Hypertensive disorders in pre-term pregnancy: management and long-term consequences
Langenveld, J.

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Josje Langenveld
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Hypertensive disorders in pre-term pregnancy: Management and long-term consequences

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Promotores: Prof. dr. B.W.J. Mol
              Prof. G.R. Saade

Co-promotor: Dr. W. Ganzevoort

Overige leden: Prof. dr. J.A.M. van der Post
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Chapter 1

General introduction and outline of the thesis
General introduction

Hypertensive disorders of pregnancy, including gestational hypertension (GH) and preeclampsia (PE) complicate 10% of all pregnancies and remain one of the leading causes of maternal mortality and morbidity, both internationally as well as in The Netherlands.\(^1\-^3\) The clinical expression of the disease ranges from mild gestational hypertension or preeclampsia at term to severe conditions in the pre-term period. The maternal syndrome of PE is associated with severe complications (especially at early gestational ages) including eclampsia or other encephalopathies, placental abruption, stroke, pulmonary oedema, liver rupture and renal insufficiency.\(^4\,^5\) The fetal or neonatal consequences include intra-uterine growth restriction, stillbirth and severe morbidity related to pre-term birth. Consequently, neonatal morbidity is strongly associated with the gestational age at onset of preeclampsia, as well as the severity of the maternal syndrome and related preterm delivery.\(^6\-^8\)

Timing of delivery in hypertensive disorders in pregnancy

The only causal treatment of hypertensive disorders in pregnancy is delivery of the placenta and inevitably delivery of the child. Signs and symptoms of GH or PE will subside within a few days after delivery.\(^5\) In the pre-term period however this results in a premature born child with related severe morbidities.\(^6\,^9\) The dilemma of maternal morbidity versus neonatal morbidity in the pre-term period has resulted in two management approaches: the interventionist approach of stabilizing and delivery, versus a more temporizing approach.\(^10\) The interventionist approach is assumed to reduce the maternal risk of deterioration and the related maternal complications and the antenatal fetal risk of stillbirth. On the other hand temporizing management results in increased gestational age and therefore in reduction of neonatal morbidity and mortality.\(^11\,^12\) Throughout gestational ages, different levels of evidence are available concerning the best management. Between 24 and 34 weeks gestational age there is a general consensus that temporizing management is justified if no severe maternal morbidity is present or anticipated and fetal condition is estimated to be satisfactory. Prolongation of pregnancy can be achieved without irreversible maternal morbidity and with improved neonatal outcome provided there is intensive monitoring of maternal and fetal well-being by experienced staff in a high-risk pregnancy care setting. This conclusion is based on several cohort studies and two randomized controlled trials.\(^11\,^13\,^14\) The difficulty of the cohort studies is the variability of inclusion criteria used per study and the different diagnostic criteria used. The two trials were
underpowered to determine safety for temporizing care. Although temporizing management seems to be permitted at early gestational ages, the exact criteria for and moment of delivery is still an area of research. After 37 weeks perinatal outcome overall is known to be good and severe maternal morbidity is therefore not acceptable.\textsuperscript{15,16} Timing of delivery of mild GH and PE has recently been investigated in the HYPITAT trial.\textsuperscript{17} This randomized controlled trial comparing interventionist care versus temporizing care between 36\textsuperscript{+0} and 41\textsuperscript{+0} weeks gestational age, showed less severe hypertensive episodes in the interventionist approach (31% versus 44%, relative risk (RR) 0.71, 95% confidence interval (CI) 0.59 to 0.86). The number of patients randomised between 36 and 37 weeks was too low to draw definite conclusions. Importantly, induction of labour in the interventionist group showed a trend to a lower cesarean section rate (RR 0.75, 95% CI 0.55 to 1.0). Perinatal outcome was not different between groups and there was a reduction in costs and better maternal quality of life.\textsuperscript{18,19} Between 34 and 37 weeks of gestational age there remains uncertainty on the best policy (temporizing versus interventionist management) as evidence for this specific group is missing. With lack of good clinical evidence on the subject and the resulting practice variation, we focus specifically on this group in this thesis.

Long term consequences

A pregnancy complicated by a hypertensive disorder is a window to assess future expected health for this mother and her child. The long term health problems are related to general health conditions and psychological consequences. General maternal health issues after a pregnancy complicated by a hypertensive disorder have been an intensively researched area in the past decade. There is abundant evidence for increased risk of cardiovascular and metabolic diseases and related death after a history of a hypertensive complication during pregnancy.\textsuperscript{20-22} Pregnancy is seen as a “stress-test” for the vascular system of the mother and having preeclampsia is an expression of a failed stress-test. Not only the future health of the mother is at state, but also the fetus may experience long-term consequences, in line with the Barker hypothesis (fetal origin of adult disease).\textsuperscript{23} Offspring from women who experienced a hypertensive disorder during their pregnancy, are at increased risk of stroke later in life.\textsuperscript{24} Additionally preeclampsia is associated with being small for gestational age (SGA) as a result of a compromised utero-placental perfusion. Being small for gestational age is related to cardiovascular disease later in life.\textsuperscript{25,26} We speculated that this SGA fetus might already have a compromised vascular system and in consequence it might have a higher risk for developing preeclampsia later in life in case of a
female fetus. With vascular reactivity studies we aimed to unravel some of the pathophysiological mechanism of preeclampsia and its long-term consequences. Apart from the general health consequences, the psychosocial impact of a pre-term delivery due to a hypertensive disorder is often huge and associated with high incidences of symptoms of post traumatic stress disorder and depression.\textsuperscript{27,28} The decision for future pregnancies largely depends on the information from the obstetrician on the estimated risk of recurrence of a pre-term delivery and the related severe maternal morbidity and serious neonatal morbidity. From a medical point of view, such counselling may influence management during the subsequent pregnancy. Information about recurrence is fragmentary and outdated, with recurrence rates ranging from as high as \textasciitilde 65\% to as low as 5\%.\textsuperscript{29,30} This variation is likely because of population selection differences and small numbers. Well numbered cohort studies that consecutively included their patients are missing, as well as individual risk prediction models. In the second part of this thesis we answered questions concerning recurrence rates and prediction of hypertensive disorders in the next pregnancy.

**Outline of this thesis**

The aim of this thesis was to address specific questions concerning long term consequences, recurrence rates and management of hypertensive disorders during pregnancy in the pre-term period. *Part I – fundamental research* – describes vascular reactivity studies in offspring using mice models with a compromised vascular system in view of the “fetal origin of adult disease” hypothesis. *Part II – clinical research* – focuses on recurrence rates, prediction and management of hypertensive disorders in the pre-term period. *Part III* contains the – summary and general discussion – describing the general considerations and focuses on future research questions.

**Part I   Fundamental research**

Chapter 2 explores the underlying pathophysiological mechanism of the impact of a compromised uterine environment on growth and vascular function of offspring and the adaptive mechanisms from successive pregnancies compared to first pregnancies.

Chapter 3 explores the underlying pathophysiological mechanism of the long-term vascular consequences on offspring who developed in a compromised uterine environment.
Part II Clinical research

Chapter 4 is an overview of existing literature on prediction and prevention of preeclampsia.

Chapter 5 is a systematic review of published evidence on recurrence risk of a delivery before 34 weeks of pregnancy due to a severe hypertensive disorder.

Chapter 6 is a report of a study on the recurrence risk of a delivery below 34 weeks due to a hypertensive disorder, after a history of an early onset hypertensive disorder and identifies parameters that predict adverse outcome.

Chapter 7 is a report of a study on the recurrence risk of a hypertensive disorders after a history of a delivery between 34 and 37 weeks of gestation due to a hypertensive disorder and identifies parameters that predict adverse outcome.

Chapter 8 explores the neonatal morbidity of children born between 34 and 37 weeks of gestation from pregnancies complicated by a hypertensive disorder.

Chapter 9 describes the study protocol of a Dutch multicentre randomized controlled trial assessing the (cost-) effectiveness of induction of labour versus expectant monitoring for gestational hypertension or mild pre-eclampsia between 34 and 37 weeks' gestation (HYPITAT-II trial).

Part III Summary and general discussion

Chapter 10 is a summary and general discussion of the previous chapters, and describes implications for future research.
References


Part I

Fundamental research
Enhanced growth and improved vascular function in offspring from successive pregnancies in endothelial nitric oxide synthase knockout mice

Monica Longo
Venu Jain
Josje Langenveld
Yuri P. Vedernikov
Robert E. Garfield
Gary D.V. Hankins
Garland D. Anderson
George R. Saade

Abstract

Objective: Transgenic mice that lack endothelial nitric oxide synthase have offspring with growth deficiency and abnormal vascular reactivity in later life. Our objective was to evaluate the role of parity in the modulation of the fetal programming of growth and vascular responses in these transgenic mice.

Study design: Oligoparous (0-2 previous pregnancies) and multiparous (5-9 previous pregnancies) nitric oxide synthase knockout (−/−KO) female mice were bred with nitric oxide synthase−/−KO and wild type (+/+WT) male mice to produce nitric oxide synthase−/−KO and maternally derived heterozygous (+/−Mat) litters. The pups were weighed weekly. Carotid arteries of the adult females from these litters were used for in vitro vascular reactivity studies.

Results: Nitric oxide synthase knockout and nitric oxide synthase synthase maternal litters that were born to oligoparous mothers had significant growth lag compared with corresponding multiparous litters. Length-tension characteristics were not different between the groups. However, optimal diameter, which is a measure of vascular tensile properties and resistance, was decreased in oligoparous compared with multiparous female offspring. Acetylcholine-mediated vasorelaxation was abolished, and contraction by phenylephrine and Ca++ was increased in oligoparous, but not multiparous, female offspring (P < .05).

Conclusion: These data support the role of abnormal uterine environment in the fetal programming of postnatal growth and vascular function in later life. Successive pregnancies may lead to maternal uterine adaptations that bypass the lack of a functional nitric oxide synthase, which leads to improvement in postnatal growth and vascular function in the offspring. Given the reported effect of parity on the risk of preeclampsia, similar mechanisms may be operative in human pregnancy.
Introduction

As hypothesized by Barker and Osmund, stimuli or insults to the fetus during the critical period of intrauterine development lead to “fetal programming” and produce adaptive changes in fetal anatomy, physiology, and metabolism that have long-term consequences. Maternal hemodynamic changes during pregnancy influence fetal growth through their impact on uteroplacental perfusion. These changes, with onset in early pregnancy, contribute to the increase in the uteroplacental blood flow. There is growth of new vessels and remodeling of existing vessels; vascular refractoriness to vasoconstrictor agents and increased production of vasodilators include nitric oxide.

Defects in the nitric oxide pathway have been implicated in the causation of various vascular pathologic states that include hypertension and intrauterine growth restriction. Three nitric oxide synthase (NOS) isoforms (ie, neuronal NOS [NOS1], inducible NOS [NOS2], and endothelial NOS [NOS3]) have been described. In the vasculature, NOS3 is the main isoform that is responsible for the production of nitric oxide. Infusion of NOS inhibitors during pregnancy causes hypertension and fetal growth restriction. Nitric oxide plays a significant role in maintaining adequate uteroplacental perfusion. NOS3-knockout mice are hypertensive and lack endothelium-dependent vasodilation.

We have shown previously that female offspring of NOS3-knockout female mice are growth restricted and have abnormal vascular function during their adult life, as compared with the genetically identical offspring of wild-type female mice. The results in this animal model of genetically induced abnormal uteroplacental perfusion indicate a fetal programming effect of the abnormal uterine environment, with impact on postnatal growth and future vascular reactivity in the offspring. Compromised uteroplacental blood flow is a common denominator in hypertensive disorders during pregnancy, especially preeclampsia. Indeed, there is a strong relationship between preeclampsia and fetal growth restriction. In addition, preeclampsia occurs more frequently in nulliparous women (3%-7%) than in multiparous women (0.8%-5%). Thus, we hypothesized that, with sequential pregnancies in the same animal, adaptive changes occur in the maternal/uterine environment that result in amelioration of the fetal programming effects in offspring of successive gestations.
Material and methods

Animals
Female mice that are homozygous for the disruption of the endothelial NOS gene (NOS3\(^{-/-}\)-KO strain: C57BL/6J-NOS3\(^{-/-}\)) and their age matched wild-type controls (NOS3\(^{+/+}\)-WT strain: C57BL/6J-NOS3\(^{+/+}\)) were obtained from Jackson Laboratory (Bar Harbor, Me). The animals were housed separately in temperature- and humidity-controlled quarters with constant light/dark cycles of 12 hours/12 hours and were provided with food and water ad libitum.

