Pre-eclampsia – Possible to Predict?

A Biochemical and Epidemiological Study of Pre-eclampsia

MARIE BOLIN
Pre-eclampsia is a major cause of maternal and perinatal morbidity and mortality worldwide. A predictor of pre-eclampsia would enable intervention, close surveillance and timely delivery, and thereby reduce the negative consequences of the disorder.

The overall aim of this thesis was to study potential predictors of pre-eclampsia by biochemical and epidemiological methods.

Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) are regulators of angiogenesis, which is important for placental development. In a prospective and longitudinal study of a low-risk population the Ang-1/Ang-2 ratio was evaluated. The Ang-1/Ang-2 ratio increased during pregnancy in all women but at gestational week 25 and 28 the ratios were significantly lower in women who later developed pre-eclampsia. The relevance of Histidine-rich glycoprotein (HRG), a protein with angiogenic properties, was furthermore evaluated. HRG levels decreased in all women, with significantly lower levels at gestational week 10, 25 and 28 in women who later developed pre-eclampsia. Thus both Ang-1/Ang-2 ratio and HRG may predict pre-eclampsia.

To evaluate the predictive value of HRG in combination with uterine artery Doppler early in pregnancy a study was performed in a high-risk population. The results revealed that the combination was better able to predict preterm pre-eclampsia than each marker individually, with a sensitivity of 91% at a specificity of 62%.

A possible association between hyperemesis gravidarum and pre-eclampsia, as well as other placental dysfunctional disorders, was investigated. Hyperemesis gravidarum may be caused by high levels of human chorionic gonadotrophin (hCG) and increased levels of hCG in the second trimester is associated with later development of pre-eclampsia. A cohort of all pregnancies in the Swedish medical birth register between 1997 and 2009 was studied. After adjustment for confounding factors an association between hyperemesis gravidarum in the second trimester and preterm pre-eclampsia, placental abruption and infants born small for gestational age was demonstrated.

In conclusion, the ratio of Ang-1/Ang-2 as well as HRG in plasma may be potential predictors of pre-eclampsia. Combination with uterine artery Doppler further increases the predictive value of HRG for preterm pre-eclampsia. Hyperemesis gravidarum in the second trimester may be considered as a clinical risk predictor of pre-eclampsia and other placental dysfunctional disorders.

Keywords: pre-eclampsia, angiopoietin, histidine-rich glycoprotein, hyperemesis gravidarum

Marie Bolin, Uppsala University, Department of Women's and Children's Health, Akademiska sjukhuset, SE-751 85 Uppsala, Sweden.

© Marie Bolin 2012

ISSN 1651-6206
ISBN 978-91-554-8523-8
urn:nbn:se:uu:diva-183394 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-183394)
To my beloved family
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


## Contents

Introduction ................................................................................................... 11
Background .............................................................................................. 11
History ...................................................................................................... 11
Definition ................................................................................................. 12
Epidemiology ........................................................................................... 13
Pathophysiology ....................................................................................... 13
Early-onset and late-onset pre-eclampsia ................................................. 16
Risk factors............................................................................................... 16
  Hyperemesis gravidarum, a possible clinical risk predictor .......... 17
Prevention and prediction of pre-eclampsia ............................................. 18
  Biochemical predictors ......................................................................... 19
  Angiopoietins ....................................................................................... 20
  Histidine-rich glycoprotein ................................................................. 21
  Accuracy of the biochemical predictors ............................................ 22
  Biophysical markers ........................................................................... 23
  Combined methods for prediction of pre-eclampsia ....................... 25
  Gene expression ................................................................................ 26
  Prophylactic treatment ...................................................................... 27

Aims .............................................................................................................. 29

Material and Methods ................................................................................. 30
  Paper I and II ....................................................................................... 30
    Study population ............................................................................ 30
    Methods .......................................................................................... 30
  Paper III .............................................................................................. 31
    Study population ............................................................................ 31
    Methods .......................................................................................... 32
  Paper IV .............................................................................................. 32
    Study population and exposure ..................................................... 32
    Outcomes ...................................................................................... 33
    Covariates .................................................................................... 34

Results ......................................................................................................... 36
  Paper I ............................................................................................... 36
  Paper II ............................................................................................. 37
  Paper III ............................................................................................ 39
  Paper IV ............................................................................................ 41
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discussion</td>
<td>44</td>
</tr>
<tr>
<td>Methodological considerations</td>
<td>44</td>
</tr>
<tr>
<td>Angiopoietins</td>
<td>45</td>
</tr>
<tr>
<td>Histidine-rich glycoprotein</td>
<td>46</td>
</tr>
<tr>
<td>Pre-eclampsia and hyperemesis</td>
<td>47</td>
</tr>
<tr>
<td>Prediction of pre-eclampsia, at present and in the future</td>
<td>49</td>
</tr>
<tr>
<td>Conclusions</td>
<td>52</td>
</tr>
<tr>
<td>Sammanfattning på svenska</td>
<td>53</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>55</td>
</tr>
<tr>
<td>References</td>
<td>58</td>
</tr>
</tbody>
</table>
Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>Alfa fetoprotein</td>
</tr>
<tr>
<td>Ang</td>
<td>Angiopoietin</td>
</tr>
<tr>
<td>AOR</td>
<td>Adjusted odds ratio</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-wide association studies</td>
</tr>
<tr>
<td>HbF</td>
<td>Foetal haemoglobin</td>
</tr>
<tr>
<td>hCG</td>
<td>Human chorionic gonadotropin</td>
</tr>
<tr>
<td>HRG</td>
<td>Histidine-rich glycoprotein</td>
</tr>
<tr>
<td>H-hCG</td>
<td>Hyperglycosylated hCG</td>
</tr>
<tr>
<td>ICD</td>
<td>International classification of diseases</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>MBR</td>
<td>Medical birth register</td>
</tr>
<tr>
<td>MoM</td>
<td>Multiples of the median</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>Pregnancy-associated protein A</td>
</tr>
<tr>
<td>PI</td>
<td>Pulsatility index</td>
</tr>
<tr>
<td>PlGF</td>
<td>Placental growth factor</td>
</tr>
<tr>
<td>PP13</td>
<td>Placental protein 13</td>
</tr>
<tr>
<td>RI</td>
<td>Resistance index</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver-operator characteristic</td>
</tr>
<tr>
<td>sFlt-1</td>
<td>Soluble fms-like tyrosine kinase-1</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>STMP</td>
<td>Syncytiotrophoblast microparticles</td>
</tr>
<tr>
<td>Tie</td>
<td>Tyrosine kinase endothelial receptor</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
</tbody>
</table>
Introduction

Background
Pre-eclampsia, a pregnancy-specific disorder, is one of the major causes of maternal and perinatal morbidity and mortality worldwide. Studies of the differences between normal pregnancies and those complicated by pre-eclampsia may give important information, useful for finding a possible predictor of pre-eclampsia. Identification of such a predictor would make intervention and close surveillance a possibility and, hopefully, reduce the negative consequences of the disorder.

History
In the pre-Hippocratic Coan Prognosis XXXI there is a description of pre-eclampsia and its severe consequence eclampsia: “In pregnancy, drowsiness and headache accompanied by heaviness and convulsions are generally bad”.¹ Later the presence of a circulating agent, a toxin of foetal or placental origin, was supposed to be the cause of eclampsia and the disease has been called “toxaemia of pregnancy”.² It was not until the middle of the 19th century that the connection between eclampsia and proteinuria was made, and then, at the end of the same century, an association with hypertension was observed.¹

We have had relatively well-functioning maternal health care in Sweden since the start of the 20th century, when blood pressure controls as well as screening for proteinuria were introduced.³ In 1948 less than 40% of the pregnant women in Sweden attended maternal health care clinics, but ten years later the attendance rate had increased to 82%.³ In Sweden, eclampsia was the prime cause of maternal mortality during the 1940s, accounting for 34% of the maternal deaths.⁴ During the 1950s maternal mortality due to eclampsia decreased significantly, which was achieved by improvements to antenatal and obstetrical care.⁴

Modern obstetric care has reduced the incidence of eclampsia in high-income countries. In Scandinavia the incidence of eclampsia is 0,05%.⁵ In low-income countries the incidence varies but a figure from WHO is 2,8%.⁶ Pre-eclampsia and eclampsia are still among the most important causes of maternal mortality, both in high- and low-income countries.⁶,⁷
Definition

There has been a lack of consistency in the definition of pre-eclampsia\(^8\) and to be able to compare different studies and results a definition of hypertension and pre-eclampsia is required. The International Society for the Study of Hypertension in Pregnancy (ISSHP) has suggested the following definitions for study purposes but has also considered a proposal for clinical diagnostic criteria.\(^9\)

**Classification of hypertension in pregnancy**
- Pre-eclampsia - eclampsia
- Gestational hypertension
- Chronic hypertension (essential or secondary)
- Pre-eclampsia superimposed on chronic hypertension

**Research definition of pre-eclampsia**
- New onset of hypertension with a systolic blood pressure $\geq 140$ and/or a diastolic blood pressure $\geq 90$ mm Hg after gestational week 20
  AND
- Proteinuria $\geq 300$ mg/24 hour or a spot urine protein/creatinine ratio $\geq 30$ mg/mmol.

The blood pressure should return to normal postpartum.

**Clinical definition of pre-eclampsia**
- New onset of hypertension with a systolic blood pressure $\geq 140$ mm Hg and/or a diastolic blood pressure $\geq 90$ mm Hg
  AND de novo appearance of one or more of the following:
  - Proteinuria $\geq 300$ mg/24 hour or a spot urine protein/creatinine ratio $\geq 30$ mg/mmol
  - Renal insufficiency (creatinine $\geq 0,09$ mmol/L or oliguria)
  - Liver disease (increased levels of liver transaminases and/or severe right upper quadrant or epigastrical pain)
  - Neurological symptoms: convulsions (eclampsia), hyperreflexia with clonus, severe headache with hyperreflexia, persistent visual disturbances (scotoma)
  - Haematological disturbances: thrombocytopenia, disseminated intravascular coagulation, haemolysis
  - Foetal growth restriction

The blood pressure should return to normal postpartum within 3 months.

**Subtypes of pre-eclampsia**
There is still an inconsistency among the definitions of different subtypes of pre-eclampsia. Various classifications are used in the definition of mild, moderate and severe\(^{10}\) as well as early- and late-onset pre-eclampsia where
the dividing line varies from gestational week 32 to 37. Another classification of pre-eclampsia is preterm and term disorder, indicating pre-eclampsia with a preterm (before gestational week 37) or term delivery (at gestational week 37 or later).

