

Emerging concepts in preeclampsia investigation

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1. ABSTRACT

Preeclampsia is a disorder that uniquely affects pregnancy and profoundly alters the short- and long-term health of the mother and fetus. The pathophysiologic processes that underlie preeclampsia can be thought of in two stages: stage 1, reduced placental perfusion, and stage 2, the maternal clinical syndrome. Multiple pathophysiologic processes influence stage one, affecting trophoblast invasion and placental function. These processes in some women result in stage two, with subsequent maternal inflammatory, metabolic, and thrombotic responses, converging to alter vascular and endothelial health. An increasingly comprehensive understanding of these factors is emerging, which we will introduce in this chapter and which will be detailed in the chapters that follow. The translation of this understanding into clinical trials and ultimately into effective preventive and therapeutic measures remains the ultimate goal of preeclampsia research.

2. DEFINITION/DIAGNOSIS

Preeclampsia is a disorder unique to pregnancy, traditionally diagnosed by new-onset hypertension and proteinuria occurring during the latter half of gestation (1). These manifestations represent easily measurable markers of a systemic disease process that is much more complex than hypertension alone and that profoundly affects multiple maternal organ systems, including placental function (2).

Research progress depends upon defining preeclampsia as specifically as possible. The likelihood that a clinical definition of preeclampsia includes more than one underlying disease process mandates attempts to identify homogeneous subsets (3). An analogous clinical scenario is that of diabetes mellitus. Imagine how our understanding of diabetes would have been hampered if all individuals with hyperglycemia were considered as one group. Attempts to identify subsets of preeclamptic women

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in whom pathophysiology might be relevantly different should be an essential component of all preeclampsia research. Until we have a better understanding of preeclampsia, studies of preeclampsia should at least approach data in a way that recognizes what seem to be potential subcategories: early versus late, with versus without IUGR, severe versus mild, and primiparous versus multiparous.

Two of these defining variables that have received recent attention are parity and hyperuricemia. The outcome of preeclampsia in first and later pregnancies is substantially different. For years there has been a strong, evidence-based plea to study these subsets of women separately (4). This distinction is not currently being carefully observed in some otherwise excellent studies. In order to achieve homogeneity, a research diagnosis of preeclampsia should include parity as one of its variables.

Among women with gestational hypertension and proteinuria, hyperuricemia is associated with adverse fetal outcomes (5). Additionally, women with isolated gestational hypertension typically have outcomes similar to normotensive pregnant women; however if gestational hypertension is accompanied by elevated uric acid the outcome is at least equivalent to that of women with hypertension and proteinuria. While the clinical utility of uric acid measurement may be limited, (6) its inclusion in a research-oriented diagnosis of preeclampsia aids in creating a uniformity of diagnosis and maximizing the homogeneity of a study group.

3. EPIDEMIOLOGY / IMPACT OF DISEASE

3.1. Maternal/Fetal Implications

Delivery of the infant and placenta remains the only curative treatment for preeclampsia. In the developed world, while antenatal care should allow for early identification of the disease and intervention via delivery, the unpredictable nature of preeclampsia limits the effectiveness of this strategy. Preeclampsia remains one of the leading causes of ICU admissions for pregnant women (7). Furthermore, development of preeclampsia before term often leads to iatrogenic preterm birth, with its associated complications. In the developing world, the lack of reliable prenatal care exacerbates the problem and leads to significant maternal mortality, reflected by an estimated 50,000 maternal deaths per year, as well as perinatal morbidity and mortality (8). The World Health Organization recently conducted a systematic review of worldwide causes of maternal mortality, providing an updated epidemiologic assessment of the magnitude and etiology of pregnancy-related death. They found that hypertensive disorders of pregnancy are among the top three specific causes of maternal death in most regions of the world, representing 16% of maternal deaths in the developed world and between 9-26% of maternal deaths in developing countries (9).

Methodological difficulties, including overlapping diagnoses, confound precise estimation of the actual proportion of perinatal deaths attributable to

preeclampsia. A Canadian database analysis suggested that approximately 10% of fetal deaths due to a specific etiology were related to preeclampsia or the combination of preeclampsia and intrauterine growth restriction (10). Prematurity-related complications also contribute, with approximately 20% of preterm births classified as indicated deliveries, primarily attributable to preeclampsia and/or intrauterine growth restriction (11).

