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Subtypes of Preeclampsia
Recognition and Determining Clinical Usefulness

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ABSTRACT: The concept that preeclampsia is a multisystemic syndrome is appreciated in both research and clinical care. Our understanding of pathophysiology recognizes the role of inflammation, oxidative and endoplasm reticulum stress, and angiogenic dysfunction. Yet, we have not progressed greatly toward clinically useful prediction nor had substantial success in prevention or treatment. One possibility is that the maternal syndrome may be reached through different pathophysiological pathways, that is, subtypes of preeclampsia, that in their specificity yield more clinical utility. For example, early and late onset preeclampsia are increasingly acknowledged as different pathophysiological processes leading to a common presentation. Other subtypes of preeclampsia are supported by disparate clinical outcomes, long-range prognosis, organ systems involved, and risk factors. These insights have been supplemented by discovery-driven methods, which cluster preeclampsia cases into groups indicating different pathophysiologies. In this presentation, we review likely subtypes based on current knowledge and suggest others. We present a consideration of the requirements for a clinically meaningful preeclampsia subtype. A useful subtype should (1) identify a specific pathophysiological pathway or (2) specifically indicate maternal or fetal outcome, (3) be recognizable in a clinically useful time frame, and (4) these results should be reproducible and generalizable (but at varying frequency) including in low resource settings. We recommend that the default consideration be that preeclampsia includes several subtypes rather than trying to force all cases into a single pathophysiological pathway. The recognition of subtypes and deciphering their different pathophysiologies will provide specific targets for prevention, prediction, and treatment directing personalized care.

Key Words: epidemiology ■ inflammation ■ prediction ■ preeclampsia ■ syndrome

In the past 30 years, there has been an explosion in our knowledge of the pathophysiology of preeclampsia. Having abandoned the approach to understanding preeclampsia based on the concept of the disorder as "Pregnancy Induced Hypertension", preeclampsia is now recognized as a syndrome encompassing far more than simply hypertension and proteinuria. The role of oxidative and endoplasmic reticulum stress, inflammation, and secondary endothelial dysfunction and the importance of angiogenic and antiangiogenic factors have all informed our understanding of the pathophysiology of preeclampsia.[1] However, despite this, our ability to prevent and predict the disorder in an actionable timely manner have not achieved a satisfactory level of success. An explanation that is receiving increasing attention is the possibility that there is >1 subtype of preeclampsia, with a variety of pathophysiological pathways leading to maternal and fetal mortality and morbidity.[2] (Figure 1).

In this presentation, we will (1) consider evidence supporting subtypes, (2) examine impediments to the recognition of subtypes, (3) address the implications of identifying subtypes, (4) review currently postulated subtypes, (5) consider how to identify other potential subtypes, and (6) present strategies to recognize and test the clinical relevance of subtypes.

Evidence for Subtypes of Preeclampsia

Several lines of evidence support the consideration that preeclampsia may not be one disorder. One obvious

Hypertension is available at www.ahajournals.org/journal/hyp

Nonstandard Abbreviations and Acronyms

ADAM12	a disintegrin and metalloprotease 12
HELLP	Hemolysis, Elevated Liver Enzymes, Low Platelets
NHS2	Nurses' Health Study 2
PAPP-A	pappalysin
PIGF	placental growth factor
PP13	placental protein 13
sFlt-1	soluble fms-like tyrosine kinase-1

finding supporting this concept is the diversity of the clinical presentation. The syndrome of new onset gestational hypertension and proteinuria or other systemic findings that we term preeclampsia includes women with an indolent disorder changing minimally with progression of pregnancy and women with an "explosive" clinical presentation who on 1 day exhibit minimal diagnostic findings and the next are critically ill. Although the concepts guiding our understanding of pathophysiology espouse reduced placental perfusion, only about one-third of infants of women with preeclampsia manifest fetal growth restriction. Further, the principal organs involved in preeclampsia vary between women, particularly in those having principally hepatic involvement with the Hemolysis, Elevated Liver Enzymes, Low Platelets variant. The laboratory findings associated with preeclampsia, whether clinically or in research studies, vary remarkably.[2] This includes the scatter of measurements of findings proposed as causally relevant to the disorder including oxidative stress and angiogenic and antiangiogenic factors (Figure 2).

Long-term implications of preeclampsia for maternal health also vary, suggesting that different subtypes of preeclampsia may presage different chronic disease risk trajectories after pregnancy. For example, preeclampsia is associated with a doubling of maternal cardiovascular risk in later life.[3] However, it would be inaccurate to consider preeclampsia as a homogenous exposure: while preeclampsia at term in the absence of SGA confers a 2-fold increased risk of maternal cardiovascular disease, term preeclampsia with SGA confers over 3-fold risk, and preterm preeclampsia over 5-fold risk of future cardiovascular disease.[4] If preeclampsia occurs before 34 weeks, that risk may be as high as 10 fold.[5]