Epidemiology

Pre-eclampsia complicates about 2-8% of all pregnancies and the syndrome results in more than 63,000 maternal deaths every year worldwide. The maternal mortality rate is highest in low- and middle income countries but pre-eclampsia is also a potentially life threatening condition in high income countries. Known complications related to pre-eclampsia are eclampsia, abruptio placentae with disseminated intravascular coagulopathy, cerebral haemorrhage, pulmonary oedema, hepatic failure, HELLP (Hemolysis Elevated Liver enzymes Low Platelet count) syndrome and acute renal failure.

Pre-eclampsia increases perinatal mortality five-fold, with most deaths caused by iatrogenic prematurity. Preterm birth in itself is responsible for the majority of neonatal deaths and nearly one half of all cases of congenital neurologic disability. In the US pre-eclampsia is responsible for 15% of premature births. Another risk for the infants is intrauterine growth restriction. A study from Norway reveals that women with pre-eclampsia have a four times higher risk of having an infant small for gestational age (SGA) compared to normal pregnancies. If the disorder occurs in early pregnancy 53% of the infants are SGA. Furthermore, to be born SGA increases the risk of hypertension, diabetes and coronary heart disease as adults.

Later in life, women who have had pre-eclampsia have an increased risk of early cardiac, cerebrovascular and peripheral arterial diseases and cardiovascular mortality. Severe and recurrent hypertensive disorders during pregnancy have a stronger association with ischemic heart disease later in life compared with mild and non-recurrent disease.

Pathophysiology

The pathophysiology of pre-eclampsia is not precisely known but a two-step model that is widely accepted has been described. The physiological change during a normal pregnancy involves spiral artery remodelling. Trophoblasts invade and replace the arteries’ endothelial cells and induce arterial smooth muscle cell apoptosis, resulting in large nonvasoactive vessels. The first step in the model has been proposed to be abnormalities of either differentiation of morula to blastocyst or the differentiation of trophoblast to cytотrophoblast or syncytiotrophoblast, which may cause
inappropriate invasion of trophoblasts into the spiral arteries, as illustrated in figure 1. The result is an inadequate placentation, followed by intermittent perfusion of the intervillous space and fluctuating levels of oxygen. Due to hypoxic-reoxygenation injuries, oxidative stress arises with widespread placental lipid and protein oxidative modifications, mitochondrial and endoplasmic reticulum stress and tissue apoptosis and necrosis. This may affect the formation of the syncytiotrophoblast as well as its turnover via its downstream regulation of syncytin and Placental Growth Factor (PlGF). The hemodynamic changes in placenta increase the volume and alter the profile of the shed debris, for instance the syncytiotrophoblast microparticles (STMP), from the placenta to the maternal vasculature.25

The second step in the model is characterized by a general systemic inflammatory response of which endothelial dysfunction is a prominent part (figure 2).\textsuperscript{26} Oxidative stress is induced, which further stimulates the inflammatory process, forming the foundation for a positive feedback system.\textsuperscript{27} A number of circulating factors that contribute to maternal endothelial dysfunction, increased vascular permeability and oxidative stress are identified; such factors are STMP, cytokines, apoptotic factors and anti-angiogenic factors.\textsuperscript{27} An activation of the coagulation cascade with formation of occlusive microthrombi is also a result of the endothelial dysfunction.\textsuperscript{16} The ability of the maternal system to handle the deficits in placentation and subsequent challenge to the maternal cardiovascular system partly depend on the immune system, as systemic inflammatory stress plays a key role in endothelial cell activation.\textsuperscript{25,27} Women with a pre-gestational endothelial dysfunction, such as pre-existing hypertension, obesity and dyslipidemi, have an increased risk of pre-eclampsia.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{The two-step model of the pathophysiology of pre-eclampsia.}
\end{figure}
Early-onset and late-onset pre-eclampsia

The heterogeneity of pre-eclampsia has drawn attention to the different characteristics of early- and late-onset pre-eclampsia with a suggested dividing line at 34+0 weeks. However the dividing line between early- and late-onset pre-eclampsia varies between different studies, from gestational week 28 to gestational week 37, which is a problem for interpreting the results. In a study from Norway early-onset pre-eclampsia, defined as delivery before 37 gestational weeks, comprises about 7% of all cases. Early-onset preeclampsia tends to be more severe for the mother and the foetus than late-onset pre-eclampsia. A stronger association with inadequate and incomplete spiral artery remodelling has been proposed for early- compared with late-onset pre-eclampsia, which might be visualized by alterations in uterine Doppler artery profiles. These findings support the theory that early-onset pre-eclampsia more often has a placental origin, while late-onset pre-eclampsia is more related to maternal constitution that is susceptible to, or suffers from, microvasculature disease.

There is morphological as well as molecular evidence that supports the theory that early- and late-onset pre-eclampsia is different with regard to pathophysiology. In a study from Egbor et al, placentas from women with early-onset pre-eclampsia were compared with late-onset examples and volume and total surface area of the terminal villi was significantly reduced in early-onset placentas compared to late-onset. Another study of placenta demonstrated a difference on molecular basis, since placentas from early-onset pre-eclampsia had a different oxygen sensing than placentas from late-onset pre-eclampsia and controls.

Risk factors

The known risk factors for pre-eclampsia are of multivariate origin. Not all studies have reported the same risks but one way to categorize the different risk factors is as in table 1.

One of the strongest risk factors is a previous pregnancy with pre-eclampsia. The recurrence rate for pre-eclampsia varies widely between studies, but a rate up to 65% is described. The risk is related to gestational age at onset, the severity of pre-eclampsia, if the foetus was growth restricted or not and whether there are any underlying medical conditions with vascular or renal implications.

Factors that reduce the risk of pre-eclampsia are a previous normal pregnancy and smoking. However, the damaging effects of smoking on general health and perinatal outcomes outweigh the positive effect of lowered incidence of pre-eclampsia.
Table 1. A categorization of risk factors for pre-eclampsia\textsuperscript{35}

<table>
<thead>
<tr>
<th>Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal-specific risk factors:</strong></td>
</tr>
<tr>
<td>History of previous pre-eclampsia</td>
</tr>
<tr>
<td>Maternal age, long interval between pregnancies</td>
</tr>
<tr>
<td>Family history</td>
</tr>
<tr>
<td><strong>Partner-related risk factors:</strong></td>
</tr>
<tr>
<td>Nulliparity/primipaternity</td>
</tr>
<tr>
<td>Limited sperm exposure, donor insemination</td>
</tr>
<tr>
<td><strong>Presence of specific underlying disorders:</strong></td>
</tr>
<tr>
<td>Chronic hypertension and renal disease</td>
</tr>
<tr>
<td>Obesity, insulin resistance, low maternal birth weight</td>
</tr>
<tr>
<td>Gestational diabetes, type-1 diabetes mellitus</td>
</tr>
<tr>
<td>Activated protein C resistance (factor V Leiden), protein S deficiency</td>
</tr>
<tr>
<td>Antiphospholipid antibodies</td>
</tr>
<tr>
<td><strong>Pregnancy-associated risk factors</strong></td>
</tr>
<tr>
<td>Multiple pregnancy</td>
</tr>
<tr>
<td>Foetus with chromosomal anomalies (trisomy 13)</td>
</tr>
<tr>
<td>Hydatidiform moles</td>
</tr>
</tbody>
</table>

Hyperemesis gravidarum, a possible clinical risk predictor

Hyperemesis gravidarum, a severe form of nausea and vomiting, is a pregnancy complication which occurs in 0.5-3% of pregnancies.\textsuperscript{38,39} It is the most common cause of hospitalization in the first half of the pregnancy and the second cause of hospitalization for pregnancy overall.\textsuperscript{40} The aetiology of hyperemesis gravidarum is not fully understood but human chorionic gonadotropin (hCG) is often stated as the most likely cause since the highest incidence of hyperemesis gravidarum coincide with the peak level of hCG at gestational week 10-12 and conditions associated with higher levels of hCG, such as twins and molar pregnancies.\textsuperscript{41} These conditions are also predisposing for pre-eclampsia.\textsuperscript{35}

As early as in an embryo with 8 cells, hCG-subunits are transcribed and are therefore suggested as possible key regulators of the implantation.\textsuperscript{42} Several variants of hCG are characterized. Hyperglycosylated hCG (H-hCG), secreted by syncytiotrophoblast, is a variant of hCG that stimulates trophoblast invasion and is the major form of hCG during the first weeks of pregnancy.\textsuperscript{43,44} HCG, secreted by trophoblasts, has many
important functions during pregnancy including promotion of progesterone production, implantation and decidualization, angiogenesis, cytotrophoblast differentiation and immune cell regulation.\textsuperscript{44}

Since hCG is of importance both for the invasion of trophoblasts and angiogenesis, which, in pregnancies complicated by pre-eclampsia and other disorders due to placental dysfunctional, are known to be insufficient, several studies have investigated the potential of hCG to predict these disorders.\textsuperscript{45-47}

Low first trimester $\beta$hCG, which is a hyperglycosylated variant of the $\beta$-subunit of hCG, and increased second trimester hCG are associated with adverse obstetrical outcome.\textsuperscript{45-50}

Elevated hCG levels in the second trimester could be due to a reduced production of (H-hCG) early in pregnancy, which results in an insufficient trophoblast migration into the spiral arteries, with a subsequent placental hypoxia that stimulates secretion of the pro-angiogenic hCG as a compensatory mechanism.\textsuperscript{51}

As hCG levels may be both a cause and an effect of placentation, time of onset of hyperemesis gravidarum may be a clinical marker of risks of pre-eclampsia and other abnormal placentation disorders. In very early pregnancy, high hCG levels may favour normal pregnancy development, while later in pregnancy, abnormal placentation may cause high hCG levels and thereby hyperemesis gravidarum.

