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The Laboratorian SM

Preeclampsia; A Clinician's Perspective

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When you're asked to write an article for a publication known as *The Laboratorian* about a clinical disease which we see commonly however the pathophysiology is still quite an enigma presents quite the challenge. My charge here is to describe a pregnancy specific disease that can have tragic and dramatic consequences for both mother and child. I will focus on laboratory parameters that may shed light on an ongoing process that contributes to this devastating pregnancy specific condition. My only hope is that scientists like you may define a pattern that will help us shed additional light on the etiology of this condition

Preeclampsia is a disorder of pregnancy characterized by the development of hypertension, edema and proteinuria in the second half of pregnancy that was not present previously. It is commonly seen as primigravid proteinuric hypertension. Non-proteinuric hypertension usually makes a case for chronic essential hypertension complicating pregnancy. In years past, the syndrome was considered when a patient presented with the triad of hypertension,

edema, and proteinuria, however minor dependant edema is so common in pregnancy, it may have little impact. We will define the impact of edema later when severity is an issue as in a patient with anasarca. Preeclampsia is characterized as either mild or severe. There is no "moderate" preeclampsia. Mild disease can be tolerated but severe needs treatment and the only effective treatment is delivery of the fetus. The pathophysiology is a multisystem, pregnancy related disease with varied risk factors, manifestations and presentations.

The etiology of this disorder has remained a mystery; however, those of us in obstetrics who see this complication often keep a very high index of suspicion when women present in pregnancy with elevated blood pressure. The hypertensive disorders of pregnancy occur in approximately 10% of pregnant women. Preeclampsia is one of the hypertensive disorders of pregnancy that also includes chronic essential hypertension and gestational hypertension. Both chronic essential hypertension and gestational hypertension probably have genetic/familial predilections that present in pregnancy due to the cardiovascular changes that occur. Preeclampsia can occur out of the blue with no predisposing risk factors and can also be seen as familial and/or "superimposed" on chronic essential hypertension which further

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Preeclampsia

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Affecting 7% to 10% of pregnancies in the United States, Preeclampsia (PE) is the most common complication of pregnancy and remains a leading cause of maternal and fetal morbidity and mortality (1). More than 200,000 American women develop this disorder each year, the rate of incidence being equivalent to that of breast cancer. PE is characterized by hypertension (systolic and diastolic blood pressure of ≥ 140 and 90 mm Hg respectively) and proteinuria (protein excretion of ≥ 300 mg in a 24 hour urine sample) which develops after 20 weeks gestation (1). Some women may develop high blood pressure without proteinuria which is referred to as pregnancy-induced hypertension. Although this too is a serious condition, it is not considered PE. Diagnosis of PE requires both elevated blood pressure (two separate BP readings must be taken at least six hours apart) and the detection of protein in the urine.

The clinical manifestation and progression of PE varies among patients. It can present strictly as a maternal disorder with normal fetal development, or it can manifest as both a maternal and fetal disorder with *in utero* growth restriction (IUGR) or sudden fetal stress. The most common, tell-tale signs of PE are hypertension and proteinuria, however, additional maternal symptoms may occur including edema, endotheliosis, and renal or liver failure. In the most severe cases, this condition can progress to eclampsia or HELLP syndrome (3). PE can have an early onset (symptoms occur prior to 32 weeks gestation) or late onset (after 32 weeks). It is worthy to note that a higher morbidity rate is associated with early onset cases (1, 4).

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UPCOMING EVENTS >>>

01/23-25	AAPPO: American Association of Preferred Provider Organizations Annual Forum, Amelia Island, FL
01/27-29	ACOG-MT: American Congress of Obstetricians & Gynecologists Montana Section Annual Clinical Meeting, Big Sky, MT
02/3-5	ACOG-MI: American Congress of Obstetricians & Gynecologists Michigan Section Annual Clinical Meeting, Canton, MI
03/27-31	ACOOG: American Congress of Osteopathic Obstetricians & Gynecologists 78 th Annual Conference, Orlando, FL

JOURNAL WATCH

De Groot CJM, van der Mast BJ, Visser W, et al. 2010. Preeclampsia is associated with increased cytotoxic T-cell capacity to paternal antigens. *Am J Obstet Gynecol.* **203**:496.e1-6.