Oligoparous (0-2 previous pregnancies) and multiparous (5-9 previous pregnancies) NOS3\(^{-/-}\)-KO female mice were mated with NOS3\(^{-/-}\)-KO and NOS3\(^{+/+}\)-WT male mice to produce 4 types of litters: oligoparous-NOS3\(^{-/-}\)-KO, oligoparous-NOS3\(^{+/+}\)-Mat, multiparous-NOS3\(^{-/-}\)-KO, and multiparous-NOS3\(^{+/+}\)-Mat (superscript Mat indicates maternal source of the nonfunctional NOS3 allele). In the oligoparous-NOS3\(^{+/+}\)-Mat and oligoparous-NOS3\(^{-/-}\)-KO litters, the pups matured in an oligoparous mother; the multiparous-NOS3\(^{+/+}\)-Mat and multiparous-NOS3\(^{-/-}\)-KO pups matured in a multiparous mother.

After delivery, female pups from the 4 groups were weighed weekly until early adulthood (week 6). Mature cycling female mice (7-8 weeks old) from the 4 litters were used for in vitro vascular reactivity experiments. The animals were killed by carbon dioxide inhalation. All procedures were approved by the Animal Care and Use Committee of the University of Texas Medical Branch.

In vitro experiments
Two-millimeter segments of carotid artery from the mice were dissected and mounted over 25-μm tungsten wires in a myograph (model 410A; J.P. Trading I/S, Aarhus, Denmark).\(^{18}\) The preparations were bathed in physiologic salt solution that was maintained at 37°C (pH approximately 7.4), and a mixture of 95% oxygen/5% carbon dioxide was bubbled continuously through the solution. Force was recorded continuously by an isometric force transducer and analyzed with Windaq software (Dataq Instruments, Akron, Ohio). With Myosight software (J.P. Trading I/S), length-tension relationship between internal circumference and wall tension (length-tension curve) was determined to characterize the viscoelastic properties of vascular rings. The slope that was obtained from the length-tension curve is a measure of elasticity of the vessel wall; the greater the slope, the more rigid the vessel wall. Optimal diameter of the vessels was calculated with the Laplace equation.\(^{18}\) In addition to being dependent on vessel elasticity, the optimal diameter of the vessels is a measure of the vascular diameter at a specific transmural pressure and therefore an estimate of vascular resistance.\(^{18}\) The rings
were contracted twice with 60 mmol/L KCl to stabilize vascular responsiveness. The second KCl response was used as the reference contraction for data analysis. Contractile responses to $\alpha$-adrenergic agonist phenylephrine ($10^{-9}$–$10^{-5}$ mol/L) were assessed. In addition, relaxant responses to endothelium-dependent relaxant acetylcholine ($10^{-9}$–$10^{-5}$ mol/L) were examined in vessels that were precontracted with phenylephrine ($10^{-7}$–$10^{-6}$ mol/L to match amplitude of contractions in the different groups). Finally and after equilibration of the vessels in high-K+ Ca++-free solution, contractile responses to cumulative concentrations of Ca$^{++}$ (0.05-5 mmol/L) were studied to evaluate responsiveness of the vascular smooth muscle.

**Data analysis**

Data are expressed as mean ± SEM. For the experiments that examined vascular elastic properties, data are expressed as the length-tension slope and optimal diameter of the vessels. For the vascular reactivity studies, the concentration-response curves to the agents that were tested were constructed. In addition, the logarithm of the concentration that produced 50% of the maximal effect (log IC$_{50}$) and the maximal effect were also compared. The Kolmogorov-Smirnov test was used to check for normality of data; 1-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparisons test were used for statistical analysis. A probability value of <.05 was considered significant.

**Drugs and solutions**

The drugs that were used in the in vitro experiments were acetylcholine hydrochloride and phenylephrine hydrochloride (Sigma Chemical Company, St Louis, Mo). Stock solutions of the drugs ($10^{-2}$ mol/L) were prepared in deionized water and stored at −20°C. The following composition of physiologic salt solution was used: NaCl, 119 mmol/L; KCl, 4.7 mmol/L; NaH$_2$PO$_4$, 1.2 mmol/L; NaHCO$_3$, 25 mmol/L; MgCl$_2$, 1.2 mmol/L; CaCl$_2$, 2.5 mmol/L; ethylenediaminetetraacetic acid, 0.026 mmol/L; and glucose, 11.5 mmol/L. In experiments that used high-K$^+$ Ca$^{++}$-free physiologic salt solution (80 mmol/L K$^+$), Ca$^{++}$ was omitted from the physiologic salt solution, and K$^+$ replaced NaCl to maintain the solution iso-osmotic.
Results

Postnatal weight gain
The weight of female pups from the oligoparous female mice (ie, oligoparous-NOS3\textsuperscript{−/−KO} and oligoparous-NOS3\textsuperscript{+/−Mat} mice) was significantly less than female pups of the multiparous female mice (ie, multiparous-NOS3\textsuperscript{−/−KO} and multiparous-NOS3\textsuperscript{+/−Mat} mice) in the first and last 2 weeks (Figure 1).

Passive characteristics of carotid artery
The length-tension curves were not significantly different among the 4 groups (Figure 2). However, the optimal diameter of the vessels was significantly smaller in the oligoparous-NOS3\textsuperscript{−/−KO} and oligoparous-NOS3\textsuperscript{+/−Mat} mice, compared

Figure 1. Postnatal weight gain (grams) up to adulthood in female homozygous NOS3 knockout (NOS3\textsuperscript{−/−KO}) and maternally derived heterozygous NOS3\textsuperscript{+/−} (NOS3\textsuperscript{+/−Mat}) offspring of oligoparous (OLIGO) and multiparous (MULTIP) NOS3\textsuperscript{−/−KO} female mice (n = 8-10 mice in each group). One-way ANOVA and Newman-Keuls multiple comparisons test were used. Asterisk, A probability value of .05 for oligoparous-NOS3\textsuperscript{−/−KO} and oligoparous-NOS3\textsuperscript{+/−Mat} versus multiparous-NOS3\textsuperscript{−/−KO} and multiparous-NOS3\textsuperscript{+/−Mat}

Figure 2. Slopes of the length-tension plots of the carotid arteries of female homozygous NOS3 knockout (NOS3\textsuperscript{−/−KO}) and maternally derived heterozygous NOS3\textsuperscript{+/−} (NOS3\textsuperscript{+/−Mat}) offspring of oligoparous (OLIGO) and multiparous (MULTIP) NOS3\textsuperscript{−/−KO} female mice (n = 8-10 mice in each group). One-way ANOVA and Newman-Keuls multiple comparisons test were used \((P > .05)\).
with multiparous-NOS3\(^{-/-}\)KO and multiparous-NOS3\(^{+/−}\)Mat mice (Figure 3), which indicates a possible increase in the vascular smooth muscle that could lead to the increased vascular resistance in the oligoparous offspring compared with the multiparous offspring.

**In vitro reactivity of carotid artery**

Phenylephrine contraction was increased significantly in oligoparous-NOS3\(^{-/-}\)KO and oligoparous-NOS3\(^{+/−}\)Mat mice, compared with multiparous-NOS3\(^{-/-}\)KO and multiparous-NOS3\(^{+/−}\)Mat mice \((P<.05; \text{Figure 4, Table 1})\). However, the IC\(_{50}\) was significantly different only in the oligoparous-NOS3\(^{-/-}\)KO mice, compared with oligoparous-NOS3\(^{+/−}\)Mat, multiparous-NOS3\(^{-/-}\)KO, and multiparous-NOS3\(^{+/−}\)Mat mice.

Acetylcholine produced vasorelaxation in a dose-dependent manner in multiparous-NOS3\(^{-/-}\)KO and multiparous-NOS3\(^{+/−}\)Mat mice. In contrast,
acetylcholine produced a small contractile effect in the oligoparous-NOS3−/−KO and oligoparous-NOS3+/−Mat mice (Figure 5, Table II).

The responses to the cumulative concentration of Ca++ were significantly higher in oligoparous-NOS3−/−KO and oligoparous-NOS3+/−Mat mice, compared with

Table I. Area under the phenylephrine concentration-response curves (AUC; arbitrary units), logarithm of the molar concentration that produces log IC50 and maximal effect (expressed as percentage of reference contraction to 60 mmol/L KCl) in the carotid arteries of female homozygous NOS3−/−KO and NOS3+/−Mat offspring of oligoparous and multiparous NOS3−/−KO female mice (n=8-10 mice in each group)

<table>
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<th>AUC</th>
<th>Log IC50</th>
<th>Maximal effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligoparous-NOS3−/−KO</td>
<td>436.93 ± 33.64*</td>
<td>−8.1 ± 0.4†</td>
<td>178.39 ± 8.6*</td>
</tr>
<tr>
<td>Oligoparous-NOS3+/−Mat</td>
<td>521.61 ± 51.60*</td>
<td>−6.9 ± 1.3</td>
<td>210.78 ± 21.3*</td>
</tr>
<tr>
<td>Multiparous-NOS3−/−KO</td>
<td>173.18 ± 16.65</td>
<td>−6.7 ± 0.07</td>
<td>92.0 ± 8.8</td>
</tr>
<tr>
<td>Multiparous-NOS3+/−Mat</td>
<td>216.57 ± 19.93</td>
<td>−6.8 ± 0.07</td>
<td>111.0 ± 6.6</td>
</tr>
</tbody>
</table>


Figure 5. Acetylcholine (ACh) concentration-responses curves in the carotid arteries of female homozygous NOS3 knockout (NOS3−/−KO) and maternally derived heterozygous NOS3+/− (NOS3+/−Mat) offspring of oligoparous (OLIGO) and multiparous (MULTIP) NOS3−/−KO female mice (n = 8-10 mice in each group). The responses are expressed as percent relaxation from the phenylephrine (PE) contraction. One-way ANOVA and Newman-Keuls multiple comparisons test were used. Asterisk, A probability value of < .05 for oligoparous-NOS3−/−KO and oligoparous-NOS3+/−Mat mice, multiparous-NOS3−/−KO mice, and multiparous-NOS3+/−Mat mice.

