

HUMAN HEALTH

ENVIRONMENTAL HEALTH

# TOWARDS EARLY DETECTION OF PRE-ECLAMPSIA

MATERNAL HEALTH  
EDUCATIONAL SERIES

  
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## What is pre-eclampsia?

Pre-eclampsia (PE) is a condition of pregnant women. It is defined as pregnancy-induced increased blood pressure (hypertension) and protein in the urine (proteinuria), which can lead to eclampsia, or convulsions. PE is estimated to affect 8,370,000 woman worldwide every year and is a major cause of maternal, fetal and neonatal morbidity and mortality.[65, 85, 96]

PE as well as intrauterine growth restriction (IUGR) is characterized by impairment of placental perfusion, which in turn leads to placental ischemia. The disorder develops early in the 1st trimester.

A state of insulin resistance (a problem that interferes with the body's ability to clear sugar from the blood and is often a precursor to Type II diabetes) has been demonstrated in active PE, and women with insulin resistance are at higher risk of developing PE during pregnancy.[66] It is also suggested that simple assessments of insulin resistance based on a single determination of fasting insulin and glucose could predict pre-eclampsia at least as well as the current gold standard for prediction of pre-eclampsia, uterine artery Doppler velocimetry.[103] Ness and Sibai have proposed that pregnancies complicated with IUGR lack the maternal metabolic syndrome (e.g. adiposity, increased insulin resistance, hyperlipidemia, excess thrombin generation) and inflammatory signals which prevent patients from developing pre-eclampsia.[99]

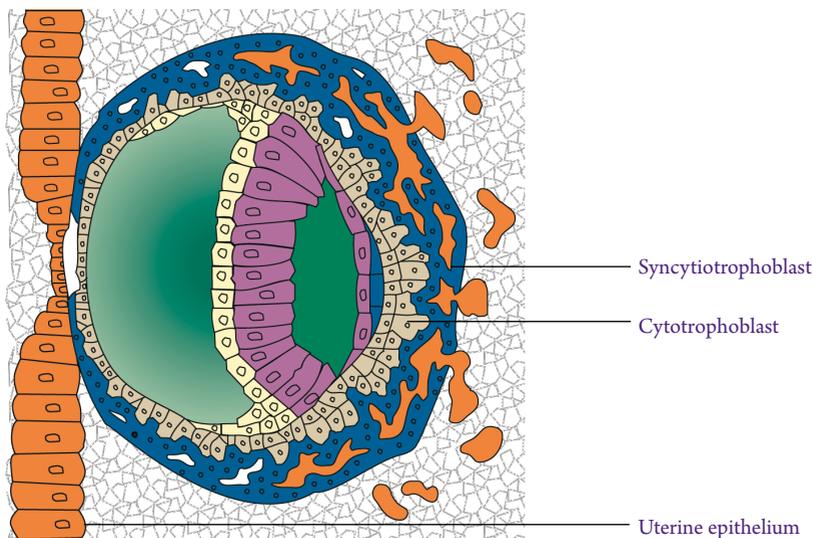


Figure 1. Trophoblasts are the first cells to differentiate from the fertilized egg in early pregnancy, becoming the outer layer of a blastocyst. They further differentiate into two layers, the inner cytotrophoblast and the outer syncytiotrophoblast. Trophoblast cells protect the fetus against the maternal immune system.

Although the causes remain unclear, the syndrome may be initiated by inadequate trophoblast invasion, releasing placental factors into maternal peripheral circulation, which in turn is believed to lead to inadequate transformation of maternal arteries to a low-pressure, high-flow system, which would allow adequate blood flow to the placenta and fetus in normal pregnancy.

The maternal vascular endothelium, which is a single cell lining that covers the luminal side of blood vessels, responds to relaxing and contracting factors that maintain vascular hemostasis. Several studies suggest that ischemic placenta contributes to endothelial dysfunction.[57] However, information on the mechanisms mediating the long-term increase in vascular resistance and arterial pressure associated with placental ischemia is lacking. Endothelial dysfunction leads to enhanced formation of endothelin and thromboxane, increased vascular sensitivity to angiotensin II, and decreased formation of vasodilators such as nitric oxide and prostacyclin.

Recent studies on placental pathology have suggested that early-onset disease is more likely to be associated with abnormal villous and vascular morphology while late-onset pre-eclampsia may be related to impaired glucose metabolism.

## **Clarification of pre-eclampsia and some related terms**

The definitions below are based on 2002 guidelines from the American College of Obstetricians and Gynecologists (ACOG), updated and verified by the international symposium on hypertension in pregnancy.[2, 74, 134]

### **Pre-eclampsia (PE)**

Pre-eclampsia is a condition based on two basic quantified signs, hypertension of > 140/90 mm Hg measured normally 4-6 hours apart in previously normotensive women, and proteinuria of at least 2+ with dipstick measurement.

Largely on the basis of these two signs, a distinction is made between mild and severe PE as outlined in Table 1. The severity of pre-eclampsia is also diagnosed according to accompanying multi-organ effects and subjective criteria such as continuous epigastric or right upper quadrant pain, headaches, blurred vision or reversible blindness, and associated with fluid retention and pulmonary edema.

Early-onset PE (also called preterm PE) is pre-eclampsia that develops early and necessitates delivery before 37 weeks of gestation. While most of these early-onset cases are severe, it is the very early ones, developed before 33-34 weeks that cause the greatest concern. They account for 12-20 % of all cases and for the majority of maternal and fetal mortality. Late-onset PE (term PE) is pre-eclampsia that develops later and does not necessitate delivery until at least 37 weeks of gestation.

Table 1. Distinction between mild and severe forms of PE on the basis of hypertension and proteinuria.

|  | Mild PE   | Severe PE   |
|--|---|---|
| Hypertension<br>systolic/diastolic (mm Hg)                   | either value $\geq 140/90$ , but<br>$< 160/110$ | Either value $\geq 160/110$   |
| Proteinuria<br>(Dipstick, 2+ = 100 mg/dL,<br>3+ = 300 mg/dL) | $\geq 2+$ , but $< 3+$                          | $\geq 3+$<br><br>Either blood pressure or<br>proteinuria as above,<br>provided the other one<br>exceeds the value set<br>for mild PE. |

## Eclampsia

Eclampsia is a dangerous form of severe PE where blurred vision, apathy, nausea and dizziness turn into convulsions, indicating the severe disorder has affected the brain and the woman is experiencing a threat to her life. [2, 74, 156]

## HELLP

HELLP is a rapidly developing life threatening condition comprising:

- Hemolysis
- Elevated liver enzymes, at least one of the following
- Lactate dehydrogenase  $> 600$  U/L
- Serum total bilirubin  $> 1.2$  mg/dL
- Serum aspartate aminotransferase (AST)  $> 70$  U/L
- Serum alanine aminotransferase (ALT)  $> 72$  U/L
- Low platelets ( $< 100,000$  cells per  $\mu\text{L}$ )

HELLP Syndrome occurs in tandem with PE, but because HELLP Syndrome's symptoms may appear before those characteristic of PE, they may be easily misdiagnosed. Partial HELLP is the term applied when a woman meets one or two of the criteria for HELLP but not all three.

The incidence of HELLP syndrome is 0.3% of all pregnancies of which 30% will occur postpartum. It increases the risk of postpartum hemorrhage. The outcome for women with PE or HELLP is not significantly different, and gestational age for delivery seems to be the decisive factor for neonatal mortality. [2, 74, 156]

### **Pregnancy induced hypertension (PIH)**

Pregnancy induced hypertension is defined according to the same blood pressure criteria as PE (with mild and severe subtypes as defined in Table 1) but excluding the proteinuria criteria.

### **Intrauterine growth restriction (IUGR)**

Intrauterine growth restriction is the failure of the fetus to realize its growth potential as a result of genetic or environmental factors. Approximately 30 % of IUGR cases are associated with chromosomal aberrations such as trisomy 18 or Turner (45,X) syndrome or more rare diseases. A number of PE cases, particularly early-onset and severe PE cases are associated with the delivery of IUGR babies.

### **Small for gestational age (SGA)**

Small for gestational age babies are those with birth weight below the 10th percentile for their gestational age. IUGR babies are invariably SGA.

### **Preterm delivery (PTD)**

Preterm delivery applies to pregnancy outcome when delivery is before 37 complete weeks of gestation.

### **Normal outcome**

Normal outcome involves delivery at term ( $\geq 37$  weeks) of a live baby with birth weight  $> 5$  %-lower percentile and with none of the symptoms associated with PE and its subtypes or eclampsia, HELLP or PIH.

### **Risk factors**

There are a number of known prior risk factors for pre-eclampsia. The following list is loosely based on that presented in Wagner.[156]

Associated with the pregnant woman:

- First pregnancy (nulliparity)
- Afro-American race
- Short inter-pregnancy interval
- High blood pressure before becoming pregnant
- Obesity
- Diabetes, kidney disease, rheumatoid arthritis, lupus or scleroderma
- Age under 20 or over 35
- Previous pre-eclamptic pregnancy
- Mother or sister had PE

Associated with the pregnant woman's husband or partner:

- First time father
- Previously fathered a pre-eclamptic pregnancy

Associated with the condition of the fetus:

- Multifetal pregnancy
- Hydrops/triploidy
- Hydatidiform mole

Contradictory results have been presented as to whether or not blood group AB raises the risk for PE.[37, 61]

Table 2. Estimates of the relative risk (RR) or the odds ratio (OR) associated with some of the pre-eclampsia risk factors mentioned in the text.

| Risk factor   | RR    | Reference | Adjusted OR<br>Early-onset-PE | Adjusted OR<br>Late-onset-PE | Reference  |
|---|-------|-----------|-------------------------------|------------------------------|--|
| Nulliparity   | 2.9   | 50        |                               |                              | Unpublished information, courtesy of K. Nicolaidis |
| Age over 40   | 2.0   |           |                               |                              |  |
| Family history of PE                                      | 2.9   |           |                               |                              |  |
| Previous PE   | 7.2   |           | 4.0                           | 2.2                          |  |
| High BMI ( $\geq 35$ kg/m <sup>2</sup> ) before pregnancy | 2.5   |           |                               | 1.1                          |  |
| Chronic hypertension (diastolic BP > 90 mm/Hg)            | 1.4   |           | 8.7                           |                              |  |
| Ovulation drugs   |       |           | 4.8                           |                              |  |
| IVF   | 2-2.7 | 12        |                               |                              |  |
| Maternal mood and anxiety disorders                       | 2.1   | 116       |                               |                              |  |

The explanation for the lower risk in second and subsequent pregnancies is thought to be immunological adaptation to the male partner's antigens. There is also some evidence for a possible viral etiology.

Recurrence risk varies from 14 % to 20 % in the next pregnancy being higher if the pre-eclampsia resulted in preterm delivery (up to 38 % if delivery before pregnancy week 28).

Small changes in maternal weight between pregnancies – as little as 10 pounds, or less than 5 kg – can alter the risk of pre-eclampsia recurrence. For a woman who loses weight, going from overweight to normal weight, the recurrence rate is as low as for someone in the normal weight category.[92]

Current diagnostic criteria for pre-eclampsia and related hypertensive disorders of pregnancy are not very clear. It is difficult to identify those who are of highest risk for adverse pregnancy outcome. Thus there is a need to find new biochemical markers to predict women at risk for subsequent disorder and to confirm the diagnosis ideally for different subsets of the disorder to guide patient management and potential future therapies.

## **Understanding of pre-eclampsia by women in pregnancy**

When a sample of 112 pregnant women were interviewed to determine their pre-eclampsia knowledge, it was found out that they had a generally poor understanding of pre-eclampsia, although improved understanding was associated with having received information about the disease.[160] Further investigation will be needed to determine how best to educate patients and whether this education can also decrease adverse outcomes associated with this syndrome.

## **The true cost of the disorder**

The incidence of PE seems to vary between countries. It is reported to affect 200,000 pregnant women in the USA, [121, 133, 157] and it is estimated to cause approximately \$10 billion in healthcare costs. The 30 % of cases that may be categorized as early-onset PE absorb 80 % of the total PE health care costs. According to the CDC, PE is the 2<sup>nd</sup> greatest cause (18 %) of maternal mortality during pregnancy [74, 133] (see also Figure 2). Hallmarks of the very severe forms of early-onset PE include incomplete trophoblast invasion into the maternal arteries, trophoblast injury, and placental degeneration.[90, 134] These severe forms of early-onset PE are usually associated with IUGR.

PE is responsible for approximately 7-9 % of neonatal morbidity and mortality. [65, 85, 96] Furthermore, the need for urgent premature delivery accounts for a 3-fold increase in the caesarean section rate among women with PE, compared to normotensive women thus prolonging the post-delivery recovery period and elevating costs.[85, 150]

Perinatal mortality as a result of PE is 5-fold greater than in non-PE pregnancies in developed countries, and seems to be higher after infertility treatment than in spontaneous gestations.