The authors of this paper propose a mechanism akin to transplant rejection is associated with the process of preeclampsia. This hypothesis unites the observations that normal pregnancies are typified by T_H2 immune responses while abnormal placentation and spontaneous abortion are T_H1 -mediated with the fact that a fetus is a semiallograft, having received half of its histocompatibility (MHC) antigens paternally. To solidify this notion, peripheral blood mononuclear samples from fourteen preeclamptic women were analyzed for the presence of cytotoxic T cells (CTL) specific to their partner's MHC antigens; fourteen gestational age and parity matched samples were used as controls. The study revealed a greater number of paternal antigen-specific CTL was associated with the preeclamptic group and postulate this outcome is a result of the woman's inability to properly suppress the immune response to these paternal factors and reach a state of immune tolerance. The authors offer the lack of chimerism, or the mixing of maternal and paternal polymorphic genes, as one possible explanation for this dichotomy.

Robinson CJ, Alanis MC, Wagner CL, et al. 2010. Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. *Am J Obstet Gynecol.* **203**:366.e1-6.

This paper evaluated a possible correlation between decreased vitamin D levels and the phenomenon of preeclampsia. The study evaluated 25-hydroxyvitamin D (25-OH-D) levels, the longer-lived but inactive form of vitamin D in 50 women experiencing early-onset severe preeclampsia (EOSPE) for comparison against 100 normal pregnancies. Each patient was categorized as normal (≥ 32 ng/mL), insufficient (< 32 ng/mL) or deficient (< 20 ng/mL). Approximately 50% of the EOSPE population was determined to be deficient in 25-OH-D, 22% were insufficient and 24% were within the normal range. Along with the vitamin D observations, this study also revealed a higher pregnancy mass body index, severe hypertension, and increased incidence of intrauterine growth retardation within the EOSPE population. Further analysis of the EOSPE population the greatest decrease on 25-OH-D levels were associated with African Americans, and the authors propose this may account for the increased rate of preeclampsia within this ethnic group. This study underscores the importance of prenatal vitamin administration and compliance.

Rose CH, McWeeney DT, Brost BC, et al. 2010. Cost-effective standardization of preterm labor evaluation. *Am J Obstet Gynecol.* **203**:250.e1-5.

Preterm labor occurs in approximately 12% of total pregnancies but, due to improper screening methodologies, results in the inappropriate hospitalization of 50% to 80% of pregnancies. The purpose of this paper was to evaluate the applicability and feasibility of implementing a standard screening protocol for the identification of women at risk of delivering preterm. The study, conducted by *physicians* at the Mayo Clinic, was based upon validated protocols from Schmitz *et al.* and Hedriana and Bliss, both of which were previously evaluated by both the March of Dimes and the Society for Maternal-Fetal Medicine. Of the 201 patients enrolled over the course of a year, 60 were excluded from the evaluation for not following the protocol accurately. From this cohort it was determined that cervical length

measurement in conjunction with fetal fibronectin analysis served to identify which patients should receive further care and which could be discharged. Briefly, if the cervix is ≥ 3 cm and there is no clinical concern for chorioamnionitis or abruption placentae then the pathogen cultures and fetal fibronectin samples can be discarded and the patient discharged. Implementation of this protocol in the exact order in which it is laid out is estimated to reduce costs by as much as 56%, which translates into approximately \$560 million dollars.

DiGiulio DB, Gervasi MT, Romero R, et al. 2010. Microbial invasion of the amniotic cavity in preeclampsia as assessed by cultivation and sequence-based methods. *J Perinat Med.* **38**(5):503-13.

Amniotic fluid is considered to be a sterile fluid and this belief has been born out for the majority of normal pregnancies. However, studies evaluating the possible breach of this microenvironment by microbial organisms have demonstrated their presence in 18% of term pregnancies with intact membranes, 34% of women experiencing prelabor rupture of membranes, 13% of preterm births, 32% of PROM cases and 9% of women diagnosed with short cervix. The authors of this paper argue that many cases go undiagnosed due to the fastidious nature of the infecting organisms and the reliance upon culturing techniques for their identification. Here they chose to explore the issue from a molecular standpoint, utilizing species discriminating PCR methodologies to determine the prevalence of microbial invasion of the amniotic cavity (MIAC) and the causative agents. To this end, amniotic fluid from 62 preterm patients was examined by PCR for the presence of bacteria or yeast, using general, non-speciating assays, as well as five species-specific assays for comparison alongside culturing. Their results identified 6 patients (9.6%) with positive amniotic fluid. Interestingly, three of these individuals harbored *Sneathia/Leptotrichia*, pathogens that rarely occur during pregnancy. While collectively these findings do not suggest MIAC as a dominant factor in the process of preterm labor, they do suggest a need for the further study of *Sneathia/Leptotrichia* and, possibly, other fastidious anaerobes in this process.