Table II. Area under the acetylcholine concentration-response curves (AUC; arbitrary units) and maximal effect (expressed as percentage relaxation of phenylephrine contraction) in the carotid arteries of female NOS3−/−KO and NOS3+/−Mat offspring of oligoparous and multiparous NOS3−/−KO female mice (n = 8-10 mice in each group)

<table>
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<tr>
<th></th>
<th>AUC</th>
<th>Log IC50</th>
<th>Maximal effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligoparous-NOS3−/−KO</td>
<td>64.39 ± 11.36*</td>
<td>NA</td>
<td>−12.5 ± 6.39*</td>
</tr>
<tr>
<td>Oligoparous-NOS3+/−Mat</td>
<td>90.30 ± 17.46*</td>
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<td>−16.3 ± 9.9*</td>
</tr>
<tr>
<td>Multiparous-NOS3−/−KO</td>
<td>238.32 ± 27.33</td>
<td>NA</td>
<td>68.7 ± 7.0</td>
</tr>
<tr>
<td>Multiparous-NOS3+/−Mat</td>
<td>309.00 ± 27.33</td>
<td>NA</td>
<td>83.2 ± 3.3</td>
</tr>
</tbody>
</table>

Our results support the hypothesis that cumulative adaptive changes occur in the uterine environment during successive gestations that may serve to counterbalance an inherent vascular perfusion defect, which improves fetal growth and future vascular function in the offspring. In humans, this is seen as a decrease in the incidence of preeclampsia and as a trend for greater birth weights in successive pregnancies.17,19 In this animal model with a genetic defect in vascular function, the fetal programming effect that is evident in the offspring of oligoparous NOS3−/−KO mice and that manifests as a delay in postnatal growth
and abnormalities in vascular function during adulthood was not seen in the genetically similar offspring of multiparous NOS3\(^{-/-}\)KO mice. Programming is defined as a permanent response to an insult or stimulus that is experienced at a critical period of fetal development, during which organogenesis and differentiation take place.\(^{20}\) During these periods of rapid growth and maturation, the fetus is vulnerable to variations in nutrient or oxygen supply and other perturbations that occur in the uterine environment. The immediate response of the fetus is to survive using its own substrate, which leads to a slowing of fetal growth.\(^{2,20}\) As such, when the fetal programming occurs during the defined critical periods of development, it may result in long-term or permanent changes to organ morphology and physiology that can lead to permanent consequences later in life. Low birth weight is known to be associated with an increased risk of the development of cardiovascular disease, coronary artery disease, and diabetes mellitus in adulthood.\(^{1}\)

The results from this study confirm our previous findings that, in the absence of maternal NOS3 expression, the abnormal uterine environment results in fetal programming, with delay in postnatal growth.\(^{13,14}\) Interestingly, this occurred in the offspring of the oligoparous, but not the multiparous, female mice, which suggests that the fetal programming effects of the abnormal uterine environment in mice that lacks functional NOS3 diminish with successive pregnancies. Similarly, we have demonstrated an increased response to contractile agents and the absence of endothelium-dependent vasodilation that are associated with decreased vascular distensibility in offspring of oligoparous mothers compared with offspring of multiparous mothers. It is unlikely that advancing maternal age may be responsible for the noted effects, because age has a negative rather than a positive effect on reproduction. These observations support a cumulative adaptive change in the uterine environment, with the deleterious effects diminishing with successive pregnancies, which lead to an improvement in the vascular function of the successive offspring in their later life. In the absence of NOS3 expression in the maternal/uterine environment, alternate pathways that may include the other NOS isoforms and other vasodilators, such as prostacyclin or endothelium-derived hyperpolarizing factor, may serve to compensate for the decreased nitric oxide production in the maternal vasculature.\(^{21}\) These changes may lead to an improvement in uteroplacental perfusion, which prevents the abnormalities in postnatal growth and future vascular function that are seen in the first pregnancies. Pregnancy-induced hypertensive disorders (which include preeclampsia) are known to occur primarily in first pregnancies, with a risk of recurrence of 47% in the second pregnancy and 21% overall.\(^{17,22}\) The beneficial effects of a previous pregnancy also have been explained by the vascular remodeling in the spiral arteries that occur early in pregnancy and can lead to increased uteroplacental
perfusion with increasing parity.\textsuperscript{23} In addition, pregnancy-induced increase in cardiac output and a decrease in peripheral vascular resistance is greater in multiparous women.\textsuperscript{24} The adaptations that occur during the first pregnancy may persist in the following pregnancies and may lower the risk of cardiovascular disease, which includes pregnancy-induced hypertension or preeclampsia in the mother.\textsuperscript{24} Further studies are needed to examine maternal cardiovascular and uteroplacental function during successive gestations in this animal model.

Despite several epidemiologic studies that have pointed to an association between fetal programming and the onset of cardiovascular disease in later life, few studies have been conducted into the long-term consequences of hypertensive disorders during pregnancy because of the intrinsic limitations of long-term retrospective and prospective studies.\textsuperscript{25} Our data raise the possibility that, in women with poor obstetric history, multiparity may reduce the associated risk of fetal programming, thereby improving fetal and postnatal growth and future vascular function of the offspring.

In conclusion, multiparity appears to circumvent the adverse effects of abnormal maternal vascular function on fetal programming, as evidenced by improved postnatal growth and future vascular function in the offspring of mice that lack a functional endothelial NOS. Further comparative studies are needed in offspring from human pregnancies that are associated with preeclampsia or intrauterine growth restriction and subsequent pregnancies to confirm the clinical relevance of our findings.
References


Enhanced growth and improved vascular function in offspring from successive pregnancies


In utero programming of adult vascular function in transgenic mice lacking low-density lipoprotein receptor

Josje Langenveld
Fanxian Lu
Egle Bytautienė
Garland D. Anderson
George R. Saade
Monica Longo

Abstract

Objective: The objective of this study was to examine the role of maternal hypercholesterolemia in fetal programming of adult vascular function using transgenic mice lacking the low-density lipoprotein receptor (LDLR).

Study Design: Homozygous LDLR knockout mice (B6.129S7-Ldlr<sup>tm1Her</sup>/J, LDLR<sup>-/-KO</sup>) and their wild-type controls (C57BL/6J, LDLR<sup>+/+WT</sup>) were cross-bred to produce 4 litter groups: LDLR<sup>-/-KO</sup>, maternally derived heterozygous (LDLR<sup>±Mat</sup>), paternally derived heterozygous (LDLR<sup>±Pat</sup>) and LDLR<sup>+/+WT</sup>. Female and male offspring were killed at 10-12 weeks of age, and carotid arteries were used for in vitro experiments.

Results: The dose responses to phenylephrine were significantly higher in LDLR<sup>-/-KO</sup> and LDLR<sup>±Mat</sup> male offspring. The contractile responses to phenylephrine in female mice were significantly increased only in the LDLR<sup>-/-KO</sup> offspring. Maximal Ca<sup>2+</sup> contraction was higher in LDLR<sup>-/-KO</sup> male and female offspring.

Conclusion: Despite being genomically similar, heterozygous offspring that developed in a hypercholesterolemic maternal environment had abnormal vascular responses later in life compared with those that developed in a normal environment.
Introduction

Epidemiologic studies have shown that the atherogenic process already begins during fetal development.\textsuperscript{1,2} Fatty streaks, the earliest lesions of arteriosclerosis, are already present in fetal arteries. The process of formation is enhanced greatly in fetuses who are born to mothers with hypercholesterolemia.\textsuperscript{3,4} This suggests that a high maternal level of cholesterol during fetal development may accelerate the atherogenesis process that occurs later in adult life.\textsuperscript{5} However, the effect of high maternal cholesterol level on the intrauterine environment and fetal vascular development is still not clear.\textsuperscript{6-8} Arteriosclerosis is characterized by a long lag time between onset and clinical manifestations.\textsuperscript{9} The endothelial dysfunction is considered an early marker of arteriosclerosis, preceding angiographic and ultrasonic evidence of arteriosclerotic plaques.\textsuperscript{10,11} Damage to the endothelium alters the balance between vasoconstricting and vasodilating agents and initiates a number of events that promote or exacerbate arteriosclerosis. In the process of arteriosclerosis, lipoprotein particles, especially low-density lipoprotein (LDL), enter the arterial wall and undergo various modifications, such as oxidation. Modified LDL is chemotactic for monocytes and macrophage. This sets off a cascade of cellular responses within the artery and induces the production of cytokines such as tumor necrosis factor \(\alpha\), interleukin-1, interleukin-6 and interleukin-8. These cytokines increase the binding of LDL to the endothelium.\textsuperscript{12-14} Oxidation of LDL has been proposed as an important mechanism in the pathogenesis of the arteriosclerotic process.\textsuperscript{15,16} Although the placenta is impermeable to the LDL particles themselves, the maternal oxidative end-product that results from the maternal hypercholesterolemic status may increase lipid peroxidation products in the fetal plasma, either directly or indirectly. Furthermore, it has been shown in animal models that fetal genes can be up- or downregulated, depending on the hypercholesterolemic status of the mother.\textsuperscript{17-20} Napoli et al\textsuperscript{19} showed an upregulation of 57 genes and a downregulation of 82 genes (such as the fibroblast growth factor binding protein gene that is involved in neovascularization and cancer growth) in the aorta of pups that were born to hypercholesterolemic mothers. The maternal cholesterolemic status can impact fetal vascular development and beyond, either through the transmission of a genetic predisposition for hypercholesterolemia or through altered fetal programming of vascular function. Epidemiologic studies may not differentiate between these 2 potential influences on vascular function in adult life.

Epidemiologic studies have their limitations. The genetic heterogeneity of the human population and the variability in diets and other confounders make it almost impossible to measure the exact interaction between the maternal environment and the genetic contribution to the fetus. Knockout animals are genetically altered...
animals, with ≥1 specific genes disrupted. With these exact gene disruptions, a knockout animal model not only give us the opportunity to identify specific interaction between environmental and genetical contribution to the fetus but also to find out the mechanism behind this specific interaction, which is a great advantage compared to epidemiologic studies. We sought to study the effect of maternal hypercholesterolemia on fetal programming using an animal knockout model. We used an LDL receptor knockout mouse model (LDLR-/-KO) lacking a functional LDL receptor. The LDL-receptor (LDLR) is involved in the clearing of lipoproteins from the circulation, and its lack leads to hypercholesterolemia and arteriosclerosis. LDLR-/-KO mice fed a normal diet develop moderate fatty streak lesions and intimal thickening with foam cells and smooth muscle cell infiltration. By crossbreeding LDLR-/-KO mice and their wild-type control mice (LDLR+/+WT), heterozygous litters that have developed in normal vs hypercholesterolemic environments, but are genomically similar, can be produced and used to determine the genetic vs environmental contributions to fetal programming.

The objective of this study was to examine the effect of maternal hypercholesterolemia on fetal vascular programming with the use of transgenic mice lacking LDLR. To assess vascular function in the offspring, we used the carotid artery, a resistance artery, and an artery with a predilection for atherosclerotic plaques and evaluated the vascular reactivity, which is 1 of the first mechanisms affected by the arteriosclerotic process.

**Material and methods**

**Animals**

Female and male homozygous knockout mice disrupted for LDLR gene (LDLR-/-KO, LDLR-/- knockout, strain: B6.129S7-Ldlr<sup>tm1Her</sup>/J) and their wild-type age-matched controls (LDLR+/+WT, LDLR+/+ wild-type strain: C57BL/6J, LDLR+/+WT) were obtained from Jackson Laboratory (Bar Harbor, ME). All of these animals were from the same bench and thus with the same genetic and environment background. The animals were housed separately in temperature- and humidity-controlled quarters with constant 12-hour light:dark cycles and were provided with food and water ad libitum. Female and male LDLR-/-KO mice and their LDLR+/+WT were crossbred to produce heterozygous pups that developed in a hypercholesterolemic knockout mother (LDLR± maternally derived pups; LDLR±<sup>Mat</sup>) or normal wild-type mother (LDLR± paternally derived pups; LDLR±<sup>Pat</sup>). Except for the parental source of the single defective allele, both groups of heterozygous pups were genetically similar and had 1 functional LDLR gene. In addition, female and male LDLR-/-KO
mice and their \( \text{LDLR}^{+/+} \text{WT} \) controls were bred to obtain \( \text{LDLR}^{-/-} \text{KO} \) and \( \text{LDLR}^{+/+} \text{WT} \) homozygous litters. Adult 3-month-old male and female offspring from these 4 groups of litters were used for the in vitro experiments. Each experimental group consisted of 6-10 offspring. The animals were killed by \( \text{CO}_2 \) inhalation in a closed chamber. The carotid arteries were dissected and used for the in vitro vascular reactivity experiments. The Animal Care and Use Committee of the University of Texas Medical Branch approved all procedures.

**Diet**

All mice were fed the same regular diet (Teklad 7012; Harlan Teklad LM-485 Mouse-Rat Sterilizable diet; Harlan Teklad, TX) that consisted of 19.92% protein, 5.67% fat, 4.37% fibers, 6.48% ash, and 53.66% nitrogen-free extract.

