>
</table>

Type of samples (blood samples should be taken from vein without ongoing intravenous infusion)

- Maternal plasma (EDTA and heparinized)
- Maternal serum
- Maternal urine
- Maternal and paternal residual whole blood for DNA
- Cord arterial and venous blood (plasma and serum as above)
- Cord residual whole blood for DNA
- Umbilical cord tissue for DNA
- Cord blood white blood cell count
- Maternal nail clippings
- Maternal and neonatal buccal swab for DNA
- Meconium

Placenta (see below for recommended method) Amniotic fluid at delivery (if can be obtained in sterilized manner)—save both pellets and supernatant

Conditions of collection and storage

- Blood: processed within 30 min of draw, in freezer by 1 h (times from draw to processing to freeze should be recorded). Store in 0.5-mL aliquots at –80°C
- Urine: centrifuge 25 mL, 5-mL aliquots of supernatant, store at –80°C
- Residual whole blood for DNA: store at –80°C

Placenta

Photograph the placenta from the chorionic and basal aspects against a scale bar

- Take a piece of umbilical cord
- Take a membrane roll 2-cm wide from the rupture site to the placental margin
- Weigh the placenta after trimming the membranes and umbilical cord to 1 cm
- Place the placenta with the basal plate uppermost, overlay a transparent grid with 2×4 sampling sites. At each site remove the basal plate by trimming with a pair of scissors. Then cut out a grape-sized piece of the exposed villous tissue, avoiding areas of infarction or other gross pathology. Wash thoroughly but gently in PBS at 4°C. Quickly divide with scissors or scalpel into pieces for metabolomics, mitochondrial respirometry, electron microscopy, RNA, protein and DNA, immunohistochemistry, and frozen sections.
- Tissue should be processed within 30 min of delivery (10 min for RNA) and record time to sampling

2. Proteinuria as exhibited by either of the following:
   a. A spot urine protein/creatinine ratio of >30 mg/mmol at any time during this pregnancy
   b. A 24-hour urine collection of ≥300-mg protein, or the equivalent from a timed collection, at any time during this pregnancy. ( Dipstick protein values should not be used unless no other measurement is available, then two readings of 1+ would exclude the individual)

3. History or current use of antihypertensive medication (including diuretics).

4. Pregestational diabetes mellitus

5. Current pregnancy as a result of in vitro fertilization

6. Regular use (more than once a week) of platelet active drugs (eg, heparin) or nonsteroidal anti-inflammatory agents affecting platelet activity (eg, ibuprofen, aspirin, Cox-1 and Cox-2 inhibitors). The use of platelet active drugs or nonsteroidal anti-inflammatory agents affecting platelet activity within 7 days (168 hours) before randomization for all studies.

7. Known fetal abnormalities (eg, neural tube defect), known chromosomal or major malformations, fetal demise, or planned termination.

8. Documented uterine bleeding within a week of screening. Unobserved self-reported bleeding with confirmed intact pregnancy on ultrasound after the bleeding episode is not an exclusion.

9. Uterine malformations

10. History of medical complications such as the following:
   a. Cancer (including melanoma but excluding other skin cancers)
   b. Endocrine disease, including thyroid disease and adrenal disease
   c. Renal disease with altered renal function (creatinine >78.6 μmol/L [0.9 mg/dL] or proteinuria [as above])
d. Epilepsy or other seizure disorder
e. Any collagen disease (lupus erythematosus, scleroderma, etc)
f. Active or chronic liver disease (acute hepatitis, chronic active hepatitis, persistently abnormal liver enzymes)
g. Hematologic disorder, including alloimmune and isoimmune thrombocytopenia but excluding mild iron deficiency anemia (Hb>90 g/L)
h. Chronic pulmonary disease, including asthma requiring regular the use of medication
i. Heart disease except mitral value prolapse not requiring medication

12. Illicit drug or alcohol abuse during current pregnancy
13. Participating in another intervention study that influences maternal and fetal morbidity and mortality or participation in this trial in a previous pregnancy.