Guller S, Tang Z, Ma YY, Di Santo S, Sager R, Schneider H. 2010. Protein composition of microparticles shed from human placenta during placental perfusion: Potential role in angiogenesis and fibrinolysis in preeclampsia. Article in press. *Placenta*:2010, doi:10.1016/j.placenta.2010.10.011.

During pregnancy proteinaceous vesicles, termed placental microparticles (MP), are shed from the outer layer of the placenta and enter the maternal blood stream directly. Studies have indicated higher levels of these MPs are associated with the occurrence of preterm labor. While there have been several reports linking the presence of MP to increased maternal blood pressure, a hallmark of preeclampsia, very few studies evaluated the composition of these particles in an attempt to explain their correlation. A few independent studies identified factors, including the tyrosine kinase sFlt-1, which binds vascular endothelial and placental growth factors, as well as soluble endoglin, which binds transforming growth factor beta, as placental proteins released with these vesicles; the association of these factors with MP prevents their binding to their cognate receptor and ultimately inhibits their function. This paper identified a mechanistic explanation linking the seemingly benign observation of increased MP production with clinical outcome by linking MPs with the processes of angiogenesis and fibrinolysis at the maternal-fetal interface and thus altering placental pathophysiology.



Balashov S, Mordechai E, Gygax SE, Adelson ME. Development of a Diagnostic Test for Screening of Mutations in *Neisseria gonorrhoeae* Conferring Resistance to Penicillin, Ciprofloxacin, Cefixime, Tetracycline, Azithromycin, and Spectinomycin. 2010 Association for Molecular Pathology Annual Meeting, San Jose, CA. November 17-20, 2010.

Peer-Reviewed Papers:

Peña KC, Adelson ME, Mordechai E, Blaho JA. 2010. Genital herpes simplex virus type 1 in women: Detection in cervicovaginal specimens from gynecological practices in the United States. *J Clin Microbiol.* **48**(1): 150-153.

Blaho, JA. 2010. Oncoapoptosis: A novel molecular therapeutic for cancer treatment. *IUBMB Life.* **62**(2): 87-91.

Ingvarsdottir K, Blaho JA. 2010. Association of the herpes virus major tegument structural protein VP22 with chromatin. *Biochim Biophys Acta*, **1799** (3-4): 200-206.

Biggs C, Walsh P, Overmyer CL, Gonzalez D, Feola M, Mordechai E, Adelson ME, Iacono, KT. 2010. Performance of influenza rapid antigen testing in influenza in emergency department patients. *Emerg Med J.* **27**(1): 5-7.



**HUMIGEN:
Peer-Reviewed Papers:**

Gallagher G, Megjugorac NJ, Yu RY, Eskdale, J, Gallagher GE, Siegel R, Tollar E. 2010. The lambda interferons: guardians of the immune epithelial interface and the Th2 response. *J Interferon Cytokine Res.* **30**(8): 603-15.

Gallagher G. 2010. Interleukin-19: Multiple roles in immune regulation and disease. *Cytokine Growth Factor Rev.* **21**(5):345-52.

Yu R, Gallagher G. 2010. A naturally occurring, soluble antagonist of Human IL-23 inhibits the development and in vitro function of Human Th17 cells. *J Immunol.* 185(12): 7302-7308.

e-Quiz

1. The hypertensive disorders of pregnancy occur in approximately _____ of pregnant women.
 - a. 5%
 - b. 10%
 - c. 15%
 - d. 20%

2. **True or False.** Globally, preeclampsia and other hypertensive disorders of pregnancy are a leading cause of maternal and infant illness and death. By conservative estimates, these disorders are responsible for 76,000 maternal and 500,000 infant deaths each year.

3. Diagnosis of PE requires:
 - a. Elevated blood pressure (two separate BP readings must be taken at least six hours apart).
 - b. The detection of protein in the urine.
 - c. Both a and b
 - d. None of the above

4. **True or False.** Preeclampsia can be cured through the administration of anti-hypersensitive drugs, corticosteroids, or magnesium sulfate.