A meta-analysis has reported an increased risk of SGA in women with hyperemesis gravidarum but pre-eclampsia was not evaluated.\textsuperscript{52} One previous study has described a weak association between pre-eclampsia and hyperemesis gravidarum but the number of women included was limited and preterm was not separated from term pre-eclampsia.\textsuperscript{53}

Prevention and prediction of pre-eclampsia

To prevent pre-eclampsia would be a very important contribution for maternal health. Prevention may be categorized into primary, secondary or tertiary, where primary prevention means avoiding occurrence of a disease, secondary prevention is interception of the process of the disease before clinical signs are observed and tertiary prevention means prevention of complications, which is more or less synonymous with treatment.\textsuperscript{35} Today, the only guaranteed primary prevention of pre-eclampsia is avoidance of pregnancy, although, there are identified risk factors, where intervention could allow primary prevention, such as maternal age, interval between pregnancies and maternal weight. Secondary prevention demands knowledge of the pathophysiological mechanism. Furthermore, the availability of techniques for early detection and intervention in the pathophysiological process are necessary. Tertiary prevention of pre-eclampsia is a proper
antenatal care which provides screening for hypertension and proteinuria, making intervention, such as timely delivery possible. With an organised antenatal care, such as is found in most high in come countries, the maternal mortality and serious morbidity have decreased since the 1950s.\textsuperscript{35}

The major value of secondary prevention would be to identify women at high risk of pre-eclampsia and make a medical intervention so that the disorder never occurs or is postponed. The ultimate predictor of pre-eclampsia should presumably identify women with an increased risk of the disorder as early as in the first trimester. The test should also be simple, rapid, non-invasive, inexpensive and the technology widely available. Furthermore it should be valid, reliable and reproducible with a high positive and a low negative likelihood ratio.\textsuperscript{54}

Biochemical predictors

The biochemical predictors of pre-eclampsia studied so far reflect our knowledge of the pathophysiology of pre-eclampsia. Several potential predictors describe the foetal and placental endocrine functions and the maternal endothelial dysfunction, some of them will be presented below:\textsuperscript{55}

\textit{Molecules from the trophoblasts}

Placental protein 13 (PP 13), expressed by the syncytiotrophoblasts, has been shown to be at a lower level in the first trimester in women who later develop early-onset pre-eclampsia compared to normal pregnancies.\textsuperscript{56} An inappropriate formation of trophoblasts may also produce a reduced amount of placenta derived proteins such as pregnancy-associated protein A (PAPP-A) in women later developing pre-eclampsia.\textsuperscript{57}

The failure of trophoblast invasion may contribute to the alteration of the surface layer of the syncytiotrophoblasts and result in leakage of alfa fetoprotein (AFP) into the maternal circulation resulting in an increased level of these proteins in women with pre-eclampsia.\textsuperscript{58}

Inhibin A and Activin A belong to the transforming growth factor β superfamily and are both elevated before the onset of pre-eclampsia. In women who develop early-onset pre-eclampsia the levels increased at an earlier gestational week than for women with late-onset pre-eclampsia.\textsuperscript{59}

Foetal cells cross the placenta during pregnancy. In pregnancies where pre-eclampsia develops the amount of foetal cells and cell-free foetal DNA and RNA have been demonstrated to be higher than in normal pregnancies. This may be explained by placental necrosis and apoptosis and an impaired DNA elimination.\textsuperscript{60,61}
Inflammatory markers
Women who later develop pre-eclampsia have, compared to healthy pregnancies, higher levels of C-reactive protein, a marker of cellular or immune activation, and Pentraxin 3, an inflammatory molecule expressed in response to inflammatory stimuli by a variety of cells including endothelial cells.\(^\text{62,63}\) Soluble tumour necrosis factor receptor 2 is another factor that reflects intra-vascular inflammation and has been shown to be increased in pre-eclampsia.\(^\text{64}\)

Angiogenic and anti-angiogenic factors
Pre-eclampsia is characterized by an imbalance between different factors that regulate vasculogenesis and angiogenesis. Vasculogenesis occurs mainly during foetal development when the formation of the vasculature derived from endothelial progenitor cells, angioblasts, form a primitive vascular network. Angiogenesis, development of new blood vessels from pre-existing ones, occurs during embryo implantation and placentation.\(^\text{65}\) In pre-eclampsia there is an imbalance in the angiogenic state where anti-angiogenesis dominates. A disturbed balance of these factors are proposed as one cause of the deterioration of the endothelial cell dysfunction and increased vascular permeability seen in pre-eclampsia.\(^\text{25}\)

A variety of pro-angiogenic factors such as Vascular Endothelial Growth Factor (VEGF) and PIGF and anti-angiogenic factors i.e. soluble fms-like tyrosine kinase-1(sFlt-1) and Endoglin have been studied as potential markers of pre-eclampsia.\(^\text{66,67}\)

In pregnancies subsequently developing pre-eclampsia, especially early-onset, lower levels of PIGF and higher levels of sFlt-1 and Endoglin have been demonstrated weeks before diagnosis. A combination of these factors has been shown to potentially improve the predictive value.\(^\text{67}\)

To achieve higher predictive power not only combination of markers are of interest, also new potential predictors are of importance.

Angiopoietins
Angiopoietins have been shown to be of importance during implantation and placentation.\(^\text{68,69}\) They are, together with the Ephrins and VEGF, important regulators of both vasculogenesis and angiogenesis.\(^\text{70}\) Angiopoietin-1 (Ang-1) regulates vascular maturation by recruiting and stabilizing attachment of pericytes.\(^\text{71-73}\) Angiopoietin-2 (Ang-2) is a natural inhibitor of Ang-1 and loosens the attachment of pericytes and stimulates angiogenesis in the presence of VEGF.\(^\text{71}\) Ang-1 and Ang-2 are expressed in syncytiotrophoblasts and endothelial cells in the placenta.\(^\text{68}\) The angiopoietins act via vascular tyrosine kinase receptors called Tie-1 and Tie-2, which are expressed predominantly in endothelial cells.\(^\text{74}\) Factors related to the angiogenic balance,
such as the angiopoietins, could potentially be used as predictive biomarkers of pre-eclampsia.

By evaluating immunohistochemical staining on placenta performed in our laboratory at the Department of Women’s and Children’s Health in Uppsala, Ang-1 and Ang-2 were found to be present in endothelial cells as well as in the trophoblasts (figure 3). No staining was found in the stroma. Their corresponding receptors Tie-1 and Tie-2 were furthermore found at the same localisations in placental tissue (unpublished data). There was also a difference in staining intensity between Ang-1 and Ang-2, where Ang-1 was visualised with a higher intensity in the syncytiotrophoblasts compared to Ang-2. The intensity was on the other hand higher for Ang-2 in endothelial cells. No difference according to staining intensity was found related to Tie-1 or Tie-2.

Figure 3. Ang-1 (A), Ang-2 (B), Tie-1 (C) and Tie-2 (D) in normal placenta.

Histidine-rich glycoprotein

In normal pregnancy, the vascular endothelial cell surface is thrombo-resistant and protects against clot formation. In pre-eclampsia however, endothelial cell dysfunction alters local anticoagulant properties, which in turn results in generally enhanced clot formation. The increased coagulability in the vasculature includes disturbances of the coagulation, as well as the fibrinolytic system. Enhanced activation of platelets and increased activity of factor VIII have been detected in pre-eclampsia.
The coagulation system involves a number of different proteins, with the enzymatic cleavage of fibrinogen to fibrin as the important end point. During normal pregnancy, the plasma level of fibrinogen increases, but in women with pre-eclampsia the fibrinogen level is even higher. Fibrinogen is known to interact with Histidine-rich glycoprotein (HRG), a multi-domain protein involved in haemostasis as well as in the angiogenic pathway that has both angiogenic and anti-angiogenic properties.

The anti-angiogenic effect of HRG has been suggested to be mediated by signal transduction targeting focal adhesions and thereby interrupting VEGF-induced endothelial cell motility. The pro-angiogenic effect could be expressed by modulation of the anti-angiogenic activity of thrombospondin. HRG is present at high levels in plasma; it is synthesized by parenchymal liver cells and transported as a free protein as well as being stored in α-granules of platelets and released after thrombin stimulation. HRG has been found in embryos and it has been suggested that the embryo produces HRG, which may be of importance for placentation.

**Accuracy of the biochemical predictors**

Despite several years of research in the field, a single test accurate enough to provide a screening tool for pre-eclampsia has not yet been found. A review of different biochemical markers for pre-eclampsia before the 25th week of gestation in cohort and case control studies revealed no test with a sensitivity and specificity over 90%. A summary of some of the biomarkers’ accuracy is presented in table 2.

When focusing on prediction of pre-eclampsia in the first trimester, a recent evaluation of biomarkers reveals that low levels of PP13, PI GF and PAPP-A and elevated levels of Inhibin A are significantly associated with the development of pre-eclampsia later in pregnancy. The sensitivity ranged from 22% to 83% at a specificity of 90%.

To improve the predictive value, a combination of biomarkers may achieve improved accuracy. An example is foetal haemoglobin (HbF)/Haemoglobin ratio and α1-microglobulin, that has demonstrated 90% sensitivity and 77% specificity for prediction of pre-eclampsia in early pregnancy.
Table 2. Biochemical predictors and their accuracy to predict pre-eclampsia

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Specificity %</th>
<th>Sensitivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>96</td>
<td>9</td>
</tr>
<tr>
<td>Fibronectin, total</td>
<td>94</td>
<td>65</td>
</tr>
<tr>
<td>Foetal DNA</td>
<td>88</td>
<td>50</td>
</tr>
<tr>
<td>hCG</td>
<td>89</td>
<td>24</td>
</tr>
<tr>
<td>Inhibin-A</td>
<td>95</td>
<td>30</td>
</tr>
<tr>
<td>Activin-A</td>
<td>89</td>
<td>61</td>
</tr>
<tr>
<td>PAPP-A</td>
<td>94</td>
<td>10</td>
</tr>
<tr>
<td>Kallikreinuria</td>
<td>98</td>
<td>83</td>
</tr>
</tbody>
</table>

Biophysical markers

Normal placentation requires a trophoblastic invasion of the maternal decidua, myometrium and blood vessels. The spiral arteries thereby lose their musculoelastic coat, which allows blood to flow with low resistance into the intervillous space. This process starts in the first trimester and is more or less completed at the end of the second trimester.

Abnormal placentation, as seen in pre-eclampsia, is associated with inadequate uteroplacental blood flow. Doppler ultrasonography might be used to assess the velocity of uterine blood flow and thereby indirectly evaluate the trophoblastic invasion of the spiral arteries. Histopathological findings of decreased endovascular trophoblast invasion have been demonstrated to be associated with high-resistance uterine artery Doppler indices.

Several different Doppler indices are used to evaluate the blood flow and the most commonly used are:

- Pulsatility Index (PI): peak systolic flow minus end diastolic flow divided by mean flow
- Resistance index (RI): peak systolic flow minus end diastolic flow divided by peak systolic flow
- Notching: presence of early diastolic notching indicating decreased early diastolic flow compared to later diastolic flow in the uterine artery (figure 4)
Low end-diastolic velocities and an early diastolic notch characterize the waveforms of uterine artery blood flow in non-pregnant and early pregnant women. Abnormal flow velocity ratios and a persistent diastolic notch after gestational week 24 have been associated with inadequate trophoblast invasion.96

Figure 4. Uterine artery Doppler without and with a notch. The notch is indicated by an arrow. Reprinted with permission from Eva Bergman, MD, PhD, Department of Women’s and Children’s health, Uppsala University.

A systematic review of the ability of uterine artery Doppler ultrasonography to predict pre-eclampsia revealed that pre-eclampsia is best predicted by an increased pulsatility index (PI) with diastolic notching in the second trimester.96 In table 3 and 4 a summary based on this review is presented.
Table 3. The best uterine artery Doppler indices and their accuracy in the first trimester.