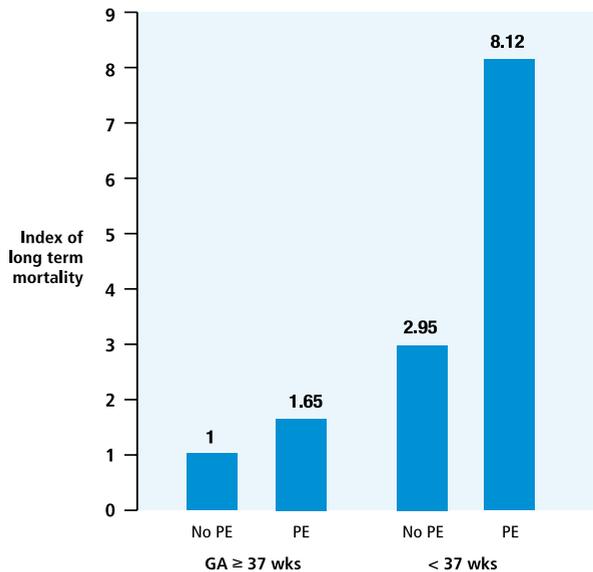
|                          |      |    |
|--------------------------|------|----|
| Australia                | 2003 | 4  |
| Bulgaria                 | 2004 | 11 |
| Canada                   | 2003 | 7  |
| Finland                  | 2005 | 7  |
| France                   | 2003 | 8  |
| Japan                    | 2004 | 6  |
| Netherlands              | 2005 | 6  |
| New Zealand              | 2003 | 9  |
| Spain                    | 2005 | 4  |
| Switzerland              | 2004 | 5  |
| United Kingdom           | 2004 | 8  |
| United States of America | 2003 | 11 |

Figure 2. Maternal deaths per 100,000 live births estimated for a selection of industrialized countries for years within the range 2003-2005. [84]

## Long term implications

Meta-analysis of almost 200,000 women who had pre-eclampsia shows a relationship between PE and an increased risk of hypertension and maternal coronary heart disease in later life.[24] Women who have PE that leads to preterm delivery have been reported to have an 8-fold higher risk of death from cardiovascular disease compared with women who do not develop PE and whose pregnancy goes to term (Figure 3.)[64]

Figure 3. Long term mortality from cardiovascular disease of women who had pre-eclampsia compared to women with no PE. Data is presented for pregnancies that continued to at least 37 weeks, and those that reached less than 37 weeks. Data from births in Norway, 1967-1992. [64]



PE shares many common pathological features with arteriosclerosis, such as impaired insulin sensitivity, dyslipidemia and coagulation disturbance.[18] However it is not clear whether PE is the direct cause of cardiovascular mortality; PE could be a result of a failed stress test (pregnancy) in a woman with predisposing factors to cardiovascular disease. Recent studies also suggest that preterm PE may be more prone to long term health issues in the mother compared to later-onset and term PE.[151] Recently it has been shown that women with PE also have a greater risk of developing diabetes even in the absence of gestational diabetes.[31]

Preterm PE is typically associated with reduced fetal growth. Babies who are small for their gestational age are more prone to diabetes or cardiovascular disease later in life.[20, 130] There is also a preliminary indication that stress during pregnancy is mediated through the placenta into the fetus and affects the baby's temperament.[117]

It has been suggested that vascular injury caused by high concentrations of sFlt-1 in PE pregnancies could be a potential mechanism for subclinical hypothyroidism.[77] Subclinical hypothyroidism, i.e. elevated TSH with normal thyroid hormone concentration, is seen in 4-10 % of all pregnancies and is more common in pregnancies with PE. According to Levine et al. subclinical hypothyroidism is not present in early pregnancy. Other reports have shown that clinical hypothyroidism itself does not predispose to PE. However, Levine et al. suggest that the vascular injury may have a long-term effect on thyroid function, possibly with an alternative pathogenic mechanism. This is supported by their findings that in PE pregnancies TSH is increased but thyroid peroxide antibody (TPO-Ab) concentrations are normal.[77]

## **The challenge to clinicians**

When PE appears in the second or third trimester of pregnancy there is no means of treatment other than delivery. However, accumulated clinical evidence begin to show, and opinion leaders believe, that early detection may enable the onset of treatment at an early stage, when the placenta is subject to re-differentiation and while maternal arteries could be remodeled.

Numerous research programs seek to identify means for identifying the risk and for the subsequent selection of medications for the prevention of PE or for reducing its severity.[2, 74, 133, 164]

In the USA, the American College of Obstetrics and Gynecology (ACOG) has issued guidelines on routine antenatal care recommending that a woman's level of risk for PE should be evaluated so that a plan for her schedule of antenatal appointments can be formulated.[2] The UK National Institute for Clinical Excellence (NICE) has followed this approach.[97]

Baker et al. have searched published literature but been unable to find a consensus on management recommendations due to wide variation of subject groups,

definitions, cut-offs and timing of tests. They concluded that there is evidence to support a stratified assessment and management of women at risk of developing pre-eclampsia into those requiring:

- Admission and/or medical management
- Enhanced community assessment
- Assessment further in the unit; those women with new hypertension or new proteinuria, no symptoms and no suspicion of fetal compromise [17]

In most of the developed countries women are screened for PE more frequently towards the end of the pregnancy. The PRECOG Guideline in the UK states that after week 20 women should be assessed for the signs and symptoms of pre-eclampsia:

- New hypertension
- New proteinuria
- Symptoms of headache or visual disturbance, or both
- Epigastric pain or vomiting or both
- Reduced fetal movements, small for gestational age infant

# Management

Serious morbidity associated with pre-eclampsia can occur from 20 weeks gestation to after delivery. Eclampsia is the most common morbidity at term while the following are more common before 32 weeks:

- Placental abruption
- Hemolysis
- Elevated liver enzymes
- Low platelet count syndrome
- Renal failure

Pre-eclampsia onset before 32 weeks leads to the most severe outcome, and on average the interval between diagnosis and delivery is 14 days (0-62 days) with a substantial number of women delivering within 72 hours.[135] In fact, 15 % of all preterm births are a consequence of pre-eclampsia.

About half of the fetuses from women with severe PE suffer from IUGR. Prenatal identification of IUGR is of importance as these fetuses are at increased risk of short term adverse outcome such as:

- Fetal distress
- Intrauterine demise
- Cerebral bleeding and seizures
- Respiratory distress
- Sepsis
- Infant death

Also, later in life babies born too small are at increased risk of type 2 diabetes, hypertension and attention deficit disorder.[23, 56, 71, 80] It has been shown that at the expense of a higher C-section rate, the risk of adverse neonatal outcome is decreased 4-fold when IUGR is diagnosed prenatally compared to undiagnosed IUGR.[80] The diagnosis of IUGR is usually made on the basis of ultrasound, and the use ultrasound-based intrauterine growth curves or “customized” growth curves to plot the estimated fetal weight is recommended. Customized growth curves take maternal and fetal characteristics into account. IUGR can also be diagnosed by using longitudinal growth charts usually with 10-day intervals between the measurements.[120]

## **Expectant vs. interventionist management of pre-eclampsia**

Management of pre-eclampsia before 34 weeks of gestation is controversial. Early delivery of the baby increases neonatal risks and thus “expectant” care aims to prolong the pregnancy with careful monitoring of indicators such as maternal hypertension and fetal heart rate.

Magee et al (2009) reviewed 72 studies, of which 28 were performed in developed and 11 in developing countries, with a total of 4650 women who were given expectant and/or interventionist care for early-onset pre-eclampsia (less than 34 weeks of pregnancy at presentation or at delivery).[83] Almost half of the studies enrolled women after a stabilization period. 40 % women failed stabilization leaving early delivery as the only option. A priori indications for delivery included uncontrolled severe hypertension, HELLP and a non-reassuring fetal heart rate. In one third of studies a specific gestational age (of 34 weeks in 10/15 studies), pulmonary edema or renal failure was the reason for the early delivery.

Interventionist studies usually described use of antihypertensive therapy, MgSO<sub>4</sub> and antenatal corticosteroids treatment. In expectant care blood pressure care, maternal/fetal monitoring and blood analysis (CBC, AST, ALT, uric acid, LDH, INR, albumin, bilirubin) were reported variably.

Expectant management was done either by hospital policy (18 %), RCT policy (31 %), or by physician preference or was unclear.

During expectant care delivery were equally often prompted by maternal or fetal indication (40 % and 36 %, respectively). In 16 % of women delivery was done due to reaching gestational age of 34 weeks. Maternal death and serious maternal complications (i.e. serious intervention and/or long-lasting consequences) were uncommon.

The conclusion from the Magee study was that expectant care is associated with pregnancy prolongation of about one or two weeks with superior outcomes for the baby and low maternal risks. Management of blood pressure is frequently complicated by highs and lows, but the incidence of other serious maternal complications is low, particularly in the absence of HELLP syndrome, but there is limited information from interventionist cohorts for comparison.[83]

In 2009 Haddad and Sibai published a review article in which they compared expectant management in pregnancies with severe PE. The article was based largely on the same clinical trials as the Magee meta-analysis discussed above, but divided the trials into those in which the management was of very early severe PE at less than 25 weeks of gestation and those in which it was at 24-34 weeks. They concluded that although pregnancy termination rather than expectant management should be seriously considered in women with severe PE at less than 24 weeks, there are many benefits associated with management beyond the period typically associated with expeditious delivery. According to criteria set by the authors, 63 % of women with severe PE were eligible for expectant management beyond the first 48 hours after admission.[60]

## **Indication for delivery and adverse outcome of pregnancy**

Indications for delivery in pre-eclampsia are often reported merely as “deteriorating maternal or fetal condition”. However, there is much published literature on the subject. The most endorsed indications are uncontrolled severe hypertension, HELLP syndrome and non-reassuring fetal heart rate.

Magee et al. recently suggested that the limited published data did not support use of the following as indication for adverse outcome of pregnancy:

- Increased proteinuria
- Elevated urine acid levels
- AEDF (Absent End-Diastolic Flow)
- Increased resistance to diastolic flow by umbilical artery Doppler velocimetry
- Uterine artery notching characteristics
- IUGR with/without oligohydramnios [83]

Thangaratnam et al. also concluded that the estimation of levels of proteinuria is not a clinically useful test to predict fetal or maternal outcomes.[41]

## **Some examples of national guidelines on screening and prevention**

### **United Kingdom**

In 75 % of UK hospitals women with suspected pre-eclampsia onset are referred to an out-patient hospital unit for assessment. There are diverse management practices and a lack of nationally recognized protocols.[17]

Guidelines for the routine care of pregnant women have been presented in an established guideline published by NICE, the National Institute for Health and Clinical Excellence.[98] A recent update on these guidelines outlines criteria for moderate and high risk pregnancies. These are summarized as follows:

#### **Moderate risk**

- Age 40 years or more
- First pregnancy
- Multiple pregnancy
- Interval since last pregnancy of more than 10 years
- Body mass index of 35 or more at presentation
- Family history of pre-eclampsia

#### **High risk**

- Chronic hypertension
- Chronic kidney disease

- Hypertensive disease during a previous pregnancy
- Diabetes
- Autoimmune disease

A woman with more than one high risk, or more than two moderate risk factors should be advised to take aspirin at a dosage of 75 mg/day starting at the age of 12 weeks.[154]

This approach does not pinpoint nulliparous women as requiring specialist consultation unless another risk for PE is present.

The PRECOG guideline complements the NICE guidelines. Women are screened at booking to identify the risk factors as listed above. For those who have risk factors, signs of PE should be checked for every 3<sup>rd</sup> week until pregnancy week 32. All women are tested for elevated blood pressure and protein in urine every other week starting at week 34. The PRECOG Guideline does not prescribe subsequent obstetric care, but states that it should be determined on an individual basis.

Recently PRECOG released more detailed recommendation for PE assessment in the hospital day unit (PRECOG II) aiming to raise awareness of the risk factors of PE, including the need for accurate diagnosis, assessment and timely referral. However, there are still uncertainties as to whom and when to refer. It is noted that more evidence is needed to support intervention or more frequent assessment or the involvement of different healthcare professionals.[88, 89]

## **Germany**

German guidelines [47] state that 2<sup>nd</sup> trimester Doppler of uterine arteries can be useful for early detection of PE with additional consideration of maternal risk factors. Low dose aspirin (75-150 mg/day, started latest at pregnancy week 16) is mentioned as being the only currently available method for the prevention of PE, especially for women with a history of severe PE. The dose established as most appropriate is 100 mg/day.

Outpatient care is recommended for mild PE cases with weekly medical inspections with blood pressure monitoring at home and elimination of additional stress factors. Indications for presentation at the clinic include diagnosis of severe PE or impending fetal distress such as:

- Abnormal CTG,
- Fetal growth restriction,
- Reduced amount of amniotic fluid
- Abnormal Doppler of uterine arteries

## France

In France expert guidelines on the multidisciplinary management of severe pre-eclampsia were published in 2008.[114] According to these, there are no clinical or biological criteria that could predict PE and thus screening of PE is not recommended. Uterine artery Doppler measurement is only recommended among high-risk patients i.e. women with previous severe or early PE.

In contrast to the UK guidelines, the French guidelines include recommendations as to how high risk patients should be treated.

- Low dose aspirin 75 – 160 mg per day is recommended for high risk patients and should be started before 20 weeks of pregnancy
- Low molecular weight heparin is not recommended for PE prevention but should only be used for those at risk of thrombosis
- Antioxidants are not recommended
- Calcium is only recommended for women with calcium deficiency

Advice is provided on hospitalization and indications for delivery and management of eclampsia, renal and hepatic disease as well as postpartum follow-up.

## Australia and New Zealand

The Society of Obstetric Medicine of Australia and New Zealand published updated guidelines in 2009.[81] Previous consensus statements were published in 1993 and 2000.

Guidelines include challenging areas like definition of severe hypertension and non-proteinuric PE, how to measure proteinuria and how to use of automated blood pressure monitors. Definitions differ slightly from those of ISSHP, for example, proteinuria is not mandatory in order to make clinical diagnosis.

Advice is given on indications for delivery and antihypertensive treatment. Low molecular heparin is recommended if there is nephrotic syndrome. Magnesium sulfate ( $MgSO_4$ ) is the drug of choice for prevention of eclampsia. However the guidelines note that routine administration of  $MgSO_4$  to pre-eclamptic women is less compelling in countries with low maternal and prenatal mortality rates. Thus individual units are advised to determine their own protocols and monitor outcomes.

Preconceptual management and prophylaxis for women at risk of pre-eclampsia is recommended:

- Aspirin in doses between 50-150 mg daily
- Calcium supplementation (1.5g daily) particularly for those with low dietary calcium intake

Guidance is not given on the means available for assessing the women who are within the high risk group. It is noted, however, that recurrence risk varies from 6 %

to 55 % with the greatest risk in women who have had early-onset pre-eclampsia and chronic hypertension. It also recommends that all women with previous PE or hypertension in pregnancy have an annual blood pressure check and regular assessment of other cardiovascular risk factors like serum lipids and blood glucose.

## **Italy**

According to recommendations by the Associazione Italiana Preeclampsia (AIPE), the Italian branch of ISSHP, the use of low-dose aspirin for prevention of pre-eclampsia is currently indicated only in patients defined as high risk (on the basis of obstetric history of pre-eclampsia and / or early-onset and severe IUGR). The recommended dose should not be less than 100 mg and the gestational time for the start of prophylaxis should certainly be before the 20th week. It is suggested that commencement at 12 weeks is probably the most appropriate time, because trophoblastic invasion is then not yet complete and changes induced by the drug can still occur.[15]

## **Canada**

In the SOGC Clinical Practice Guideline of March 2008,[82] the evidence that severe gestational hypertension (or PE specifically) many have some adaptive function.[155] is remarked. For example, neonatal morbidity is lower and neuro-developmental outcome better among SGA babies whose mothers become hypertensive compared to those whose mothers do not.[86] In recognition of this, the Canadian guidelines aim not only at the prevention of PE, but equally at the prevention of the complications associated with pre-eclampsia.

With regard to prediction, at booking for antenatal care, women with markers of increased risk for pre-eclampsia should be offered obstetric consultation, and should be considered for risk stratification involving a multivariable clinical and laboratory approach.

Recommendations for treatment during pregnancy are then available for women at low risk, intermediate risk, and high risk of PE in the current pregnancy. One of the preventive treatments recommended in women at increased risk (but not in low risk women) is low-dose aspirin (75–100 mg/d), administered at bedtime. This treatment should start pre-pregnancy or from diagnosis of pregnancy but before 16 weeks' gestation, and continue until delivery.