Attempts to prevent preeclampsia share a common evolution. Small early studies are successful[6] but subsequent larger studies are not.[7,8] One obvious explanation is publication bias with the only small studies considered for publication being those that are successful. An alternative consideration is that small studies are from homogeneous populations while larger, characteristically multicenter studies, are much more heterogeneous in relation to ethnicity, socioeconomic status, age, environmental and other influences. In a disorder with several pathophysiological pathways, a therapy is most likely to be successful if the pathophysiology at which it is directed is present in a homogeneous population. Support for this comes from NIH studies of aspirin (ASA) to prevent preeclampsia in low and high risk women.[9,10] Although these multicenter center studies did not support a preventive effect of ASA when data from all centers were combined, in 2 of the centers, ASA was effective in both studies. The effect of ASA at these 2 centers could have been masked by failure in other centers with subjects that did not share a pathophysiology countered by aspirin therapy.

Impediments to the Recognition of Subtypes

Despite this substantial variability, the concept of the preeclampsia syndrome as one disorder with a common pathophysiology persists. Part of this is due to the "Occam's Razor" approach to understanding disease pathophysiology. This concept suggests that the simplest, least complex, explanation for a disease most likely pertains. One can argue that in this age of personalized medicine this may or may not be pertinent for complex diseases, but this most certainly is
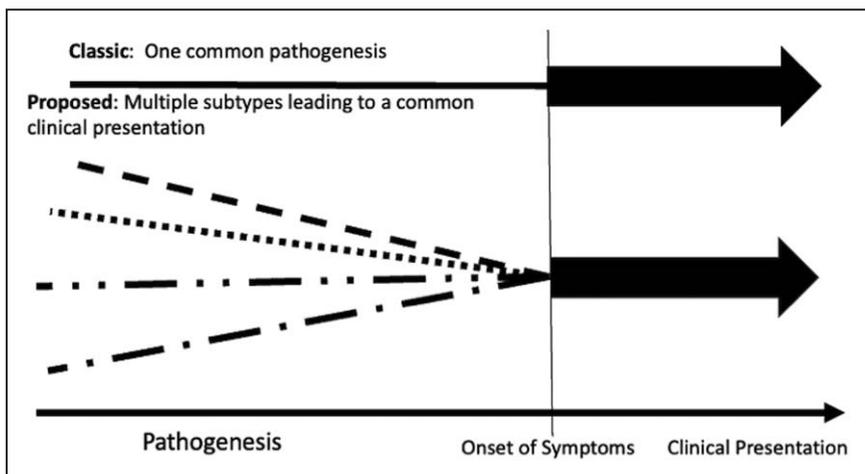

Figure 1. Multiple pathophysiological pathways.
The classic pathway to preeclampsia is considered as a unified pathophysiological pathway resulting in the preeclampsia syndrome. In this presentation, we propose that several pathways can result in the common clinical presentation of preeclampsia.
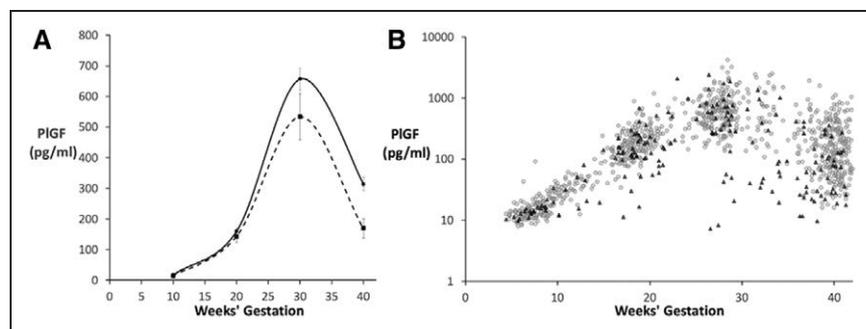
Figure 2. PlGF (placental growth factor) measured longitudinally across pregnancy.
PlGF was measured in 50 women who developed preeclampsia and 300 who did not. The data are arrayed in **A** as mean and SEM (square and dashed line preeclampsia, circle and solid line control). In **B**, the same data are arrayed as a scatter plot (preeclampsia black triangle, control gray circle) clearly indicating the heterogeneity of the data that is not evident in **A**.

unlikely for a syndrome such as preeclampsia. The convention that gestational proteinuria and hypertension were diagnostic of preeclampsia was not based on the importance of these findings in the pathophysiology of disorder. Rather, they were serendipitous observations in the late 19th century of clinical findings which preceded the seizures of eclampsia.[11] All of these attitudes led to a thought process that sought to squeeze all of these diversities into a single disorder, ignoring obvious exceptions. This was supported by the use of data presentation which tended to mask variability. Recently, Weissgerber et al[12] have emphasized that the most common approach to present continuous data in figures as bars with SD or even more so with SE is very effective at masking variability. This has led to the plea, especially pertinent in a syndrome such as preeclampsia, to present data in a manner that reveals, rather than hides, outliers.[12] The same objection applies to other data arrays and presentation that do not provide an indicator of variability (Figure 2).