<table>
<thead>
<tr>
<th>Population</th>
<th>Pre-eclampsia</th>
<th>Doppler indices</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive likelihood ratio</th>
<th>Negative likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>Overall</td>
<td>PI</td>
<td>95</td>
<td>5.4</td>
<td>0.78</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>PI</td>
<td>40</td>
<td>90</td>
<td>4.0</td>
<td>0.67</td>
</tr>
<tr>
<td>High risk</td>
<td>Overall</td>
<td>Notching</td>
<td>91</td>
<td>46</td>
<td>1.7</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>Notching</td>
<td>91</td>
<td>46</td>
<td>1.7</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Table 4. The best uterine artery Doppler indices and their accuracy in the second trimester.

<table>
<thead>
<tr>
<th>Population</th>
<th>Pre-eclampsia</th>
<th>Doppler indices</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive likelihood ratio</th>
<th>Negative likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>Overall</td>
<td>PI and Notching</td>
<td>23</td>
<td>99</td>
<td>7.5</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>PI</td>
<td>78</td>
<td>95</td>
<td>15.6</td>
<td>0.23</td>
</tr>
<tr>
<td>High risk</td>
<td>Overall</td>
<td>Notching</td>
<td>96</td>
<td>20.2</td>
<td>0.17</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>RI</td>
<td>80</td>
<td>78</td>
<td>3.7</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Combined methods for prediction of pre-eclampsia

Since pre-eclampsia is characterized by a complex pathophysiology with heterogeneous clinical and laboratory findings, it may not be realistic to search for a single marker to predict the disorder. A combination of two or more independent markers, each representing separate pathophysiological processes, should theoretically improve the possibility for predicting pre-eclampsia with a high degree of accuracy.

A systematic review by Giguere et al. showed that a combination of biochemical and ultrasonographic markers might improve the prediction of pre-eclampsia. In low risk populations several combinations, including PP13, PAPP-A, a disintegrin and metalloproteinase (ADAM), activin A or inhibin A measured in the first or early second trimester, combined with uterine artery Doppler investigation, showed a sensitivity of 60-80% and a
specificity of >80%. A combination of PP13 and uterine artery Doppler showed a sensitivity of 90% and a specificity of 90 % for severe pre-eclampsia in a high risk population.

Another review by Kuc et al, 90 where first trimester predictors were evaluated, concluded furthermore that the combination of biomarkers and uterine artery Doppler ultrasound predicted pre-eclampsia better than a single predictor. The sensitivities varied between 38% and 100% at a specificity of 90%

Taking into account maternal characteristics such as previous pregnancy with pre-eclampsia, maternal age, diabetes mellitus, hypertension and obesity, prediction of pre-eclampsia might be improved in the first trimester.98 Women developing pre-eclampsia later have higher systolic blood pressure and mean arterial pressure (MAP) before the onset of clinical disease.99 In a review, MAP has been demonstrated to be a better predictor of pre-eclampsia than systolic and diastolic blood pressure.100 The combination of maternal characteristics in the first trimester, including mean arterial pressure, uterine artery pulsatility index and the biochemical markers PAPP-A and PlGF in the first trimester has revealed predictive values with 95% specificity and 93% sensitivity for early-onset and 36 % for late-onset pre-eclampsia.101 Another large study combine maternal characteristics, including mean arterial pressure, uterine artery pulsatility index and the biochemical markers PAPP-A, PlGF, PP13, sEndoglin, Inhibin-A, Activin-A, Pentraxin 3 and P-Selectin. The result at 95% specificity was, for early-onset 91% sensitivity, intermediate onset 79% sensitivity and late-onset pre-eclampsia 61% sensitivity.102

Gene expression

Prevalence of pre-eclampsia – eclampsia in certain families indicates a genetic contribution to the disorder.103 Whether the susceptibility for pre-eclampsia depends on a single gene or multiple ones has been debated.104

The technique of whole genome microarrays opens up new possibilities in the search for gene(s) contributing to the development of pre-eclampsia. Through genome-wide association studies (GWAS) it is possible to find single nucleotide polymorphism (SNP) that is associated with a disease and indicates a region of the human genome which influences the risk of the specific disorder. Studies of populations with different ancestors have demonstrated a common maternal pre-eclampsia locus on chromosome 2, although at different positions.105-107

To evaluate which genes in the genome are expressed, studies of RNA transcriptions are performed. A comparison of maternal blood from women developing early onset severe pre-eclampsia with blood from women with healthy pregnancies, has demonstrated 72 genes either up- or down-regulated in women with pre-eclampsia.108 The differential gene expression
of placentas with inadequate placentation, according to existing notch with Doppler ultrasonography, indicates that the progression of pre-eclampsia may be driven by induction of pro-inflammatory genes, in contrast to those pregnancies that did not develop pre-eclampsia.\textsuperscript{109}

To predict pre-eclampsia, genetic studies could focus on SNPs associated with well known risk factors, such as vascular disease, to find a possible SNP that could predict pre-eclampsia\textsuperscript{110} but, at present, no specific SNP has yet been identified for this role.

Another approach is to further investigate the protein products of the genes of interest. Up-regulated HbF genes and accumulation of HbF protein in placentas from women that developed pre-eclampsia have been demonstrated.\textsuperscript{111} These findings support the case for further studies of HbF as a possible predictor of pre-eclampsia.

**Prophylactic treatment**

The pathophysiology of pre-eclampsia is not sufficiently well characterized to provide a specific prophylactic treatment. However, several studies have investigated the potential effect of antiplatelet agents, i.e. low dose aspirin <75 mg.\textsuperscript{112,113} Antiplatelet drugs have anti-inflammatory properties and affect the imbalance of prostacyclin and tromboxane seen in pre-eclampsia.\textsuperscript{114} A Cochrane review described that aspirin was associated with an overall 17% reduction in the risk of pre-eclampsia, with a number needed to treat of 72. The risk reduction in a high risk population was higher, with a number needed to treat of 19.\textsuperscript{115} Another review\textsuperscript{113} revealed a major reduction of preterm pre-eclampsia when treatment with low dose aspirin was initiated before or at 16 weeks of gestation. Treatment with low dose aspirin seems, furthermore to be safe, with few known side effects on the mother and child.\textsuperscript{112,116}

Another possible prophylactic treatment for pre-eclampsia is calcium supplementation. In a Cochrane review it was concluded that a daily intake of at least 1g calcium reduced the risk of pre-eclampsia by about 50%.\textsuperscript{117} The effect was most obvious in women with a predicted high risk according to known risk factors and also in women with a low dietary calcium intake. Although the incidence of pre-eclampsia was reduced, there was no clear reduction in severe pre-eclampsia, eclampsia or admission to intensive care.

Supplementation of calcium seems to reduce blood pressure and may also affect the uteroplacental blood flow by lowering the resistance index in uterine and umbilical arteries.\textsuperscript{118} Supplementation of calcium is relatively cheap and the substance is already available on the market. It is likely to be safe for both the woman and the foetus, though more studies are required.\textsuperscript{117}

In placentas from women with pre-eclampsia, thrombotic lesions have been observed.\textsuperscript{119} To prevent the vascular pathology, the possible prophylactic role of anti-thrombotic agents, such as low molecular weight
heparin, have been studied. Promising results, with a reduction of pre-
eclampsia and eclampsia, have been described but the number of studies and
participants included were small.\textsuperscript{120} To date, prophylactic treatment is not
recommended but further research is required.

In conclusion, medical intervention, with aspirin as well as calcium,
seems to be possible, indicating that a screening test would be of importance.
Aims

• to analyse whether there is a difference in the ratio between Ang-1 and Ang-2 throughout pregnancy between women who develop pre-eclampsia and those who do not and, furthermore, to determine if the ratio between Ang-1 and Ang-2 can be used as a predictor of pre-eclampsia in a low risk population

• to analyse whether there is a difference in circulating levels of HRG throughout pregnancy between women who develop pre-eclampsia and those who do not and, furthermore, to determine if HRG can be used as a predictor of pre-eclampsia in a low risk population

• to determine if the combination of HRG and uterine artery Doppler ultrasonography in early pregnancy can be used as a predictor of pre-eclampsia

• to investigate if there is an association between hyperemesis gravidarum in the first or second trimester and pre-eclampsia as well as other placental dysfunction disorders
Material and Methods

Paper I and II

Study population
A cohort of healthy pregnant women (n = 469) was enrolled in gestational week 8–12 at five participating prenatal centres in the county of Värmland, Sweden, during autumn 2004–spring 2007. Only women with singleton pregnancies were recruited. Women with a concurrent diagnosis such as chronic hypertension, episodes of high blood pressure before pregnancy, persistently elevated blood pressure before the 20th week of gestation, upper urinary tract infection, pre-existing renal disease, diabetes mellitus, and drug abuse were not included.

Plasma samples were collected in lithium/heparin containing tubes at gestational weeks 10, 25, 28, 33 and 37.

Pre-eclampsia was defined as new-onset hypertension (≥140/90 mm Hg) observed on at least two separate measurements ≥6 h apart, combined with proteinuria (≥2 on a dipstick or in a 24h urine sample showing ≥300 mg/24 h). Clinical and laboratory routine parameters were registered.

The studies were approved by the regional Ethics Committee of the Medical Faculty of Uppsala University, and informed consent was obtained from each patient included in the study.

Methods

Measurement of Ang-1, Ang-2 and HRG in plasma
Plasma samples were analysed for levels of Ang-1 and Ang-2 by enzyme-linked immunosorbent assay (ELISA) using commercially available kits.

HRG was analysed by an ELISA set up in the laboratory of the Department of Women’s and Children’s Health, Uppsala University.
Statistics

All statistical analysis was performed by the SPSS 15.0 for Windows software package (SPPS, Chicago, IL). Among the background variables a chi-square test was used for proportions. For comparisons of median values a Mann–Whitney \( U \) test was used for independent samples and for mean values students t-test was used.

The Kaplan-Meier method was used to illustrate time to onset of pre-eclampsia in gestational week 25 by using a cut-off value of the ratio.

All significance tests were two-tailed. P-values \( \leq 0.05 \) were considered as statistically significant. Receiver–operator characteristic (ROC) curves were constructed to test arbitrarily chosen Ang-1/Ang-2 and HRG cut-off values for predicting pre-eclampsia.

Paper III

Study population

The patients were recruited between 2002 and 2005 as part of an ongoing prospective study of Doppler ultrasound and serum markers for pre-eclampsia in women attending a routine antenatal care visit at St. George’s Hospital Obstetric Unit, London. Multiparas with a high risk profile, such as essential hypertension, previous pregnancy with pre-eclampsia or intrauterine growth restriction and all primiparas were included. Pregnancy outcomes were obtained from the delivery suite database or from general practitioners.

The study was designed as a case-control study and involved 175 women who were randomly selected from the cohort: 86 women with an uncomplicated pregnancy (controls) and 89 women who developed pre-eclampsia (cases).