Recommendations are also made for postpartum care, future pregnancy and long-term cardiovascular health.

## **USA**

ACOG Guidelines published in 2002 [48] state that no single screening test has been found to be reliable and cost effective. Uric acid has been one of the most commonly used tests but only with 33 % positive predictive value.

The guidelines recommend continued observation/expectant management for the women with a preterm fetus only if she has mild PE. Therapy consists of maternal and fetal evaluation.

No randomized trials have determined the best tests for fetal evaluation. The ACOG working group recommended:

- Weekly non-stress tests, biophysical profiles, or both, which should be repeated as indicated according to maternal condition
- Testing twice weekly for suspected IUGR or oligohydramnios
- Ultrasound examination for fetal growth and amniotic fluid assessment every three weeks
- Daily fetal movement assessment

Maternal evaluation consists of evaluation of worsening PE including laboratory tests (platelet count, liver enzymes, renal function and protein in urine) weekly or more often if disease progression is questionable.

Hospitalization is often initially recommended for women with new-onset PE. Subsequent management may be continued in the hospital at a day-care unit or at home based on initial assessment.

The American Society of Hypertension (ASH) has published guideline articles in 2008 and 2010 [78, 79] which include solicited review advice from the ACOG. There are also reviews of the status of the tests available to predict PE as well as strategies to prevent and manage PE. Currently in the USA no Food and Drug Administration (FDA) cleared or approved test exists for the detection of pre-eclampsia.

## **Mexico**

Mexican guidelines are similar to NICE guidelines in using obstetric history criteria for classifying pregnant women for high risk. No recommendations will be made for supplements given to prevent PE before multicenter studies have shown the usefulness.[34]

## **Atypical PE**

Eclampsia is not always associated with high blood pressure, in a UK population study 34% of eclamptic women had a maximum of diastolic blood pressure of less than 100 mm Hg.[49]

Sibai and Stella recently reviewed nonclassic and atypical pre-eclampsia and described diagnosis and treatment of patients.[136]

Table 3. Guidelines for prediction and prevention of PE.

| Country                   | Screening method   | Prevention  | Reference/year  |
|---------------------------|--|---|---|
| UK                        | Based on risk factors (see page 16)                                  | 75 mg ASA daily from week 12 until delivery   | 98 (NICE) 2008<br>88 (PRECOG) 2005<br>89 (PRECOGII) 2009<br>154 (NICE) 2010 |
| France                    | Based on maternal history  | 75-160 mg ASA daily before wk 20 / Calcium for women with calcium deficiency                              | 114 (CNGOF, SFAR, SFMP, SFNN) 2009  |
| Australia and New Zealand |  | 50-150 mg ASA daily preconceptionally for women at risk / 1.5 g calcium for women with low dietary intake | 81 (SOMANZ) 2009  |
| Germany                   | Based on maternal history<br>2 <sup>nd</sup> trimester<br>uA Doppler | 100 mg ASA daily for women with a history of severe PE before week 16                                     | 47 (DGGG) 2010  |
| Italy                     | Based on risk factors  | At least 100 mg/day ASA before week 20 and preferably starting week 12                                    | 15 (AIPE) 2007  |
| Canada                    | Based on risk factors  | 75-100 mg/day ASA before week 16 for women at increased risk  | 82 (SOGC) 2008  |
| USA                       |  | MgSO <sub>4</sub> for severe PE to prevent seizures<br>ASA and calcium not recommended                    | 48 (ACOG) 2002  |
| Mexico                    | Based on risk factors  |   | 34 (SALUD) 2007   |

# Prophylaxis

## Aspirin treatment

Placental damage caused by pre-eclampsia is thought to lead to activation of platelets and the clotting system. Women with PE produce excess thromboxane, a vasoconstrictor and stimulant of platelet aggregation. Aspirin potentially inhibits platelet production of thromboxane. Aspirin has been tried for both prevention and treatment. The CLASP trial showed that it significantly reduced preterm birth but did not affect proteinuric PE, IUGR or neonatal mortality. It was concluded that low-dose aspirin (60 mg/day was used in the CLASP trial) may be justified in women with early-onset PE that will necessitate preterm delivery.[38]

Subsequently it was shown that the start of treatment might need to be in the first trimester of pregnancy [146] and also higher doses (100-150 mg/day) [72] were suggested. One study suggested that aspirin should not be given to all pregnant women as it might cause gastroschisis, [70] but further work has apparently not substantiated the connection. Low-dose aspirin is generally thought to be safe.[42]

Coomarasamy et al.[43] performed a meta-analysis of 14 placebo controlled clinical trials, including a total of 12,416 women and concluded that aspirin reduces the risk of perinatal death and pre-eclampsia in women with historical risk factors such as previous history of pre-eclampsia, chronic hypertension, diabetes, and renal disease.

Meta-analysis of 32,217 women mostly with low or moderate risk for pre-eclampsia, showed moderate (10%) but consistent reduction in risk of PE preterm delivery before 34 weeks of gestation and having a pregnancy with serious adverse outcome [14]. It also suggested that multiparous women with a history of hypertensive disorder of pregnancy may derive a larger benefit.

## The importance of starting treatment before 16 weeks

In 2009 Bujold et al. described a meta-analysis to assess the influence of gestational age at the time of starting the aspirin treatment on the incidence of pre-eclampsia in women at increased risk on the basis of abnormal uterine artery Doppler. Nine randomized controlled trials with a total of 1317 women were included in the analysis. Women were randomized for low-dose aspirin (50-150 mg) and either for placebo or no treatment (usual care). They found that there was 52% reduction in the risk of pre-eclampsia compared with the control group when aspirin treatment was started before 16 weeks of pregnancy. When aspirin treatment was started after week 16 there was no significant reduction in pre-eclampsia risk. Use of aspirin was not associated with significant changes in the rate of preterm births regardless of gestational age at the initiation of treatment, but the IUGR rate was reduced by 49% in the subgroup of women treated before 16 weeks of pregnancy compared to 18% decrease in the studies overall.[29]

An additional, larger meta-analysis involving 11,348 women was subsequently performed. In this work the original assessment of risk was not limited to abnormal uterine artery, but involved women assessed as being at risk according to the various criteria used in the original studies.

Table 4. The effect of low-dose aspirin on the development of pre-eclampsia and other maternal and fetal outcomes.

| Study (year)       | Development of PE   | Preterm delivery       | Neonatal death           | SGA                                | Risk of abruption bleeding         | Reference |
|--------------------|---|------------------------|--------------------------|------------------------------------|------------------------------------|-----------|
| Duley (2001)       | Moderate risk patients: 15% reduction<br><br>High risk patients: 15% reduction<br>NNT=100 | 8% reduction<br>NNT=72 | 14% reduction<br>NNT=250 | 8% reduction                       | not reported                       | 51        |
| Coomarasamy (2003) | 14% reduction   | 14% reduction          | 21% reduction            | 215 g weight gain in aspirin group | not significant difference in risk | 43        |
| Ruano (2005)       | Low risk patients: no significant reduction<br><br>High risk patients: 13% reduction      |                        |                          |                                    | not reported                       | 126       |
| Askie (2007)       | 10% reduction   | 10% reduction          | 9% reduction             | 10% reduction                      | not significant difference in risk | 14        |
| Cochrane (2007)    | 19% reduction overall<br>NNT=69<br><br>NNT=118 (moderate risk)<br><br>NNT=18 (high risk)  |                        |                          |                                    |                                    | 52        |

The study confirmed the importance of starting aspirin treatment before a cut-off gestational age of 16 weeks, and the authors concluded that low-dose aspirin initiated in early pregnancy is an efficient method of reducing the incidence of pre-eclampsia and IUGR.[30]

Baschat et al. presented similar results showing 40 % reduction of both early and late-onset pre-eclampsia when aspirin treatment was started during the 1st trimester of pregnancy among high risk pregnancies. Risks were calculated by combining maternal risk factors with uterine artery Doppler PI and maternal serum PAPP-A using a risk calculation algorithm.[21]

## **A review of studies relating to other treatments**

### **The use of magnesium**

Magnesium is an essential mineral for optimal metabolic function. Studies estimate that a substantial number of Americans do not receive the recommended dietary amount of magnesium, [55] and there is increasing interest in investigating whether magnesium deficiency can be linked to medical conditions such as arrhythmia, asthma, migraine and eclampsia/pre-eclampsia and in the possible effectiveness of magnesium supplements.

Magnesium sulfate is shown to block convulsions associated with eclampsia. A multicenter, placebo controlled Magpie trial [13] found a greater than 50 % reduction in the rate of eclampsia in a group receiving magnesium sulfate. This effect was not found among those women from the western world, though the number of women in this group who were tested was small.

In many countries magnesium sulfate is used to treat severe PE, but there is little evidence of its benefits in mild pre-eclampsia. Magnesium sulphate does not affect maternal and perinatal mortality and morbidity if given maximally 24h before delivery, [137] though the Magpie trial showed that it increased pulmonary depression of the pregnant women. A number of studies have revealed negative effects of magnesium therapies on newborns: reduced bone density, increased troponin T in fetal blood, reduced fetal heart rate, reduced Apgar scores etc.[91]

### **The use of calcium**

The Cochrane review [16] of trials found that calcium supplementation during pregnancy is a safe and relatively cheap means of reducing the risk of high blood pressure in women at increased risk, and women from communities with low dietary calcium. No adverse effects were found, but further research is needed to find the ideal dosage for supplementation and to confirm the results, which stem from several rather small trials. A large multicenter trial performed between 1992 and 1995, CPEP, did not find a risk reduction in healthy nulliparous women [73].

The World Health Organization calcium supplementation trial among low-calcium-intake women [152] showed that calcium supplementation did not prevent PE, but it did reduce its severity and maternal and neonatal morbidity.

An ancillary study performed in parallel with the WHO trial concluded that calcium supplementation among low-intake Argentinean mothers resulted in lower uterine artery Doppler PI and RI when measured in 20-36 weeks of pregnancy.[32]

Nifedipine, a calcium blocker and blood pressure decreasing agent could potentially prevent pre-eclampsia.[119]

### **The use of folic acid**

Some studies have shown that supplementation with multivitamins containing folic acid might reduce the pre-eclampsia risk. A Canadian study reported a 63 % reduction in risk in those women who had started taking folic acid supplements before or early in pregnancy and continued until the 3<sup>rd</sup> trimester. Women used twice the recommended level (1 mg) for prevention of neural tube defects. However, the researchers were unable to claim a cause-and-effect relationship.[158]

### **Anticoagulant treatment**

Data from the literature indicates that angiotensin-converting enzyme (ACE) polymorphism affects the recurrence of PE. ACE is involved in key events of hemostasis and of inflammatory processes related to PE. Low-molecular-weight heparin (LMWH) has been reported to reduce the incidence of PE and IUGR and the severity of these pregnancy events in thrombophilic women. A recent study also showed that in women with a previous history of PE without thrombophilic factors, but with homozygote ACE D allele, LMWH administration reduced the adverse outcomes.[87] The ACE DD genotype was suggested as possibly identifying a thrombophilic condition. The favorable effects of LMWH have been hypothesized to be due to its anti-inflammatory activities and modulatory function on growth factors like epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF). There is also evidence for a direct positive effect of heparin and aspirin on the placenta. [26]. In a presentation at the 2009 FMF Congress in Slovenia, Hassam Shehata proposed that all pregnant women with BMI over 40 be administered LMWH during pregnancy.

### **Prevention of angiogenesis inhibition in placenta**

According to research from the National Institute of Child Health and Human Development of the National Institutes of Health it also might be possible to develop a treatment for pre-eclampsia, by supplying at risk women with additional placental growth factor (PlGF) and VEGF. Theoretically, these substances would bind to soluble fms-like tyrosine kinase 1 (sFlt-1), allowing the PlGF and VEGF made by the body to be used by the blood vessel cells that require them.[3]

Table 5. Large clinical trials for prevention of pre-eclampsia.

| Trial  | References                           | Study design   | Prophylaxis   | No of women |
|--|--------------------------------------|--|---|-------------|
| CLASP<br>(Collaborative Low-dose Aspirin Study in Pregnancy) | 38                                   | Randomized multi-center study  | Daily doses of 60 mg of aspirin between 12 and 32 weeks of gestation  | 9,367       |
| CPEP<br>(Calcium for Pre-eclampsia Prevention)               | 73, 74                               | Randomized, multi-center, double blind study performed in 1992-1995. | 2 g calcium daily. Supplementation given from 13-21 weeks until the end of pregnancy                          | 4,589       |
| Calcium supplementation study                                | 152                                  | Randomized, multicenter, study performed in 2001-2003                | 1.5 g calcium daily given before 20 weeks till the end of pregnancy   | 8,325       |
| MAGPIE<br>(Magpie Trial Group in 33 countries)               | 13<br>www.<br>magpietrial.<br>org.uk | Randomized, multicenter placebo controlled study                     | Timing of infusion and doses of magnesium supplementation varied  | 10,110      |
| VIP<br>(Vitamins in Pre-eclampsia)                           | 113                                  | Randomized placebo controlled study performed in 2003-2005           | 1000 mg vitamin C and 400 IU vitamin E daily starting from 16-21 weeks of gestation till the end of pregnancy | 2,410       |
| ACTS<br>(Australian collaborative Trial of Supplements)      | 127                                  | Randomized multi-center placebo controlled study in 2001-2005        | 1000 mg vitamin C and 400 IU vitamin E daily starting from 15-19 weeks of gestation till the end of pregnancy | 1,877       |

| Trial   | References | Study design  | Prophylaxis   | No of women |
|---|------------|---|---|-------------|
| WHO<br>(Vitamin supplements in populations of low nutritional status) | 153        | Randomized multi-center placebo controlled study in 2004-2006 | 1000 mg vitamin C and 200 IU vitamin daily starting from 14-22 weeks of gestation till the end of pregnancy | 1,365       |
| Oak Birth Cohort Study  | 158        | 2002-2005   | 1 mg folic acid before or at 12-20 weeks of gestation till the 3 <sup>rd</sup> trimester                    | 2,951       |
| DNBC<br>(The Danish National Birth Cohort)                            | 69         | 1996-2001   | Dietary and supplemental vitamin C and E at weeks 21-25 of gestation  | 57,346      |

### Antioxidant supplementation

Several groups have reported on the administration of vitamin C and vitamin E supplements to pregnant women in randomized, placebo controlled trials. The VIP trial concluded that high doses of antioxidants did not reduce the incidence of PE, but increased the rate of babies born with a low birth weight and thus these antioxidants would not be justified in pregnancy.[113] The ACTS Study Group came to the same conclusion regarding the ineffectiveness of the supplements to reduce the incidence of PE, but they did not see any detrimental effect on birth weights.[127] A multicenter WHO trial was done among low socio-economic and nutritional status populations following a research protocol used in the VIP trial. Conclusions were similar to those derived by the ACTS study group.[153]

Klemmensen et al. estimated vitamin C and E intake from a food frequency questionnaire, which was completed in gestational week 25 for both diet and supplements in the preceding 4 weeks. They found that there was a decreasing trend of severe PE/eclampsia/HELLP with increasing dietary vitamin C and a small increase in the incidence of severe disease with a high intake of vitamin E. Supplementary vitamin C showed a less clear protective effect. In general, vitamin levels were lower than in the randomized controlled trials listed in table 5.[69]

Rumiris et al. studied women with low dietary intake of vitamins who received daily supplements of antioxidants starting at 8-13 weeks of gestation. Supplementation was associated with better maternal (PE, abortion, hypertension) and perinatal (PTD, IUGR) outcomes.[128]

There is an on-going clinical trial (DAPIT study) to determine whether supplementation with vitamin C and vitamin E from early pregnancy will reduce the risk of developing PE in pregnant women with type 1 diabetes.