The Importance of Subtypes

We will present several suggestions to begin to consider as different kinds of preeclampsia. The search for subtypes within a disease can be classified along a variety of dimensions. These dimensions could include classic epidemiological variables such as the timing of onset, disease severity, anatomic or pathophysiologic differences in affected organ systems, just to suggest several categories.[13] However, the important fact is that these may point us to relevant differences in the pathophysiologic pathways to maternal and infant morbidity and mortality, acute and long term. In other medical disorders, this has become the rule. For example, how successful would diabetes therapy be if we did not recognize the existence of different pathological pathways in Type 1 and Type 2 diabetes? In this presentation, we present findings to begin to mine potential subtypes for such clinically relevant pathways. The importance of identifying these potential pathophysiological subtypes cannot be overemphasized. The existence of several subtypes of preeclampsia makes it unlikely that one diagnostic or one preventive therapy will be effective. However, the recognition of subtypes and deciphering their different pathophysiologies will provide specific targets for diagnosis and prevention directing personalized care and management.

CURRENTLY SUSPECTED SUBTYPES OF PREECLAMPSIA

Several subtypes of preeclampsia have been proposed. We will consider these suspected subtypes as early targets for testing the hypothesis of preeclampsia variants and present information demonstrating the challenges associated with discriminating subtypes.

Early and Late Onset Preeclampsia and Severe and Mild Preeclampsia

Historically, in clinical practice, preeclampsia has been defined as early (<34 weeks' gestation) or late onset (>34 weeks) (despite the definition based on the label suggesting onset, these would be better described as early and late delivery, since onset is rarely known) or as mild or severe (based on blood pressure, clinical findings, and degree of proteinuria). Although severe preeclampsia is primarily a subjective clinical assessment and difficult to clearly differentiate from less severe forms of the disorder, early onset preeclampsia can easily be separated from late onset preeclampsia. It manifests clear differences from the late onset disorder. Early onset preeclampsia is associated with an increase of growth restricted infants[14] and pathological evidence of placental malperfusion.[15] This is not the case with later onset of the disorder which is also less likely to predict later life cardiovascular disease. Furthermore, available biomarker predictors for preeclampsia are more successful for early than late onset preeclampsia.[16] This has led to general agreement that the occurrence of preeclampsia early or late likely indicates distinct variants.

It also seems likely that the diverse risk factors predicting preeclampsia, including previous preeclampsia, chronic hypertension, preexisting diabetes, multifetal gestation, and obesity would be mediated through different genes, metabolites, and pathways to preeclampsia.

However, neither early versus late onset preeclampsia nor the disorder in women with different risk factors

can be discriminated by conventional laboratory assessment of biomarkers such as PlGF (placental growth factor), sFlt-1 (soluble fms-like tyrosine kinase-1), PP13 (placental protein 13), ADAM12 (a disintegrin and metalloprotease 12), PAPP-A (pappalysin).[17] This raises the challenge that there is likely overlap in the several pathways to the preeclampsia syndrome. This should not be considered to diminish the relevance of different routes to the disorder. A principal demonstrated in geriatric medicine is that with disorders of individuals that result from the interaction of several pathways to a common disease effect, normalizing even one of these can reverse the overall disorder.[18]

Gestational Hypertension

In considering the different suspected subtypes of preeclampsia, it is important to consider gestational hypertension. To many and for many years, gestational hypertension has been considered as simply the mildest form of preeclampsia. However, Chesley[11] long ago pointed out that, while gestational hypertension might be an early (mildest) form of preeclampsia (as many as 25% of cases proceed to preeclampsia), it was just as likely to be chronic hypertension masked by the normal reduction of blood pressure in early pregnancy. Finally, it might be an altogether different form of pregnancy hypertension. Now that other organ system involvement is considered with hypertension to define preeclampsia,[19] gestational hypertension is probably best considered as nonsyndromic increased blood pressure during pregnancy. This is not to diminish its importance. Blood pressure elevation in pregnancy with hyperuricemia even without proteinuria is as good a predictor of growth restriction or iatrogenic preterm birth as hypertension with proteinuria.[20] However, hypertension with neither elevated uric acid nor proteinuria was no different than normal pregnancy in relationship to these outcomes. Nevertheless, despite minimal impact on fetal outcome, the relationship of gestational hypertension to increased later life cardiovascular disease is at strong as preeclampsia.[4,21] Thus, gestational hypertension may merit its own subtyping and should not be considered simply a mild form of preeclampsia.

Other Suspected Subtypes

Only about one-third of the cases of preeclampsia are associated with growth restriction. As mentioned, these tend to be present with early onset preeclampsia. However, even in this setting not all women with preeclampsia have growth restricted infants. It is possible that these pregnancies may differ from those with normal fetal growth.

It is now clearly established that the signs and symptoms of preeclampsia can occur for the first time days to weeks after the termination of pregnancy. In one study, 0.5% of delivered women returned with new onset postpartum preeclampsia.[22] Almost nothing is known about the pathophysiology or long-range prognosis of this condition, but conventional pathways such as placental malperfusion are unlikely.