4. OVERVIEW OF RESEARCH – TRANSLATIONAL APPROACH

4.1. Leon Chesley's Mandate

Preeclampsia has been dubbed “the disease of theories.” (12). Dr. Leon Chesley, in an address in 1975 to a group that would later become the International Society for the Study of Hypertension in Pregnancy, implored those involved in both the care of women with preeclampsia and the investigation of the disease to continue to persevere in an attempt to uncover its true pathophysiology. He spoke of the “legion” of medical treatments that have been tried, and he advised against “empiric..., symptomatic” treatments not founded in scientifically elucidated understanding. His own work was without exception based on careful observation and studies of well characterized cases of the disorder (13). Chesley's approach embodies the currently favored focus on bedside to bench to bedside, translational methods of investigating medical conditions, and it demonstrates extraordinary foresight. As decades of investigation into the nature of preeclampsia have begun to yield incremental insights and ultimately may lead to a unified understanding of the disease, it is clear that this approach is absolutely fundamental.

With a translational model in mind, the following manuscripts will focus on the mechanistic and pathophysiologic concepts relevant to preeclampsia, always mindful of the clinical and therapeutic applications of this emerging understanding. In this introductory chapter, we will review a schema that we have come to use to aid in understanding and studying preeclampsia and introduce some of the contributing factors that will be elaborated in the chapters that follow. In keeping with the approach to therapy based upon evidence, in addition to therapy guided by experimental evidence we will also describe relevant clinical trials and investigations, the final steps in scientifically based therapeutic decision-making.

5. OVERALL SCHEMA: TWO-STAGE DISEASE

5.1. Stage One

Impaired placental perfusion, frequently due to abnormal placentation, underlies preeclampsia. The physiologic process of endovascular invasion of uterine spiral arteries by extravillous trophoblast is associated with vascular remodeling and the creation of a low resistance and nonreactive blood supply to the uteroplacental circulation. Failure to achieve this results in impaired placental perfusion and persistent sensitivity to vasoactive stimuli (14).

Clinical evidence supports the concept that impaired placental perfusion predisposes women to

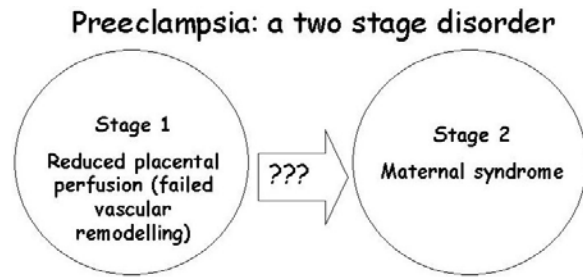


Figure 1. Schematic of a two-stage model for preeclampsia. Stage one represents impaired placental perfusion, often resulting from abnormal spiral artery remodeling. Stage two represents the maternal manifestations of disease. The linkage between these stages remains incompletely elucidated.

preeclampsia, originating from the classic example of molar pregnancy (15). Further evidence that large placental size disproportionate to perfusion increases risk of preeclampsia comes from multiple gestations (16). Also, microvascular diseases such as diabetes, hypertension, thrombophilias, and collagen vascular diseases that limit systemic perfusion confer an elevated risk of preeclampsia (1). Support for the importance of relative placental ischemia also comes from animal models of preeclampsia that result from experimental reduction of uterine blood flow (17).

Successful trophoblast invasion likely depends on appropriate immunologic function and response to changes in placental oxygen tension. At approximately 10-12 weeks after conception, oxygen tension in the intervillous space increases dramatically (18). This relative hyperoxia causes a phenotypic change of trophoblast from primarily proliferative to primarily invasive or endothelial-like (19, 20). Concurrently, this also results in a significant increase in local reactive oxygen species. Maternal antioxidant capacity appears to determine the ability of the decidual/trophoblast interface to accommodate to this challenge (21). Preeclampsia is associated with an impaired ability of trophoblasts to undergo this phenotypic change (22). This may be due to an inherent cellular defect or to a relatively lower capacity to repair oxidative injury to these cells in preeclampsia.