A large vitamin C and E trial (CAPPS study) among low risk women in USA has begun with an anticipated sample of 10,000 low-risk women.

## **Statins**

Statins, which have been recognized to be beneficial to cardiovascular patients, have recently been shown to inhibit sFlt-1 and sEng production in vitro and in another study to reduce blood pressure in a murine model of obesity during pregnancy.[46, 53] While statins are contraindicated during pregnancy these animal studies might lead to reinvestigation of their benefits in management of PE.

## **Other possibly preventive supplements**

### **Dark chocolate**

There is evidence that a high intake of certain flavonoids may be associated with a low risk of coronary heart disease and stroke. Cocoa flavonoids have been shown to have antioxidant effects, decrease LDL-cholesterol oxidation, reduce platelet aggregation and enhance endothelial function. Dark chocolate has been shown to induce nitric oxide mediated vasodilatation and to reduce insulin-resistance.[22, 59] Two recent studies have shown the association between increased consumption of chocolate and reduced likelihood of pre-eclampsia [129, 145] but Klebanoff et al. were unable to confirm this association.[68]

### **Coenzyme Q10**

An Equadorian research group has randomly assigned 235 pregnant women for Coenzyme Q10 supplements or placebo from 20 weeks of pregnancy until delivery and found that women who were given supplements had a lower rate of PE.[140]

### **Omega 3-fatty acids**

There is evidence that the required intake of omega 3-fatty acids does not meet requirements and that omega 3-fatty acids have a potential importance for pregnancy. Current data from randomized controlled trials are not sufficient to recommend supplementation. Nevertheless, several organizations have made recommendations for fish consumption or supplementation (e.g. Food Standard Agency (FSA) in UK, FDA/EPA in the USA and the European community). It should be noted that in most of the trials reported so far supplementation was started after 16 weeks of pregnancy.[63]

# Screening

Screening is performed to identify those apparently healthy members of a population who are at significant risk of a disease. Since large numbers of people are screened, the screening tests need to be inexpensive and easy to perform. Limited or expensive health care measures such as monitoring or possible preventive treatment may then be applied to those pregnancies found to be at high risk.

Unlike a diagnostic test, a screening test does not provide confirmation of the presence of a disorder, but rather, an indication of the risk of that disorder being present. Screening is of value because it focuses attention and resources on only those pregnancies where the additional attention will be the most beneficial.

The traditional method of screening for PE is based on maternal history. The likelihood of developing PE is increased in:

- Women of African rather than Caucasian ancestry
- Nulliparous vs. multiparous pregnancies
- Those with high BMI
- Those with a prior or family history of PE

Among women considered as high-risk by risk factors, approximately 25 % will go on to develop PE compared with 5 % in the general population.[2, 90, 97] The disadvantage of this approach is quite clear since 30-40 % of all pregnancies are first pregnancies. Not only is the knowledge of the pregnancy history lacking, but also first pregnancies have a 1.5-3 times higher risk of developing PE compared to subsequent pregnancies. It is not practical to consider all first pregnancies as high-risk. The problem of identifying which of the first time pregnancies are at risk is another reason why good tools to predict the risk of developing PE are needed. Various markers and marker combinations have been proposed and estimates of their performance in screening for early-onset pre-eclampsia are presented in Table 6.

## Uterine artery Doppler

In the past, the best way to estimate the risk of pre-eclampsia was to consider only the maternal history. Unfortunately, this is a fairly unreliable method. This is especially true in primigravid women, the very population where the incidence of pre-eclampsia is the highest. Since the development of pre-eclampsia is thought to include abnormal placentation and its vascular supply, it logically follows that evaluation of the uterine artery blood flow resistance in pregnancy may be helpful in establishing this risk. Some success in predicting preeclampsia was achieved by measuring uterine artery blood flow in the second trimester. Studies on screening using this marker at 20-24 weeks of gestation have reported that the detection rate of pregnancies that subsequently develop early-onset PE is 80-90 %, with a false positive rate of 30-35 %.[100]

Table 6. Estimates of performance in screening for early-onset pre-eclampsia using various markers and marker combinations.

| DR% at 5% FPR | History | MAP | uA Doppler | PAPP-A | PIGF | Reference          |
|---------------|---------|-----|------------|--------|------|--------------------|
| 20            |         |     |            | x      |      | Foidart 2010 [54]  |
| 22            |         |     |            | x      |      | Poon 2009 [106]    |
| 23            |         |     |            | x      |      | Akolekar 2009 [8]  |
| 24            |         |     |            | x      |      | Akolekar 2008 [11] |
| 26            |         |     |            |        | x    | Foidart 2010 [54]  |
| 28            |         |     |            |        | x    | Akolekar 2008 [11] |
| 30            | x       |     |            |        |      | Foidart 2010 [54]  |
| 32            | x       |     |            |        |      | Poon 2009 [106]    |
| 33            | x       |     |            |        |      | Yu 2005 [162]      |
| 33            | x       |     |            |        |      | Akolekar 2011 [9]  |
| 37            | x       |     |            |        |      | Poon 2009 [108]    |
| 37            | x       |     |            |        |      | Poon 2010 [107]    |
| 38            |         |     | x          |        |      | Akolekar 2008 [11] |
| 38            |         |     | x          |        |      | Poon 2009 [106]    |
| 39            | x       |     |            |        |      | Akolekar 2008 [11] |
| 40            | x       |     |            |        |      | Poon 2010 [112]    |
| 41            | x       |     |            | x      |      | Poon 2009 [106]    |
| 45            | x       |     |            | x      |      | Poon 2010 [107]    |
| 47            | x       |     |            | x      |      | Foidart 2010 [54]  |
| 47            | x       |     |            | x      |      | Akolekar 2011 [9]  |
| 47            |         |     | x          | x      |      | Poon 2009 [106]    |
| 48            | x       |     |            |        |      | Akolekar 2009 [8]  |
| 48            | x       |     |            |        | x    | Foidart 2010 [54]  |
| 49            | x       |     |            |        |      | Poon 2010 [107]    |
| 50            |         |     | x          |        |      | Akolekar 2009 [8]  |
| 52            | x       |     |            | x      |      | Akolekar 2008 [11] |

| DR% at 5% FPR | History | MAP | uA Doppler | PAPP-A | PIGF | Reference          |
|---------------|---------|-----|------------|--------|------|--------------------|
| 54            | x       |     |            |        | x    | Akolekar 2011 [9]  |
| 55            | x       |     |            |        | x    | Akolekar 2008 [11] |
| 60            | x       |     | x          | x      |      | Foidart 2010 [54]  |
| 60            |         |     | x          |        |      | Foidart 2010 [54]  |
| 62            | x       |     | x          | x      |      | Akolekar 2009 [8]  |
| 63            | x       |     | x          | x      |      | Poon 2009 [106]    |
| 63            | x       |     | x          |        |      | Foidart 2010 [54]  |
| 65            | x       |     | x          |        |      | Akolekar 2009 [8]  |
| 65            | x       |     | x          |        |      | Poon 2010 [107]    |
| 65            | x       | x   |            | x      |      | Poon 2010 [107]    |
| 66            | x       |     | x          |        |      | Poon 2009 [106]    |
| 67            | x       | x   | x          |        |      | Akolekar 2011 [9]  |
| 68            | x       |     | x          | x      |      | Poon 2010 [107]    |
| 69            | x       |     | x          |        |      | Akolekar 2008 [11] |
| 69            | x       |     | x          | x      |      | Akolekar 2008 [11] |
| 69            | x       |     |            | x      |      | Akolekar 2009 [8]  |
| 76            | x       |     | x          |        | x    | Akolekar 2008 [11] |
| 76            | x       |     | x          | x      | x    | Akolekar 2008 [11] |
| 78            | x       |     | x          |        | x    | Foidart 2010 [54]  |
| 78            | x       | x   | x          |        |      | Poon 2010 [112]    |
| 78            | x       | x   | x          |        |      | Poon 2009 [111]    |
| 78            | x       | x   | x          |        |      | Poon 2010 [107]    |
| 78            | x       | x   | x          | x      | x    | Akolekar 2011 [9]  |
| 84            | x       | x   | x          | x      |      | Poon 2010 [107]    |
| 93            | x       | x   | x          | x      | x    | Poon 2009 [109]    |

Moving screening for pre-eclampsia to the first trimester appears to improve the detection rate. Furthermore, since this is done at a time when the process of placentation is less advanced, the chance of any future preventative steps succeeding is increased.

The Fetal Medicine Foundation has evaluated the utility of combining maternal history, uterine artery pulsatility index (UAPI), maternal mean arterial pressure (MAP), maternal serum pregnancy-associated plasma protein-A levels (PAPP-A), and maternal serum placental growth factor levels (PIGF) in screening for pre-eclampsia in the first trimester.[39, 107, 108, 110] The specific maternal factors that appear to play the most significant role in adjusting the risk of pre-eclampsia are maternal BMI, age, ethnicity, smoking and parity. In a study, which included 7,797 patients, the combination of these parameters predicted early severe pre-eclampsia in 93 % cases, late pre-eclampsia in 36 % of the cases, and 18 % of the cases of gestational hypertension with a 5 % false positive rate.

In comparison, using maternal history alone predicts only 30 % of early severe pre-eclampsia and 20 % of late pre-eclampsia for a 5 % false positive rate.

### Measuring uterine artery PI using Doppler at 11-13+6 weeks' gestation

Identification of the uterine arteries begins by obtaining a sagittal view of the lower uterine segment and the cervix. The cervical canal is visualized and the endocervix

is identified at the junction of the canal and the lower uterine segment. The uterine artery is generally found in the paracervical tissue at the level of the endocervix. Therefore, the transducer is directed to this region and the uterine artery may be found there with the aid of color Doppler.

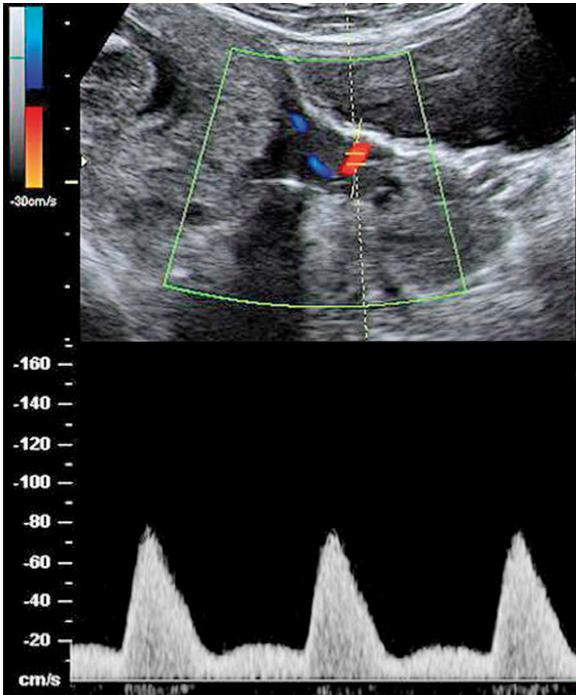


Figure 4. Color Doppler of uterine arteries (upper) and uterine artery waveform (lower) obtained using the conditions described in the text. Images courtesy of Cathy Downing, Fetal Medicine Foundation, USA.

In order to obtain the best and reproducible UAPI measurements, the Doppler gate should be set at 2 mm and the angle of insonation should be less than 30° with respect to the longitudinal axis of the uterine artery.

The image should be significantly magnified. This aids in the positive identification of the uterine artery and allows the operator to place the Doppler gate within its lumen accurately. At least three waveforms that are similar in shape should be obtained. The PI should be measured in both uterine arteries and the lowest PI value is used for risk assessment.

An interesting approach has been to study the possibility of using Doppler ultrasound in a contingency screening strategy. In a prospective screening study for PE in 3107 singleton pregnancies, Plasencia et al measured UAPI values at 11 + 0 to 13 + 6 weeks, and then again at 21 + 0 to 24 + 6 weeks. They found that UAPI declines more rapidly in unaffected pregnancies than in those developing PE [104] (Table 7).

If contingency screening were to be applied, women participating in normal first trimester screening could also have uterine artery blood flow measurement at that time. In the pregnancies with the highest risk, the Doppler assessment would be repeated in the second trimester.[104]

Table 7. Proportion of those pregnancies in which UAPI was above 90 % centile at 11 + 0 to 13 + 6 weeks where it was also above the 90 % centile at 21 + 0 to 24 + 6 weeks. Early PE here means delivery before 34 weeks gestation. Late PE means delivery at or after 34 weeks gestation. Data abstracted from reference 104.

|  | Early PE | Late PE | No PE |
|--|----------|---------|-------|
| n  | 22       | 71      | 3014  |
| $\frac{21^{+0} \text{ to } 24^{+6} \text{ weeks UAPI above 90 \% centile}}{11^{+0} \text{ to } 13^{+6} \text{ weeks UAPI above 90 \% centile}} \times 100$ | 94%      | 74%     | 37%   |

It has recently been suggested that there are differences in maternal cardiac function and uterine artery resistance between pregnancies with normal outcome, pre-eclampsia without SGA (small for gestational age), pre-eclampsia with SGA and those with SGA without pre-eclampsia.[67] In this study, it was found that in an apparently healthy, low-risk nulliparous population, heterogeneity between the groups was evident by 11–14 weeks gestation, well before the onset of clinical disease (Table 8).