It is also important to compare the route to preeclampsia in high and low resource settings. Although obesity, a leading risk factor for preeclampsia,[23] is increasing in many low resource settings, undernutrition is characteristically associated with preeclampsia in these sites. There are few in-depth studies of unique preeclampsia pathogenesis in low resource settings. However, it seems reasonable that preeclampsia with under and over nutritional intake might not be similar. Further, different microbiome, qualitative nutritional intake, levels of activity, and sexual practices including pregnancy timing could also dictate different pathways to preeclampsia.

The presence of outliers should also not be ignored. It is rare for any preeclampsia predictor or component of manifest disease not to be highly scattered with outliers and considerable overlap with findings in women without preeclampsia. It should be considered and if possible tested that the diversity is due to different kinds of preeclampsia. This is demonstrated in Figure 2, in which arraying data as mean and SE completely mask the diversity of the preeclampsia finding which when examined longitudinally identified subsets of preeclamptic subjects.[24]

IDENTIFICATION OF OTHER POTENTIAL CANDIDATE SUBTYPES

Hypothesis Driven

In addition to the above proposed variants of preeclampsia, available data suggest other subtypes might be successfully explored.

Involvement of Different Maternal Organ Systems: an Indicator of Subtypes?

Organs involved in the clinical presentation of preeclampsia are quite variable. While the primary defining feature is maternal hypertension, there is variable involvement of different maternal organ systems including the renal, hepatic, vascular, hemopoietic, coagulation and fibrinolytic systems, the brain, and the immune system.[17,25] Is it possible that the different organs involved might indicate different pathways to preeclampsia?

The variable involvement of differing organ systems suggests that biomarkers affiliated with these systems may identify subtypes[17,25]; however, most of the evidence implicating specific organs is derived from cross-sectional studies of women who present with preeclampsia, making it difficult to decipher cause from effect; this was the reasoning behind longitudinal prediction studies beginning

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early in gestation.[26] While such studies failed to show sufficient predictive power of late first trimester biomarker measurements, for example, PLGF, sFlt-1, they did show increased predictive capacity later in gestation and with serial measurement of biomarkers[27,28] but still not reaching clinical utility. Perhaps, carefully collected data from larger populations will indicate different linkages to different organ systems, providing specific targets.

Placental or Maternal Preeclampsia?

The placenta is considered central to the pathophysiology of preeclampsia as delivery of the placenta alleviates the condition. Involvement of defects in extravillous trophoblast invasion and adaptation of spiral arteries in early onset preeclampsia[29] are now generally accepted; however, there is still controversy as to the involvement of this mechanism in late onset preeclampsia. There is a relative dearth of standardized studies of the placental bed in late onset preeclampsia; however, there is evidence that in such cases, there is normal trophoblast invasion and adaptation of the vasculature supplying the placenta.[30] What then might be the underlying pathophysiology? Redman and Staff[15] have suggested that the abnormal placentation of early gestation and the failure of the placenta in late gestation to maintain its own adequate oxygen supply due to increase in trophoblast growth results in a common insult, syncytiotrophoblast stress, a common convergence point of 2 separate insults. These alternative routes to placental dysfunction, one present from early gestation and one not manifest until late gestation should suggest different predictors and preventive strategies.

An alternative explanation is that late onset preeclampsia is largely because of maternal factors. The increased incidence of preeclampsia in women with preexisting vascular dysfunction (previous preeclampsia, chronic hypertension, and pregestational diabetes) is strong evidence that maternal vascular condition is a predisposing factor.[31] Additionally, the knowledge that women who have experienced preeclampsia are at increased risk for cardiovascular disease and stroke in later life suggests that vascular stress test of pregnancy may have exposed women with underlying subclinical cardiovascular disease such that they developed preeclampsia. Hence, in some women, the placenta may be "normal" but the stress of pregnancy interacts with predisposing suboptimal maternal vascular condition to cause preeclampsia.[17,25,31] However, data do not support the hypothesis that maternal factors are more important with late onset preeclampsia. The effect of prepregnancy maternal risks is indistinguishable for early and late onset preeclampsia, while the relationship to late life cardiovascular is far greater for early than late onset preeclampsia.[5]

It is simplistic to consider separate maternal and placental variants of preeclampsia. The placenta is always involved, but it is likely that maternal genetics, epigenetics, behavior, and environment interact with the placental factors modifying the route to placental dysfunction or the response to placental dysfunction. These variable maternal responses raise important questions in our considerations of subtypes and outcomes. It is evident with current observations that 2 women with similar laboratory findings, BMI ethnicity, etc, may have diverse clinical outcomes. Part of this will be censoring by clinical care (eg, earlier delivery) but part will be different maternal response to the placental insult. It is quite possible that the several placental syndromes, abruption, early pregnancy loss, stillbirth, fetal growth restriction and preterm birth, may all represent different maternal responses to the same insult.