Immunologic interactions are probably central in enabling effective trophoblast invasion. A specific population of uterine natural killer (uNK) cells function as the primary decidual immune cells. These cells express killer immunoglobulin receptors (KIRs) that recognize specific HLA molecules, including HLA-C, HLA-E, and HLA-G, expressed by extravillous trophoblast. In support of the role of immune response in normal implantation specific genotypic combinations of maternal KIR subtypes and fetal HLA-C that would be expected to impede implantation confer an increased risk of preeclampsia (23, 24).

5.2. Stage Two

The clinical manifestations that comprise the

maternal syndrome of preeclampsia are termed "Stage 2" in this model. A common thread to explain the maternal expression of preeclampsia is endothelial dysfunction (25). Physiologically, the endothelial monolayer maintains vasodilation and thereby modulates vascular tone, and acts in anti-inflammatory and anti-thrombotic capacities (26). Disruption of this protective mechanism yields precisely the systemic findings seen in preeclampsia: excessive peripheral vascular resistance, increased vascular permeability, generalized inflammation, and a tendency toward thrombosis, manifest at its worst as disseminated intravascular coagulopathy.

Reduced intravascular volume and vasoconstriction, which results from hypersensitivity to endogenous pressors, (27) in combination with the formation of microthrombi, (28) lead to reduced systemic perfusion. Autopsy findings from women dying with preeclampsia/eclampsia corroborate the primacy of impaired perfusion, with findings of hepatic, adrenal, and intracranial hemorrhage, infarction, and necrosis. Endocardial necrosis, also a classic finding of inadequate organ perfusion, characterizes cardiac changes (29). Glomerular endotheliosis, notably the renal finding characteristic of preeclampsia, is characterized by endothelial hypertrophy and obstruction of the glomerular capillary lumen, also consistent with reduced renal perfusion (30).

This endothelial process would be predicted to result in the multi-organ system manifestations of preeclampsia. Women with preeclampsia develop hypertension refractory to pharmacotherapy. They may hemolyze red blood cells and consume their platelets, causing anemia and placing them at risk for severely impaired oxygen carrying capacity. They can develop hepatic failure, renal failure, or seizures. Impaired placental function can result in fetal morbidity or mortality, or, unpredictably, in placental abruption.

Factors contributing to the endothelial dysfunction of preeclampsia include those that damage the endothelium as well as those that alter its function or impede its repair. It is quite likely that the endothelial activation is only part of a more generalized inflammatory response that characterizes normal pregnancy and is increased in preeclampsia. Neutrophils and monocytes activated as part of this inflammatory response likely contribute to endothelial damage, as does the characteristic dyslipidemia of preeclampsia (31). Angiogenic factors, derived both from the placenta and from other sources, ordinarily favorably impact the function of the endothelium. However, in preeclampsia the positive effects of these factors is inhibited by increased concentrations of soluble antagonists of these activities, soluble fms-like tyrosine kinase-1 (sFlt-1), which inhibits the activity of VEGF and PlGF, (32, 33) and a soluble form of the TGF-beta receptor, soluble endoglin (sEng) that reduces the activity of TGF-beta (34). Both of these molecules act to inhibit normal endothelial physiology.

It is also important to recall that the net health of

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the endothelium is determined by not only increased injury but also reduced repair. This component of endothelial health is receiving increased attention in atherosclerosis and also seems to be aberrant in preeclampsia. Repair was once thought to occur solely by local regrowth of endothelial cells; more recently, the pivotal role of bone marrow derived endothelial stem cells has become increasingly clear. Impaired endothelial health can occur secondary to a reduced number or function of bone marrow-derived endothelial progenitor cells; a phenomenon recognized as a component of cardiovascular disease (35) and emerging as a candidate factor in preeclampsia.