Table 8. Where PE is accompanied with SGA, and also in pregnancies with SGA without PE, 1st trimester UAPI has been found to be raised compared to uncomplicated pregnancies. [67]

|  | PE without SGA | PE with SGA | SGA without PE |
|--|----------------|-------------|----------------|
| UAPI compared to uncomplicated pregnancy | Similar        | Raised      | Raised         |

## Mean arterial pressure

Accurate measurement of blood pressure in pregnant women is particularly important when attempting to identify early signs of pre-eclampsia. As a means of prediction it has been suggested that the mean arterial pressure (MAP), whether measured in the first or second trimester, is better than systolic blood pressure, diastolic blood pressure, or an increase of blood pressure.[40]

In clinical practice MAP measurement in the first trimester may not make a clinical impact in isolation but could be suitable for use with other markers, including maternal serum markers, to improve the accuracy for estimating risk of pre-eclampsia. Already it has been shown following a large prospective study that maternal variables such as ethnic origin, body mass index, and personal history of PE, combined with MAP at 11+0 to 13+6 weeks is able to identify a group at high risk for pre-eclampsia.[105]

### How MAP is measured

The Fetal Medicine Foundation recommends the following protocol for the measurement of blood pressure (see also the FMF's automatic calculator on <https://courses.fetalmedicine.com/calculator/map?locale=en>). The measurement should be made when the gestational age is between 11 and 13+6 weeks and when the crown rump length is between 45 and 84 mm.

Figure 5. Measurement of mean arterial pressure (MAP).



- An automated device should be used and this should be calibrated at regular intervals.
- The woman should be in a seated position with her arm supported at the level of the heart.
- A small (<22 cm), normal (22-32 cm) or large (33-42) adult cuff should be used depending on the mid-arm circumference.
- After rest for 5 minutes the blood pressure (BP) should be measured in both arms simultaneously and a series of recordings made at 1-minute intervals until variations between consecutive readings fall within 10 mmHg systolic and 6 mmHg in diastolic pressure (DBP) in both arms.
- The mean arterial pressure MAP should be calculated for each arm as the average of the last two measurements. The arm with the highest final MAP should be considered in assessment of risk.

## **Biomarkers**

Since early-onset PE cases account for by far the greater part of the total PE health care costs, a major need is the revelation of just these early-onset cases. Intensive efforts are being directed towards identifying new non-invasive biomarkers that allow both early and accurate prediction of PE. Risk detection within the first 12 weeks of pregnancy leaves a maximum of 28 weeks for tailoring prevention strategies.

With the advent of a suitable marker or markers, a screening approach may be envisaged in which the prior risk is modified by likelihood ratios derived from the frequency distribution of one or more biochemical markers.

Efficient screening to define high risk pregnancies for PE would not only help to plan the pregnancy monitoring of a high risk pregnancy and to avoid unnecessary healthcare visits of a low risk pregnancy, but also would help in designing more efficient clinical trials for prevention of PE.

### **An ideal biomarker for PE should:**

- Be easily measured and easily integrated with routine testing already performed as part of prenatal testing
- Delineate the risk of developing PE in the 1st trimester, thus creating a wide window of opportunity to implement treatment strategies, which may facilitate normal placental development. This needs to occur at an early stage when the placenta is capable of responding to corrective or moderating medications
- Ideally, the biomarker might also serve as a plausible mediator of the underlying pathogenesis (which is currently incompletely understood).

Several possible markers of PE have been proposed. Some may have certain value as late markers after week 24, while other markers show real potential as early markers.

The most promising candidates for markers are those specific molecules produced by the placenta in association with the pathological processes that lead to PE.

### **VEGF, sFlt-1, sVEGFR-2, sEng and PlGF**

Alteration in circulating angiogenic factors have been implicated in the pathogenesis of PE. VEGF, an endothelial cell-specific growth factor, and PlGF promote angiogenesis, acting through the tyrosine kinase receptors, VEGF receptor 1 (VEGFR-1) and VEGF receptor 2 (VEGFR-2). sFlt-1, which is a splice variant of VEGFR-1, binds to circulating VEGF and PlGF and prevents their interaction with endothelial cell receptors; especially inhibiting VEGF stimulation of the nitric oxide system through VEGFR-2.[149] sFlt-1 has been shown to be elevated in pregnant women with PE. Levine et al.[76] have reported elevated levels in maternal serum five weeks before clinical symptoms appeared. Increased levels of sFlt-1 are accompanied associated by decreased levels of free VEGF and PlGF.

Recently sVEGFR-2 has been shown to be decreased in maternal plasma in pre-eclamptic pregnancies which contradicts with the view that recombinant sVEGFR-2 has anti-angiogenic activity [36] The mean plasma concentration of sVEGFR-2 is about 10 times higher than that of sFlt-1 in maternal serum, but it has lower affinity to VEGF in normal pregnancies.

PlGF is expressed at high levels by trophoblast cells in the placenta. Low levels of free PlGF have been reported in the 1<sup>st</sup> and 2<sup>nd</sup> trimester in women developing pre-eclampsia.[11, 109, 124, 141, 142, 147] Vatten et al. suggest that low increase in PlGF concentration in early pregnancy independent of change in sFlt-1 is associated with highly increased risk for preterm pre-eclampsia.[147] Also, lower urinary level of PlGF has been reported at pregnancy weeks 25-28 following later development of PE.

PlGF has also been reported to have value in screening for trisomy 21 and other major chromosomal abnormalities.[45, 162]

Soluble endoglin (sEng) is another anti-angiogenic factor that has been implicated in pathogenesis of PE showing higher plasma sEng levels after pregnancy week 23-26 or approximately 8 weeks before the onset of clinical preterm pre-eclampsia. TGF- $\beta$  interacts with endoglin receptor, which has been shown to be upregulated in the placenta in pre-eclampsia leading to increased secretion of the soluble form into maternal circulation. Placenta specific sEng appears to play a role in trophoblast invasion and differentiation [75, 124, 149]. Rana et al. suggested that changes in sFlt-1 and sEng from first to second trimesters may be potentially useful for screening patients at high risk for subsequent development of preterm pre-eclampsia.[118]

At present it is unclear which factors trigger the excessive upregulation of placental sFlt-1 production. One proposed model has been hypoxic condition in pre-eclamptic placenta causing endothelial dysfunction and impaired angiogenesis. However, Savvidou et al. suggest that there is no direct causal effect between decreased level of second trimester maternal serum PlGF, increased level of sEng and the endothelial dysfunction assessed by flow-mediated dilation of the brachial artery.[132]

There are supportive findings that fetuses of pre-eclamptic mothers do not have high circulating concentrations of sEng nor sFlt1, which is said to be consistent with the idea that fetuses do not experience proteinuria or hypertension like the mothers because they are not exposed to high concentrations of antiangiogenic factors.[139]

### **IGF-1 and IGFBP-1**

Insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 1 (IGFBP-1) are modulators for placental growth. Reduced levels of IGF-1 and IGFBP-1 during the late 1st trimester have been reported.[101] Also, an IGF-1 increase from the first to second trimester has been reported to be associated with preterm PE while low levels of IGFBP-1 is associated with term PE.[148]

### **Ang-1, Ang-2 and Tie-2**

Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) are both ligands for tyrosine kinase endothelial cell receptor, Tie-2. Ang-1 causes endothelial maturation and vascular stabilization and Ang-2 acts as an antagonist of Ang-1 leading to angiogenesis. An anti-angiogenic state has been described in patients with pre-eclampsia, SGA fetuses and fetal death. Changes in the concentration of circulating angiogenic and anti-angiogenic factors can precede the clinical recognition of pre-eclampsia and SGA by several weeks.

Lower plasma concentrations of soluble Tie-2 has been reported in 3<sup>rd</sup> trimester in patients with pre-eclampsia and SGA.[58] Increased plasma levels of Ang-1 and Tie-2 and reduced levels of Ang-2 were reported in pregnancies suffering from PIH. Ang-2 serum levels were also reported to be decreased in established PE.[95] However, Akolekar et al (2009) did not find significantly different serum Ang-2 levels during the 1st trimester when considering pregnancies developing PE later in pregnancy compared to normal pregnancies.[5]

### **sP-Selectin**

Selectins are adhesion molecules regulating interactions of the leucocytes, platelets and endothelial cells, thus they are essential components of the immune response. P-selectin also has specific roles in the inflammatory and haemostatic functions of the platelets. P-selectin acts as a receptor that supports binding of leucocytes to activated platelets and endothelium. Recently, circulating forms of

selectins have been described and soluble L and P selectin may inhibit leukocyte adhesion to cytokine stimulated endothelium. The soluble form of P-selectin (sP-selectin) might represent a proteolytic fragment or a soluble splice variant of 140 kDa P-selectin, lacking the transmembrane domain.

Endothelial injury and neutrophil activation is common to pathophysiology of pre-eclampsia (transmigration of neutrophils through the endothelium).

In several studies it has been shown that sP-selectin concentration is higher in pre-eclampsia compared to normal pregnancy. Acar et al. (2001) suggested that elevated selectin levels are a non-specific consequence of endothelial injury which is common to all pathologies like coronary heart disease, rheumatoid arthritis rather than being a cause. They measured sP-selectin from citrate plasma samples after 20 weeks of gestation.[1]

Bosio et al. (2001) stated that citrate plasma P-selectin has potential as a 1st trimester clinical marker of pre-eclampsia. They performed longitudinal study of 400 pregnancies at 10-14, 20-24, 28-32, 34-36 and 37 weeks of gestation and 6-8 weeks postpartum. 20 PE cases and 24 GH cases were analyzed. Mean concentration of sP-selectin of women who later developed pre-eclampsia was higher than in normotensive pregnancy. No differences in plasma levels were found between GH and normotensive groups in the 1st trimester.[27]

Banzola et al. (2007) and Akolekar et al (2010) also found higher sP-selectin plasma levels at 1st trimester in those pregnancies developing PE later on.[10, 19] The latter group concluded that in pregnancies that develop pre-eclampsia there is evidence of platelet activation from the first trimester, but there is no direct link between the degree of impaired placentation and platelet activation

### **PTX-3**

PTX-3 is a recently described inflammatory molecule that belongs to the same family as C-reactive protein. PTX is expressed by different cells such as endothelial cells, monocytes, macrophages and fibroblasts exposed to inflammatory processes. It binds to angiogenic growth factor (fibroblast growth factor 2). PTX3 plasma levels are increased in vascular disorders.

Cetin et al. (2006) studied 20 pregnancies complicated by pre-eclampsia and 16 pregnancies with IUGR at weeks 9-11, 17-23 and 29-36. Higher values were found with pregnancies complicated with PE. In pregnancies suffering from IUGR there was no significant increase. Only 3<sup>rd</sup> trimester values were reported: 13.8 ng/mL in PE cases vs. 2.2 ng/mL in normal pregnancies.[35]

Rovere-Querini et al. (2006) published a paper where they studied women with preeclampsia (n =30), women with uncomplicated pregnancies (n =66), age-matched healthy women (n= 50), women who developed acute bacterial infections (n= 20), and women with rheumatoid arthritis (n =20).

Samples were collected at weeks 33-34. Serum levels of PTX3 were higher in pre-eclampsia compared with normal control pregnancies ( $5.08 \pm 1.34$  and  $0.59 \pm 0.07$  ng/mL, respectively,  $P < .001$ ).[125]

Akolekar et al. reported plasma PTX3 values significantly higher in the early-onset PE group (1.44 MoM;  $p < 0.0083$ ) but not in late-onset PE (1.11 MoM) or GH (1.10 MoM) compared to the controls (0.97 MoM) at pregnancy weeks 11-13.[4]

### **Inhibin A and Activin A**

Inhibins ( $\alpha$ - $\beta$  dimers) and activins ( $\beta$ - $\beta$  dimers) are glycoprotein hormones belonging to the transforming growth factor  $\beta$  superfamily. These proteins are mostly produced in placenta, deciduas and fetal membranes. During pregnancy, inhibin A levels in maternal serum are considerably higher than in serum of non-pregnant women. Maternal serum levels of inhibin A increase during the first trimester and decline after about 12 weeks. Levels remain stable at 15 to 25 weeks and then increase, reaching peak at term.[94] The fetoplacental unit appears to be the major source of increased circulating concentrations of inhibin A and activin A in early pregnancy.[93] Although the function of inhibin A in pregnancy is unknown, inhibin A may be involved in fetal and placental development.

Activin A is present in serum free and bound to follistatin. Normally total activin A is measured after disrupting the complex. Circulating levels of activin A are the same during the first and second trimester of pregnancy, and rise in the 3<sup>rd</sup> trimester with a high increase at term.

Elevated levels plasma and serum of inhibin A and activin A have been reported during the first trimester in pregnancies which subsequently development of pre-eclampsia.[7, 8, 138]

### **Adhesion molecules**

Results for adhesion molecules like E-selectin, VCAM-1 and ICAM-1 during the 2<sup>nd</sup> trimester have been conflicting and abnormal levels later in pregnancy can be considered to be a secondary effect of PE rather than the cause for it.

### **Total fibronectin**

Total fibronectin has been reported to have potential value in predicting PE among high risk pregnancies, but also at a relatively late stage of pregnancy.

Its positive predictive value among the general population has not been shown to be useful in the prediction of PE.

### **Other endothelial cell associated substances**

Other endothelial cell associated molecules like plasminogen activator, plasminogen activator inhibitor-1 and 2 (PAI-1 and PAI-2) and von Willebrand Factor, nitric oxide (NO) production and nitric oxide synthase inhibitor, and asymmetric

dimethylarginine (ADMA) have been studied as suitable markers for functional status of the uterine and systemic vasculature.[6, 56]

### **Thrombophilic factors**

Factor V Leiden does not seem to be a risk factor for PE according to a recent study.[61]

Other factors including Protein S and Protein C have shown conflicting results.

### **SHBG**

Insulin resistance is implicated in the pathogenesis of PE. Increased early pregnancy insulin resistance, marked by reduced first trimester SHBG levels, has been reported to be associated with increased risk of subsequent PE. Also lower second trimester values of SHBG have been reported in PE pregnancies compared to normal cases.[159, 161]

### **Neurokinin B**

Neurokinin B has been reported to be increased in PE pregnancies already in weeks 9-13 of gestation.[102]

### **Cell-free circulating fetal DNA**

Increased levels of cell-free circulating fetal DNA in maternal plasma might be indicative for PE. It has been suggested that oxidative stress in the placenta increases apoptosis and necrosis of trophoblast cells which leads to increased levels of cell-free DNA into maternal circulation. Also, circulating fetal RNA has been reported to be in maternal plasma.[115]

### **SERPINA 1**

SERPINA1 is an abundant plasma protein with antiproteolytic activity and is synthesized by liver, macrophages, neutrophils and trophoblasts. Increases in serum values occur in rheumatoid arthritis, vasculitis and infections. Studies have shown that increased levels of serum SERPINA1 are associated with the development of arterial hypertension and an increased risk for cardiovascular disease. Recent proteomic experiments showed that women with severe PE have higher serum and urine immunoreactivity and it is suggested that in the future, the pattern of SERPINA1 fragmentation present in urine may be used for differentiating PE from other pre-existing hypertensive proteinuric disorders during gestation by using mass spectrometry.[28]

### **PP13**

PP13 (placental protein 13) is a potential marker for early detection of PE in low risk groups. It is a homo-dimer of 16-18 kDa subunits linked by disulphide bonds, and was first isolated from human placenta and characterized in 1983.[25]

Due to its homology with members of the galectin family, the protein has been designated galectin-13. There is a single conserved carbohydrate recognition domain and a strong binding affinity to sugar residues widely expressed in the placenta.