**Drugs and solutions**

The drugs that were used in the experiments were acetylcholine hydrochloride, phenylephrine hydrochloride, serotonin hydrochloride, and calcium from Sigma (St. Louis, MO). Stock solutions of all drugs (10\(^{-2}\) mol/L) were prepared in deionized water and stored at -20°C. The Krebs solution that was used was prepared in the following manner: glucose, 11.5 mmol/L; NaCl, 119.0 mmol/L; KCl, 4.7 mmol/L; MgSO\(_4\), 1.2 mmol/L; KH\(_2\)PO\(_4\), 1.2 mmol/L; NaHCO\(_3\), 25.0 mmol/L; ethylenediaminetetraacetic acid, 0.026 mmol/L; and CaCl\(_2\), 2.5 mmol/L. In the experiments that used high \( \text{K}^+ \) \( \text{Ca}^{2+} \)-free solution (80 mmol/L \( \text{K}^+ \)), \( \text{Ca}^{2+} \) was omitted from the physiologic salt solution, and \( \text{K}^+ \) replaced NaCl to maintain osmolality of the solution.

**In vitro experiments**

Two-millimeter segments of the carotid artery from the 4 groups of offspring were isolated and mounted in a wire myograph (Multi Myograph System-610M; J.P. Trading J/S, Aarhus, Denmark) over 25-μm tungsten wires. The preparations were bathed in Krebs solution and maintained at 37°C (pH 7.4), and a mixture of 95% \( \text{O}_2 \) and 5% \( \text{CO}_2 \) was bubbled continuously through the solution. The force was recorded continuously by an isometric force transducer and analyzed with Power Lab data acquisition and playback software (ADI Instruments, Colorado Springs, CO). The experiments were performed at a ring diameter of the vessel equal to 0.9 of the optimal diameter. The optimal diameter is an estimate of the vascular diameter in situ at a specific transmural pressure. The optimal diameter and the passive tension applied to the vessels (3.5 mN) were determined in previous experiments that were done in our laboratory. After stabilization of the vascular tone, the rings were contracted twice with 60 mmol/L KCl to enhance the vascular
responsiveness. The second response was used as reference contraction for data analysis. After equilibration in Krebs solution, contractile responses to cumulative concentrations of the α-adrenergic agonist phenylephrine (10^-10 to 10^-5 mol/L) and serotonin (10^-10 to 10^-5 mol/L) were performed and followed by a single dose of acetylcholine (10^-6 mmol/L) to evaluate endothelial function. Only experiments with intact endothelium were used in our final analysis. We also evaluated the relaxant response to the endothelium-dependent acetylcholine (10^-10 to 10^-5 mmol/L) after precontraction with phenylephrine (10^-7 to 10^-6 mmol/L). After equilibration of the vessel in high-K⁺ Ca²⁺-free solution, contractile responses to cumulative concentrations of Ca²⁺ (0.05-5 mmol/L) were studied to evaluate the responsiveness of the vascular smooth muscle.

Data analysis

Data are expressed as mean ± SEM, with “n” representing the number of animals used in each experiment. For the vascular reactivity studies, the concentration-response curves to the agents that were tested were constructed. Responses at each concentration and the maximal effect were compared among the different groups. The 4 groups of the female animals were compared, and a comparison was made among the 4 groups of male animals. One-way ANOVA followed by Newman-Keuls post-hoc test were used for statistical analysis. A probability value of < .05 was considered significant.

Results

In vitro vascular reactivity

Concerning the reaction of phenylephrine among the 4 male groups, the dose-responses to phenylephrine in the carotid artery were significantly higher in the LDLR⁻/⁻KO and LDLR±Mat male offspring, compared with LDLR+/+WT and LDLR±Pat mice. Maximal effect in %: LDLR⁻/⁻KO (N = 6) 102.9 ± 12.17 mN, LDLR±Mat (N = 7) 80.2 ± 6 mN vs LDLR+/+WT (N = 8) 40.70 ± 4 mN and LDLR±Pat (N = 6) 56.7 ± 8.1 mN. (P < .05; Figure 1, A). The contractile responses to phenylephrine among the 4 groups of female mice were significantly increased only in the LDLR⁻/⁻KO offspring, compared with the other groups. Maximal effect in %: LDLR⁻/⁻KO (N = 7) 102.7 ± 11.69 mN, LDLR±Mat (N = 10) 74.4 ± 9.2 mN vs LDLR+/+WT (N = 8) 61.5 ± 3.8 mN and LDLR±Pat (N = 6) 64.8 ± 8.0 mN. (P < .05; Figure 1, B).
Concerning the vascular reactivity to calcium among the male groups, the dose response curve to cumulative concentrations of calcium was significantly higher in the LDLR−/−KO adult male offspring (n = 6) at 1 mmol/L, 1.5 mmol/L, and 2 mmol/L concentration, compared with the other groups of homozygous control male mice (n = 8), heterozygous maternal male mice (n = 7), and heterozygous paternal male mice (n = 6; Figure 2, A).

The maximal effect among the 4 groups of female adult offspring mice to Ca²⁺ was higher in the LDLR−/−KO mice, compared with the other groups: LDLR−/−KO (N = 6), 2.16 ± 0.27 mN; LDLR+/−Mat (n = 10), 1.32 ± 0.09 mN; LDLR+/+WT (n = 8), 1.46 ± 0.21 mN; and LDLR−/−Pat (n = 6), 1.37 ± 0.3 mN; P < .05; Figure 2, B).

The responses to cumulative concentrations of serotonin did not differ among the 4 groups of offspring, in either the male or the female groups. The same numbers of pups were used in each group as in the experiments with phenylephrine and calcium. Similarly, there were no differences in the responses to cumulative concentrations of acetylcholine in either male or female offspring (Figure 3, A and B).

Comment

In this study, we used a knockout mouse lacking the LDLR gene as a model to study the role of maternal hypercholesterolemia in fetal programming of vascular function.
With our cross-breeding scheme, we were able to compare the contractile properties of the carotid arteries between mice completely lacking a functional LDLR allele, wild-type mice, mice heterozygous for the LDLR gene disruption that developed in a normal maternal environment, and heterozygous mice developing in a maternal environment completely lacking a functional LDLR gene. In both

**FIGURE 2.** Calcium dose-response and concentration-response curves
A. Calcium dose-response curves in the carotid arteries from male LDLR-/-KO, maternally derived heterozygous LDLR± (LDLR±Mat), paternally derived heterozygous LDLR± (LDLR±Pat), and LDLR+/+WT offspring. The asterisk denotes a probability value of < .05, compared with the corresponding responses in the other groups. B. Calcium concentration-response curves in the carotid arteries from female LDLR-/-KO, maternally derived heterozygous LDLR± (LDLR±Mat), paternally derived heterozygous LDLR± (LDLR±Pat), and LDLR+/+WT offspring. The asterisk denotes a probability value of < .05, compared with the corresponding responses in the other groups.

**FIGURE 3.** Acetylcholine concentration-response curves
A. Acetylcholine concentration-response curves in the carotid arteries of male mice LDLR-/-KO, maternally derived heterozygous LDLR± (LDLR±Mat), paternally derived heterozygous LDLR± (LDLR±Pat), and LDLR+/+WT. The responses are presented as percent relaxation of the phenylephrine contraction. B. Acetylcholine concentration-response curves in the carotid arteries of female mice LDLR-/-KO, maternally derived heterozygous LDLR± (LDLR±Mat), paternally derived heterozygous LDLR± (LDLR±Pat), and LDLR+/+WT. The responses are presented as percent relaxation of the phenylephrine contraction.

function. With our cross-breeding scheme, we were able to compare the contractile properties of the carotid arteries between mice completely lacking a functional LDLR allele, wild-type mice, mice heterozygous for the LDLR gene disruption that developed in a normal maternal environment, and heterozygous mice developing in a maternal environment completely lacking a functional LDLR gene. In both
male and female homozygous LDLR\(^{-/-}\)KO offspring, a lack of expression of a functional LDLR allele was associated with increased contractile responses to phenylephrine and calcium in the isolated carotid artery in vitro. Interestingly, the contractile response to phenylephrine in the heterozygous maternally derived male offspring was similar to the knockout mice; the heterozygous paternally derived male offspring had a contractile response that was similar to the wild-type mice, despite the genomically similar background of the heterozygous offspring. This difference between the genomically similar heterozygous offspring can be due to altered fetal programming of adult vascular function by a maternal environment lacking a functional LDLR allele. Interestingly this could not be confirmed in the female mice. Although there was a trend toward a difference in vascular reactivity between the heterozygous maternally derived female mice compared with the knockout mice, this difference was not statistically significant.

These findings support a role for maternal hypercholesterolemia during fetal vascular programming in the early onset of altered vascular responses in later life.\(^2,3,19\) The process of arteriosclerosis seems to be a chronic inflammatory condition that is converted to an acute clinical event by the induction of plaque rupture, which in turn leads to thrombosis. The process may take years to become clinically evident. However, our results show that the process may start very early in life. The earliest lesions, the fatty streaks, which by themselves may not be of major clinical significance, develop at various sites predicated by hemodynamic and mechanical factors. The LDL enters the arterial wall and undergoes modification including oxidation, which leads to the production of inflammatory mediators like the cytokines.\(^12,16\) The monocytes in the vessel wall take up the oxidized lipoproteins and induce their differentiation into lipid-laden foam cells. Damage to the endothelium will result in altered vascular reactivity, which is an early marker of this process.\(^10,11,16\) The vascular responses to phenylephrine in the LDLR\(^{-/-}\)KO and the LDLR\(^{\pm}\)Mat offspring confirm an early vascular dysfunction as a consequence of a high maternal cholesterol level.

The higher than normal vascular response to calcium in the male and female LDLR\(^{-/-}\)KO mice may be explained by the process of proliferation and migration of vascular smooth muscle cells. The vascular smooth muscle cells respond to the cascade of events that are associated with the process of arteriosclerosis, and as discussed earlier, with proliferation and migration from the media and into the intima. The increased migration and proliferation increases the vessel’s contractile potential.\(^27,28\) The vascular contractility in the LDLR\(^{\pm}\)Mat and the LDLR\(^{\pm}\)Pat offspring to calcium was not significantly different from that of the wild-type offspring. We would have expected a higher contractile reaction of the heterozygous LDLR\(^{\pm}\)Mat offspring to calcium, as was seen in the LDLR\(^{-/-}\)KO offspring. This can be explained by the fact that vascular smooth muscle cell
proliferation and migration is a process that takes a long time and the use of older offspring may have shown a difference. The offspring were also fed a normal diet. On this diet, these transgenic mice produce moderate fatty streaks, although a western diet with higher cholesterol content may have accelerated the process of arteriosclerosis. In the cascade of events that leads to arteriosclerosis, the process of vascular smooth muscle cells proliferation and migration occurs at a later stage then foam cell formation. This may explain the reason that we did see similar exaggerated responses to phenylephrine in the LDLR\(^{-/-}\)KO and the LDLR\(^{\pm Mat}\) but not yet in the reaction to calcium. Further research may shed more light on the vascular responses of LDLR\(^{\pm Mat}\) offspring later in life, particularly when the offspring are fed a high cholesterol diet (eg, western-type diet).

We also found gender difference in the response to phenylephrine between heterozygous LDLR\(^{\pm Mat}\) offspring, with increased contractile responses in males but not females. This could be due to the protective role of estrogen. Further research in ovariectomized animals may further our understanding.