Although endothelial dysfunction explains many features of preeclampsia, other factors contribute not only to endothelial dysfunction but also directly to aspects of pathophysiology. Increased vascular sensitivity to pressors could clearly occur due to endothelial dysfunction; however an agonistic autoantibody to the angiotensin subtype 1 receptor (AT1) has also been noted in circulation in women with preeclampsia. This could explain increased sensitivity to angiotensin, one of the first differences clearly identified to precede manifest preeclampsia. Furthermore, the ability of these agonistic antibodies to activate NADPH oxidase could contribute to oxidative stress (36) and the activation of tissue factor by the antibody (37) could contribute to the coagulation abnormalities of Stage Two.

Factors other than endothelial activation also contribute to the abnormal activation of the coagulation cascade characteristic of preeclampsia. Genetic and acquired thrombophilias may also increase the risk of preeclampsia, and alterations in the normal circulating levels of several factors, notably protein S, protein C, and antithrombin III have been noted in preeclampsia (38).

Other components of Stage Two of preeclampsia are features of the metabolic syndrome. Insulin resistance, elevated triglycerides, free fatty acids, uric acid and reduced HDL are all present in preeclampsia (39). Free fatty acids and triglycerides alter endothelial function, while uric acid is associated with more severe preeclampsia even with gestational hypertension without proteinuria (5). Recent findings from animal experiments suggest that uric acid may also be a direct pathogen (40).

In addition to a role in pathogenesis of preeclampsia these findings of the metabolic syndrome in preeclampsia emphasize the similarities between preeclampsia and later life atherosclerosis. Other similarities include the pathological finding in decidual vessels termed atherosis that is quite similar to atherogenic changes in systemic vessels outside of pregnancy. The similarity of risk factors for the two disorders further supports the relationship. With the fascinating exception of smoking, which is addressed in a later chapter, all of the risk factors for later life cardiovascular disease (endothelial dysfunction, black race, obesity, hyperextension, diabetes, elevated homocysteine, etc.) increase preeclampsia risk (39).

These risk factors for preeclampsia are relevant to another feature of preeclampsia. That is that reduced placental perfusion is not sufficient to result in Stage Two. Although impaired placentation appears to contribute to preeclampsia, the same spiral artery remodeling defect is also seen in isolated intrauterine growth restriction (41) and preterm birth (42). It would follow that the maternal manifestations of preeclampsia develop as a result of a particular maternal response to the impaired placentation process. The factors involved in this response include the risk factors cited but also depend on maternal genetic factors that shape the maternal inflammatory response, antioxidant capacity, and metabolic makeup. Furthermore, maternal behavior, stressors, and environmental exposures also influence this predisposition. The heterogeneous expression of preeclampsia also indicates that the balance of the contribution of these factors varies. With profoundly reduced perfusion only minimal maternal sensitivity is required while with extreme maternal sensitivity only minor reduced perfusion is necessary.

Not surprisingly Stage Two of preeclampsia predicts later life cardiovascular disease (43-45). This is present in all women with preeclampsia including those in whom it is only diagnosed in first pregnancies, (44) but is strikingly increased in women with preeclampsia occurring before 37 weeks and in women with recurrent preeclampsia (43).

5.3. Link between stages one and two

A particularly relevant question for therapy is, what is the linkage between the two stages of preeclampsia? (See Figure 1.) If this linkage was identified, a unified therapy could be used to treat/prevent the disorder. Failing this, the heterogeneous nature of preeclampsia described in the following chapters would dictate specific therapy for specific subsets of women, many yet to be identified.

Several possible linkages have been proposed. In the past the search for unique materials produced by the placenta in response to reduced perfusion has been futile (46). Current thinking favors an excess of usual placental products either pathological or perhaps adaptive. The capacity of the placenta to, for example, produce inflammatory cytokines is well recognized and detailed in a subsequent chapter. Recent evidence of increased microparticles of placental origin and increased fetal DNA suggest that the release of placental fragments, probably secondary to apoptosis, could be a link (47). *In vitro* studies support this concept. Isolated placental microparticles can activate monocytes, (48) injure endothelium (49) and alter vascular function. Some other candidates include the increased production of sflt and endoglin in response to hypoxia, increased leptin production (50) and excess inflammatory cytokines from non-placental sources (51, 52).