5. Which of the following clinical factors may increase the risk for preeclampsia:
 - Maternal age
 - Primigravid status
 - Multiple pregnancies (twins or higher order multiples)
 - Obesity
 - Pre-existing hypertension
 - Renal disease
 - Collagen vascular diseases

For results to the electronic Epidemiology Quiz, please visit www.mdlab.com and click on the e-Quiz link.

Quality Assurance Q&A

Q: My office keeps receiving Specimen Discrepancy Notification's to verify the patient. We print out a copy of the patient's demographics sheet from our Electronic Medical Records (EMR) system and staple it to the MDL test requisition form. Isn't this enough?

A: When attaching a print out of patient demographics to an MDL test requisition form, you must, at a minimum, write the patient name on the test requisition form. This enables our laboratory to confirm that the tests marked on the test requisition form are being ordered for that patient's specimen. Demographics print out are an acceptable way to supply the additional information required.

Preeclampsia; A Clinician's Perspective

complicates the well-being of the mother and fetus.

Before embarking on a discussion of the hypertensive disorders of pregnancy, one must discuss the cardiovascular changes that accompany normal pregnancy at the earliest stages of gestation. From just after conception, there are hormonal and cardiovascular adjustments that include decrease in blood pressure, tachycardia, decrease in peripheral vascular resistance, and the beginnings of a dramatic increase in both cardiac output and intravascular volume. As blood pressure is the product of cardiac output times the total peripheral resistance, the blood pressure decreases in early normal pregnancy can only come about as a result of a dramatic decrease in total peripheral resistance.

It is due to many of these changes that we may see a decrease in an individual's blood pressure very early in gestation that can mask previously existing minor blood pressure elevations caused by chronic essential hypertension. As pregnancy advances, intravascular volume increases and blood pressure may increase. If we previously haven't known of the existence of chronic essential blood pressure, we may be inclined to think that this non-proteinuric blood pressure elevation is due to early preeclampsia rather than the natural course of chronic essential hypertension in pregnancy. This distinction is not just academic as women with chronic essential hypertension have an additional risk for developing superimposed preeclampsia. This decrease in blood pressure that occurs early in pregnancy will often mask mild chronic essential hypertension that may have predated the pregnancy. As obstetricians generally deal with younger women who may have never had their blood pressures monitored, this could be missed. Therefore, if patients present at the end of the first trimester of the pregnancy, their blood pressure may have never been taken and may be significantly lower than it was prepregnancy. If their pressures are above the range usually seen as "normal" (120/80), they may have the genetic predisposition for chronic essential blood pressure. Normotensive young women will have blood pressures in the range somewhat less than 120/80 in that first trimester. It has been shown that mean arterial pressures (MAP) >90 is where perinatal mortality starts to increase. (120/80 converts to a MAP of 93.3) Superimposed preeclampsia occurs in a patient with chronic essential hypertension that develops proteinuria as the pregnancy advances.

I will attempt to describe the hypertensive disorders of pregnancy and how, through laboratory and clinical evaluations, we can hone in on the more specific diagnosis of preeclampsia.

Much of what we will discuss here shows preeclampsia to be a multi-system total body disease with clinical findings that include hypertension edema and proteinuria with other signs and symptoms that could include headache, abdominal pain, thrombocytopenia, elevated liver enzymes, as well as complications that directly affect the fetus including abruptio placenta and intrauterine growth restriction.

Laboratory evaluation in the pregnant patient with chronic essential hypertensive or preeclampsia may be rather vague. When preeclampsia occurs, the patient usually develops proteinuria, sometimes as much as nephrotic range proteinuria, and vasoconstriction which is often reflected in a higher hemoglobin and hematocrit. The usual pregnant patient has a "physiologic anemia" due to increases in serum greater than red cell mass early in pregnancy. Elevations in hemoglobin and hematocrit

usually as a result of vasoconstriction and can predate hypertension. Other laboratory signs including liver enzyme elevations, uric acid elevation, increases in BUN and creatinine often are subtle indices for preeclampsia.

In addition to proteinuria, the patient's urine may show signs of red cell casts and her peripheral smear may show abnormal red cell morphology adding to the intrigue of an immune or inflammatory etiology.