In conclusion we have shown that maternal hypercholesterolemia seems to accelerate the process of arteriosclerosis in their offspring and in consequence alters their vascular reactivity. This animal model of maternal hypercholesterolemia supports the role of an adverse intrauterine environment on fetal vascular programming, which leads to an abnormal vascular function in later life. Hypercholesterolemia during pregnancy, such as seen in LDLR knockout mice, leads to change in vascular reactivity of the offspring early in life, even in those offspring that receive a normal diet.
References


Part II

Clinical research
Prediction and primary prevention of pre-eclampsia

Shakila Thangaratinam
Josje Langenveld
Ben Willem J Mol
Khalid S Khan

Abstract

Pre-eclampsia is associated with increased maternal and perinatal mortality and morbidity. Early recognition of women at risk of pre-eclampsia will enable to identify the high risk women who may benefit from enhanced surveillance and prophylaxis. This chapter summarises the accuracy of various tests performed to predict the onset of pre-eclampsia and the effectiveness of preventative treatment. The tests to predict pre-eclampsia include clinical history, examination findings, laboratory and haemodynamic tests. In general, tests in early pregnancy for predicting later development of pre-eclampsia have better specificity than sensitivity, as BMI (Body Mass Index) >34, alpha-fetoprotein, fibronectin and uterine artery Doppler (bilateral notching) all have specificities above 90%. Only uterine artery Doppler resistance index and combinations of indices have a sensitivity of over 60%. Tests like kallikreinuria and SDS page proteinuria, not used in clinical practice have shown high sensitivity above 80%, without compromising specificity, and require further investigation. None of the tests are sufficiently accurate to recommend for routine use in clinical practice. The various treatment options to prevent pre-eclampsia include pharmacological agents, dietary supplementation and lifestyle modification. Antiplatelet agents, primarily low-dose aspirin reduce the risk of pre-eclampsia by 10% (RR 0.90, 95% CI 0.84 to 0.97). Calcium effectively prevents pre-eclampsia (RR 0.45; 95% CI 0.31 – 0.65); the beneficial effect being observed in the high risk group (RR 0.22; 95% CI 0.12 – 0.42) and in the group with low nutritional calcium intake (RR 0.36; 95% CI 0.20 -0.65). Pharmacological agents like low molecular weight heparin, progesterone, nitric oxide donors, anti-hypertensives and diuretics are not effective in preventing pre-eclampsia. Dietary supplements like magnesium, anti oxidants, marine oils and folic acid do not reduce the incidence of pre-eclampsia. There is paucity of evidence to demonstrate that lifestyle interventions like rest, exercise and reduced dietary salt intake prevent pre-eclampsia.
Background

Pre-eclampsia is associated with adverse maternal and fetal outcomes. The incidence of pre-eclampsia varies with the risk factor ranging from 2% to 8% of pregnancies.\(^1\) In unselected women the incidence of pre-eclampsia is reported as 2.5% (95% CI 1.9-3.4%).\(^2\) The incidence is much higher in women at high risk for pre-eclampsia and estimated to be 10% (9.3 - 10.8%).\(^3\)\(^-\)\(^8\) Women at high risk of pre-eclampsia include those with chronic hypertension, chronic kidney disease, autoimmune disease like systemic lupus erythematosis, type 1 and 2 diabetes and those with hypertension in a previous pregnancy. Pre-eclampsia can develop into severe pre-eclampsia and/or eclampsia. Overall, 15% to 25% of women with gestational hypertension progress to pre-eclampsia.\(^9\)

Screening is undertaken on a healthy population. It identifies those more at risk from a disease. It assumes that early detection through confirmatory tests is possible and that treatment improves outcome. Typically it involves a series of tests: Initial (screening) test, which if positive, leads to a confirmatory or gold standard test for disease. Only after confirmation of the second test treatment would be instituted. Hence classical screening may be carried out by relatively poor initial tests. This is because the screening test guides confirmation and the confirmatory test guides treatment. In pre-eclampsia this is not so (see Fig). The intention here is to apply primary preventative treatment straight after initial screening. Therefore in pre-eclampsia we require screening performance far superior to be expected for other screening tests.

Screening for pre-eclampsia is an important part of routine antenatal care. Identification of women at risk of pre-eclampsia will help to judiciously allocate resources for close monitoring and prophylactic treatment to minimise adverse maternal and fetal outcomes. This will depend on the accuracy of the tests performed to predict the onset of pre-eclampsia. Ideally, a test should perform well in both sensitivity and specificity. Often there is a trade off between sensitivity and specificity and the preferred test performance depends on the prevalence and the severity of the condition i.e. pre-eclampsia and associated complications and the effectiveness, safety and cost of the prophylactic treatment.

On one hand, the consequences of false positive results include the costs of intensive monitoring and treatment associated morbidity among normal women who would not develop pre eclampsia, it is important that test specificity is suitably high. On the other hand, falsely negative results miss women who develop pre-eclampsia at a later stage, leading to additional costs and morbidity of cases left untreated necessitating a test with high sensitivity. Presence of effective, cheap and safe interventions favours a test with high sensitivity than specificity.
The risk status of pregnant women to develop pre-eclampsia is currently assessed at the first antenatal booking visit through clinical history and examination. Routine screening for pre-eclampsia is based on measurement of blood pressure and urinalysis for proteinuria.

**Sources of information**

The evidence for accuracy of tests and effectiveness of treatment for prediction and prevention of pre eclampsia has been obtained by review of reviews including Cochrane reviews, HTA reports, relevant guidelines, and primary studies in Medline (1966 – to date). The tests evaluated in the prediction pre-eclampsia and the preventative treatment methods are provided in Table 1a.
### Table 1a. Tests evaluated for prediction of pre-eclampsia

<table>
<thead>
<tr>
<th>PREDICTION OF PRE-ECLAMPSIA</th>
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<tr>
<td><strong>CLINICAL HISTORY</strong></td>
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<td>Parity</td>
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<td>Family history of pre-eclampsia</td>
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<td>Multiple pregnancy</td>
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<td>Pre eclampsia in previous pregnancy</td>
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<td>History of autoimmune disease, thrombophilia, diabetes, chronic hypertension</td>
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<td>Molar pregnancy</td>
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<td><strong>CLINICAL EXAMINATION</strong></td>
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<td>Body Mass Index (BMI)</td>
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<td>- BMI ≥ 30</td>
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<td>- BMI ≥ 35</td>
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<td>Blood pressure in the first trimester and second trimester</td>
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<td>- Mean arterial pressure</td>
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<td>- Systolic blood pressure</td>
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<td>- Diastolic blood pressure</td>
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<td><strong>LABORATORY TESTS</strong></td>
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<td>Proteinuria</td>
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<td>- Total 24 hours proteinuria</td>
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<td>- Microalbuminuria</td>
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<td>- Spot albumin:creatinine ratio (ACR)</td>
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<tr>
<td>- Spot protein:creatinine ratio (PCR)</td>
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<tr>
<td>- Urinary Kallikrein</td>
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<td>Plasma fibronectin (FN)</td>
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<td>- Cellular FN</td>
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<td>- Total FN</td>
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<tr>
<td>Angiogenic biomarkers</td>
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<tr>
<td>- Placental Growth Factor (PIGF)</td>
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<tr>
<td>- soluble fms-like tyrosine kinase 1 (sFlt-1)</td>
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<td>- soluable Endoglin</td>
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<tr>
<td><strong>OTHER TESTS</strong></td>
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<td>Human chorionic gonadotrophin</td>
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<td><strong>HAEMODYNAMIC INVESTIGATIONS</strong></td>
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<td>- pulsatility index</td>
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<tr>
<td>- Unilateral / bilateral notching</td>
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<td>- Resistance index</td>
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<td>- S/D ratio</td>
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<td>- A/C ratio</td>
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<td>- Notch index</td>
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Predictors of onset of pre-eclampsia

Clinical history

The risk factors assessed at booking for pre-eclampsia include age, parity, history of pre-eclampsia in a previous pregnancy especially if severe or early onset,18;18-20 a family history of pre-eclampsia,21;22 multiple pregnancy,23;24 duration between pregnancies and pre-existing medical conditions like diabetes18;23;25, chronic hypertension,18;23;26 renal disease,27 thrombophilias and autoimmune disease.28 Obstetric factors associated with high risk are a current hydropic29 or molar pregnancy.30 A systematic review of studies showed that the risk of pre-eclampsia is almost doubled in women over 40 years of age (RR 1.7; 95% CI 1.2, 2.3 in primiparous women and RR 2; 95% CI 1.3, 2.9 in multiparous women).31 The risk of pre-eclampsia is tripled in nulliparous women (RR 2.9; 95% CI 1.3, 6.6), women with a family history of pre-eclampsia (RR 2.9; 95% CI 1.7, 4.9) and in women with multiple pregnancies (RR 2.9; 95% CI 2.0, 4.2 in twins and RR 2.8; 95% CI 1.3, 6.4 in triplet pregnancy).31 The risk is even increased seven times in women who had pre-eclampsia in their previous pregnancy (RR 7.2; 95% CI 5.9, 8.8).31 In women with insulin dependent diabetes, the risk of developing pre-eclampsia is quadrupled (RR 3.6; 95% CI 2.5, 5).31 Women with a history of autoimmune disease and thrombophilia are at significantly increased risk of developing pre-eclampsia with RR of 6.9 (95% CI 1.1, 42.3) and 9.7 (95% CI 4.3, 21.8) respectively.31

Clinical examination

Body Mass Index

Obesity in pregnancy is considered to be a risk factor for developing pre-eclampsia. The body mass index (BMI) is a standard for obesity measurement adjusting bodyweight for height (weight [kg]/height squared [m^2]). BMI is categorised as underweight (BMI<20), normal weight (BMI 20-25), overweight (BMI>25) and obese (BMI>30). A systematic review of 36 studies involving 1,699,073 women evaluated the accuracy of BMI in predicting pre-clampsia.32 Pooled estimates for all studies with overweight and obese women (18 studies) were 47% (33–61) for sensitivity and 73% (64–83) for specificity. For a BMI ≥ 30 (19 studies), these estimates were 19% (19–20) and 90% (88–93), and for a BMI ≥ 35 (4 studies), the estimates were 21% (12–31) and 92% (89–95). The corresponding LRs were 1.7 (95% CI 0.3–12) for BMI ≥ 25 and 0.73 (95% CI 0.22–2.5) for BMI < 25, 1.9 (95% CI 0.3–12) for BMI ≥ 29 and 0.88 (95% CI 0.61–1.3) for BMI < 29, and 2.7 (95% CI 1.0–7.3) for BMI ≥35 and 0.86 (95% CI 0.68–1.07) for BMI < 35. BMI (at any cutoff), pre-pregnancy or at booking, was a fairly weak predictor for pre-eclampsia.
Blood Pressure

Blood pressure measurement is routinely performed in antenatal care to diagnose or predict hypertensive disease. A systematic review of 34 studies with 60,599 women evaluated the role of blood pressure as a predictor of pre-eclampsia. The areas under the summary receiver operating characteristic curves for blood pressure measurement in the second trimester were 0.76 (95% CI 0.70 - 0.82) for mean arterial pressure, 0.68 (95% CI 0.64 - 0.72) for systolic blood pressure and 0.66 (95% CI 0.59 - 0.72) for diastolic blood pressure. A similar trend was noticed for blood pressure measurements in the first trimester.

A mean arterial pressure of 90 mm Hg or more showed a pooled sensitivity of 62% (95% CI 35 - 89%) and a pooled specificity of 82% (95% CI 72 - 92%); which corresponds with derived likelihood ratio of a positive test 3.5 (95% CI 2 - 5) and likelihood ratio of a negative test of 0.46 (95% CI 0.16 - 0.75). For a specificity of 90% the sensitivities of diastolic blood pressure and systolic blood pressure were 35% and 24% respectively. In high risk populations a diastolic blood pressure of 75 mm Hg or more at 13 to 20 weeks’ gestation best predicted pre-eclampsia, although the accuracy of prediction was modest (likelihood ratio of positive and negative test of 2.8 and 0.39, respectively). Mean arterial pressure appears to be a better predictor for pre-eclampsia than systolic blood pressure, diastolic blood pressure, or increased blood pressure. Blood pressure measurements in the first and second trimester at the first antenatal visit for healthy normotensive women do not help predict pre-eclampsia.

Laboratory tests

Uric acid

The association between high blood uric acid levels and pre-eclampsia was reported in 1917. Renal impairment and an increased breakdown of purines in the ischaemic placenta leading to overproduction of uric acid may explain increased serum uric acid levels in pregnant women destined to develop pre-eclampsia. The role of uric acid as a predictor of pre-eclampsia was evaluated in a systematic review that included five studies with 514 women. The sensitivity ranged from 0.0% to 55.6% and the specificity from 76.9% to 94.9%. There was significant clinical heterogeneity and poor reporting in the included studies. The accuracy of the available evidence is insufficient to recommend uric acid as a predictor of pre-eclampsia.