Different Long-Range Outcomes of Preeclampsia As Indicators of Subtypes

Both preeclampsia and gestational hypertension predict future cardiovascular and cardiometabolic risk in mothers, including chronic hypertension, type 2 diabetes, myocardial infarction, heart failure, and stroke.[32,33] Yet, only roughly half of the women with preeclampsia develop chronic hypertension by age 50 to 60.[3,34] There may be differences in the preeclampsia of women who do, and do not, later develop CVD. For example, preterm preeclampsia and recurrent preeclampsia are especially powerful predictors of maternal CVD, suggesting clinically severe preeclampsia is the most likely to predict CVD; yet, to date, studies large enough to examine CVD end points have lacked detail on clinical severity of the preeclampsia episode, so this has yet to be confirmed.

As we examine preeclampsia as an exposure predicting future maternal health, various preeclampsia subtypes may be revealed; we may even conclude that certain subtypes of preeclampsia might be better grouped with other pregnancy complications predictive of maternal cardiovascular disease. For example, spontaneous preterm delivery and placental abruption[35–38] are also associated with heightened CVD risk. Preeclampsia shares some clinical and histological features with these pregnancy complications. These observations might prompt us to think outside the hypertensive box and consider grouping preeclampsia with other features of pregnancy that result from, or predict, maternal cardiometabolic health.

Furthermore, we may surmise novel preeclampsia subtypes by examining long-term maternal health outcomes other than cardiometabolic disease. For example, in the NHS2 (Nurses' Health Study 2), women with a history of hypertensive pregnancy had increased risks of respiratory, neurological, and infectious disease mortality than did women with normotensive pregnancies, even after adjustment for factors such as body mass index.

Such later life implications of preeclampsia beg the question of whether chronic disease risk factors predate

preeclamptic pregnancy but go undetected because they are rarely tested in young women, or because the risk factors rarely exceed clinical thresholds in a young population. Subclinical elevations of blood pressure, fasting glucose, abdominal obesity, inflammation, dyslipidemia, markers of poor hemodynamic function, and liver enzymes indicative of endothelial dysfunction are sometimes evident in the years before preeclamptic pregnancy.[39–44] Similarly, much—though not all—of the risk of CVD associated with preeclampsia history is accounted for by the emergence of traditional cardiovascular risk factors, such as hypertension, after pregnancy.[34,45] Both the existence and variability in prepregnancy and postpregnancy cardiometabolic risk factors may suggest useful preeclampsia subtypes.

Understanding preeclampsia's association with later CVD may yield new insights into the vascular and metabolic types of preeclampsia: perhaps preeclampsia can be usefully subcategorized by endothelial dysfunction, insulin resistance, inflammation, or angiogenesis. Some omics studies are identifying characteristics of preeclampsia that are familiar to cardiologists, including alterations in specific lipids and carnitines.[46] As we interrogate omics studies, it may be fruitful to pursue omic signatures associated with CVD as a priori hypotheses.

Immunologically Determined Subtypes

During pregnancy, the fetal allograft has to be tolerated by the maternal immune system.[47] There has long been the thought that preeclampsia, and other disorders of gestation, are associated with a breakdown of this immune tolerance. The major effectors at the maternal fetal interface are uterine natural killer cells that are not cytotoxic but regulate trophoblast invasion and spiral artery remodeling. They recognize self-major histocompatibility complexes of the mother and nonself-allogenic major histocompatibility complexes from the paternal genotype. Uterine natural killer cells express KIRs (killer cell Ig-like receptors)[48] while extravillous trophoblast express the KIR ligand polymorphic HLA-C.[49] Every pregnancy expresses a unique combination of maternal KIR and fetal HLA-C (has paternal component), which affects the success of placentation and pregnancy.[50] Inhibition of uterine natural killer cell responses by major histocompatibility complexes-self recognition may lead to defective spiral artery remodeling. Also, certain maternal KIR haplotypes can protect against preeclampsia while others confer risk.[50,51] These immunologic interactions determine a specific immune subtype, which can be predicted by maternal and paternal genes, and which may someday be amenable to intervention.

On the other hand, we have long been aware that pregnancies resulting from the use of assisted reproductive technologies are at higher risk of developing preeclampsia.[52] This is thought to be the result of immunologic interactions and of asynchrony between the development of the blastocyst and endometrium at the time of implantation (the seed and the soil hypothesis)[53,54] due to the use of hormonal regimens to facilitate egg collection. This mismatch may alter placental development and extravillous trophoblast invasion as seen in early onset preeclampsia. It would seem these interactions would provide a profitable area to explore as a source of subtypes with implications for all pregnancies.

Discovery-Driven Candidate Subtypes

Another strategy to identify alternative routes to the preeclampsia is to utilize the power of discovery science (Data Supplement: Clustering of subtypes[1–9]). Current powerful analytical strategies have allowed agnostic determination of disease subtypes. The rapid development of the "-omic" technologies has dramatically expanded the number of dimensions that can be added to the more traditional categories with associated increases in both physiological insight but also analytic complexity. Regardless of the source of the information, however, the underlying assumption will be that individuals sharing a disease subtype will also share similar disease pathophysiologies.[55,62] The search for subtypes then becomes an attempt to identify the presence of these groups, or "clusters", that represent similar pathophysiological patterns.