Maternal blood was collected in non-heparinized tubes. The blood samples were on average collected at gestational week 14.

Patient characteristics including demographics, smoking status, and obstetric and medical history were obtained from women at the first hospital visit and entered into a foetal medicine unit database.

Pre-eclampsia was defined as two recordings of blood pressure of 140/90 mmHg or greater at least 4 hours apart and proteinuria of 300 mg or more within 24 hours, or two readings of at least 2 on dipstick analysis of urine if a 24-hour collection was not available, and developed after 20 weeks of gestation.
Women with pre-eclampsia who were delivered in gestational week 37 or later were defined as term pre-eclampsia and women who were delivered before 37 weeks of gestational age were defined as preterm pre-eclampsia.

Approval was obtained for the study from the local Research Ethics Committee at Wandsworth, UK, and all women gave written, informed consent prior to inclusion.

Methods

Doppler ultrasound
As part of routine antenatal scans, uterine artery PI was abdominally measured in the first or second trimester of those women who gave their permission. The ultrasound examination was, on average, performed at gestational week 14+6 and in 159 of the 175 women Doppler indices were registered.

The uterine artery PI was expressed as multiples of the median (MoM) to correct for changes in the normal values with gestational age.

Measurement of HRG in serum
HRG was analysed by an ELISA set up in the laboratory of the Department of Women’s and Children’s Health, Uppsala University.

Statistics
All statistical analyses were performed by SPSS 18.0 for Windows software pack (SPSS, Chicago, IL, USA).
Comparisons between different continuous variables were made with Student’s t-test and a Chi-square-test was used for proportions. Non-normally distributed variables were compared by Mann-Whitney U test. A receiver-operator characteristic (ROC) curve was constructed to determine the best cut-off values of HRG and PI for prediction of pre-eclampsia. Two tailed p-values<0.05 were considered as statistically significant.

Paper IV
Study population and exposure
From the Swedish Medical Birth Register (MBR) a cohort was created of singleton births, born at a gestational age of 22 weeks or more, where the pregnancy was estimated to have started January 1st 1997 or later and resulted in a birth December 31st 2009 or earlier. The MBR includes prospectively collected information, including demographic data,
reproductive history and complications during pregnancy, delivery and the neonatal period among more than 98% of all births in Sweden. Complications during pregnancy and delivery are classified according to the International Classification of Diseases (ICD), as noted by the responsible doctor at discharge from hospital after delivery. Individual record linkage between the MBR and other registers is possible through each individual’s unique personal registration number.

1,156,050 pregnancies were included. The exposure variable was admission to hospital because of hyperemesis gravidarum. Information on exposure was collected through linkage to the nation-wide Patient Register, which includes information on dates of hospital admissions and discharges and diagnoses, which are classified according to ICD codes.

13,287 pregnancies with at least one admission for hyperemesis gravidarum were identified. Pregnancies with a first admission to hospital for the exposure at 22 gestational weeks or later (n=1,017) were excluded.

Pregnancies with hyperemesis gravidarum were stratified into first trimester hyperemesis gravidarum, defined as first admission to hospital before 12 completed gestational weeks (n=10,186), and second trimester hyperemesis gravidarum with a first admission between 12 and 21 completed gestational weeks (n=2,084).

The final study population included 1,155,033 pregnancies, of whom 12,270 (1.1%) were exposed to hyperemesis gravidarum before 22 gestational weeks (figure 5).

Outcomes

The outcomes were pre-eclampsia, placental abruption, stillbirth and SGA. Pre-eclampsia was categorized into preterm (birth before 37 gestational weeks) and term (birth at 37 gestational weeks or later). We had no information on gestational age at onset of pre-eclampsia in the MBR. Gestational age was assessed by ultrasound scans or, if no early second trimester ultrasound scan was available, the last menstrual period was used to calculate gestational age at delivery.

Pre-eclampsia was defined as a blood pressure more than or equal to 140/90 mm Hg combined with proteinuria (≥ 0.3 g/24 hours) occurring after 20 weeks of gestation.

Placental abruption was defined as premature separation of the placenta and identified by ICD codes. Stillbirth was defined as a foetal death occurring at 28 weeks of gestation or later. SGA was defined as a birth weight of 2 standard deviations or more below the mean birth weight for gestational age according to the sex-specific Swedish foetal growth curve. When analyzing risk of SGA, we excluded stillbirths and births with missing or misclassified information on birth weight and/or gestational age (n=5,036), leaving 1,146,142 births in the study population.
Covariates

Information on maternal body mass index (BMI), height, cigarette consumption and cohabitation with infant’s father was collected from the first antenatal visit. Data on maternal age, parity and infant’s sex was collected at delivery. To obtain information on the mothers’ country of birth and highest level of formal education, individual linkage with the Register of Total Population and the Education Register (December 31st 2010) was performed.
Information on presence of maternal hyperthyreosis was collected at the discharge from the delivery hospital. Further, information on pre-gestational diabetes and chronic hypertension was collected from the first antenatal visit and at discharge from delivery hospital.

Statistical analysis
Risks of pre-eclampsia, placental abruption, stillbirth and SGA were calculated for women admitted to hospital because of hyperemesis gravidarum, using women without admission for hyperemesis gravidarum as reference. Odds Ratios (OR) with 95% confidence intervals (CI) were calculated using the generalized estimation equation (PROC GENMOD) method, since observations are not independent in women who delivered more than once during the study period. Adjustments were made for maternal factors associated with risks of the exposure and outcomes, including maternal age, parity, BMI, height, smoking, cohabitation with infant’s father, infant’s sex, mother’s country of birth, education, presence of hyperthyreosis, pre-gestational diabetes and chronic hypertension. Adjustments were also made for year of infant birth, categorized into 1997-2001, 2002-2005 and 2006-2009.

All statistical analyses were performed with Statistical Analysis Software version 9.1 (SAS Institute, Inc., Cary, NC).
Results

Paper I

Nineteen women who developed pre-eclampsia and 43 women with a normal pregnancy were randomly selected from the study population. There were no significant differences between the groups according to maternal age, parity and body mass index.

Plasma levels of Ang-1 and Ang-2

The levels of Ang-1 and Ang-2 varied during pregnancy. The median Ang-1/Ang-2 ratio increased in all women as shown in figure 6. The ratios were significantly lower at gestational week 25 and 28 in women who later developed pre-eclampsia than in normal pregnant women (1.48 compared to 2.19 and 2.12 compared to 3.54, p<0.05 and p<0.05). After gestational week 28 the Ang-1/Ang-2 ratio was still lower in women who developed pre-eclampsia compared to women with normal pregnancy, but the difference was not statistically significant.

![Graph showing median values of the Ang-1/Ang-2 ratio](image.png)

**Figure 6.** Median values of the Ang-1/Ang-2 ratio in women with normal pregnancies (filled circles) and in women developing pre-eclampsia (unfilled circles) at different gestational weeks.
Cut-off value for prediction of pre-eclampsia

ROC curves regarding the prediction of pre-eclampsia at arbitrarily chosen Ang-1/Ang-2 cut-off values were constructed for gestational week 25 and week 28, as illustrated in figure 7. A cut-off value of 1.41 for the Ang-1/Ang-2 ratio at gestational week 25 showed a sensitivity of 47% and a specificity of 87% to predict pre-eclampsia later in pregnancy. A cut-off value of 1.84 in gestational week 28 for the Ang-1/Ang-2 ratio resulted in a sensitivity of 50% and a specificity of 80%.

Figure 7. ROC curve illustrating the Ang-1/Ang-2 ratio (a) in gestational week 25 and (b) in gestational week 28. Area under the curve (AUC) is 0.71 and 0.72 respectively.

Paper II

From the cohort, 20 women who developed pre-eclampsia and 44 women randomly selected from the group of normal pregnancies delivered at term were included. There were no significant differences between the groups according to maternal age, parity and body mass index.
Plasma levels of HRG during pregnancy

The results indicated that the mean HRG levels decreased as the pregnancy proceeded in all women, irrespective of whether they developed pre-eclampsia or not (figure 8). However, in the group of women who developed pre-eclampsia the levels of HRG were significantly lower in gestational week 10, 25 and 28 compared to women with a normal healthy pregnancy (40.1 µg/mL vs 55.9 µg/mL p<0.05, 30.1 µg/mL vs 42.8 µg/mL p<0.05 and 25.6 µg/mL vs 33.8 µg/mL p<0.05).

Figure 8. Mean levels of HRG in women with normal pregnancies (filled circles) and in women developing pre-eclampsia (unfilled circles) at different gestational weeks.

Cut-off values for prediction of pre-eclampsia

ROC curves regarding the prediction of pre-eclampsia at arbitrarily chosen HRG cut-off values were constructed, as demonstrated in figure 9. A cut-off value of 49.1 µg/mL for HRG at gestational week 10 showed a sensitivity of 79% and a specificity of 44% to predict pre-eclampsia later in pregnancy, with an accuracy of 79%. For gestational week 25 a cut-off value of 32.7 µg/mL showed a sensitivity of 79% and a specificity of 57%. At gestational week 28 a cut-off value of 27.5 µg/mL gave a sensitivity of 82% and a specificity of 64%.
Paper III

Of the 89 women who developed pre-eclampsia, 62 were delivered at term while 27 delivered preterm. The controls were all delivered at term. Women who developed pre-eclampsia did not differ from controls in terms of maternal age or smoking habits at enrolment, but they had a higher BMI and were less often nulliparous. However, HRG levels were not associated with either BMI or parity.

*Serum levels of HRG and uterine artery Doppler*

Women who developed preterm pre-eclampsia had significantly lower levels of HRG compared to controls (45.0 μg/mL vs 82.7 μg/mL, p=0.001) (figure 10). However, for the entire group of women who developed pre-eclampsia, no difference in comparison with controls was found (80.2 μg/mL vs 82.7 μg/mL, p=0.7). Correlation analyses were performed to test HRG against gestational age using the Pearson correlation test but no correlation was observed (P =0.2, r =-0.1).

PI, expressed as multiples of the median (PI MoM), was significantly higher in the pre-eclampsia group, especially among women who developed...
preterm pre-eclampsia compared with controls (1.1 vs 1.0, p<0.05 and 1.6 vs 1.0, p<0.001) (figure 10).

**Figure 10.** Boxplots showing the median serum levels of HRG (µg/ml) and PI MoM for controls and preterm pre-eclampsia. The top and the bottom of the boxes represent the third and the first quartiles. The horizontal line within the box represents the median value. The bars on the side of the box represent the highest and the lowest value. Extreme values are withdrawn but included in the statistical analysis.

**Cut-off value for prediction of pre-eclampsia**

ROC curves for HRG, PI MoM and a combination of HRG and PI MoM were constructed (figure 11). Sensitivities at different specificity levels for prediction of preterm pre-eclampsia were obtained. The AUC was 0.72 (95% CI, 0.61-0.83; p=0.001) for HRG, 0.79 (95% CI, 0.67-0.90; p<0.001) for PI MoM and 0.85 (95% CI 0.77-0.93; p<0.001) for the combination of HRG and PI MoM.