Proteinuria

Routine proteinuria urinalysis is conducted in antenatal clinics from first booking for pre-eclampsia prediction. Early detection of proteinuria in women with new...
onset hypertension helps to differentiate those women with pre-eclampsia from gestational hypertension, thereby influencing further management. Proteinuria is usually evaluated by dipstick (visual or automated) or by 24 hour urinary total protein excretion. The use of spot protein: creatinine ratio (PCR) and spot albumin: creatinine ratio (ACR) has been recently used in clinical practice. A review of diagnostic accuracy of proteinuria in predicting pre-eclampsia onset included 11 studies (4,388 women). The pooled estimates of sensitivity and specificity were for total proteinuria 35% (95% CI 13 - 68%) and 89% (95% CI 79 - 94%); for microalbuminuria 62% (95% CI 23 - 90%) and 68% (95% CI 57 - 77%); ACR 19% (95% CI 12 - 28%) and 75% (95% CI 73 - 77%). The excretion of urinary kallikrein is lower than in normotensive pregnancy with sensitivity greater than 80% and specificity greater than 90%.

Cellular and total fibronectin
Women who develop pre-eclampsia are reported to have higher plasma fibronectin (FN) concentrations than pregnant controls. FN is a glycoprotein of which several subtypes exist. Inflammation, vascular injury and malignancy are generally associated with increased expression of the ED-A (also called ED-1+ or oncofetal FN) and ED-B (also called ED-2+) forms of FN, particularly in the blood vessel walls. ED-A and ED-B are both called cellular FN and represents only 5% of all FN in plasma whilst total FN contains all subtypes of FN. A review on the accuracy of FN as a predictor of pre-eclampsia onset included three studies. For cellular FN the highest specificity of 96% (95% CI 79 - 99%) was achieved in the 2nd trimester at a cut-off value of 5.0 μg/ml with a sensitivity of 50% (95% CI 29.9 - 70.1%). For total FN the highest specificity was 94% (95% CI 86 - 98%) with a sensitivity of 65% (95% CI 44 - 83%) at a cut-off value of 293 μg/m.

Angiogenic Biomarkers
Vascular endothelial growth factor (VEGF) is crucial for vascular development, angiogenesis, maintenance of the vasculature and normal kidney function. Pathogenesis of pre-eclampsia is considered to be related to an imbalance between proangiogenic factors, like VEGF or placental growth factor (PIGF), and antiangiogenic factors, like soluble fms-like tyrosine kinase 1 (sFlt-1, a splice variant of VEGF receptor-1) and the soluble form of endoglin. Circulating levels of VEGF and maternal serum concentrations of PIGF are significantly lower in preeclamptic patients when compared with healthy controls. sFlt-1 and soluble endoglin have been shown to be increased in the maternal circulation in pre-eclampsia even before the onset of disease. While sFlt-1 and PIGF provide a degree of discrimination between normal pregnancies and those destined to develop pre-eclampsia, their combination (ratio of sFlt-1 to PIGF) may provide
superior performance. For a diagnostic cut-off of 38.46 for sFlt-1/PlGF ratio, the positive and negative predictive values were 88.5%, positive likelihood ratio was 7.7 and negative likelihood ratio was 0.13. For a cut off value of 85 for sFlt-1/PlGF ratio, the highest sensitivity was 82% and specificity was 95%.

Other tests
Maternal serum alpha-fetoprotein (AFP) and human chorionic gonadotrophin (HCG) are routinely used to screen for fetal aneuploidy and anomalies. A review of diagnostic accuracy of AFP in predicting pre-eclampsia included 12 studies (137,097 women) with pooled estimates of sensitivity and specificity of 9% (95% CI 5-16%) and 96% (95% CI 94-98%). The evidence for diagnostic accuracy of maternal HCG included 16 studies (72,732 women) with pooled estimates of sensitivity and specificity of 24% (16%-35%) and 89% (86%-92%) respectively.

Haemodynamic investigations
Pre-eclampsia is characterised by abnormal placentation, resulting in inadequate uteroplacental blood flow. Uterine artery Doppler ultrasound has been demonstrated to be a reliable, non-invasive method of examining uteroplacental perfusion. The velocity of uterine artery blood flow assessed by the Doppler ultrasound has been evaluated as part of routine ultrasound screening for pre-eclampsia. Uterine artery Doppler findings may be reported unilateral or bilateral notching, single ratios such as S/D ratio, A/C ratio, and Notch Index, pulsatility index or resistance index.

A systematic review included 74 studies (n=79,547) in which uterine artery Doppler ultrasonography was used to predict pre-eclampsia. In low risk women, an increased pulsatility index with diastolic notching in the second trimester (>16 weeks) was the best predictor of pre-eclampsia (likelihood ratio of a positive test LR+ 7.5, 95% CI 5.4–10.2; likelihood ratio of a negative test LR- 0.59, 95% CI 0.47–0.71). A second trimester finding of increased pulsatility index (LR+ 15.6, 95% CI 13.3–17.3; LR- 0.23, 95% CI 0.15–0.35) and bilateral notching (LR+ 13.4, 95% CI 8.5–17.4); LR- 0.4 (95% CI 0.2–0.6) in the second trimester also predicted the onset of severe pre-eclampsia in low risk women. In high-risk women, the best predictor of pre-eclampsia was unilateral notching (LR+ 20.2, 95% CI 7.5–29.5; LR- 0.17, 95% CI 0.03–0.56) and an increased pulsatility index with notching (LR+ 21.0, 95% CI 5.5–80.5; LR- 0.82, 95% CI 0.72–0.93) in the second trimester. Use of Doppler to predict severe pre-eclampsia in high-risk patients had low diagnostic accuracy (LR+ 3.7). Pulsatility index and bilateral notching in the second trimester appear to be the most promising Doppler indices for prediction of pre-eclampsia. Placental profile including uterine artery doppler imaging and placental morphology in the second trimester and maternal serum screening in the first
trimester have been evaluated as predictors of pre-eclampsia. Abnormal placental morphological condition included shape or texture or both. The odds ratio of developing pre-eclampsia or HELLP syndrome in the group with normal placental profile compared to women with ≥ abnormal profile test results was 0.2 (95% CI 0.1, 0.4).

Prevention of pre-eclampsia

Current strategies for prevention focus on pharmacological therapy, dietary interventions and modification of lifestyle (Table 1b).

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<tr>
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Pharmacological treatment

*Antiplatelet agents*

Activation of platelets, as well as an imbalance of thromboxane:prostacyclin activity occurs during the pre-clinical phase of pre-eclampsia. Thus it is possible that agents could prevent or delay the development of pre-eclampsia. Aspirin, the most widely used anti-platelet agent has been evaluated for its prophylactic role...
in the prevention of pre-eclampsia at dose between 50-150mg/day (mostly either 60mg/day or 100mg/day).\(^{10}\)

A Cochrane review of 43 randomised trials including 32,590 women reported on the effectiveness of anti-platelet agents in preventing pre-eclampsia.\(^{10}\) It showed that anti-platelet agents reduce the risk of pre-eclampsia by 17% (RR 0.83; 95% CI 0.77–0.89). Aspirin was the agent evaluated in 38 of the 43 included studies. The subgroup analysis by maternal risk showed that there was a statistically significant reduction in the risk of pre-eclampsia in both high risk (RR 0.75; 95% CI 0.66 - 0.85) and moderate risk (RR 0.86; 95% CI 0.79 - 0.95) women. Risk factors like first pregnancy, BMI over 35, abnormal uterine artery Doppler, multiple pregnancy, family history of pre-eclampsia or being a teenager confer moderate risk. Meta-analysis of individual patient data confirmed the reduction in the risk of pre-eclampsia with anti-platelet agents, although with less estimated treatment effect (RR 0.90; 95% CI 0.84–0.97).\(^{44}\) A recent meta analysis of 27 trials (11,348 women) evaluated the influence of gestational age at which aspirin treatment was started in preventing pre-eclampsia in women at risk of pre-eclampsia.\(^{45}\) Commencement of aspirin early in gestation was associated with a greater reduction in the incidence of pre-eclampsia than treatment beginning in late gestation. The RR for prevention of pre-eclampsia was 0.47 (95% CI 0.34 to 0.65) for aspirin commenced before 16 weeks, and 0.81 (95% CI 0.63 to 1.03) when commenced after 16 of gestation.\(^{45}\) Analysis of the optimal dosage of the drug by the NICE Guideline Development Group showed that 75 mg of aspirin is the optimal dosage for prevention of pre-eclampsia.\(^{16}\) The increase in benefit was not observed for higher doses.

Current NICE guidelines recommend high risk women (those with one or more risk factors like previous severe pre-eclampsia, diabetes, chronic hypertension, kidney disease or autoimmune disease) and women with 1 or more moderate risk factors (first pregnancy, multiple pregnancy, interpregnancy interval over 10 years, age more than 40, family history of pre-eclampsia BMI ≥ 35 at first visit) to take 75 mg of aspirin daily from 12 weeks of pregnancy until the birth of the baby.

**Nitric oxide agents**

Nitric oxide is an endothelium-derived vascular relaxation factor. Nitric oxide is continuously produced by the endothelium and contributes to the physiological vascular adaptations of normal pregnancy by mediating vasodilatation and inhibition of platelet aggregation.\(^{46}\) A relative reduction in nitric oxide may contribute to the pathophysiology of pre-eclampsia. Drugs that are converted by the body into nitric oxide may improve uterine artery blood flow and placental perfusion and prevent the onset of pre-eclampsia. Commonly used nitric oxide
Donors include glyceryl trinitrate, isosorbide mononitrate, isosorbide dinitrate, sodium nitroprusside and S-nitroglutathione. The effectiveness of nitric oxide donors and precursors in preventing pre-eclampsia was evaluated in 4 RCTs (170 women). They did not show a statistically significant reduction in the incidence of either pre-eclampsia (RR 0.83, 95% CI 0.49 -1.41) or severe pre-eclampsia (RR 0.10 95% CI 0.01 – 1.87). There is insufficient evidence to recommend routine clinical use of nitric oxide donors for the prevention of pre-eclampsia.

**Low Molecular Weight Heparin**

In pre-eclampsia, the extravillous trophoblastic infiltration and the resulting transformation of high resistance spiral arteries to low resistance uteroplacental blood vessels is affected. Ischaemic thrombotic lesions have been observed in the placenta of women with pre-eclampsia. The possible prophylactic role of anti-thrombotic agents like low molecular weight heparin (LMWH) to prevent the vascular pathology in pre-eclampsia, have been studied. A review of two RCTs of high risk women studied LMWH in preventing pre-eclampsia (100 women). Both studies commenced LMWH after pregnancy was confirmed. While the pooled estimate showed a reduction in the incidence of pre-eclampsia (RR 0.23; 95% CI 0.08 – 0.68), available evidence is insufficient to recommend LMWH as pre-eclampsia prophylaxis outside the setting of a clinical trial.

**Anti-hypertensives for mild to moderate hypertension**

Treatment with anti-hypertensives was considered in women with mild and moderate hypertension in pregnancy to minimise the worsening of the disease. A Cochrane review of 22 RCTs (2702 women) did not show any beneficial effect of antihypertensive drugs compared to placebo/no treatment in preventing pre-eclampsia with an RR of 0.97 (95%CI 0.83-1.13). Subgroup analysis by gestation, type of hypertensive disease and use of placebo showed no difference between the groups.

**Progestrone**

The use of progestrone has been evaluated in 1 RCT (128 women) for the prevention and treatment of pre-eclampsia. There was no beneficial effect observed in the reduction of pre-eclampsia (RR 0.21, 95% CI 0.03 – 1.77). There is a paucity of high quality evidence to recommend progestrone for the prevention of pre-eclampsia.
Diuretics

Management of hypertension in non-pregnant women often involves the use of diuretics. With increasing evidence of the reduction of plasma volume in pre-eclampsia, there were concerns that diuretics might worsen the existing hypovolaemia in women with pre-eclampsia, thereby adversely affecting the mother and baby.51

Four RCTs (1,391 women) compared the use of thiazide diuretics with placebo or no treatment in the prevention of pre-eclampsia. There is no significant reduction in pre-eclampsia by treatment with diuretics (RR 0.68, 95% CI 0.45 – 1.03).52 Given the lack of benefit and the potential adverse side effects of diuretics to the mother and fetus, their use in pregnancy should not be recommended.