High-Risk Subjects
Studies of women who are at high risk for development of preeclampsia should be sufficiently powered to determine the efficacy of therapy or prediction on obviously disparate risk groups separately.

Confounding Factors to be Considered in All Studies

Obesity
Obesity has a profound effect on the incidence of preeclampsia with the incidence typically doubling with each 5 to 7 kg/m² increase in prepregnancy body mass index. Indeed the dramatic increase in obesity in the United States for the past 10 years means that obesity has become a major pathophysiologic factor, probably via its associated inflammatory milieu, in the development of preeclampsia. Because obesity is also more prevalent in black and Hispanic populations, it needs to be taken into account as a confounding factor in studies. If possible, prepregnancy body mass index should be recorded together with body mass index in the first trimester and at delivery. Weight gain throughout pregnancy should be calculated.

Smoking
The incidence of smoking is decreasing slowly in the United States but varies by region and by socioeconomic status. Paradoxically smoking exerts a protective effect on the development of preeclampsia although preeclampsia that develops in smokers is usually more severe. Data on whether the patient was ever or never a smoker should be obtained as well as whether the patient smoked during the index pregnancy.

Sex of the Fetus
There is a strong influence of sexual dimorphism across many aspects of reproductive physiology, particularly those involving inflammatory mechanisms. There is a well-known association of a male fetus with adverse perinatal outcomes, particularly those related to delivery at early gestational age. The presence of a male fetus (and a male placenta) is associated with a slightly greater overall risk (1.02) of development of preeclampsia than that of a female fetus. However, preeclampsia that develops early in gestation is predominantly more associated with a female rather than that with a male fetus (relative risk, 0.7 at 26 weeks). Whether this effect is because of a disproportionate delivery of male fetuses for other causes at this time that removes them from the population that will develop preeclampsia remains unknown. However, the presence of a sexually dimorphic effect means fetal sex should be recorded.

Outcomes
The outcome variables recorded for studies will be dependent on whether the study is a clinical trial of an intervention or whether it is a study evaluating a predictor. In addition, for clinical intervention studies, demographic factors will influence the outcome studied. In developing countries, the focus is on maternal outcomes, whereas in developed countries the focus will be more on fetal outcomes. With this in mind outcomes on both mother and fetus should be collected, and composite outcomes combining fetal and maternal outcomes are discouraged.

Standardized Data Collection
The value and strength of any clinical study is proportional to the amount and quality of data collected. We provide in Table 1 the minimum data set we consider necessary for collection in a study of preeclampsia. This would allow combination and comparison with other data sets to enable meta-analyses to be performed. In Table 2, we provide the optimal data set that could be collected in a comprehensive approach when studies involving determination of pathophysiologic mechanisms are proposed. Guidelines for specifying date and time using International Standard ISO 8601 and for the use of SI units are presented in the in the online-only Data Supplement.

Perspective
Despite many years of clinical and basic science studies and of many small-scale and several large-scale interventional studies, we have not been able to predict, prevent, or treat preeclampsia. There is now a growing realization that under the umbrella of the preeclampsia syndrome, there may be several different phenotypes that may be predicted by distinct biomarkers, presented with different features, and potentially treated by different therapies. Previously, using a standard clinical definition of preeclampsia, these phenotypes have been merged within large cohorts contributing to the lack of success in predicting and treating preeclampsia. The lack of standardization in study design and clinical data acquisition has prevented combination of studies. We offer here an outline that can be used for study design and clinical trials. In addition, we present the minimum requirements for a data set that will facilitate comparisons, whereas collection of a more comprehensive or optimal data set will allow in-depth investigation of pathophysiology. We are now at the point of being able to define phenotypes of preeclampsia by clinical and biochemical criteria and thus tremendously increase our understanding of pathophysiology. Knowledge of distinct pathophysiologies will to lead to more specific therapeutic approaches.

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Disclosures
None.

References

Novelty and Significance

What Is New?