For prediction of preterm pre-eclampsia a suggested optimal cut-off value for HRG<67.8 µg/mL had a sensitivity of 74% and a specificity of 66%, whereas a cut-off value for PI MoM>1.3 had a sensitivity of 78% and a specificity of 81%. The highest values for prediction were found using the combination of HRG and PI MoM with a sensitivity of 91% and a specificity of 62%. As an alternative to the optimal cut-off value the predictive value of the combination of HRG and PI MoM could be presented as a detection rate of 39% and 61%, at a false positive rate of 5% and 10% respectively (table 5).
Figure 11. ROC curve for HRG and PI with AUC 0.72 for HRG, 0.79 for PI and 0.85 for the combination of HRG and PI.

Table 5. Prediction of preterm pre-eclampsia at different specificity levels

<table>
<thead>
<tr>
<th>Specificity (%)</th>
<th>HRG Sensitivity (%)</th>
<th>PI Sensitivity (%)</th>
<th>HRG and PI Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>11</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>90</td>
<td>30</td>
<td>44</td>
<td>61</td>
</tr>
<tr>
<td>80</td>
<td>56</td>
<td>78</td>
<td>70</td>
</tr>
<tr>
<td>70</td>
<td>67</td>
<td>78</td>
<td>87</td>
</tr>
</tbody>
</table>

Paper IV

In pregnancies with hyperemesis gravidarum women with a first admission to the hospital in the first trimester, were generally older, more often multi-gravida and overweight/obese (BMI>25.0), non-smoking, expecting a female infant and from a non-Nordic country, than women with a first admission to the hospital in the second trimester.

Compared with pregnancies without hyperemesis gravidarum, pregnancies with hyperemesis gravidarum had a slightly increased risk of pre-eclampsia, especially preterm pre-eclampsia. When stratifying the exposure
into first and second trimester hyperemesis gravidarum, the strongest association between hyperemesis gravidarum and pre-eclampsia was observed between second trimester hyperemesis gravidarum and preterm pre-eclampsia, where a more than two-fold increased risk was seen (table 6). Compared with pregnancies without hyperemesis gravidarum, pregnancies with hyperemesis gravidarum were associated with an almost 50% increased risk of placental abruption and a slightly increased risk of an SGA birth. When stratifying the exposure into first and second trimester hyperemesis gravidarum, the strongest risks were again observed for second trimester hyperemesis gravidarum, which was associated with a more than three-fold increased risk of placental abruption and a 39% increased risk of SGA. First trimester hyperemesis gravidarum was not significantly associated with placental abruption and SGA (table7). No association between hyperemesis gravidarum and stillbirth was found.

Table 6. Hyperemesis gravidarum and risk of preterm and term pre-eclampsia

<table>
<thead>
<tr>
<th>Hyperemesis gravidarum</th>
<th>Preterm (&lt;37 weeks)</th>
<th>Term (≥37 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Rate, %</td>
</tr>
<tr>
<td>No</td>
<td>7,322</td>
<td>0.6</td>
</tr>
<tr>
<td>Yes</td>
<td>101</td>
<td>0.8</td>
</tr>
<tr>
<td>First trimester</td>
<td>72</td>
<td>0.7</td>
</tr>
<tr>
<td>Second trimester</td>
<td>29</td>
<td>1.4</td>
</tr>
</tbody>
</table>

aAOR adjusted odds ratio. Adjustments were made for maternal age, parity, BMI, height, smoking, cohabitation with infant’s father, infant’s sex, mother’s country of birth and years of formal education, presence of hyperthyreosis, pre-gestational diabetes or chronic hypertension and year of birth of infant.
Table 7. Hyperemesis gravidarum and risk of placental abruption and giving birth to an infant born SGA

<table>
<thead>
<tr>
<th>Hyperemesis gravidarum</th>
<th>Placental abruption</th>
<th>Small for gestational age&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Rate, %</td>
</tr>
<tr>
<td>No</td>
<td>4652</td>
<td>0.41</td>
</tr>
<tr>
<td>Yes</td>
<td>64</td>
<td>0.52</td>
</tr>
<tr>
<td>First trimester</td>
<td>42</td>
<td>0.41</td>
</tr>
<tr>
<td>Second trimester</td>
<td>22</td>
<td>1.06</td>
</tr>
</tbody>
</table>

<sup>a</sup>Defined as a live born infant with a birth weight for gestational age < - 2 standard deviations below the sex-specific Swedish fetal growth.

<sup>b</sup>AOR adjusted odds ratio. Adjustments were made for maternal age, parity, BMI, height, smoking, cohabitation with infant’s father, infant’s sex, mother’s country of birth and years of formal education, presence of hyperthyreosis, pre-gestational diabetes or chronic hypertension and year of birth of infant.
Discussion

Methodological considerations

Pre-eclampsia is one of the major causes of maternal and perinatal morbidity and mortality worldwide. A predictor of pre-eclampsia would make intervention, close surveillance, early diagnosis and timely delivery possible and thereby reduce the negative consequences of the disorder.\textsuperscript{91}

When new biomarkers of pre-eclampsia are investigated, where differences throughout pregnancy are likely to occur, a longitudinal approach to the collection of samples is preferable. In our studies presented in papers I and II, women from a low-risk population were prospectively included at enrolment to maternal care and blood samples were collected in gestational weeks 10, 25, 28, 33 and 37, according to general procedures for antenatal care in Sweden. This longitudinal approach is a major strength of our studies since it allowed us to follow the women through the pregnancy and, furthermore, allowed us to investigate whether there were specific differences in women who developed pre-eclampsia compared to healthy controls. We could, in addition, identify at which gestational week the differences between the groups seemed to happen, which was how we evaluated whether the marker was a potential predictor of pre-eclampsia or not. In our total study population 469 women were included. Of these, 22 were diagnosed with pre-eclampsia and 44, out of 302 women with healthy pregnancies delivered at term, were randomly selected as controls. The study population selected is rather unique, since, compared with other studies where biomarkers of pre-eclampsia have been investigated, cohorts with a cross-sectional approach dominate. With a cross-sectional cohort, it is not possible to detect changes throughout pregnancy, although the number of patients in these studies can be larger than in longitudinal studies. One limitation with our studies is that the number of women included was relatively small and larger prospective studies are needed to determine whether our findings related to the Ang-1/Ang-2 ratio, as well as levels of HRG, can be used as clinically relevant predictors. In follow-up studies it might be relevant to study analyses related to early- and late-onset pre-eclampsia, as well as levels of the potential predictors in a gestational week between week 10 and 25.

Major strengths of the study presented in paper IV are the large sample size and the nationwide population-based design, where data was collected
prospectively which precludes recall biases. The large sample size made it possible to stratify hyperemesis gravidarum by trimester of hospitalization, and to study rare adverse outcomes, such as preterm pre-eclampsia and placental abruption. We were also able to control for a substantial number of possible confounders even if we cannot exclude that our findings may partly be due to unmeasured confounding.

One weakness of our study presented in paper IV is the lack of information on debut of symptoms of hyperemesis gravidarum. Information on maternal diet, weight loss or insufficient weight gain during pregnancy was not available. Weight loss/low weight gain during pregnancy has been shown to be a risk factor for delivery of an SGA infant, but has a negative association with pre-eclampsia risk why we doubt that the results regarding pre-eclampsia are influenced by the weight loss. There are no national guidelines for hospitalization of women with hyperemesis gravidarum and the threshold for admission to hospital could vary between different regions, hospitals and doctors. However the proportions of women hospitalized due to hyperemesis was about the same throughout the study years which indicate that the handling of these patients was rather constant and not influenced by changes in the health care system.

Angiopoietins

Angiopoietins are important in both vasculogenesis and angiogenesis and are involved in both implantation and placental development. Since pre-eclampsia seems to be a disorder characterized by an imbalance in the angiogenic – anti-angiogenic state the relation between Ang1 and Ang 2 is of interest. According to our study presented in paper I, there is a significant difference at gestational week 25 and 28 in the Ang-1/Ang-2 ratio in normal pregnancies and pregnancies later developing pre-eclampsia.

Our theory that the levels of the angiopoietins differ in normal pregnancies compared to pregnancies that develop pre-eclampsia is supported by research in the placental field. VEGF has been shown to be of importance in vasculogenesis, angiogenesis and the development of placenta. A number of publications have focused on the importance of the angiopoietins which act together with VEGF. Increased levels of both VEGF and Ang-1 mRNA have been demonstrated in the placenta during normal pregnancy, but the level of Ang-2 mRNA was decreased. It has also been reported that the angiopoietins and their receptors are crucial in the development of the early placenta. Furthermore, in consistent with the theory of inadequate placentation leading to subclinical placental hypoxia, a reduction of pO2 has been shown to up-regulate Ang-2 and VEGF mRNA, but not the expression of Ang-1 or the Tie-2 receptor.
In a study with a cross-sectional design, where women were included at onset of clinical signs of pre-eclampsia, levels of Ang-2 in serum were analysed and a significant difference in women with pre-eclampsia compared to normal pregnancy was demonstrated. These results are not comparable to ours since their blood samples were collected at the onset of clinical signs of pre-eclampsia while ours are collected prospectively and longitudinally before the onset of clinical symptoms.

In another study where levels of angiopoietins at gestational week 12-15 and 16-20 were evaluated, women with subsequent pre-eclampsia compared with controls had a higher level of Ang-2 in gestational week 16-20, while the Ang-1 levels did not differ. When subgroup analyses were made, the increased level of Ang-2 was only found in women with a later, severe pre-eclampsia and women with a subsequent intrauterine growth-restricted foetus. These results support our findings of a lower Ang-1/Ang-2 ratio in women who later develop pre-eclampsia.

Our results indicate that the angiopoietins might be involved in the development of pre-eclampsia and support the theory of an angiogenic imbalance. In the current field of research where biomarkers of pre-eclampsia are investigated, early prediction of pre-eclampsia is the main focus. It would be interesting to perform further studies that describe the Ang-1/Ang-2 ratio between gestational weeks 10 and 25 to find when the difference becomes significant between women who develop pre-eclampsia and healthy controls.

**Histidine-rich glycoprotein**

In addition to the angiogenic imbalance, demonstrated by the angiopoietins above, an excessive inflammatory response and hypercoagulability have been documented in pregnancies complicated by pre-eclampsia. HRG is a protein that interacts with all these processes, a fact that stimulates interest for further investigation into its role in the development of pre-eclampsia, as well as its potential as a predictor of pre-eclampsia. Our results in paper II revealed that plasma levels of HRG decrease during pregnancy in general, with significantly lower levels in gestational weeks 10, 25 and 28 in women who later develop pre-eclampsia, compared to women with a normal pregnancy.