Nutritional supplementation

Calcium

Women with high intake of calcium have been observed to have a low incidence of hypertensive disorders in pregnancy.53 This led to the hypothesis that an increase in calcium intake during pregnancy might reduce the incidence of pre-eclampsia, especially in women with low calcium intake. Calcium acts by reducing smooth muscle contractility and vasoconstriction by its effect on parathyroid and intracellular calcium. It might also have an indirect effect on smooth muscle function by increasing magnesium levels. Recently, calcium has been shown to affect uteroplacental blood flow by reducing resistance in the uterine and umbilical arteries.54

A recent systematic review of thirteen RCTs (15,730 women) assessed the effectiveness of calcium in preventing pre-eclampsia.12 About two thirds of the women had a low dietary calcium intake (10,678 women) and 587 women were at high risk. The average daily calcium intake in the studies was 1.5 – 2 grams of various preparations including calcium carbonate (eight RCTs), elemental calcium (three RCTs) and calcium gluconate (one RCT). Calcium was effective in the prevention of pre-eclampsia (RR 0.45; 95% CI 0.31 – 0.65). The beneficial effect was mainly observed seen in the high risk group (RR 0.22; 95% CI 0.12 – 0.42) and in the group with low nutritional calcium intake (RR 0.36; 95% CI 0.20 - 0.65). The subgroup analysis for women with adequate dietary calcium intake showed no significant effect on the incidence of pre-eclampsia (RR 0.62, 95% CI 0.32 - 1.2). It is possible that the beneficial effect observed in the group with low calcium intake is due to the epiphenomenon of blood pressure reducing effect of calcium in the active arm of the trials involving these women. A daily intake of at least 1 gram (e.g. 1.5 – 2 gram) of calcium reduces the incidence of pre-eclampsia in high risk women and women with low dietary intake of calcium.
Anti-oxidants

Abnormal placental development in pre-eclampsia leads to reduced placental perfusion, and mediates a state of oxidative stress. Anti-oxidants (such as vitamin C, vitamin E, lycopene and selenium) have been proposed to protect proteins and enzymes from oxidation and destruction by free radicals, and help to maintain cellular membrane integrity. Dietary supplementation with anti-oxidants may limit the endothelial damage observed in pre-eclampsia.

A Cochrane review evaluated the effectiveness of anti-oxidants for preventing pre-eclampsia included 9 trials and 5,446 women. There was no evidence that anti-oxidants prevent pre-eclampsia (RR 0.73, 95% CI 0.51 – 1.06). Since the publication of this Cochrane review, 4 large RCTs have studied the effectiveness of anti-oxidants in preventing pre-eclampsia in both low risk and high risk women. There was no decrease in the incidence of pre-eclampsia in either low risk or high risk women taking anti-oxidant supplementation. There is no evidence that anti-oxidants are effective in preventing pre-eclampsia and there have been consistent concerns raised in the adequately powered trials about potential perinatal harm.

Folic acid

The effect of folic acid supplementation in the second trimester on pre-eclampsia has been evaluated in 1 prospective cohort study. The dose of folic acid was over 1 mg and used in combination with multivitamins. There was no statistically significant reduction in the incidence of pre-eclampsia with folic acid supplementation alone (RR 0.46; 95% CI 0.16-1.31). When taken in combination with multivitamins, there was a reduction in the incidence of pre-eclampsia (RR 0.37; 95% CI 0.18-0.75).

Magnesium

Meta-analysis of two RCTs (474 women) comparing magnesium supplementation with placebo did not show any effect on the incidence pre-eclampsia (RR 0.87, 95% CI 0.57 -1.32). There is not enough good quality evidence to support magnesium supplementation for prevention of pre-eclampsia.

Marine oil and other prostaglandin precursors

Marine oils are rich sources of n-3 long chain polyunsaturated fatty acids (omega-3 fatty acids). Marine oil fatty acids have the potential to down regulate vasoconstriction and endothelial damage responses of pre-eclampsia through direct competition with the thromboxane A2 precursor, arachidonic acid. Other agents, such as evening primrose oil, contain a fatty acid called gamma-linolenic acid that has a similar mechanism of action as omega-3 fatty acids.
Meta-analysis of 4 RCTs (1,683 women) comparing fish-oil or primrose oil with placebo, did not show a reduction in pre-eclampsia (RR 0.86; 95% CI 0.59 -1.27). 

Subgroup analysis in low risk (RR 1.0; 95% CI 0.52 – 1.98) and high risk patients (RR 0.80; 95% CI 0.50 - 1.29) did not observe any benefit for reducing pre-eclampsia. There is not enough evidence to support the routine use of marine oil, or other prostaglandin precursor, supplements during pregnancy to reduce the risk of pre-eclampsia.

**Life style interventions and diet**

Life style preferences like exercise, bed rest and type of diet may influence the risk of hypertension and pre-eclampsia. They are mostly influenced by the choice of the woman and there is a need for robust evidence prior to making any recommendations.

**Rest**

Rest has been proposed to have a beneficial effect on the prevention of pre-eclampsia based on few case control studies. Furthermore, the observation of increase in systolic blood pressure in women walking or moving about, compared to when they have been sitting for some time has led to randomised studies in this area.

Two small trials (106 women) have studied the effectiveness of rest compared with restricted activity in preventing pre-eclampsia. There was a reduction in risk of pre-eclampsia with four to six hours rest per day (one trial, 32 women; RR 0.05; 95% CI 0.00 - 0.83), compared with normal activity. Rest of 30 minutes per day plus nutritional supplementation was associated with a reduction in the risk of pre-eclampsia (one trial, 74 women; RR 0.13, 95% CI 0.03 - 0.51). There is insufficient evidence to recommend rest to women to reduce pre-eclampsia.

**Exercise**

Regular exercise is associated with an increase in plasma volume and cardiac output, lower plasma triglycerides, inflammatory cytokines and insulin resistance. This led to the hypothesis that exercise during pregnancy might reduces the risk on pre-eclampsia. The meta-analysis of 2 RCTs (45 women) comparing moderate intensity aerobic exercise with normal physical activity during pregnancy in woman at moderate to high risk of pre-eclampsia showed no significant difference in the incidence of pre-eclampsia (RR 0.31; 95% CI 0.01 – 7.09). There is no evidence that exercise is effective in preventing pre-eclampsia.
**Altered dietary salt**

In the past, women have been advised that lowering their salt intake might reduce their risk on developing pre-eclampsia. Two RCTs (631 women) compared the advice of reducing dietary salt intake with normal diet in pregnancy and found no difference in the incidence of pre-eclampsia (RR 1.11; 95% CI 0.46 - 2.66). Salt consumption during pregnancy should remain a matter of personal preference and not as a recommendation.

**Energy and protein intake**

Nutritional advice in pregnancy has no effect on the incidence of pre-eclampsia (RR of 0.89; 95% CI 0.42-1.88). Isocaloric balanced protein supplementation (RR 1.00; 95% CI 0.57-1.75) and balanced protein/energy intake (RR 1.2; 95% CI 0.77 – 1.89) are also ineffective in preventing pre-eclampsia. From the limited available evidence, there is no indication that changing energy/protein intake has a preventing effect on the incidence of pre-eclampsia.

**Garlic**

Garlic has been suggested to lower blood pressure, reduce oxidative stress, and/or inhibit platelet aggregation with a potential to prevent pre-eclampsia. Results from the randomised trial (100 women) comparing garlic tablets and placebo showed no significant reduction in pre-eclampsia was observed in women taking garlic tablets (RR 0.78; 95% CI 0.31 -1.93). There is insufficient evidence to recommend increased garlic intake for preventing pre-eclampsia.

**Summary**

Tests in early pregnancy for the prediction of onset of pre-eclampsia have better specificity than sensitivity, with wide variation in the precision of accuracy estimation (Table 2). No single test has been shown to robust evidence to predict pre-eclampsia. Given the quality, level and precision of the accuracy evidence, no single test has emerged as a front runner in the quest to predict and prevent pre-eclampsia. Tests like BMI >34, AFP, fibronectin and uterine artery Doppler (bilateral notching) have high specificity. They have the potential to minimise unwarranted inconvenience, expense and morbidity associated with false positive results when disease would not have developed later in pregnancy anyway. Tests with high sensitivity, e.g. Doppler (resistance index and combinations), have low false negative results with the potential to reduce costs and morbidity of cases left untreated associated. The test that seems to offer the promise of both high sensitivity and high specificity is kallikreinuria and requires further investigation.
## Table 2. Accuracy of tests in predicting pre-eclampsia

<table>
<thead>
<tr>
<th>Test</th>
<th>RR (95% CI)</th>
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<tr>
<td><strong>CLINICAL HISTORY</strong></td>
<td></td>
</tr>
<tr>
<td>Maternal age &gt; 40</td>
<td></td>
</tr>
<tr>
<td>Primigravida</td>
<td>1.7 (1.2 – 2.3)</td>
</tr>
<tr>
<td>Multiparida</td>
<td>2.0 (1.3 – 2.9)</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>2.9 (1.3 – 6.6)</td>
</tr>
<tr>
<td>Family history of pre-eclampsia</td>
<td>2.9 (1.7 – 4.9)</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td></td>
</tr>
<tr>
<td>- Twins</td>
<td>2.9 (2.0 – 4.2)</td>
</tr>
<tr>
<td>- Triplets</td>
<td>2.8 (1.3 – 6.4)</td>
</tr>
<tr>
<td><strong>Pre-eclampsia in previous pregnancy</strong></td>
<td></td>
</tr>
<tr>
<td>History of autoimmune disease</td>
<td>7.2 (5.9 – 8.8)</td>
</tr>
<tr>
<td>History of thrombophilia</td>
<td>6.9 (4.3 – 42)</td>
</tr>
<tr>
<td><strong>CLINICAL EXAMINATION</strong></td>
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<tr>
<td>Body Mass Index (BMI)</td>
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<tr>
<td>- BMI ≥ 25</td>
<td>47% (33 – 61) 73% (64 – 83)</td>
</tr>
<tr>
<td>- BMI ≥ 30</td>
<td>19% (19 – 20) 90% (88 – 93)</td>
</tr>
<tr>
<td>- BMI ≥ 35</td>
<td>21% (12 – 31) 92% (89 – 95)</td>
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<tr>
<td><strong>Blood pressure in the first trimester</strong></td>
<td></td>
</tr>
<tr>
<td>- Mean arterial pressure ≥ 90 mmHg</td>
<td>62% (35 – 89) 82% (72 – 92)</td>
</tr>
<tr>
<td><strong>LABORATORY TESTS</strong></td>
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<tr>
<td>Uric Acid</td>
<td>36% (22 – 53) 83% (73 – 90)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td></td>
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<tr>
<td>- Total 24 hours proteinuria</td>
<td>35% (13 – 68) 89% (79 -94)</td>
</tr>
<tr>
<td>- Microalbuminuria</td>
<td>62% (23 – 90) 68% (57 – 77)</td>
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<tr>
<td>- Spot albumin:creatinine ratio</td>
<td>19% (12 – 28) 75% (73 – 77)</td>
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<tr>
<td>Plasma fibronectin (FN)</td>
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<tr>
<td>- Cellular FN</td>
<td>50% (30 – 70) 96% (79 – 99)</td>
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<tr>
<td>- Total FN</td>
<td>65% (44 – 83) 94% (86 – 98)</td>
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<tr>
<td>Alpha-fetoprotein</td>
<td>9% (5 – 16) 96% (94 – 98)</td>
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<tr>
<td>Human chorionic gonadotrophin</td>
<td>24% (16 -35) 89% (86 – 92)</td>
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<td>Trimester</td>
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<tr>
<td>Low risk women</td>
<td>23% (14 – 35) 99% (98 – 99)</td>
</tr>
<tr>
<td>- High pulsatility index and notching</td>
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<tr>
<td>High risk women</td>
<td>83% (36 – 100) 96% (90 – 99)</td>
</tr>
<tr>
<td>- Unilateral notching</td>
<td>19% (5 -42) 99% (97 – 100)</td>
</tr>
<tr>
<td>- High pulsatility index and notching</td>
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</table>
Anti-platelet agents, mainly aspirin show a statistically significant effect in preventing pre-eclampsia (Table 3). Pharmacological agents like low molecular weight heparin, progesterone, nitric oxide donors, anti-hypertensives and diuretics are not effective in preventing pre-eclampsia. Dietary supplementation with calcium has been beneficial in women with low calcium intake and in those who are at high risk for pre-eclampsia. Dietary supplements like magnesium, anti-oxidants, marine oils and folic acid do not reduce the incidence of pre-eclampsia. There is paucity of evidence to demonstrate that life interventions like rest, exercise and reduced dietary salt intake prevent pre-eclampsia.