• This study presents a strategy for adherence to standardized protocols to study preeclampsia

• This may allow identification of subtypes of the syndrome and allow comparison and combination of different studies by standardized data and biosample collection.

What Is Relevant?

• Because preeclampsia remains a major problem worldwide for mothers and babies, with no improvement in management or early recognition, there is a need to standardize the approach to study the complex syndrome.

Summary

We present the minimum requirements for a data set to facilitate comparisons in a study of preeclampsia together with a comprehensive or optimal data set to allow in-depth investigation of pathophysiology. In addition, standards for sample collection are presented.
Strategy for Standardization of Preeclampsia Research Study Design
Leslie Myatt, Christopher W. Redman, Anne Cathrine Staff, Stefan Hansson, Melissa L. Wilson, Hannele Laivuori, Lucilla Poston and James M. Roberts

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A STRATEGY FOR STANDARDIZATION OF PREECLAMPSIA RESEARCH STUDY
DESIGN

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*For the Global Pregnancy CoLaboratory (COLAB)
**Presentation and use of data**

Much of the heterogeneity of the pathophysiology of preeclampsia in studies is hidden by standard deviation and standard errors bars. This data array leads to the conclusion that for a particular marker of pathophysiology all women with preeclampsia are moderately or slightly different than normal pregnant women. The true relationship as demonstrated in Figure S1 is that there is enormous overlap with many women with preeclampsia no different than normal women. Thus, whether one posits oxidative stress, antiangiogenic factors or inflammation as the links between the abnormal placenta and maternal systemic disease laboratory assessment shows there are many exceptions.

**Identification of preeclampsia subtypes**

Better definition of phenotypes of preeclampsia and increased understanding of pathophysiology will inevitably lead to not one, but several criteria for diagnosis.

We should begin to exploit differences in preeclamptic women with different laboratory findings. For example in studies focusing on prediction does a particular laboratory finding predict a specific outcome? Currently it is clear that a reduction in circulating angiogenic factors or an excess of antiangiogenic factors is a better predictor of preterm than term preeclampsia. Recently measurement of placenta-associated biomarkers, including angiogenic factors, in the maternal circulation has been proposed as a way to better subclassify preeclampsia groups 1. Do these factors also predict IUGR (with or without preeclampsia)? Is blood pressure higher, is proteinuria more common or are there other associated pathophysiological findings? From a demographic perspective are these findings more likely to be abnormal in obese women, or women with twins or pre-existing hypertension or recurrent preeclampsia? These questions should be considered in all studies of the pathophysiology of preeclampsia.

Another useful finding has been obscured by the use of huge biobanks assembled from residual serum and plasma from early pregnancy screening for aneuploidy. These studies have identified useful correlations with later pregnancy preeclampsia but by definition these biobanks do not include samples at the time of clinical preeclampsia. Is it possible that women whose preeclampsia is not predicted by an early pregnancy pathophysiological marker never have this pathophysiology? In other words are there women whose preeclampsia is not associated with altered early gestational profiles of placentally derived factors including angiogenic markers (or those indicating increased inflammation or oxidative stress)? These are questions that require an answer.

**Subtyping by clinical definition**

Preeclampsia can be conveniently classified into four basic subtypes based on severity and gestational age of diagnosis. The majority of data defining early vs. late preeclampsia has been generated in relation to time of delivery, as this data is easily available in retrospect from the clinical chart. It has thus mainly used <37 or >37 weeks gestation as the definition of early vs late onset preeclampsia. Perinatal morbidity and mortality is greatest in deliveries <34 weeks gestation 2 so we suggest the definition of
early onset preeclampsia be <34 0/7 weeks gestation and late onset be >34 0/7 weeks gestation. This data again can be easily obtained from the chart but of course is predicated by previous accurate determination and recording of gestational age.