There are numerous clinical factors that increase the risk for preeclampsia. They can include maternal age, primigravid status, multiple pregnancies (twins or higher order multiples), obesity, pre-existing hypertension, renal disease, and collagen vascular diseases. Physical findings and symptoms can also be associated with many of these risk factors. The onset of headache, scotomata, right upper quadrant abdominal pain, suggestive of liver swelling, and total body water and salt overload often result in anasarca. Cardiovascular overload can present as pulmonary edema and congestive heart failure. It is not uncommon to see cardiac decompensation to the extent that one may have to consider a peripartum cardiomyopathy, another potentially lethal pregnancy related disease.

The antiphospholipid antibody syndrome can produce a syndrome very similar to preeclampsia and also can consist of maternal thrombosis, fetal growth restriction and other multisystem complaints. The autoimmune component or inflammatory reaction at the endothelial level may all be common presentations with severe preeclampsia. Differentials also include thrombotic thrombocytopenic purpura (TTP).

Some individuals have invoked an inflammatory cause however the etiology is still very much unknown. That being said however there is no question that there is intense vasoconstriction that often can be evaluated with the use of Doppler ultrasound. Characteristic waveforms reflecting vasospasm in the maternal uterine artery may be an early screen, well before blood pressure increases, for the later development of this vasospastic condition we know as preeclampsia.

It was shown by Dr. Norman Gant years ago that a primigravida's blood pressure would increase with a very small dose of vasoconstrictor substance administered at mid pregnancy in women who were ultimately destined to become preeclamptic. Women who would ultimately have normotensive pregnancies would need larger doses of vasoconstrictive type drugs to increase their blood pressure than their counterparts with impending preeclampsia. This "preeclampsia predictor" defined individuals who were to become preeclamptic up to eight weeks prior to their blood pressures increasing suggesting there is a fundamental process ongoing well before the onset of hypertension. This phenomenon suggested that there was a vascular sensitivity in women destined to become preeclamptic not seen in women who remained normotensive or a physiologic vasodilation in normotensive individuals that was blunted in those becoming preeclamptic. This effect could be mediated as part of an adjustment of the rennin-angiotensin system but this has not been able to be demonstrated. Women who are destined to become preeclamptic are known to have changes ongoing well before they have elevation of their clinically obtained blood pressure.

Even though the vast majority of women who developed preeclampsia become symptomatic later in pregnancy, this constellation of symptoms can occur early when the pregnancy is complicated by trophoblast disease including molar pregnancies. The above mentioned



Doppler studies and sensitivity to vasopressors both suggest an early pre-clinical process ongoing that becomes a dramatic multisystem disease later. Conditions like molar pregnancy may, in some way, stimulate an clinical manifestation.

Blood pressure in a pregnant woman is monitored regularly throughout their prenatal care. It is important to recognize that the technique in obtaining these blood pressures can produce variation. Variables like the size of the patient's arm and the cuff that is used must be appropriately sized. The difference between a blood pressure obtained using a stethoscope and listening to Korotkov sounds may be different significantly from the blood pressure taken by a digital readout that usually measures pulsations. The onset and disappearance of pulsations may be vastly different than the onset and disappearance of the Korotkov sounds. This difference is dramatically demonstrated in the blood pressures obtained from digital readout appliances versus a stethoscopically obtained blood pressure. The position that patient is in when her blood pressures taken also is significant. The blood pressure cuff should be at the level of the patient's heart.

Proteinuria, one of the hallmarks of preeclampsia, is predicated on a glomerular lesion in the kidney. Because renal changes are a physiologic aspect of pregnancy, normotensive healthy pregnant women will spill more protein than her nonpregnant healthy counterpart. Therefore, the high limit of normal for protein excretion in the urine is as high as 300 mg per 24-hour urine. Proteinuria into the nephrotic range of 5000 mg per 24-hour urine would categorize the diagnosis as severe preeclamptic. Other criteria for severe preeclampsia can include headache, scotomata, upper right quadrant pain and thrombocytopenia in the mother as well as growth restriction and oligohydramnios in the fetus. Once the patient has deteriorated to severe preeclampsia, plans for delivery should be underway.