PP13 exhibits weak lysophospholipase activity associated with calcium mobilization, while free fatty acid liberation and elevation of prostaglandins suggested a possible role in regulating vasoconstriction/vasodilatation balance and maternal artery remodeling.[14]

It has been proposed that the unique dimerization via disulphide bonds might affect the activity of PP13 upon the oxygenization changes in the placenta.

As other galectins are involved in immunobiological functions, PP13 and its placental homologues might also have immune functions at feto-maternal interfaces.[143]

Low serum levels of PP13 have been reported during the 1st trimester of pregnancy in women developing pre-eclampsia.[44, 123]

### **Cystatin C**

Some nested case-control studies have suggested that Cystatin C could be a potential markers in mid-gestation in combination with other markers.[144]

### **25-hydroxyvitamin D**

Vitamin D deficiency has been linked to adverse pregnancy outcomes. Lower 25-hydroxyvitamin levels were found in women with early-onset PE.[122]

### **Neopterin**

Maternal serum neopterin concentrations have been measured to be higher in women developing PE than women with normal pregnancy.[33]

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# Abbreviations

|                |  |
|----------------|--|
| <b>ACE</b>     | Angiotensin-converting enzyme                                    |
| <b>ACOG</b>    | American College of Obstetrics and Gynecology                    |
| <b>ACTS</b>    | Australian Collaborative Trial of Supplements                    |
| <b>ADMA</b>    | Asymmetric dimethylarginine                                      |
| <b>AEDF</b>    | Absent End-Diastolic Flow  |
| <b>AIPE</b>    | Associazione Italiana Preeclampsia                               |
| <b>ALT</b>     | Alanine aminotransferase   |
| <b>Ang</b>     | Angiopoietin   |
| <b>ASA</b>     | Acetylsalicylic acid (aspirin)                                   |
| <b>ASH</b>     | American Society of Hypertension                                 |
| <b>AST</b>     | Aspartate aminotransferase                                       |
| <b>BMI</b>     | Body mass index  |
| <b>CAPPS</b>   | Combined Antioxidant and Preeclampsia Prediction Studies         |
| <b>CBC</b>     | Complete blood count   |
| <b>CBD</b>     | Carbohydrate binding domain                                      |
| <b>CLASP</b>   | Collaborative Low-dose Aspirin Study in Pregnancy                |
| <b>CNGOF</b>   | Collège national des gynécologues et obstétriciens français      |
| <b>CPEP</b>    | Calcium for Preeclampsia Prevention (study group)                |
| <b>CTG</b>     | Cardiotocograph  |
| <b>DAPIT</b>   | Diabetes and Pre-eclampsia Intervention Trial                    |
| <b>DGGG</b>    | Deutsche Gesellschaft für Gynäkologie und Geburtshilfe           |
| <b>DBP</b>     | Diastolic blood pressure   |
| <b>DNBC</b>    | Danish National Birth Cohort                                     |
| <b>EGF</b>     | Epidermal growth factor  |
| <b>FMF</b>     | Fetal Medicine Foundation  |
| <b>FSA</b>     | Food Standard Agency   |
| <b>GH</b>      | Gestational hypertension   |
| <b>HELLP</b>   | Hemolysis, elevated liver enzymes and low platelet count         |
| <b>ICAM-1</b>  | Intercellular adhesion molecule-1                                |
| <b>IGF-1</b>   | Insulin-like growth factor 1                                     |
| <b>IGFBP-1</b> | Insulin-like growth factor binding protein 1                     |
| <b>IL</b>      | Interleukin  |
| <b>INR</b>     | International normalized ratio                                   |
| <b>ISSHP</b>   | International Society for the Study of Hypertension in Pregnancy |
| <b>IUGR</b>    | Intrauterine growth restriction                                  |
| <b>LDH</b>     | Lactate dehydrogenase  |

|                                |  |
|--------------------------------|--|
| <b>LMWH</b>                    | Low-molecular-weight heparin                                 |
| <b>MAGPIE</b>                  | Magnesium Sulphate for Prevention of Eclampsia (study group) |
| <b>MAP</b>                     | Mean arterial pressure                                       |
| <b>MoM</b>                     | Multiple of median   |
| <b>NICE</b>                    | UK National Institute for Clinical Excellence                |
| <b>NNT</b>                     | Number needed to test  |
| <b>NO</b>                      | Nitric oxide   |
| <b>OR</b>                      | Odds ratio   |
| <b>PAI-1</b>                   | Plasminogen Activator Inhibitor-1                            |
| <b>PAPP-A</b>                  | Pregnancy-associated plasma protein A                        |
| <b>PIH</b>                     | Pregnancy induced hypertension                               |
| <b>PIGF</b>                    | Placental growth factor                                      |
| <b>PP13</b>                    | Placental protein 13   |
| <b>PTD</b>                     | Preterm delivery   |
| <b>RR</b>                      | Relative risk  |
| <b>RCT</b>                     | Randomized controlled trial                                  |
| <b>SBP</b>                     | Systolic blood pressure                                      |
| <b>sEng</b>                    | Soluble endoglin   |
| <b>SFAR</b>                    | Société française d'anesthésie et de réanimation             |
| <b>sFit-1</b>                  | Soluble fms-like tyrosine kinase 1                           |
| <b>SFMP</b>                    | Société française de médecine périnatale                     |
| <b>SFNN</b>                    | Société française de néonatalogie                            |
| <b>SGA</b>                     | Small for gestational age                                    |
| <b>SHBG</b>                    | Sex hormone binding globulin                                 |
| <b>SOCCG</b>                   | Society of Obstetricians and Gynaecologists of Canada        |
| <b>STBM</b>                    | Syncytiotrophoblast microvillous membrane                    |
| <b>TGF-<math>\beta</math></b>  | Transforming growth factor-beta                              |
| <b>Tie-2</b>                   | Tyrosine kinase endothelial cell receptor 2                  |
| <b>TNF-<math>\alpha</math></b> | Tumor necrosis factor-alpha                                  |
| <b>TPO-Ab</b>                  | Thyroid peroxide antibody                                    |
| <b>TSH</b>                     | Thyroid stimulating hormone                                  |
| <b>UAPI</b>                    | Uterine artery pulsativity index                             |
| <b>VEGF</b>                    | Vascular endothelial growth factor                           |
| <b>VCAM-1</b>                  | Vascular cell adhesion molecule-1                            |
| <b>VEGFR</b>                   | Vascular endothelial growth factor receptor                  |
| <b>VIP</b>                     | Vitamins in Preeclampsia (study group)                       |
| <b>WHO</b>                     | World Health Organization                                    |



## References

1. Acar A, Altinbaş A, Oztürk M, Koşar A, Kirazlı S. (2001): Selectins in normal pregnancy, pre-eclampsia and missed abortus. *Haematologia* 31(1): 33-38.
2. ACOG practice bulletin (2002): Diagnosis and management of preeclampsia and eclampsia. *Obstet Gynecol* 99(l): 159-67.
3. Ahmad S, Ahmed A. (2004): Elevated Placental Soluble Vascular Endothelial Growth Factor Receptor-1 Inhibits Angiogenesis in Preeclampsia. *Circ Res* 95: 884 - 891.
4. Akolekar R, Casagrandi D, Livanos P, Tetteh A, Nicolaides KH. (2009): Maternal plasma pentraxin 3 (PTX3) at 11 to 13 weeks of gestation in hypertensive disorders of pregnancy. *Prenat Diagn* 29(10): 934-938.
5. Akolekar R, Casagrandi D, Skufta E, Ahmed AA, Nicolaides KH. (2009): Maternal serum anti-opietin-2 at 11 to 13 weeks of gestation in hypertensive disorders of pregnancy. *Prenat Diagn* 29(9): 847-851.
6. Akolekar R, De Jesus Cruz J, Perez Penco JM, Y Zhou, Nicolaides KH. (2010): Maternal Plasma Plasminogen Activator Inhibitor-2 at 11 to 13 Weeks of Gestation in Hypertensive Disorders of Pregnancy. *Hypertension in Pregnancy, Early Online*:1-10, 2010d.
7. Akolekar R, Etchegaray A, Zhou Y, Maiz N, Nicolaides KH. (2009): Maternal Serum Activin A at 11-13 Weeks of Gestation in Hypertensive Disorders of Pregnancy. *Fetal Diagn Ther* 25: 320-327.
8. Akolekar R, Minekawa R, Veduta A, Romero XC, Nicolaides KH. (2009): Maternal plasma inhibin A at 11-13 weeks of gestation in hypertensive disorders of pregnancy. *Prenat Diagn* 29: 753-760.
9. Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. (2011): Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. *Prenat Diagn* 31: 66-74.
10. Akolekar R, Veduta A, Minekawa R, Chelemen T, Nicolaides KH. (2010): Maternal Plasma P-selectin at 11 to 13 Weeks of Gestation in Hypertensive Disorders of Pregnancy. *Hypertens Pregnancy* Mar 8. [Epub ahead of print]
11. Akolekar R, Zaragosa E, Poon LCY, Pepes S, Nicolaides KH. (2008): Maternal serum placental growth factor at 11+0 to 13+6 weeks of gestation in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 32: 732-739.
12. Allen VM, Wilson RD, Cheung A (2006): Pregnancy outcomes after assisted reproductive technology. *J Obstet Gynaecol Can* 28: 220-250.
13. Altman D, Carroli G, Duley L, Farrell B, Moodley J, Neilson J, Smith D. Magpie Trial Collaboration Group (2002): Do women with pre-eclampsia, and their babies, benefit from magnesium sulphate? The Magpie Trial: a randomised placebo-controlled trial. *Lancet* 359(9321): 1877-90.
14. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA. (2007): Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. *The Lancet* 369:1791-98.
15. Associazione Italiana Preeclampsia (AIPE), Italian branch of ISSHP. (2007): Linee guida per il management preeclampsia dell'ipertensione in gravidanza.

16. Atallah AN, Hofmeyr GJ, Duley L. (2006): Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. *The Cochrane Database of Systematic Reviews Issue 1*. John Wiley & Sons Ltd.
17. Baker P, Black R, Blicowce J, Cooper C, de Swiet M, Fletcher G, Jokinen M, Milne F, Moran PA, et al. (2006): A new evidence-based guideline for the out-patient assessment and management of women with new hypertension or proteinuria to differentiate those at risk of worsening disease. *Hypertension in Pregnancy 25: Plenary Session Oral Communications p. 61*.
18. Baker P. (2004): *Pre-eclampsia - Current Perspectives on Management*, John Kingdom (Ed.) Taylor & Francis.
19. Banzola I, Farina A, Concu M, Sekzawa A, Purwosunu Y, Strada I, Arcelli D, Simonazzi G, Caramelli E, Rizzo N. (2007): Performance of a panel of maternal serum markers in predicting preeclampsia at 11–15 weeks' gestation. *Prenatal Diagn 27(11): 1005-1010*.
20. Barker DJ. (1997) The long-term outcome of retarded fetal growth. *Clin Obstet Gynecol 40(4): 853-63*.
21. Baschat A, Poon L, Adams I, Blitzer M, Nicolaidis K, Harman C. (2008): Impact of first trimester aspirin therapy on the population prevalence of pre-eclampsia. *Am J Obstet Gynecol, Supplement S79*.
22. Bayard V, Chamorro F, Motta J, Hollenberg NK. (2007): Does Flavanol Intake Influence Mortality from Nitric Oxide-Dependent Processes? Ischemic Heart Disease, Stroke, Diabetes Mellitus, and Cancer in Panama. *Int J Med Sci 4: 53-58*.
23. Bele JO, Mykletun A, Daltvik AK, Rasmussen S, Dahl AA. (2006): Outcomes in adulthood for children with foetal growth retardation. A linkage study from the Nord-Trondelag health study (HUNT) at the medical birth registry of Norway. *Acta Psychiatr Scand 113: 501-509*.
24. Bellamy L, Casas JP, Hingorani AD, Williams DJ. (2007): Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ 335: 974-86*.
25. Bohn NG, Krause W, Winckler W. (1983): Purification and characterization of two new soluble placental tissue proteins (PP13 and PP17). *Oncodev Biol Med 4: 343-350*.
26. Bose P, Black S, Kadyrov M, Weissenborn U, Neulen J, Regan L, Huppertz B. (2005): Heparin and aspirin attenuate placental apoptosis in vitro: Implications for early pregnancy failure. *Am J Obstet Gynecol 192(1): 23-30*.
27. Bosio RM, Cannon S, McKenna PJ, O'Herlihy CO, Conroy R, Brady H. (2001): Plasma P-selectin is elevated in the first trimester in women who subsequently develop pre-eclampsia. *BJOG 109: 709-715*.
28. Buhimschi IA, Guomao Z, Funai EF, Harris N, Sasson IE, Bernstein IM, Saade GR, Buhimschi CS. (2008): Proteomic profiling of urine identifies specific fragments of SERPINA1 and albumin as biomarkers of preeclampsia. *Am J Obstet Gynecol 199: 551e1-551.e16*.
29. Bujold E, Morency A-M, Roberge S, Lacasse Y, Forest J-C, Giguere Y. (2009): Acetylsalicylic Acid for the Prevention of Preeclampsia and Intra-uterine Growth Restriction in women with Abnormal Uterine Artery Doppler: A systematic Review and Meta-analysis. *J Obstet Gynaecol Can 31(9): 818-826*.