HRG has previously been measured, although with another method of analysis, in normal pregnancies where blood samples from different women at gestational weeks 27 to 42 were analysed. In that study, levels of HRG decreased during the third trimester, which is consistent with our findings. In addition, a study of women in the third trimester described lower HRG levels for women who had developed pre-eclampsia compared to normal
pregnancy, but without a significant difference.\textsuperscript{129} We did not have a significant difference in HRG levels between normal pregnancies and pregnancies later complicated by pre-eclampsia at neither gestational week 33 nor 37. However, our study has increased understanding of HRG levels, since we followed women with both normal pregnancies and those later complicated by pre-eclampsia through the whole pregnancy.

We know that HRG is expressed in embryos\textsuperscript{89} and is present in placenta\textsuperscript{80} but its exact function remains to be elucidated. Our results in paper II revealed a possibility that HRG could be a predictor as early as in gestational week 10.

The suitability of HRG for predicting pre-eclampsia might be improved when combined with uterine artery Doppler measurements or in combination with other markers. In cooperation with St George’s University we were able to perform the study presented in paper III, where we demonstrated that separate measurements of HRG and PI may predict preterm pre-eclampsia, but by combining HRG and PI the accuracy increased. These results are in line with studies proposing a combination of biochemical and biophysical markers for a successful prediction of pre-eclampsia in early pregnancy.\textsuperscript{90}

Early-onset pre-eclampsia has been proposed to have a stronger association with inadequate and incomplete trophoblast invasion and spiral artery remodelling than late-onset pre-eclampsia.\textsuperscript{30,31} In a previous study an association between placental HRG and early-onset pre-eclampsia has been demonstrated\textsuperscript{80}, whereas in this study we present an association between HRG serum level and preterm, but not term, pre-eclampsia.

Both uterine artery Doppler and HRG concentration may reflect the impaired trophoblast invasion per se but since HRG is a negative acute phase reactant, the lower levels could also be a reflection of the inflammatory response described in pre-eclampsia. A combination of tests reflecting the same pathogenic phase in pre-eclampsia may improve the accuracy, but a combination of tests that mirror different aspects of the disorder could achieve an even higher predictive power. Further studies are needed to confirm our results and the use of additional markers might further improve the predictive values.

**Pre-eclampsia and hyperemesis**

Hyperemesis gravidarum is, according to our results in Paper IV, associated with increased risks of pre-eclampsia, placental abruption, and SGA birth. These risks were especially associated with hospitalization due to hyperemesis gravidarum in the second trimester. As pre-eclampsia, placental abruption and SGA birth are associated with abnormal placentation,\textsuperscript{130,131}
our findings indicate an association between abnormal placentation and hyperemesis gravidarum manifested in the second trimester.

The finding of an increased risk of pre-eclampsia for women with hyperemesis gravidarum is in agreement with one former study. Our study has increased understanding about the association between the disorders, since we separated preterm from term pre-eclampsia. The discovery of a stronger association between hyperemesis gravidarum and preterm rather than term pre-eclampsia suggests that hyperemesis gravidarum is associated with abnormal placentation. In addition, after stratifying hyperemesis gravidarum by hospitalization in the first or the second trimester, we could clearly show that it is hyperemesis gravidarum in the second trimester that is associated with preterm pre-eclampsia risk and accordingly with abnormal placentation.

Earlier studies report an association between high levels of total hCG in the second trimester and risk of pre-eclampsia and SGA birth. Our findings are in agreement with these studies, since hyperemesis gravidarum is associated with high levels of hCG. The high levels of hCG in the second trimester could be a compensatory mechanism to an insufficient early trophoblast migration and invasion of the spiral arteries, which could be due to a low level of H-hCG, the variant of hCG that stimulates invasion in very early pregnancy. Thus, abnormal placentation may cause high levels of hCG in the second trimester, which then causes hyperemesis gravidarum with a late onset.

There are also other possible explanations to our findings. Hyperemesis gravidarum is a classical example of an interaction of biological and psychosocial factors. There are associations with both high thyroxin and estradiol levels. HCG is structurally similar to thyroid-stimulating hormone and an increased production of thyroxin is associated with hyperemesis gravidarum. Women with second trimester hyperemesis might have a prolonged or delayed stimulation of thyroxin, compared to women with first trimester hyperemesis. This might affect placentation, since former studies have shown an association between hyperthyreosis and placental dysfunction disorders. Pre-eclampsia is mostly associated with low estradiol levels, and we therefore doubt that estradiol is a link between these disorders.

An epidemiological study cannot explain why hyperemesis gravidarum with a late onset could be associated with placental dysfunction disorders but hyperemesis gravidarum in the second trimester may be a clinical risk predictor to consider when predicting the risk of pre-eclampsia and other disorders associated with abnormal placentation.
Prediction of pre-eclampsia, at present and in the future

Prediction of pre-eclampsia, which would make possible early detection and give the opportunity to intervene by correcting the pathophysiological changes, demands increased knowledge about the process that leads to the development of pre-eclampsia. Through studies of potential predictors we have learned more about the pathophysiology of pre-eclampsia.

A reliable predictive test would help us to individualize the level of surveillance during pregnancy. It is, though, important to remember that there are a number of, mainly low-income, countries around the world, where the majority of the maternal and perinatal complications related to pre-eclampsia appear, that would benefit from just a better and well-functioning maternal care. However, an easy and sensitive predictive test would help to save lives, even in poorer countries, since this could help the midwife to recommend the woman to come for more frequent controls and maybe also to be delivered at a hospital. In high-income countries a predictor of pre-eclampsia could be used to prevent or postpone the disorder by prophylactic treatment.

To be able to intervene with defects in implantation and placentation that might lead to increased risk of pre-eclampsia, the ultimate predictor should be able to identify women with an increased risk as early as in the first trimester. Since pre-eclampsia is a very heterogenous disorder, a predictive test should be constructed by using different markers of importance that reflect different aspects of the pathogenesis. Potential components of such a combination could be anamnestic risk factors, angiogenic, inflammatory and other biochemical factors, uterine artery Doppler and MAP. We postulate that the biochemical markers HRG and the Ang-1/Ang-2 ratio that we have studied would be interesting to study in a combined model for prediction of the disease. Hyperemesis gravidarum in the second trimester as a clinical risk predictor could also be considered, as demonstrated in our epidemiological study.

It has been discussed which category of patients a test should be capable of identifying and, since women with early-onset pre-eclampsia often have worse symptoms and a more severe disease, it might be postulated that this category would be of interest to focus on. This would make it possible to offer prophylactic treatment and increased surveillance, which presumably should be more cost effective with a reduced maternal and foetal morbidity and mortality as a consequence. Potentially, it should be possible to construct a predictive test for pre-eclampsia of a placental origin by including biochemical factors that reflect different steps of inadequate implantation and/or placentation in combination with uterine artery Doppler. Pre-eclampsia with a maternal origin could possibly be identified with a relatively high sensitivity and specificity by a combination of known risk factors and MAP.
The combination of biophysical methods and several other factors makes the tests more expensive and several of the tests use technologies that are not widely available. In low-income countries there is a need for more studies of potential predictors that use simple and inexpensive technology, as well as non-invasive screening methods such as dipstick tests of urine. Some studies have investigated the potential to use urinary analyses for prediction of pre-eclampsia\textsuperscript{136-138} although the results have varied.

The theory that hyperemesis gravidarum in the second trimester is induced by high levels of hCG, which is increased as a compensatory mechanism to low H-hCG early in pregnancy, has aroused interest in H-hCG as another potential predictor of pre-eclampsia. In contrast to hCG produced by syncytial trophoblast, extravillous trophoblast secretes H-hCG that has been demonstrated to stimulate invasion.\textsuperscript{43} H-hCG levels, in serum and in urine, seem to be a reliable test to diagnose pregnancies after in vitro fertilisation treatment as early as six days after embryo transfer, and, in addition, to separate whether a pregnancy is a clinical pregnancy or a biochemical pregnancy.\textsuperscript{139} As a predictor of pre-eclampsia, lower levels of H-hCG in the urine have been demonstrated in the second trimester for women who later developed pre-eclampsia compared with normal pregnancies.\textsuperscript{136} Since H-hCG is of importance from the very beginning of the implantation and is the most common form of hCG during the first weeks afterward, it is an interesting potential predictor. An advantage is the possibility for detecting H-hCG in urine in very early pregnancy. Further studies of the ability for H-hCG to predict pre-eclampsia early in pregnancy would be of interest.

In the search for new potential predictors of pre-eclampsia, GWAS offers, new possibilities to screen the human genome and further evaluate different gene expressions. The result of genetic studies could either be a specific SNP as a predictor of pre-eclampsia or a protein or molecule that the different gene expressions are coding for.

For screening tests in general a premium is placed on a low rate of false positive results but for pre-eclampsia a false negative result could pose a high risk and therefore a somewhat lower specificity than usual should be accepted.\textsuperscript{91} Suggested cut-off values out of the ROC curves in study II and III were chosen to prioritize a high sensitivity, even if relatively low specificity may cause increased concern and anxiety for the women.

With a low specificity, several women who would never have developed pre-eclampsia would be offered prophylactic treatments and increased surveillance. This fact increases the need for the treatment to be safe and without any side-effects. The level of surveillance also has to be acceptable for the women. The possibilities of prophylactic treatment are limited, since no specific treatment is provided, but for a high risk group antiplatelet drugs, i.e. low dose aspirin, seems to have a relative good effect in preventing or postponing the disorder, at least if the treatment is started early in pregnancy.\textsuperscript{113} It is also important to evaluate other different possible
prophylactic treatments, where a substance such as heparin might be of interest. Intervention as early and as specific as possible in the development of pre-eclampsia is recommended. The ultimate would be to start treatment before the trophoblast invasion of the myometrium happens. It might also, in the future, be possible to start a potential prophylactic treatment even before the pregnancy begins, based on genetic testing and counselling. The possibility of over-expressing angiogenic factors in viral vectors for injection in the myometrial wall to optimize the angiogenic status has also been discussed but there are no such studies on-going on humans yet.

At present there are several predictive tests that do reach rather high sensitivity and specificity. The next step is to evaluate whether these tests, in combination with prophylactic treatment and/or increased surveillance, reduce the incidence or postpone diagnosis and improve the maternal and neonatal outcome. To perform these studies a multicenter approach with randomized controlled trials has to be made to achieve a decent number of patients at risk of pre-eclampsia.

The title of this thesis is “Pre-eclampsia – possible to predict?” The answer is “Yes, pre-eclampsia might be possible to predict”. To investigate whether there are any clinical benefits related to prediction, further studies are needed.
Conclusions

Ang-1/Ang-2 ratio increases during pregnancy in all women, but the ratios are significantly lower at gestational weeks 25 and 28 in women who later develop pre-eclampsia compared with normal pregnancies.