The degree of proteinuria is usually consistent with the degree of renal glomerular pathology. The classic renal lesion for preeclampsia has been capillary glomerular endotheliosis. The etiology of this classic lesion is unknown however the swelling of the capillary endothelial cells not only impacts on the degree of proteinuria but on significant decreases in glomerular filtration rate and renal plasma flow. Laboratory abnormalities including BUN, creatinine, and uric acid occur. One must recognize however that the healthy pregnant woman with her 50% increase in renal function from very early in pregnancy will have a BUN and serum creatinine normally quite low. It is a good general rule that BUN and creatinine should be 50% of the nonpregnant norm. If the pregnant patient has BUN and creatinine levels in the normal range, this would clearly be abnormal.

When faced with a clinical situation of the patient developing hypertension, defined as 140/90, with proteinuria, there are maternal and fetal issues that need to be anticipated. Severe preeclampsia which is defined as blood pressures over 160/110 offers additional risks for both the mother and fetus. This total body disease can affect, among other systems, the central nervous system causing seizures. Seizures defines the progression from preeclampsia to eclampsia. Other maternal stressors on the cardiovascular system can put the patient in congestive heart failure or pulmonary edema. Aggressive medical therapy and delivery are the only modalities known to reverse this sometimes rapidly progressing and at times fatal disease.

The part of the triad of hypertension edema and proteinuria that is most vague is edema. The normal increase in total body salt and water brings with it a certain amount of dependent edema in pregnancy. However pathologic edema which includes edema of the face and upper torso to include anasarca is uncommon and suggestive of a poor prognostic sign of multisystem failure.

Summary case study

Let's review some of the specific issues that occur in a woman with severe preeclampsia or even eclampsia. Let's say a previously normal pregnant woman presents with a blood pressure of 160/110, 4+ proteinuria, headache, scotomata, anuria, and is somnolent. The primary focus at this point is to ensure the maternal well-being and depending on the stage of gestation, secondarily monitor the fetal well-being. Aggressive control of blood pressure, seizure prophylaxis and plans for delivery are all components of good care. Renal shutdown, congestive heart failure, coagulopathy, and seizures as well as fetal distress all impact on the need for expeditious management.

Laboratory evaluation on this young woman demonstrates a hemoglobin of 13, thrombocytopenia at 32,000, elevated liver enzymes, uric acid of 8.6, BUN of 12 and a serum creatinine of 0.9. After the patient's first urine sample showing 4+ proteinuria, she was not able to void any further. A Foley catheter was placed and there was no urine in the bladder. This patient had a rather rapid and dramatic cardiorespiratory arrest, full code, emergent perimortem cesarean section, extended resuscitation and ultimately the patient succumbs. The premature and small infant was lethargic at birth but ultimately did well. The postmortem identifies the multisystem aspect of this disease. The cerebral spinal fluid was bloody, petechial hemorrhages are seen in the central nervous system. There was evidence of disseminated intravascular coagulation. The lungs and liver all showed evidence of petechial hemorrhage and congestion. A subcapsular hematoma of the liver had ruptured and there was free blood in the peritoneum.

This may be a fictional case but one that every obstetrician has seen except for the rupture of the subcapsular hepatic hemorrhage. We can see from this scenario that the disease known as severe preeclampsia affected the central nervous system, cardiopulmonary and splanchnic organs. Disseminated coagulation combined with a subcapsular hematoma rupture and exsanguination all could have contributed to this maternal death. The fetus was delivered and was significantly growth restricted. In addition to the numerous maternal systemic abnormalities, this fetus had undergone uteroplacental insufficiency and growth restriction for some time.

How do we prevent this type of tragic outcome?

Routine prenatal care along with risk factor identification could identify individuals as being at risk. These risk factors can include heredity, previous history and maternal medical diseases including pre-existing hypertension, collagen vascular disease, inflammatory diseases and renal disease. Early and high index of suspicion can go a long way to predicting a slowly developing case. There will always be those cases with rapid deterioration generally presenting with acute convulsive episodes not anticipated. Risk factors and surveillance may identify the more indolent cases. Fetal growth restriction, when present, is a disorder due to maternal or placental issues approximately 80% of the time and is an important risk factor and predictor of severity of preeclampsia. It is not uncommon for us to follow a woman with progressive fetal growth restriction that ultimately will become preeclamptic. Women of advanced maternal age who may also have chronic essential hypertension may be at risk to even a greater degree as superimposed preeclampsia carries an inordinately high maternal morbidity and mortality.

High index of suspicion along with clinical and laboratory parameters that demonstrate appropriate cardiovascular, renal, hepatic and blood pressure changes in pregnancy may go a long way to implicate the early diagnosis and treatment of this potentially tragic disease for both mother and infant.