Oxidative stress secondary to excess placental generation of reactive oxygen species is an attractive common denominator. Oxidative stress or associated

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hypoxia/reperfusion generating oxidative stress could stimulate apoptosis leading to the release of placental fragments and increase s-f1t, endoglin, cytokine and leptin production. The placenta in preeclampsia is at risk for hypoxia/reperfusion by the effect of vasoactive agents upon the reactive spiral arteries that persist due to failed vascular remodeling. In addition, normal pregnancy is associated with profound physiological alterations in uterine and hence placental perfusion in response to posture, exercise and meals.

Oxidative stress and maternal antioxidant capacity could contribute to the development of preeclampsia at both Stage One and Stage Two. Evidence of oxidative stress in preeclampsia has been demonstrated locally in the placenta as well as systemically in maternal circulation. In the placenta, several antioxidant systems show lower activity, coincident with elevations in several lipid markers of oxidation in the placenta decidua and maternal circulation. Lipid oxidation findings may be confounded by alternative sources of lipid peroxides and *in vitro* oxidation of lipids (53). However, a very stable marker of oxidation, nitrotyrosine, the product of reactions between the prooxidant peroxynitrite and tyrosine residues of protein is increased in tissues and blood in preeclampsia. Placentas from preeclamptic patients show higher levels of nitrotyrosine staining in the villous endothelium, vascular smooth muscle, and stroma than placentas from controls (54-56). Nitrotyrosine is also increased in the maternal blood and in maternal blood vessels (57). Additional evidence of oxidative stress comes from consumption of antioxidants, and reduced ascorbate concentrations are associated with clinical preeclampsia and also precede clinically evident disease (58).

The following chapters address specific aspects of the processes that contribute to the overall pathophysiology of preeclampsia. Each may impact the impaired placentation process, the maternal predisposition to the clinical manifestations of the disease, or the link between the two stages.

6. TRANSLATION INTO CLINICAL PRACTICE

Clinical ability to predict, prevent, and treat preeclampsia has been partly empiric and but more recently increasingly informed by our understanding of the underlying pathophysiology. This historic observation underlies the question asked regarding every woman who presents for care with preeclampsia: do the maternal and fetal risks of disease progression outweigh the neonatal risks of delivery at the current gestational age? This is of most concern in the earliest and most severe cases, when both mother and fetus are at highest risk. Even in this population, there is support for expectant management and close observation rather than immediate delivery, provided that the trajectory of disease progression remains stable (59, 60).

Eclampsia is the longest recognized and perhaps most feared complication of preeclampsia. Investigations into the treatment and prevention of eclamptic seizures

have shown fairly definitively that magnesium is superior to other anticonvulsants and that its overall preventive efficacy is quite good (61-63). The support for magnesium therapy is an example of a purely empiric therapy adapted absolutely by one group of clinicians and dismissed as absolutely inappropriate by others that was eventually (much later than it should have been) tested by clinical trials. Although the question still exists as to what degree of severity warrants therapy, the evidence is clear that magnesium is the drug of choice for the prevention and treatment of eclamptic seizures. Globally, magnesium therapy is becoming the standard of care for treatment and prevention of seizures in severe preeclampsia; in the developed world, where surveillance for magnesium side effects and toxicity is feasible, prophylaxis is also standard of care for mild disease. Magnesium appears to work directly on the cerebral cortex to block neuronal calcium influx through glutamate channels.

Clinical strategies built upon our understanding of the preeclampsia disease process have tended to take more of a preventive bent. Trials of varying quality but some extremely well done have examined nutrient supplementation, antiplatelet therapy, and the use of antioxidants. Even though success has been limited with these trials much has been learned from several excellent studies that are now guiding trials of other agents.

The use of calcium supplementation was originally directed by epidemiological observation of increased risk of preeclampsia in populations with low calcium intake (64). Many small studies in developing countries suggested benefit (65). but a large NIH trial showed no benefit in an US population (66). Findings in these studies raised the question pertinent to other studies as to whether therapy was nutrient supplementation or replacement. It was hypothesized that the difference in results was secondary to inadequate dietary calcium in developing countries that was not the case in the US (67). This was tested in a trial administering calcium to women in areas established to have low calcium intake (68). The results did not demonstrate a reduction in the incidence of preeclampsia but indicated a reduction in severity. Another lesson from these studies was that using nutrient supplements may not have the same effect as improving diet.