Ang-1/Ang-2 ratio is a possible predictor for later onset of pre-eclampsia in a low-risk population.

The level of HRG decreases during pregnancy in all women, but the levels are significantly lower at gestational weeks 10, 25, and 28 in women who later develop pre-eclampsia compared with normal pregnancies.

HRG may be a possible predictor for later onset of pre-eclampsia in the first trimester in a low-risk population.

The combination of HRG and uterine artery Doppler in early pregnancy is a better predictor of preterm pre-eclampsia than each marker individually and may be a possible predictor for later onset of preterm pre-eclampsia.

An association between hyperemesis gravidarum in the second trimester and preterm pre-eclampsia, as well as other placental dysfunction disorders, is demonstrated.
Preeklampsi, som är en graviditetsspecifik multiorgan sjukdom, utgör en av de vanligaste orsakerna till maternell och perinatal morbidity och mortalitet världen över. I Sverige drabbas omkring 3% av alla gravida men i många andra länder är förekomsten högre. Orsaken till preeklampsi är inte fullständigt klarlagd men, framför allt vid tidigt debuterande sjukdom, kan en möjlig förklaring vara en defekt infästning av moderkakan vilket i sin tur orsakar en ökning av substanser som är skadliga för moderns blodkärl. Vid preeklampsi har förändrade nivåer i blodet, av bland annat blodkärlsnybildande faktorer och inflammatoriska markörer, konstaterats flera veckor innan sjukdomsdebut vilket kan användas för att prediktera vilka kvinnor som har en ökad risk att utveckla preeklampsi. Möjligheten att kunna förhindra, eller att modifiera utvecklingen av preeklampsi, ökar ju tidigare som skillnaden mellan en normal graviditet, och en som senare utvecklar sjukdomen, upptäcks.

Blodkärlsnybildning regleras bland annat av Angiopoietin-1 och Angiopoietin-2 och påverkas även av Histidin rikt glykoprotein (HRG) som också är involverad i inflammatoriska processer. Ett annat ämne som påverkar infästningen av moderkakan och blodkärlsnybildningen är Humant chorton gonadotropin (hCG). Tidigare studier har visat att höga nivåer av hCG i andra trimestern är kopplat till utvecklingen av preeklampsi. Höga nivåer av hCG anses även kunna orsaka graviditetsillamående.

Målet med detta avhandlingsarbete ha r varit att uppnå ökad förståelse för bakomliggande orsaker till preeklampsi, att hitta ett tillförlitligt sätt att prediktera preeklampsi med enbart biokemiska markörer eller i kombination med en biofysisk markör såsom dopplerundersökning av arteria uterina samt att undersöka om det finns en korrelation mellan svårt graviditetsillamående och inadekvat infästning av moderkakan.

Till det första delarbete includerades tidigare helt friska kvinnor i samband med inskrivning på mödravården. Blodprover samlades därefter in i graviditetsvecka 10, 25, 28, 33 och 37. Blodproverna från 19 kvinnor som utvecklade preeklampsi och 43 kvinnor med normala graviditeter analyserades med avseende på nivån av Angiopoietin-1 (Ang-1) och Angiopoietin-2 (Ang-2), med hjälp av kommersiellt tillgängliga enzyme-linked immunosorbet assay (ELISA) kit. Analysen visade att kvoten Ang-1/Ang-2 steg under graviditeten för alla kvinnor men i graviditetsvecka 25 och 28 var den kvoten signifikant lägre för kvinnor som senare utvecklade

Sammanfattning på svenska
preeklampsi. Kvoten Ang-1/Ang-2 är således en möjlig prediktor för preeklampsi.

I samma population som beskrivits ovan analyserades dessutom nivåerna av Histidin rikt glykoprotein (HRG) med hjälp av ELISA utvecklat på Institutionen för kvinnors och barns hälsa i Uppsala. Resultatet visade att HRG sjönk under graviditeten för alla kvinnor men nivåerna var signifikant lägre i graviditetsvecka 10, 25 och 28 för kvinnor som senare utvecklade preeklampsi. HRG är alltså en möjlig tidig prediktor för preeklampsi.

För att utvärdera om HRG i kombination med Dopplerundersökning av arteria uterina skulle förbättra den prediktiva förmågan genomfördes en studie i samarbete med St George’s hospital i London. Blodprovstagnning och Dopplerundersökning genomfördes i genomsnitt i graviditetsvecka 14. 175 kvinnor med ökad risk för att utveckla preeklampsi bestående av förstföderskor, kvinnor med essentiell hypertoni, tidigare preeklampsi eller med barn födda små för gestationsåldern (SGA) deltog. 89 kvinnor utvecklade preeklampsi, 27 förlöstes före graviditetsvecka 37 och 62 i fullgången tid. 86 kvinnor hade en normal graviditet. Resultatet visade att kombinationen av HRG och Dopplerundersökning av arteria uterina var bättre på att förutsäga vilka kvinnor som skulle drabbas av preeklampsi före graviditetsvecka 37 än varje markör för sig, med en sensitivitet på 91% och en specificitet på 62%.

Ett eventuellt samband mellan inläggningskravande svårt graviditetsillamående och preeklampsi, samt andra sjukdomar orsakade av defekt infästning av moderkakan, undersökte en kohort bestående av alla singelgraviteter i Medicinska födelse registret mellan åren 1997 och 2009 (n=1,156,050). En länkning till patientregistrerades genomfördes. Riskerna för preeklampsi, moderkaksavlossning, dödföddhet och SGA jämfördes med graviditeter utan inläggningskravande graviditetsillamående som referens samt justerades för moderns ålder, paritet, BMI, längd, rökning, sammanboende med barnafadern, barnets kön, moderns fäktelse, utbildningsnivå, hypertyreos, diabetes mellitus, kronisk hypertoni och år då barnet föddes. Analysen visade att kvinnor med ett första vårdtillfälle på grund av graviditetsillamående i andra trimestern har en mer än fördubblad risk för prematur preeklampsi, tre gånger ökad risk för moderkaksavlossning och en 39%-ig riskökning för SGA. Resultatet talar före ett samband mellan svårt graviditetsillamående och andra sjukdomar och senare insjuknande i sjukdomar associerade med inadekvat infästning av moderkakan.

Sammanfattningsvis har våra studier visat att kvoten mellan Ang-1/Ang-2 samt nivåerna av cirkulerande HRG i plasma avviker hos kvinnor som senare utvecklar preeklampsi. Denna skillnad innebär en möjlighet att prediktera vilka kvinnor som kommer att drabbas av preeklampsi. HRG i kombination med Dopplerundersökning av arteria uterina förbättrar den prediktiva förmågan av tidigt debuterande preeklampsi. Hyperemesis i andra trimestern är en klinisk prediktor att beakta gällande preeklampsi och andra tillstånd orsakade av inadekvat infästning av moderkakan.
Acknowledgement

This thesis was conducted at the Department of Women’s and Children’s Health, Uppsala University.

I would like to thank the women who participated in this study and all those who supported, assisted and encouraged me during this process. I especially wish to express my gratitude to:

**Helena Åkerud**, Associate Professor at the Department of Women’s and Children’s Health, Uppsala University, and my main supervisor, for sharing your great knowledge and enthusiasm about science with me. Your intelligence and how you manage a large family, research, clinical work and training is impressive. Besides, you are a generous and wise person who has opened your home for me during my stays in Uppsala and offered interesting discussions about all things important in life.

**Anna-Karin Wikström**, Associate Professor at the Department of Women’s and Children’s Health, Uppsala University and my co-supervisor, for being so supportive and understanding. You have introduced me into epidemiology with great competence and patience. Your warmth and openness has rendered me many laughs.

**Inger Sundström Poromaa**, Professor at the Department of Women’s and Children’s Health, Uppsala University and my co-supervisor, for your intelligent remarks and interesting discussions and for, together with **Alkistis Skalkidou**, arranging an intensive week of research in Crete which offered both hard work and a hard walk.

**Basky Thilaganathan**, Professor at the Division of Clinical Development Sciences, Department of Obstetrics and Gynecology, St George’s University of London, for your generosity with material, competence and encouragement.

**Ove Axelsson**, Professor Emeritus of the Department of Women’s and Children’s Health, Uppsala University, for valuable support and fruitful discussions.
Jan Gustafsson, Professor and head of the Department of Women’s and Children’s Health, Uppsala University, for giving me the opportunity to perform my PhD-studies in Uppsala.

All coauthors for their contributions to this thesis.

Marju Dahmoun, Kenneth Challis and Kicki Rapp, present and former heads of the Department of Women’s health in Sundsvall, for providing work conditions that enabled me to start this project, encouraging me and giving me the opportunity to develop.

The Department of Research and Development in Sundsvall for financial and practical support and Erling Englund for statistical guidance.

Eva Bergman and Birgitta Segebladh, former colleagues in Sundsvall and now working in Uppsala, for initiating this thesis by introducing me to Helena Åkerud, and for being living examples of how to combine clinical work in Sundsvall with research in Uppsala.

Jesper Agrell, Sahruh Turkmen and Per Kempe, colleagues and friends, in the obstetrical section of the Department of Women’s health in Sundsvall, for close and smooth collaboration with challenging discussions as well as good laughs.

Lotta Andréen, Anna Enander, Anna Palm, Heléne Dalemo Lundin, Lena Enesund and all other colleagues and friends, at the Department of Women’s health in Sundsvall, for the combination of hard work, interesting discussions and an enjoyable atmosphere with many laughs which always has made me go to work with a light step.

Ingela Danielsson and Helena Blom, senior research fellow and PhD student at the Department of Research and Development in Sundsvall, for encouragement and for giving me other perspectives of my research.

Peter, Erik, Gustav, Axel and Astrid Åkerud for letting me be a part of your everyday life and always making me feel welcome.

Helena Nilsson, Karin Langlet, Catarina Haag, Petra Waleij, Lotte Wargentin, Nina Hagwall and Catharina Björkman, the very best friends I can imagine, for better or for worse. I do wish we could meet more often.

Hjalmar och Margret Bolin, my parents in law, for all the help and support throughout the years as well as many nice dinners.
Yngve och Vivi, my parents, for your endless love, support, encouragement and for always being there when needed.

Fredrik, Annika and Sara, my family bonus, for your openness, warmth, wit and generosity. Your talents, although very different, are impressive!

Göran, my beloved husband and closest friend, for sharing my life and stick with me through thick and thin.

Axel, Märta and Theodor, my children and treasures, for being just those you are which make me full of joy and pride. You are the meaning of it all and simply the best!
References


120. Dodd JM, McLeod A, Windrim RC, Kingdom J. Antithrombotic therapy for improving maternal or infant health outcomes in women considered at risk of placental dysfunction. *Cochrane* 2010(6):CD006780.