Preeclampsia

To date, no preventative measures or effective treatments have been identified for preeclampsia (PE). Anti-hypersensitive drugs, corticosteroids, or magnesium sulfate may be administered to handle the symptoms of PE and prevent progression to eclampsia. This does not, however, provide a cure; rather, the treatments are adopted to extend the pregnancies to allow for the delivery of a more mature fetus. The only true resolution of PE is achieved through delivery of the fetus and removal of the placenta via induced labor or caesarean section (3).

The precise etiology of preeclampsia is yet to be determined, but it is likely the result of several factors. Despite the fact that there are many questions left unanswered, one certainty does exist, research has shown a central role of the placenta in the pathology of this disease (3). Associations have been identified with impaired angiogenesis, changes in local oxygen tension, and immunological alterations in the early placental microenvironment (5). Additional factors that may be involved in the origin of PE include genetic disposition, obesity, and autoimmune disorders (3).

In 2000, Odegard and colleagues identified a correlation between the development of PE and loss of the invasive phenotype in trophoblasts. During the first trimester of pregnancy, the outer layer of the blastocyst, the trophoblast, forms and anchors villi in addition to forming a network of lacunae between these villi. The trophoblast is also responsible for invading its maternal surroundings and remodeling the uterine spiral arteries to provide an adequate blood supply to the fetus. Impaired trophoblast invasion may lead to deficient implantation, alterations in placental development, and inadequate blood supply and transport of nutrients (5).

In a review focused on examining the immunological factors associated with the origin of PE, Laresgoiti-Servitje *et al.*, reported a shift toward Th1 responses and the increased production on IFN- γ in preeclamptic pregnancies. The source of IFN- γ remains elusive, however, its role in inhibiting trophoblast invasion is well established. The authors propose the observed shift towards a Th1 phenotype may also be the result of elevated IFN- γ . Further studies are necessary to identify the cells or antigens regulating the immune responses in PE as well as the precise mechanisms involved (5).

Recently, a group of researchers from New York identified a novel mouse model of immunologically-mediated pre-eclampsia. Ahmed and colleagues examined the CBA/J x DBA/2 mouse model of recurrent miscarriages and found similarities to the clinical manifestation of human PE. Females spontaneously developed symptoms of PE including proteinuria, renal glomeruloendotheliosis, and hypersensitivity to vasoconstrictor agents such as angiotensin II. The absence of hypertension is noted as a key feature in the human pathology of this disease; however, the researchers argue that this is still a valuable model because it recapitulates a pregnancy-induced disorder with preeclamptic features (4). Additionally, Ahmed's group used the CBA/J x DBA/2 mouse model to examine the effects of pravastatin in preventing the onset of PE. Pravastatin is an anti-inflammatory drug shown previously to protect CBA/J x DBA/2 pregnancies from spontaneous abortion. Drug-treated mice showed no signs of renal endothelial damage, proteinuria, or hypertension in response to angiotensin II. Furthermore, increased vascular endothelial growth factor (VEGF) release from mouse trophoblasts was observed with increasing concentrations of pravastatin. As such, the authors of this study suggest that pravastatin may prevent PE and its related complications by restoring angiogenic balance and placental development through increasing VEGF levels. In conclusion, they recommend further analysis of pravastatin as a candidate therapy to prevent PE (4).

VEGF, placental growth factor (PLGF), soluble VEGF receptor-1 (sFlt1), and soluble endoglin (sEng) are released from the placenta and regulate placental development and function. Anton and colleagues examined the effects of angiotensin II and angiotensin-(1-7), a vasoconstrictor and vasodilator respectively, on the release of VEGF, PLGF, sFlt1, and sEng. Elevated concentrations of sFlt1 and sEng were observed in preeclamptic chorionic villi. The authors propose an inhibitory role of angiotensin on sFlt1 in normal pregnancy. Loss of this regulation in PE would allow for increased sFlt1 and anti-angiogenesis (1).

A number of studies have explored the genetic aspects of this disease as well. In 2008, Sun *et al.*, screened 47,000 genes by means of microarray analysis (6). One hundred and forty (140) genes were found to be differentially expressed between the peripheral leukocytes of healthy and preeclamptic patients, revealing a significant difference in gene expression between the two phenotypes (6). In another study by Founds and colleagues, quantitative real-time PCR was used to validate eight candidate genes identified in microarray analysis of first trimester preeclamptic placentas (7). While further analysis and replication is required for the confirmation of candidate genes as biomarkers, the researchers here show a significant down-regulation of one particular gene, FSTL3, which may therefore have a role in PE (7).