Microparticle-Associated Protein Clustering in Preeclampsia

One of our groups analyzed plasma microparticle proteomic data gathered at 12 weeks' gestation from 23 women with early onset preeclampsia of a nature severe enough to require delivery in or before the 35th week of gestation.[64] Analysis indicated 2 subgroups. Proteins in one cluster were associated with platelet function, while those in the second were associated with complement activity. Interestingly, the second cluster was associated with a more severe clinical course with earlier average gestational age at delivery, higher blood pressures, and more severe laboratory perturbations. This unsupervised search for disease subgroups within women diagnosed with preeclampsia was internally validated by its associated with distinct biological processes and clinically validated by the differences in clinical characteristics and outcome associated with each cluster. At least regarding early preeclampsia with sufficient severity to require delivery during or before the 35th week of gestation, these clusters may identify distinct subsets with different pathophysiologies within the disease.

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Evidence of Subtypes From Genomic Studies

A bioinformatics approach has been used to extract from the published literature and organize, using cluster analysis and gene ontology, genes and variants associated with different clinical phenotypes of preeclampsia.[65] There was distinct segregation of genes by severity and timing of preeclampsia, with associated conditions such as growth restriction and Hemolysis, Elevated Liver Enzymes, Low Platelets syndrome and by biological source (maternal, fetal, or both). Subsequent placental genomic studies suggest at least 3 subgroups of preeclampsia. Using the DNA methylation signatures from a mixed group of early onset preeclampsia, late onset preeclampsia, and preterm controls, Wilson et al[66] performed hierarchical clustering analysis and found 2 separate clusters. Within early onset preeclampsia, they also found evidence of 2 subgroups, however, without strong correlations to clinical phenotypes.

In an attempt to unravel the possible involvement of the placenta in the heterogeneous subtypes of preeclampsia, Leavey et al[67] adopted a genome-wide microarray analysis of gene expression profiles in placental tissue from normotensive versus preeclamptic women. This revealed 5 distinct clusters of placental gene expression: (1) maternal preeclampsia with placental gene expression profiles similar to healthy pregnancy, (2) "Canonical" preeclampsia with high expression of angiogenic genes, those related to hypoxia and altered hormone secretion, (3) immunologic preeclampsia with an over-representation of immune and proinflammatory genes, (4) preterm normotensive women with overexpression of genes related to cell proliferation and stress responses, and (5) those with no strong clinical, gene expression, or epigenetic associations, probably being chromosomal abnormalities. Subsequently this group performed additional studies[68] and found a 65% concordance between placental gene expression and histopathologic phenotype and strong evidence of 3 distinct forms of placental disease underlying preeclampsia. Cluster 1 developed preeclampsia with very little evidence of placental involvement, Cluster 2 had the most severe placental pathology with evidence of maternal vascular malperfusion, distal villous hypoplasia, infarctions, advanced villous maturation and increased syncytial knots and preponderantly early onset preeclampsia. The immune subtype, cluster 3, again had significant placental pathology with profound immune gene activation, for example, overexpression of TNFa, IFNg, chemokine ligand-10. Cluster 4 showed the lesions of histological chorioamnionitis but were normotensive, and cluster 5 (the chromosomal abnormality group) had no enrichment of placental lesion.

Overall, current literature confirms the heterogeneity of preeclampsia at the molecular level. The variable clinical presentation, biomarkers of different organ systems that can be measured and the differing placental gene expression and histological subtypes point toward at least 2 different subtypes of Canonical or Immune susceptibility, with other possible less prevalent subtypes and the exact number of subtypes are yet to be confirmed by additional large-scale cohort studies at the multiomics level.[69] However, omics approaches are discovery driven science, and it has proven clinically insightful in treating other complex diseases, such as cancers. It has become clear that this field will continue to accumulate more data to be organized into databases such as Data and Specimen Hub in NICHD. This will ultimately lead to more comprehensive and unbiased identification of molecular subtypes of preeclampsia with corresponding clinical phenotypic differences.

DETERMINING THE RELEVANCE OF CANDIDATE SUBTYPES

Clinical Relevance

Currently available and emerging data support that the preeclampsia syndrome may be divided into several subtypes. However, which of these are clinically significant? There are features which indicate whether a particular subtype is relevant to management:

1. Subtype identifies a pathway amenable to therapy.
   - The subtype identifies a specific targetable pathophysiological pathway.
2. Or—the purported subtype predicts radically different outcomes for mother and/or baby (Figure 3).
3. A subtype must be recognizable:
   - It must be feasible to identify the subtype in time to direct prediction, prevention and therapy. This is particularly relevant given that many discovery studies are performed on maternal or fetal tissues, including placenta, at the end of pregnancy.
4. Findings must be reproducible and generalizable.
   - Reproducibility should be tested for the subtype in similar populations. Once reproducibility is established, generalizability should be examined in disparate populations. We suspect most subtypes will be present in most populations but possibly with radically different frequencies. This would explain the difficulty of demonstrating successful prediction or prevention in heterogenous populations. We would urge that assessing generalizability will include low resource settings in which the vast majority of infant and maternal mortality occur.
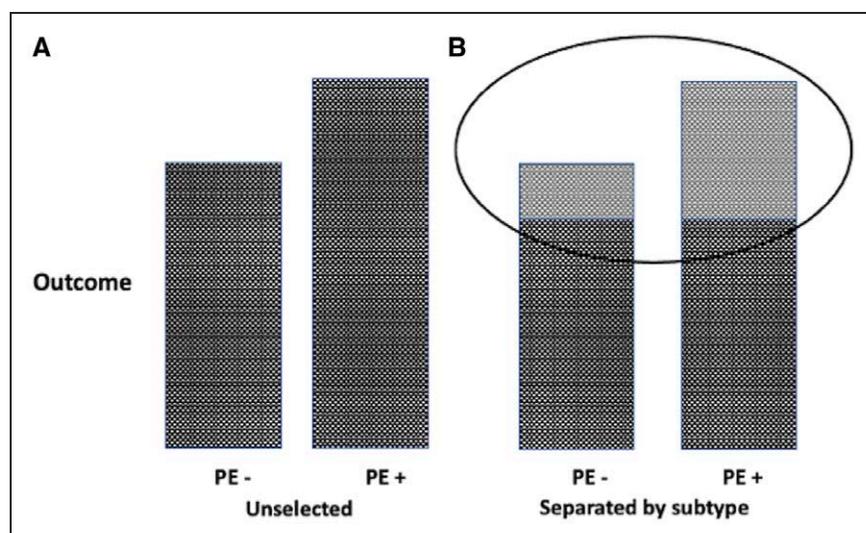
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Figure 3. Increased information on adverse outcome determined by subtype.
A, Indicates the overall adverse outcome in women with or without preeclampsia. There is a slight to moderate increase in the adverse outcome in all women with preeclampsia. In **B**, the circled area indicates that within these same women those within a specific cluster or subtype (the shading indicating the specific cluster is missing) manifest a greater difference in the proportional occurrence of the outcome.

Evaluation of Clinical Relevance

Conventional epidemiological relationships may provide evidence for the features cited for subtype relevance.

1. Subtype identifies a pathway amenable to therapy:
   – If a particular therapy is almost universally effective in a subtype this directs attention to a particular pathway(s).
2. Or—the subtype predicts outcome:
   – The maternal and fetal outcome with the subtype should be compared with that in the general preeclamptic population (Figure 3).
3. A subtype must be recognizable to predict outcome early enough to direct therapy.
   – The correlation of the subtype with conventional clinical findings, which in some cases will have identified the subtype (obesity, race, early pregnancy blood pressure, etc) must be sufficiently specific to justify modifying management. Similarly, biomarkers, again which may have been used to identify a particular subtype, must possess similar specificity.
4. Findings must be reproducible and generalizable.
   – Reproducibility and generalizability should be tested for the subtype in similar and different populations including in subjects from low resource settings

Harnessing the Power of Big Data to Draw Inference

The use of big data analyses to complement standard epidemiological approaches provides special insights. In addition to being used to identify previously unrecognized subsets, those identified or suspected from clinical findings or hypotheses can be examined to determine the existence and specificity of particular pathways. Given the heterogeneity of this syndrome, we propose that a big data driven approach is much needed to draw inference on preeclampsia subtypes.

1. Subtype identifies a pathway amenable to therapy:
   – Systems analysis of clusters may indicate a specific pathway to the syndrome (eg, inflammation). If such analysis does not clearly indicate a specific pathway, analyses of risk and response to therapy of women with the cluster can nonetheless provide useful management information.[70]
2. Or—the subtype predicts outcome:
   – Clusters even if not part of a discernable pathway can be related to outcome (Figure 3).[70]
3. A subtype must be recognizable:
   – A molecular subtype is credible and potentially useful clinically to direct therapy or indicate outcome if collectively the patients in that cluster are enriched with some clinical phenotypes. For a molecular subtype that is lacking clear phenotypic correspondence, additional pathological findings, biomarkers correlation, or association with risk factors (environmental, behavioral or genetic) may provide guidance to its implications for clinical management.
4. Findings must be reproducible and generalizable.
   – Once the best clusters are identified, one should use additional similar cohorts to validate that indeed these subgroups are reproducible, rather than being specific only to the cohort where the discovery is made. For this purpose, supervised learning methods can be employed. When reproducibility is established, the presence and frequency of these clusters in disparate populations including low resource setting should be examined.