30. Bujold E, Roberge S, Lacasse Y, Bureau M, Audibert F, Marcoux S, Forest J-C, Giguère Y. (2010): Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. *Obstet Gynecol.* 116: 402-14.
31. Carr DB, Newton KM, Utzschneider KM, Tong J, Gerchman F, Kahn SE, Easterling TR, Heckbert SR. (2009): Preeclampsia and risk of developing subsequent diabetes. *Hypertension in Pregnancy* 28: 435-447.
32. Carroli G, Merialdi M, Wojdyla D, Abalos E, Campodonico L, Yao S-E, Gonzalez R, Deter R, Lindheimer M, Van Look P. (2010): Effects of calcium supplementation on uteroplacental and fetoplacental blood flow in low-calcium-intake mothers: a randomized controlled trial. *Am J Obstet Gynecol* 202: 45-54.
33. Cayci EO, Kurt YG, Aydin I, Alacam H et al. (2010): Cord blood and maternal serum neopterin concentration in patients with pre-eclampsia. *Clin Chem Lab Med* 48(8): 1127-1131.
34. Centro Nacional de Equidad de Género y Salud Reproductiva (SALUD) (2002): Prevención, diagnóstico y Manejo de la Preeclampsia/Eclampsia. Lineamiento Técnico.
35. Cetin I, Cozzi V, Pasqualini F, Nebuloni M, Garlanda C, Vago L, Pardi G, Mantovani A. (2006): Elevated maternal levels of the long pentraxin 3 (PTX3) in preeclampsia and intrauterine growth restriction. *AJOG* 194: 1347-53.
36. Chaiworapongsa T, Romero R, Gotsch F, Espinoza J, Nien JK, Goncalves L, Edwin S, Kim YM, Erez O, Kusanovic JP, Pineles BL, Papp Z, Hassan S. (2008): Low maternal concentrations of soluble vascular endothelial growth factor receptor-2 in preeclampsia and small for gestational age. *J Maternal-Fetal & Neonatal Med* 21(1): 41-52.
37. Clark P, Wu O. (2008): ABO(H) blood groups and pre-eclampsia. A systematic review and meta-analysis. *Thromb Haemost.* 100(3): 469-74.
38. CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) Collaborative Group. (1994): CLASP - a randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. *CLASP* 343(8898): 619-29.
39. Cnossen JS, Morris RK, Riet G, Mol BW, van der Post JA, Coomarasamy A, Zwinderman AH, Robson SC, Bindels PJ, Kleijnen J, Khan KS. (2008): Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. *CMAJ* 178:701-711.
40. Cnossen JS, Vollebregt KC, de Vrieze N, ter Riet G, Mol BWJ, Franx A, Khan KS, van der Post JA. (2008): Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. *BMJ* 336: 1117-1120.
41. Coomarasamy A, O'Mahony A, Sharp S, Zamora J, Khan KS, Ismail KMK. (2009): Estimation of proteinuria as a predictor of complications of preeclampsia: A systematic review. *BMC Med* 7: 10.
42. Coomarasamy A, Brauholtz D, Song F, et al. (2003): Individualizing use of aspirin to prevent pre-eclampsia: a framework for clinical decision making. *BJOG* 110: 882-888.
43. Coomarasamy A, Honest H, Papaionnou S, Gee H, Khan KS. (2003): Aspirin for prevention of preeclampsia in women with historical risk factors: a systematic review. *Obstet Gynecol* 101: 1319-1332.

44. Cowans NJ, Spencer K, Meiri H. (2008): First-trimester maternal placental protein 13 levels in pregnancies resulting in adverse outcomes. *Prenatal Diagn* 28(2): 121-5.
45. Cowans NJ, Stamatopoulou A, Spencer K. (2010): First trimester maternal serum placental growth factor in trisomy 21 pregnancies. *Prenat Diagn.* 30(5): 449-53.
46. Cudmore M, Ahmad S, Al-Ani B et al. (2007): Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. *Circulation* 115(13):1789-1797.
47. Deutsche Gesellschaft für Gynäkologie und Geburtshilfe (DGGG). (2010): Diagnostik und Therapie hypertensiver Schwangerschaftserkrankungen AWMF-Leitlinien-Register Nr. 015/018.
48. Diagnosis and Management of Preeclampsia and Eclampsia. (2002): ACOG Practice Bulletin No 33, January.
49. Douglas KA, Redman CW. (1994): Eclampsia in the United Kingdom. *BMJ* 309: 1395-1400.
50. Duckitt K, Harrington D. (2005): Risk factors for PE at antenatal booking: systematic review of controlled studies. *BMJ* 330: 565.
51. Duley L, Henderson-Smart DJ, Knight M, King JR. (2001): Antiplatelet drugs for prevention of pre-eclampsia and its consequences: systematic review. *BMJ* 322: 329-333.
52. Duley L, Henderson-Smart DJ, Knight M, King JR. (2003): Antiplatelet agents for prevention of pre-eclampsia and its complication. *Cochrane Database Syst Rev* 4: CD004659.
53. Elahi MM, Cagampang FR, Anthony FW, Curzen N, Ohri SK, Hanson MA. (2008): Statin treatment in hypercholesterolemic pregnant mice reduces cardiovascular risk factors in their offspring. *Hypertension* 51(4): 939-944.
54. Foidart JM, Munaut C, Chantraine F, Akolekar R, Nicolaidis KH. (2010) Maternal plasma soluble endoglin at 11-13 weeks' gestation in pre-eclampsia. *Ultrasound Obstet Gynecol.* 35: 680-7.
55. Ford ES, Mokdad AH (2003): Dietary magnesium intake in a national sample of U.S. adults. *J Nutr.* 133: 2879-82.
56. Geva R, Eshel R, Leitner Y, Fattal Valevski A, Harel S. (2006): Neuropsychological outcome of children with intrauterine growth restriction: a 9 year prospective study. *Pediatrics* 118: 91-100.
57. Gilbert JS, Ryan MJ, LaMarca BB, Sedeek M, Murphy SR, and Granger JP. (2008): Pathophysiology of hypertension during preeclampsia: linking placental ischemia with endothelial dysfunction. *Am J Physiol Heart Circ Physiol* 294: H541-H550.
58. Gotsch F, Romero R, Kusanovic JP, Chaiworapongsa T, Dombrowski M, Erez O, Than NG, Mazaki-Tovi S, Mittal P, Espinoza J, Hassan SS. (2008): Preeclampsia and small-for-gestational age are associated with decreased concentrations of a factor involved in angiogenesis: soluble Tie-2. *J Matern Fetal Neonatal Med.* 21(6): 389-402.
59. Grassi D, Lippi C, Necozione S, Desideri G, Ferri C. (2005): Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *Am J Clin Nutr* 81(3): 611-614.
60. Haddad B, Sibai BM. (2009): Expectant management in pregnancies with severe pre-eclampsia. *Semin Perinatol* 33: 143-151.

61. Hiltunen LM, Laivuori H, Rautanen A, Kaaja R, Kere J, Krusius T, Paunio M, Rasi V. (2009): Blood group AB and factor V Leiden as risk factors for pre-eclampsia: a population-based nested case-control study. *Thromb Res* 124(2): 167-73.
62. Hirokoshi K, Maeshima Y, Kobayashi K, Matsuura E, Sugiyama H, Yamasaki Y, Masuyama H, Hiramatsu Y, Makino H. (2005): Increase of serum angiopoietin-2 during pregnancy is suppressed in women with preeclampsia. *Am J Hypertens* 18(9.1): 1181-8.
63. Hösli I, Zanetti-Daellenbach R, Holzgreve W, Lapaire O. (2007): Role of omega 3-fatty acids and multivitamins in gestation. *J Perinatal Med* 35:19-24.
64. Irgens HU, Reisaeter L, Irgens LM, Lie RT, Roberts JM. (2001): Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. Pre-eclampsia and cardiovascular disease later in life: who is at risk? *BMJ* 323, 1213-1217.
65. Jones WK. (2000): Improving Women's Health: National Strategy is needed. *Chronic Disease Notes & Reports*. National Center for Disease Prevention and Health Promotion 13: 1-7.
66. Kaaja R, Laivuori H, Laakso M, Tikkanen MJ, Ylikorkala O. (1999): Evidence of a state of increased insulin resistance in preeclampsia. *Metabolism* 48:892-896.
67. Khaw A, Kametas N, Turan O, Bamfo J, Nicolaides K. (2008): Maternal cardiac function and uterine artery Doppler at 11–14 weeks in the prediction of pre-eclampsia in nulliparous women. *BJOG* 115: 369–376.
68. Klebanoff MA, Zhang J, Zhang C, Levine RJ. (2009): Maternal Serum Theobromine and the Development of Preeclampsia. *Epidemiology* 20(5) 727-732.
69. Klemmensen ÅK, Tabor A, Osterdal ML, Knudsen VK, Hallorsson TI, Mikkelsen TB. (2009): Intake of vitamin C and E in pregnancy and risk of pre-eclampsia: prospective study among 57,346 women. *BJOG* 116: 964-974.
70. Kozar E, Nikfar S, Costei A, Boskovic R, Nulman I, Koren G. (2002): Aspirin consumption during the first trimester of pregnancy and congenital anomalies: a meta-analysis. *Am J Obstet Gynecol* 187: 1623–1630.
71. Lawlor DA, Hübinette A, Tynelius P, Leon DA, Smith GD, Rasmussen F. (2007): Associations of gestational age and intrauterine growth with systolic blood pressure in a family-based study of 368,485 men in 331,089 families. *Circulation* 115.
72. Leitich H, Egarter C, Hussein P, Schemoer M. (1997) A meta-analysis of low dose aspirin for the prevention of intrauterine growth retardation. *Br J Obstet Gynaecol* 104: 450–459.
73. Levine RJ, Esterlitz JR, Raymond EG, DerSimonian R, Hauth JC, Ben Curet L, Sibai BM, Catalano PM, Morris CD, Clemens JD, Ewell MG, Friedman SA, Goldenberg RL, Jacobson SL, Joffe GM, Klebanoff MA, Petruilis AS, Rigau-Perez JG. (1996): Trial of Calcium for Preeclampsia Prevention (CPEP): rationale, design, and methods. *Control Clin Trials* 17(5): 442-69.
74. Levine RJ, Hauth JC, Curet LB, Sibai BM, Catalano PM, Morris CD, DerSimonian R, Esterlitz JR, Raymond EG, Bild DE, Clemens JD & Cutler JA. (1997): Trial of calcium to prevent preeclampsia. *N Eng J Med* 337: 69-76.

75. Levine RJ, Lam C, Qian C. (2006): Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 355: 992-1005.
76. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai, BM, Sukhatme VP, Karamanchi SA. (2004): Circulating antigenic factors and the risk for preeclampsia. *New Engl. J. Med* 350: 672-683.
77. Levine RJ, Vatten LJ, Horowitz GL. Qian C, Romundstad PR, Yu KF, et al. (2009): Pre-eclampsia, soluble fms-like tyrosine kinase 1 and the risk of reduced thyroid function: nested case-control and population based study. *BMJ* 339: b4336.
78. Lindheimer MD, Taler SJ, Cunningham FG. (2008): Hypertension in pregnancy. *J Am Soc Hypertens* 2(6): 484-94.
79. Lindheimer MD, Taler SJ, Cunningham FG. (2010): ASH Position Article. Hypertension in Pregnancy. *J Am Soc Hypertens* 4(2): 68-78.
80. Lindquist PG, Molin J. (2005): Does antenatal identification of small-for-gestational age fetuses significantly improve their outcome? *Ultrasound Obs Gyn* 25: 258-264.
81. Lowe S, Brown M, Dekker G, et al. (2009): Guidelines for the management of hypertensive disorders of pregnancy. Australia and New Zealand. *Aust N Z J Obstet Gynaecol* 49: 242-246.
82. Magee LA, Helewa M, Moutquin J-M, von Dadelszen P. (2008): Diagnosis, Evaluation, and Management of the Hypertensive Disorders of Pregnancy. SOGC CLINICAL PRACTICE GUIDELINE No. 206 JOGC 30 (3).
83. Magee LA; Yong PJ; Espinosa V Côté AM; Chen I, von Dadelszen P. (2009): Expectant management of pre-eclampsia remote from term: A structured systematic review. *Hypertens Pregn* 25: 1-36.
84. Maternal Mortality in 2005. Estimates developed by WHO, UNICEF, UNFPA and the World Bank. World Health Organization, Geneva.
85. Mathews TJ, Curtin SC, MacDorman MF. (2000): Infant mortality statistics from the 1998 period linked birth/infant death data set. *National Vital Statistics Reports*, 48 (12). Hyattsville, Maryland: National Center for Health Statistics.
86. McCowan LM, Pryor J, Harding JE. (2002): Perinatal predictors of neurodevelopmental outcome in small-for-gestational-age children at 18 months of age. *Am J Obstet Gynecol* 186: 1069-75.
87. Mello G, Parretti E, Fatini C, Riviello C, Gensini F, Marchionni M, Scarselli GF, Gensini GF, Abbate R. (2005): Low-Molecular-Weight Heparin Lowers the Recurrence Rate of Preeclampsia and Restores the Physiological Vascular Changes in Angiotensin-Converting Enzyme DD Women. *Hypertension* 45: 86-91.
88. Milne F, Redman C, Walker J, et al. (2005): The pre-eclampsia community guideline (PRECOG): how to screen for and detect onset of pre-eclampsia in the community. *BMJ* 330: 576-580.
89. Milne F, Redman C, Walker J, et al. (2009): Assessing the onset of pre-eclampsia in the hospital day unit: summary of the pre-eclampsia guideline (PRECOG II). *BMJ* 339: 626-8.
90. Miodovnik M , Myatt L. (1999): Prediction of Preeclampsia, *Seminars in Perinatology* 25(1): 45-57.

91. Mittendorf R, Covert R, Boman J, Khoshnood B, Lee KS, Siegler M. (1997): Is tocolytic magnesium sulphate associated with increased total paediatric mortality? *Lancet* 350: 1517–1518.
92. Mostello D, Chang J, Allen J, Luehr L, Shyken J, Leet T. (2010): Recurrent Preeclampsia: The Effect of Weight Change Between Pregnancies. *Obstet & Gynecol* 116(3): 667-672.
93. Muttukrishna S, Child TJ, Groome NP, Ledger WL. (1997): Source of circulating levels of inhibin A, pro alpha C-containing inhibins and activin A in early pregnancy. *Human Reprod* 12: 1089-1093.
94. Muttukrishna S, George L, Fowler PA, Groome NP, Knight PG. (1995): Measurement of serum concentrations of inhibin-A ( $\alpha$ - $\beta$ A dimer) during human pregnancy. *Clin. Endocrinol* 42: 391-397.
95. Nadar SK, Karalis I, Al Yemeni E, Blann AD, Lip GY. (2005): Plasma markers of angiogenesis in pregnancy induced hypertension. *Thromb Haemost* 94(5): 1071-6.
96. National Center for Health Statistics, 2000 final Natality Data Report.
97. National Institute for Clinical Excellence (commissioned by). (2003): Antenatal care routines for the healthy pregnant woman. Clinical Guideline. National Collaborating Centre for Women's and Children's Health. October 2003.
98. National Institute for Clinical Excellence. NICE guideline 62. Antenatal care – routine care for the healthy pregnant woman. London: NICE, 2003 and 2008. [www.nice.org.uk/CG062](http://www.nice.org.uk/CG062).
99. Ness RB, Sibai BM. (2006): Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *Am J Obs Gynecol* 195: 40-49.
100. Nicolaides KH, Papageorgiou AT, Yu CKH, Bindra R, Pandis G. (2001): Multicenter screening for pre-eclampsia and foetal growth restriction by transvaginal uterine artery Doppler at 23 weeks of gestation. *Ultrasound Obstet Gynecol* 18: 441-449.
101. Ning Y, Williams MA, Vadachkoria S, et al. (2004): Maternal plasma concentration of insulin-like growth factor-1 and insulinlike growth factor-binding protein-1 in early pregnancy and subsequent risk of preeclampsia. *Clin. Biochem* 37: 968-973.
102. Page NM, Woods RJ, Gardiner SM, Lomthaisong K, Gladwell RT, Butlin DJ, Manyonda IT, Lowry PJ. (2000): Excessive placental secretion of neurokinin B during the 3rd trimester causes pre-eclampsia. *Nature* 405: 797-800.
103. Parretti E, Lapolla A, Dalfrà MG, Pacini G, Mari A, Cioni R, Marzari C, Scarselli G, Mello G. (2006): Preeclampsia in lean normotensive normotolerant pregnant women can be predicted by simple insulin sensitivity indexes. *Hypertension* 47: 449–453.
104. Plasencia W, Maiz N, Poon L, Yu C, Nicolaides KH. (2008): Uterine artery Doppler at 11+0 to 13+6 weeks and 21 + 0 to 24 + 6 weeks in the prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 32: 138-146.
105. Poon LC, Kametas NA, Pandeva I, Valencia C, Nicolaides KH. (2008): Mean arterial pressure at 11+0 to 13+6 weeks in the prediction of preeclampsia. *Hypertension* 51(4): 1027-33.
106. Poon LC, Maiz N, Valencia C, Plasencia W, Nicolaides KH. (2009) First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. *Ultrasound Obstet Gynecol* 33:23-33.