More relevant information, particularly in regard to pathophysiology, may be gleaned by definition of time of onset of clinical symptoms of preeclampsia rather than time of delivery. Recording of all blood pressure and urinary protein measurements throughout gestation will allow determination of the earliest time at which the earliest symptom of preeclampsia (elevated blood pressure or proteinuria) in a patient who goes on to develop preeclampsia or the earliest time at which the definition of preeclampsia (hypertension plus proteinuria) is met by a patient. This time of onset may then be hours, days or weeks before delivery with expectant management but would represent time of onset of the syndrome rather than time of delivery due to the syndrome, itself subject to the vagaries of clinical practice. However the precision of defining time of onset is dependent on the frequency of antenatal visits at which measurements can be made and samples collected.

Phenotyping in relation to biochemical and biophysical variables

The rationale for consideration of pre-eclampsia subtypes has been outlined above. We recommend that sub classification should be according to specific organ targets and important underlying pathophysiologies for example angiogenic, inflammatory or oxidative stress. Investigators are encouraged to thoughtfully assess their data with attention to other potential subsets.

Allocation of patients to different subtypes, either clinical or biochemical, will provide enriched populations for study. Not every patient needs to be assigned to a subgroup and feasibly some patients might qualify for more than one subtype, however this enrichment will improve our ability to understand subtypes.

Recommendations for data recording

It is recommended that International Standard ISO 8601 be used to avoid confusion when specifying numeric representations of date and time. The international standard date notation is YYYY-MM-DD where YYYY is the year in the usual Gregorian calendar, MM is the month of the year between 01 (January) and 12 (December), and DD is the day of the month between 01 and 31. For example, the fourth day of February in the year 1995 is written in the standard notation as 1995-02-04.

Units of Measurement: Laboratory values should be expressed as Système International (SI) units of measure. The metric system is preferred for the expression of length, area, mass, and volume. To change from conventional units of measure the Units of Measure conversion table on the website for the AMA Manual of Style should be used:

Calculation of Gestational age

A. If no ultrasound has been performed previously, this procedure must be performed before patient enrollment.

1. The first day of the last menstrual period (LMP) is determined, and a judgment made as to whether or not the patient has a ‘sure’ LMP date.

2. If the LMP date is unsure, the ultrasound measurements obtained at the patient’s first ultrasound examination (preferably first trimester) are used to determine the project gestational age, by the standard method of ultrasound gestational age determination at that institution.

3. If the date of her LMP is sure, and the ultrasound confirms this gestational age within the number of days specified in Table S1, then the LMP derived gestational age is used to determine the project gestational age.

4. If the ultrasound determined gestational age does not confirm the LMP generated gestational age within the number of days specified in Table S1, then the ultrasound is used to determine the project gestational age.
Table S1. Recommendations for Accurate Determination of Gestational Age

<table>
<thead>
<tr>
<th>Gestational age by LMP at first ultrasound</th>
<th>Ultrasound measurement</th>
<th>Ultrasound agreement with LMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>up to 13 6/7 weeks</td>
<td>crown-rump length</td>
<td>± 5 days - use LMP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 6 days - use ultrasound</td>
</tr>
<tr>
<td>16-22 weeks</td>
<td>Biometry based on biparietal diameter, abdominal circumference, and femur length</td>
<td>± 10 days – use LMP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 11 days – use ultrasound</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td>Base on ultrasound, preferably first trimester.</td>
</tr>
</tbody>
</table>

* Adapted from Spong et al ³ with permission of the American Medical Association

Literature Cited


**Figure S1**

The heterogeneity of laboratory findings in preeclampsia: Scattergrams indicate two well-established pathophysiological findings in preeclampsia. **A.** Malondialdehyde concentrations before and 24 h after delivery in women with preeclampsia (PE) or without preeclampsia (Norm). **B.** The concentration of s-Flt in women with severe or mild preeclampsia and normal pregnancy. The wide scatter of the findings, and the overlapping of the data from women with and without preeclampsia, is typical of findings with many analytes in preeclampsia.

* (A) reprinted from Hubel et al. (1996)⁴, with permission from Elsevier. (B) reprinted from Powers et al. (2005)⁵, with permission from Elsevier.