CHALLENGES AND SOLUTIONS

Recognition of Subtypes

A major challenge to the recognition of preeclampsia as more than 1 disorder is the approach of many investigators to studies of preeclampsia. In certain investigative

areas, such as cancer, it has become dogma that diseases with phenotypic similarities do not necessarily share common pathogenesis. In 2015, Wang et al[69] stated, "… it is increasingly necessary to consider pathogenesis and inherent heterogeneity of any given health condition and outcome. As the unique disease principle implies, no single biomarker can perfectly define disease." This has not been the perception of most investigators in preeclampsia. Efforts are made repeatedly to make all cases of preeclampsia fit into to a particular pathogenic pathway (eg, angiogenesis)[71] or some other model of preeclampsia, such that in some cases, it is stated that if the particular marker is not present the disorder is not preeclampsia.[71] This approach needs to be modified with the default assumption that there is more than 1 subtype. In keeping with this, data must be evaluated and presented in a manner that emphasizes rather than hides variability.[72]

A tremendously valuable addition to our understanding of preeclampsia comes from discovery science, which not only identifies previously unrecognized subsets but also has the capacity to decipher pathways and fine tune risk factors. Given the heterogeneity of the syndrome, it is possible that the same molecular subtype exhibit different profiles at the gene or DNA methylation or metabolite levels; however, their commonality is manifested by similarly altered biological functions. Thus, a holistic view for defining molecular subtype needs to be recognized.

High-Quality Data in Large Quantities

A major challenge to the search for subtypes is the availability of large amounts of high-quality data. Electronic medical records would seem to be a readily available source of large amounts of data. Unfortunately, although the amount of data is large and of reasonable quality, the data being collected emphasizes billing rather that research and vital information often is not present. Attempts to mine birth certificates and administrative data provide large amounts of data, but this often is not high quality and again often lacks adequate detail. A virtually untapped source of high-quality data is research studies; however, even very large single studies do not provide adequate numbers of subjects for state of the art analyses. An obvious solution is data sharing; however, this provides its own challenges.[73] A major challenge is the mind set of investigators, funding agencies, and academic institution for whom individual credit is the major consideration. In addition, even in well-designed studies, data fields, including outcomes, are not well characterized since there is currently no accepted standardization. Data collection and storage and management present other challenges. Very few databases are designed with the ability to share data as a priority. Trying to combine different databases is expensive and time consuming and again made less valuable because there are no agreed upon standards.

There are some encouraging signs of progress. Funding agencies, (https://www.sciencemag.org/news/2019/11/why-nih-beefing-its-data-sharing-rules-after-16-years) and academic institutions are increasingly appreciating and encouraging sharing of data. Efforts are also being made to encourage investigators to appreciate the value of sharing as indicated in joint efforts of groups studying Covid.[74] The Global Pregnancy Collaboration (CoLab) has provided suggestions to facilitate sharing[73] and recommended data fields for the study of preeclampsia,[75] and the International Collaboration to Harmonise Outcomes in Preeclampsia has suggested outcomes to be included in preeclampsia studies.[76] To attempt to facilitate sharing and merging of data, the CoLab has also prepared a preeclampsia database (COLLECT) available to all investigators for a modest cost with no charge to investigators from low resource or beginning investigators who do not have funding. This harmonized database is web based, modular, and with data only accessible to the investigator.[77] However, when choosing to do so, individuals using this database can easily share data with each other.

CONCLUSIONS/RECOMMENDATIONS

Conclusions

There is abundant evidence that preeclampsia exists as >1 subtype (eg, early and late onset preeclampsia). Our current understanding of the variable presentation and prognosis of preeclampsia also directs testing of the hypotheses that some cases of preeclampsia (eg, with and without obesity, affecting different organ systems, or with or without later life consequences) may also present clinically useful subtypes. These findings are complemented by biomedical informatics techniques enabling new discoveries of preeclampsia subtypes.

Recommendations

1. We should recognize subtypes of preeclampsia as the default option and celebrate rather than ignore outliers.
2. Efforts should be directed at testing the clinical usefulness of purported subtypes and ultimately design subtype-specific prediction, prevention, and therapy.
3. Discovery science should be exploited to identify and refine subtypes, pathways, risk factors, and outcome. Since several different subtypes based on different omics (eg, gene expression, DNA methylation) are proposed, it is important to consolidate these conclusions aided by advanced bioinformatics algorithms that can integrate multiple types of data.

4. It is critical to establish consistent and standard protocols for sample collection, sample processing, data generation, and data analysis pipelines. Equally importantly, data harmonization and sharing is urgently needed to enable the "Big Data" approach. The pregnancy research community, similar to other biomedical domains, needs to rapidly adopt to the "data sharing" and "open data" mind-set to allow knowledge discovery and re-discovery.[78]

5. We should modify data collection, sharing, and analysis to support future "perfect" studies but should also begin now, as best possible with materials available, to discriminate preeclampsia subtypes.

Analytical strategies directed at characterizing and utilizing heterogeneity that have become part of the armamentarium of investigators in other areas should begin to be applied to the study of preeclampsia. Tools are available to identify clinically useful subtypes. The importance of recognizing and using these subtypes cannot be overemphasized. With these approaches, we will identify rational, individualized approaches to prediction, prevention, and perhaps even therapy.

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REVIEW