107. Poon LC, Stratieva V, Piras S, Piri S, Nicolaides KH (2010): Hypertensive disorders in pregnancy: combined screening by uterine Doppler, blood pressure and serum PAPP-A at 11-13 weeks. *Prenat Diagn* 30: 216-23.
108. Poon LCY, Kametas NA, Chelemen T, et al. (2010): Maternal risk factors for hypertensive disorders in pregnancy: a multivariate approach. *J Hum Hypertens* 24: 104-10.
109. Poon LCY, Kametas NA, Maiz N, Akolekar R, Nicolaides KH (2009): First-trimester prediction of hypertensive disorders in pregnancy. *Hypertension* 53: 812-818.
110. Poon LCY, Staboulidou I, Maiz N, et al. (2009): Hypertensive disorders in pregnancy: screening by uterine artery Doppler at 11-13 weeks. *Ultrasound Obstet Gynecol.* 34: 142-8.
111. Poon, LCY, Karagiannis G, Leal A, et al. (2009): Hypertensive disorders in pregnancy: screening by uterine artery Doppler and blood pressure at 11-13 weeks. *Ultrasound Obstet Gynecol* 34: 497-502.
112. Poon LC, Akolekar R, Lachmann R, Beta J, Nicolaides KH.. (2010) Hypertensive disorders in pregnancy: screening by biophysical and biochemical markers at 11-13 weeks. *Ultrasound Obstet Gynecol* 35:662-70.
113. Poston L , Briley AL, Seed PT, Kelly FJ, et al. (2006): Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. *Lancet* 367, 1145-54.
114. Prise en charge multidisciplinaire de la prééclampsie. Recommandations formalisées d'experts communes (2009): *Annales Francaises d'Anesthesie et de Reanimation* 28: 275-281.
115. Purwosunu Y, Sekizawa A, Okazaki S, et al. (2009): Prediction of pre-eclampsia by analysis of cell-free messenger RNA in maternal plasma. *Am J Obstet Gynecol* 200: 386-393.
116. Qui C, Williams MA, Calderon-Margalit R, Cripe SM, Sorensen TK. (2009): Pre-eclampsia risk in relation to maternal mood and anxiety disorders diagnosed before or during early pregnancy. *Am J Hypertens* 22: 397-402.
117. Räikkönen K, Pesonen AK, Järvenpää AL, Strandberg TE. (2004): Sweet babies: chocolate consumption during pregnancy and infant temperament at six months. *Early Hum Dev* 76(2): 139-45.
118. Rana S, Karumanchi SA, Levine RJ, Venkatesha S, Rauh-Hain JA, Tamez H, Thadhani R. (2007): Sequential changes in antiangiogenic factors in early pregnancy and risk of developing pre-eclampsia. *Hypertension* 50:137-142.
119. Redman CWG. (2003): Hypertension in Pregnancy, in Warrell DA, *Oxford textbook of medicine* Volume 3, chapter 13.4 pp. 396-403.
120. Richter J, van Mieghem T, Devlieger R. (2010): Clinical and ultrasound work-up and follow-up of preeclampsia. *Acta Clinica Belgica* 65-2.
121. Roberts JM, Cooper DW. (2001): Pathogenesis and genetics of preeclampsia. *Lancet* 357: 53-56.
122. Robinson CJ, Alanis MC, Wagner CL, Hollis BW, Johnson DD. (2010): Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. *Am J Obstet Gynecol* 203(4): 366.

123. Romero R, Kusanovic JP, Than NG, Erez O, Gotsch F, Espinoza J, Edwin S, Chfetz I, Gomez R, Nien JK, Sammar M, Pineles B, Hassan SS, Meiri H, Tal Y, Kuhnreich I, Papp Z, Cuckle HS. (2008): First-trimester maternal serum P13 in the risk assessment for preeclampsia. *AJOG* 199(2): 122. e1-122e11.
124. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, Kusanovic JP, Gotsch F, Erez O, Mazzaki-Tovi S, Gomez R, Edwin S, Chaiworapongsa T, Levine RJ and Karumanchi A. (2008): A longitudinal study of angiogenic (placental growth factor) and antiangiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. *J Maternal-Fetal & Neonatal Med* 21(1): 9-23.
125. Rovere-Querini R, Antonacci S, Dell'Antonio G, Angeli A, Almirante G, Dal Cin E, Valsecchi L, Lanzani C, Sabbadini MG, Doglioni C, Manfredi AA, Castiglioni MT. (2006): Plasma and tissue expression of the long pentraxin during normal pregnancy and preeclampsia. *Obstet Gynecol* 108: 148-55.
126. Ruano R, Fontes RS, Zugaib M. (2005): Prevention of preeclampsia with low-dose aspirin - a systematic review and meta-analysis of the main randomized controlled trials. *Clinics* 60: 407-414.
127. Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS, for the ACTS Study Group. (2006): Vitamins C and E and the Risks of Preeclampsia and Perinatal Complications *N Eng J Med* 354: 1796-1806.
128. Rumiris D, Purwosunu Y, Wibowo N, Farina A, Sekizawa A. (2006): Lower rate of pre-eclampsia after antioxidant supplementation in pregnant women with low antioxidant status. *Hypertens Pregnancy* 25: 241-253.
129. Saftlas, A.F. et al. (2010): Does chocolate intake during pregnancy reduce the risk of pre-eclampsia and gestational hypertension. *Ann. Epidemiol.* 20: 584-591.
130. Sallout B, Walker M. (2003): The fetal origin of adult diseases. *J Obstet Gynaecol* 23(5): 555-60.
131. Savvidou MD, Hingorani AD, Tsikas D, et al. (2003): Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop preeclampsia. *Lancet* 361: 1511-1517.
132. Savvidou MD, Noor M, Anderson JM, Hingorani AD, Nicolaides KH. (2008): Maternal endothelial function and serum concentrations of placental growth factor and soluble endoglin in women with abnormal placentation. *Ultrasound Obstet Gynecol* 32: 871-876.
133. Sibai B, Dekker G, Kupferminc M. (2005): Pre-eclampsia, *Lancet*, 4, 365(9461): 785-99.
134. Sibai BM, Lindheimer M, Hauth J, et al. (1998): Risk factors for preeclampsia, abruptio placentae, and adverse neonatal outcomes among women with chronic hypertension. *N Eng J Med* 339: 667-671.
135. Sibai BM, Mercer BM, Schiff E, Friedman SA. (1994): Aggressive vs. expectant management of severe pre-eclampsia 28 to 32 weeks gestation: a randomised controlled trial. *AJOG* 171: 818-22.
136. Sibai BM, Stella CL. (2009): Diagnosis and management of atypical preeclampsia-eclampsia. *Am J Obstet Gynecol* 200: 481.

137. Sibai BM. (2004): Magnesium sulfate prophylaxis in preeclampsia: Lessons learned from recent trials. *Am J Obstet Gynecol* 190: 1520-6.
138. Spencer K, Cowans NJ, Nicolaides KH. (2008): Maternal serum inhibin-A and activin-A levels in the first trimester of pregnancies developing pre-eclampsia. *Ultrasound Obstet Gynecol* 32: 622-626.
139. Staff AC, Braekke K, Johnsen GM, Karumanchi SA, Harsem NK. (2007): Circulating concentrations of soluble endoglin (CD105) in fetal and maternal serum and in amniotic fluid in pre-eclampsia. *AJOG* 197(2): 176.e1-6.
140. Teran E, Hernandez I, Nieto B, Tavera R, Ocampo JE, Calle A. (2009): Coenzyme Q10 supplementation during pregnancy reduces the risk of pre-eclampsia. *Int J Gynaecol Obstet* 105(1): 43-5.
141. Thadhani R, Ecker JL, Mutter WP, Wolf M, Smirnakis KV, Sukhatme VP, Levine RJ, Karumanchi SA. (2004): Insulin resistance and alterations in angiogenesis: additive insults that may lead to preeclampsia. *Hypertension* 43(5): 988-92.
142. Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, Ecker J, Karumanchi SA. (2004): First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. *J Clin Endocrinol Metab* 89(2): 770-775.
143. Than NG, Pick E, Bellyei S, Szigeti A, Burger O, Berente Z, Janaky T, Boronkai A, Kliman H, Meiri H, Bohn H, Than GN, Sumegi B. (2004): Functional analysis of placental protein 13/galectin-13. *Eur J Biochem* 271: 1065-1078.
144. Thilaganathan B, Wormald B, Zanardini C, Sheldon J, Ralph E, Papageorgiou AT. (2010): Early-pregnancy multiple serum markers and second-trimester uterine artery Doppler in predicting preeclampsia. *Obstet Gynecol* 115(6): 1233-8.
145. Triche EW, Grosso LM, Belanger K, Darefsky AS, Benowitz NL, Bracken MB. (2008): Chocolate Consumption in Pregnancy and Reduced Likelihood of Preeclampsia. *Epidemiology* 19(3): 459-464.
146. Vainio M, Kujansuu E, Iso-Mustajärvi M, Mäenpää J. (2002): Low dose acetylsalicylic acid in prevention of pregnancy-induced hypertension and intrauterine growth retardation in women with bilateral uterine artery notches. *BJOG* 109(2): 161-167.
147. Vatten LJ, Eskild A, Nilsen TI, Jeansson S, Jenum PA, Staff AC. (2007): Changes in circulating level of angiogenic factors from the first to second trimester as predictors of preeclampsia. *Am J Obstet Gynecol*. 196(3): 239. e1-6.
148. Vatten LJ, Nilsen TI, Juul A, Jeansson S, Jenum PA, Eskild A. (2008): Changes in circulating level of IGF-1 and IGF-binding protein-1 from the first to second trimester as predictors of preeclampsia. *Eur J endocrinol* 158: 101-5.
149. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdoah Y, Lim KH, Yuan HT, Libermann TA. et al. (2006): Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat. Med* 12: 642-649.
150. Ventura SJ, Martin JA, Curtin SC, Menacker F, Hamilton BE. (2001): Vital Statistics Births: Final Data for 1999. 49(1): 1-99.

151. Vikse BE, Irgens LM, Leivestad T, Skjaerven R, Iversen BM. (2008): Preeclampsia and the risk of end-stage renal disease. *N Engl J Med* 359(8): 800-809.
152. Villar J, Abdel-Aleem H, Merialdi M, Mathai M, Ali MM, Zavaleta N, Purwar M, Hofmeyr J, Ngoc NN, Campdonico L, Landoulsi S, Carroli G, Lindheimer M. (2006): World Health Organization randomized trial of calcium supplementation among low calcium intake pregnant women. *Am J Obstet Gynecol* 194(3): 639-49.
153. Villar J, Purwar M, Merialdi M, Zavaleta N, thi Nhu Ngoc N, Anthony J, et al. (2009): World Health Organisation multicentre randomised trial of supplementation with vitamins C and E among pregnant women at high risk for pre-eclampsia in populations of low nutritional status from developing countries. *BJOG* 116: 780-8.
154. Visintin C, Muggleston MA, Almerie MQ, Nherera LM, James D, Walkinshaw S; Guideline Development Group. (2010) Management of hypertensive disorders during pregnancy: summary of NICE guidance. *BMJ* 340:c2207.
155. von Dadelszen P, Magee LA, Taylor EL, Muir JC, Stewart SD, Sherman P, et al. (2005): Maternal Hypertension and Neonatal Outcome Among Small for Gestational Age Infants. *Obstet Gynecol* 106: 335-9.
156. Wagner LK (2004): Diagnosis and management of preeclampsia. *Am Fam Physician* 70: 2317-24.
157. Walker JJ (2000): Preeclampsia. *Lancet* 356: 1260-1265.
158. Wen SW, Chen X-K, Redger M, White R, Yang Q, Smith GN, Sigal RJ, Perkins SL, Walker MC. (2008): Folic acid supplementation in early second trimester and the risk of preeclampsia. *Am J Obstet Gynecol* 198: 45-47.
159. Wolf M, Sandler L, Munoz K, Hsu K, Ecker JL, Thadhani R. (2002): First trimester insulin resistance and subsequent preeclampsia: A prospective study. *J Clin Endocrinol Metab* 87(4): 1563-68.
160. You WB, Wolf M, Bailey SC, Pandit AU, Waite KR, Sobel RM, Grobman W. (2010): Factors Associated with Patient Understanding of Preeclampsia. *J Hypertens*, Sep 22. [Epub ahead of print]
161. Yu CJH, Papageorghiou AT, Bindra R, Spencer K, Nicolaides KH. (2007): Second-trimester sex hormone-binding globulin and subsequent development of pre-eclampsia. *J Maternal-Fetal & Neonat Med* 16(3):158-162.
162. Yu CK, Smith GC, Papageorghiou AT, Cacho AM, Nicolaides KH. (2005) An integrated model for the prediction of preeclampsia using maternal factors and uterine artery Doppler velocimetry in unselected low-risk women. *Am J Ostet Gynecol*. 193 429-436.
163. Zaragoza E, Akolekar R, Poon LCY, Pepes S, Nicolaides KH. (2009): Maternal serum placental growth factor at 11-13 weeks in chromosomally abnormal pregnancies. *Ultrasound Obstet Gynecol* 33: 382-386.
164. 2000 Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. (2000): *Am J Obstetrics Gynecol* 183: S1-S22.









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