Despite a considerable amount of research in recent years, the precise etiology of PE remains unclear. Additionally, there is no effective treatment for this condition. Being that this has a fairly high rate of incidence, continued research examining factors involved in the etiology and pathology of PE, as well as potential therapeutics will be of great value to the fields of gynecology and obstetrics.

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LEGAL CORNER

New CMS Physician Signature Requirements for Test Requisitions

By: Mark A. Lieberman, Esq., General Counsel and Theresa K. Begly, Esq., Compliance Officer

On November 2, 2010, the Centers for Medicare & Medicaid Services ("CMS") finalized its proposed CY 2011 Medicare Physician Fee Schedule Rule which states that *all test requisitions must be signed by the ordering physician in order to be reimbursed under the Clinical Laboratory Fee Schedule.*

While CMS's finalized rule states that this policy will go in to effect on January 1, 2011, CMS has announced a delay to the implementation of this policy until April 1, 2011.

As of April 1, 2011, ALL test requisitions paid under the Clinical Laboratory Fee Schedule must be signed by the ordering clinician

This new signature policy reverses CMS's prior position that test requisitions do not need to be signed by the physician as long as the physician clearly documents in the patients' medical records his or her intent that the test be performed.

The reasoning behind CMS's decision is that this new policy to

require physician signatures on all laboratory test requisitions will eliminate all prior confusion surrounding the issue. Further, CMS stated that if the physician is already filling out the test requisition, there is no additional burden on the physician to sign the test requisition.

The laboratory community has been vocal in expressing its displeasure with CMS's new policy and has requested that CMS push back the effective date of the new policy so that laboratories have sufficient time to educate their physician clients on the new policy and to ensure that the laboratory has sufficient administrative staff to handle the large volume of tests that will need to be put on hold due to a lack of physician signature on the test requisition.

Please note that the new signature requirements do not affect electronic orders or orders taken over the telephone. MDL appreciates your assistance in helping us comply with these new CMS requirements.



Medical Diagnostic Laboratories, L.L.C. *New Tests Announcement*

Now Available only on the **UroSwab®** (Urine specimens only)

Test 176 Urinary Pathogens Antibiotic Resistance (*E. coli*, *Klebsiella* species, *Proteus mirabilis*)

Available when one of the following tests performed are positive:

Test 141 *Escherichia coli* by Real-Time PCR

Test 172 *Klebsiella* species by Real-Time PCR (Reflex to Speciation by Pyrosequencing)

Test 146 *Proteus mirabilis* by Real-Time PCR

As of January 1, 2011, the following test replacement will take effect:

Discontinued	148	<i>Klebsiella pneumoniae</i> by Real-Time PCR
Replacement	172	<i>Klebsiella</i> species by Real-Time PCR (Reflex to Speciation by Pyrosequencing)

The following Test is being discontinued as of January 1, 2011:

Test 113 Herpes simplex virus (HSV) viral load by Real-Time PCR** (#126 Req.)

New Tests Available Beginning January 1, 2011:

Available on **OneSwab®**

Test 172 *Klebsiella* species by Real-Time PCR (Reflex to Speciation by Pyrosequencing)

Test 174 *Pseudomonas aeruginosa* by Real-Time PCR

Test 175 *Eggerthella* species by Real-Time PCR

Available on **NasoSwab®**

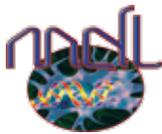
Test 174 *Pseudomonas aeruginosa* by Real-Time PCR

Available on **UroSwab®** (males only)

Test 174 *Pseudomonas aeruginosa* by Real-Time PCR

Available on **UroSwab®** (males & females)

Test 172 *Klebsiella* species by Real-Time PCR (Reflex to Speciation by Pyrosequencing)



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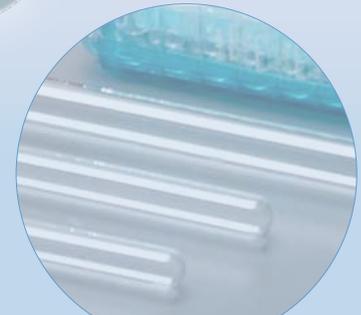
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