As mentioned, abnormal coagulation appears to underlie either the first and/or the second stage of preeclampsia. One manifestation of this is an imbalance in prostacyclin and thromboxane A₂. For this reason, it was hypothesized that aspirin therapy could help to maintain the normal balance of these factors and prevent the development of preeclampsia. Though individual studies of low-dose aspirin for the prevention of preeclampsia have generally shown limited or no benefit, (69) meta-analyses have suggested that, particularly in populations at high risk for the development of preeclampsia, aspirin may confer modest benefit (70).

Important lessons came from calcium and aspirin studies that have guided subsequent trials. First, therapy

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must be begun before clinically evident disease to be effective. Secondly, reducing the diagnosis of preeclampsia is less important than reducing fetal and/or maternal morbidity. Furthermore, close attention must be directed at the effect of treatment on the fetus and studies must be powered to look at important endpoints. Finally, it is mandatory as a component of large trials to collect information to increase understanding of preeclampsia. Recognizing the heterogeneity of preeclampsia, this should include information on whether there are subsets of subjects in whom the particular therapy may be more or less effective. Also, quite importantly the impact of therapy on markers of underlying pathophysiology should be assessed, as it the mechanistic understanding of the disease process that originally informed the study concept and design. Even in the absence of positive results, such studies can inform our understanding of disease process in unique ways, and the collection of this information remains crucial.

The most recent therapeutic area of investigation in the prevention of preeclampsia has focused on antioxidant supplementation. This direction of research was prompted by studies supporting the potential role of oxidative stress as the linkage of the two stages of preeclampsia. These studies have been guided by the principles above with power to determine important maternal and fetal outcomes. Whether the evidence of oxidative stress noted in preeclampsia can translate into an effective preventive strategy via antioxidant supplementation may or may not be resolved. A small trial published in 1999 suggested that the incidence of preeclampsia might be decreased by the administration of vitamins C and E (71). This finding prompted several large-scale trials. The Vitamins in Preeclampsia (VIP) study was a multicenter trial conducted in the United Kingdom that randomized high-risk women between 14 and 22 weeks gestation to supplementation with vitamins C and E or placebo. They found that preeclampsia incidence was no different between the groups. The investigators also raised concerns for adverse pregnancy outcomes, as the treatment group showed a higher incidence of low birth weight (though not small for gestational age) and perhaps an increased risk of stillbirths over 24 weeks (72). Subsequently, the Australian Collaborative Trial of Supplements trial of antioxidants versus placebo in low risk nulliparous women between 14 and 22 weeks gestation corroborated the VIP finding of no difference in preeclampsia rate, but reassuringly did not show any differences in adverse outcomes among the groups (73). Another investigation in the United States remains ongoing. The CAPPS trial, in contrast to other trials, will address whether treatment in early pregnancy prior to the oxidative stress associated with the establishment of intervillous blood flow at 8-10 weeks gestation will be effective. In this study 40% of women are beginning therapy before 12 weeks while enrollment in the other studies began at 18 weeks gestation on average. All of these studies are to be commended for the major efforts to accumulate data as part of the study for the reasons cited above.

7. CONCLUSIONS

Preeclampsia represents a significant threat to maternal and fetal health and life in the short term. In general, the acute signs of disease reverse with delivery of the placenta. However, in the longer term, a higher risk of later life cardiovascular disease is associated with preeclampsia, particularly in its recurrent or severe forms.

While the understanding of this disease process remains incomplete, decades of investigation have provided a great deal of insight into its pathophysiology. Ultimately, translating this understanding into prevention and therapy will enable us to alleviate both acute morbidity and mortality as well as to potentially reduce the burden of chronic cardiovascular disease.

Clinically, women with preeclampsia present with highly variable symptoms and signs, reflecting the wide spectrum of this disease. Ultimately, the lack of uniformity in the pathophysiologic mechanisms noted to underlie preeclampsia may be the reason for the clinical variability. Many roads may lead to similar, though not exactly the same place. Pursuing these differences in the fashion suggested to us by Chesley may ultimately lead to therapies that are individualizable according to a particular patient's specific disease process.

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