<table>
<thead>
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<td>Nitric oxide agents</td>
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<tr>
<td>Low molecular weight heparin</td>
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<tr>
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<tr>
<td>Progesteron</td>
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<td>Diuretics</td>
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<tr>
<td><strong>NUTRITIONAL SUPPLEMENTATION</strong></td>
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<tr>
<td>Calcium</td>
</tr>
<tr>
<td>Anti-oxidants</td>
</tr>
<tr>
<td>Folic acid</td>
</tr>
<tr>
<td>Magnesium</td>
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<td>Marine oil and prostaglandin precursors</td>
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<tr>
<td><strong>LIFESTYLE INTERVENTIONS</strong></td>
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<td>Rest</td>
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<tr>
<td>Exercise</td>
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<td>Altered dietary salt</td>
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<td>Energy and protein intake</td>
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<td>Garlic</td>
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</table>

**Practice points**

- Given the generally low sensitivities of the tests evaluated, clinicians need to consider refraining from testing for predicting pre-eclampsia, but initiate preventative treatment.
- Aspirin should be commenced at 12 weeks of pregnancy for women at high risk of pre-eclampsia or with more than one moderate risk factor.
- Calcium supplementation should be considered in those with low dietary calcium intake.
It is not advisable to recommend low molecular weight heparin, progesterone, nitric oxide agents, anti-hypertensives and diuretics for the prevention of pre-eclampsia.

There is no strong evidence to recommend dietary supplementation with magnesium, anti-oxidants, marine oils, and folic acid to prevent pre-eclampsia.

Life style interventions like rest, exercise and diet requirements like low salt intake should be based on personal preferences alone.

**Research agenda**

- To design robust test accuracy studies with sufficient power to estimate test sensitivity of newly developed tests and markers
- To evaluate the added value of new tests using statistical analyses that incorporate information which physicians document through the clinical history (risk profile)
- To undertake individual patient data diagnostic meta-analyses to improve the power and validation of so developed predictive models
- To evaluate the clinical and cost effectiveness of calcium supplementation in women at moderate and high risk of pre-eclampsia
- To assess the effectiveness of lifestyle interventions with good quality, adequately powered randomised controlled trials which measure all relevant clinical outcomes, side effects, costs and acceptability to women
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Recurrence risk of a delivery before 34 weeks of pregnancy due to a severe hypertensive disorder: a systematic review

Josje Langenveld
Sofie Jansen
Joris A. van der Post
Hans Wolf
Ben Willem J Mol
Wessel Ganzevoort

Abstract

Objective: Early-onset hypertensive disorders of pregnancy are associated with adverse maternal and neonatal outcome. The risk of recurrence influences parents choices on subsequent pregnancies and the counselling obstetrician, but evidence so far has been limited. We performed the first systematic review on the risk of recurrence of hypertensive disorders that had caused delivery < 34 weeks.

Study Design: We searched MEDLINE, EMBASE and the Cochrane Library for articles published until September 2009 that report on pregnancy outcome after an earlier pregnancy complicated by early hypertension, pre-eclampsia or HELLP [hemolysis elevated liver enzymes low platelets] syndrome, which resulted in a delivery before 34 weeks. Recurrence rates of premature deliveries due to hypertensive disorders were calculated for each study separately. Pooled data were calculated.

Results: The search retrieved 36 relevant articles, of which eleven fulfilled the inclusion criteria. These eleven studies reported on 2377 patients (range 18 – 1754 patients per study), who had 2461 deliveries. Seven studies were included for further calculation. The pooled risk of a delivery before 34 weeks due to recurrence of hypertension, pre-eclampsia or HELLP was 7.8% (95% confidence interval 6.7% to 9.0%).

Conclusion: Opposed to some previous studies, the overall recurrence rate is generally low. The pooled recurrence risk of an early-onset hypertensive disorder is approximately 8%.
Recurrence risk of a pre-term delivery: a systematic review

Introduction

Early-onset hypertensive disorders of pregnancy (pre-eclampsia, hemolysis elevated liver enzymes low platelets [HELLP] syndrome) resulting in a delivery before 34 weeks gestational age occur in approximately 1% of pregnancies in nulliparous woman. Early onset pre-eclampsia is associated with an increased risk of adverse maternal and perinatal outcomes. Perinatal outcome is mainly correlated with gestational age at time of delivery and fetal growth restriction (FGR). Gestational age at admission is the most important factor in the prediction of adverse infant outcome. Maternal complications are placental abruption, coagulopathy, HELLP syndrome, pulmonary edema, acute renal failure, eclampsia, liver failure or hemorrhage, stroke or even death and remote cardiovascular morbidity.

The impact of a severe hypertensive complication of pregnancy is often significant, leading to anxiety of women before and during next pregnancies. Van Pampus et al reported that around 30% of women abstain from further pregnancies due to anxiety, because the risk of recurrence of hypertensive disorders in a subsequent pregnancy is known to be increased. The psychosocial condition of the mother after delivery is mainly associated with the gestational age at diagnose, the earlier the worse. Counselling of these patients is a difficult task for the obstetrician. Evidence on the recurrence risk of hypertensive disorders is important for several reasons. From the patient perspective, the decision for future pregnancies will partly depend on this knowledge. From a medical point of view, such knowledge may influence management during the subsequent pregnancy. Information about recurrence was until recently fragmentary and outdated, with the most quoted study of Sibai et al. dating from 1991. Recently more studies have been performed on this subject. As systematic knowledge on data on the recurrence rate of pre-eclampsia leading to preterm delivery is lacking we performed a systematic review on the recurrence of a hypertensive disorder before 34 weeks.

Methods

Literature search

We performed a search in the electronic libraries PubMed (MEDLINE), EMBASE and Cochrane Library. Language restrictions or restrictions on publication date were not applied. The search covered all records until September 2009. The following terms: “pre-eclampsia”[Mesh] AND [early or severe or pre-term or early onset or 32 or 34] AND [history or previous or secondary or subsequent or
recurrence] were used. Cross-references of the selected studies were checked to identify other studies.

We included all studies that described cohorts of women with a history of a hypertensive disorder that resulted in a delivery before 34 weeks of gestation. A hypertensive disorder was defined as gestational hypertension (GH), pre-eclampsia or HELLP syndrome. A hypertensive disorder was defined as severe, if delivery was before 34 weeks. Chronic hypertension was not an exclusion criterion. Gestational hypertension was defined as a diastolic blood pressure of at least 90 mmHg on two occasions, 4-6 hours apart. Pre-eclampsia was defined as hypertension with a persistent high diastolic blood pressure (≥ 90 mm Hg) or systolic blood pressure (≥ 140 mm Hg), in combination with significant proteinuria defined as ≥1+ (0.3g/L) or more on proteinuria dipstick test, a protein/creatinine ratio of 30 mg/mmol or more on a random sample or a urine protein excretion of 300 mg or more per 24 hrs after 20 weeks of pregnancy in a formerly normotensive woman. HELLP syndrome was defined as follows: haemolysis by abnormal peripheral smear (schistocytes, burr cells, echinocytes) and/or elevated serum bilirubin (indirect form) and/or low serum haptoglobin levels and/or elevated lactate dehydrogenase (LDH) levels (threshold of 180–600 U/L), or significant drop in hemoglobin levels; elevated liver enzymes by abnormal serum levels of aspartate transaminase (ASAT) ≥70 U/L and low platelets < 100,000/mm³.

Quality assessment and data extraction

Two authors (JL and SJ) independently screened titles and abstracts of identified studies. Relevant articles were selected and studied full text. Selection was performed by inclusion criteria and data extraction. Disagreements were resolved by consensus or by a third reviewer (BWM). Studies with data on the recurrence risk of hypertension, pre-eclampsia or HELLP in subsequent pregnancy after a premature delivery due to a hypertensive disorder were selected for further reading. Data-extraction was independently completed by JL and SJ by using a data-extraction form, which was partly based on the QUADAS (quality assessment of diagnostic accuracy studies) criteria. The assessment of the methodological quality of the article was partly based on the QUADAS tool and textbook guidelines. The following items were scored; (1) clearly description of selection criteria (in- and exclusion criteria), (2), if possible confounders were mentioned,(3) how selection of patients was performed (prospective cohort study versus case control) and (5) if the selection of patient was done in a consecutive manner. The quality of an article was defined as “good”, when all items were scored, “mediocre”, when one or two of these criteria were missing and “poor”, when more than two of these criteria were missing.
From the selected publications the following data were extracted: publication year, setting, begin and end of inclusion period, selection of patients, study design and number of patients that were lost to follow-up. We also recorded inclusion and exclusion criteria as well as possible confounders (chronic hypertension, body mass index, smoking habits, pre-existing diseases like diabetes, thrombophilia). The total number of women that delivered before 34 weeks of gestation due to a hypertensive disorder in their index pregnancy was listed. Either the exact gestational age at time of delivery had to be reported, or there had to be explicit information that there had been a delivery before 34 weeks of gestation. The use of medication that was intended to prevent a recurrence, such as anticoagulants was noted. For the subsequent pregnancies similar data were extracted. To be included, at least gestational age at delivery in the subsequent pregnancy or a subdivision between patients with a delivery before or after 34 weeks had to be reported. If the information on gestational age was not available the study was excluded.

**Data analysis**

The primary outcome was a recurrence of gestational hypertension, pre-eclampsia or HELLP leading to a delivery before 34 weeks of gestation. Secondary outcomes were the recurrence of a hypertensive disorder in pregnancy independently of gestational age. For each study we constructed a table with the number of patients in the index pregnancy, the number of patients who delivered before 34 weeks of gestation, between 34 and 37 weeks of gestation and after 37 weeks in their subsequent pregnancy due to a hypertensive disorder, and the number of patients with a completely uncomplicated pregnancy. For each study separately we calculated the absolute risk of recurrence on a premature delivery, with a 95% confidence interval (CI) for proportion.

Subsequently, data of all studies that included an unselected patient population were pooled using the Mantel-Haenszel method. If a study made a more stringent selection (tighter gestational age groups, or only HELLP-syndrome) than the inclusion criteria of this study, the study was excluded for pooling. From the pooled data, the absolute risk of recurrence with a 95% CI for proportion was calculated.

**Results**

The results of the search are shown in figure 1. The computerised Pubmed Embase and Cochrane Library search for pre-eclampsia identified 1447 publications. Among these studies were 416 duplicates. Of the 1061 unique titles we screened abstracts, 1025 were excluded because they did not match the inclusion criteria.
Thus, 36 articles were potentially relevant and the full text of these articles was screened. Checking of cross-references did not reveal further studies. Of these 36 articles, 25 were excluded. The main reason for exclusion was lacking information on the gestational age at delivery of the index pregnancy or of the subsequent pregnancy. Therefore they did not match the inclusion criteria of a delivery under 34 weeks of gestation or recurrence could not be calculated. Eleven articles on women pregnant after pre-eclampsia were relevant and included in this review (figure 1).

![Flowchart Search PE](image)

**Figure 1** Flowchart Search PE

The 11 included studies reported on 2377 patients (range 18 - 1754 patients per study). Three studies included all subsequent deliveries, i.e. a total of 255 deliveries in 171 patients\textsuperscript{23,24,25}, whereas the other eight studies only reported on the first subsequent pregnancy, i.e. a total of 2,240 subsequent deliveries in 2,240 patients. Table 1 shows the risk of recurrence of hypertension, pre-eclampsia or HELLP resulting in delivery before 34 weeks in the eleven studies.

Of the 11 studies, four studies describe a selected patient population (Sullivan\textsuperscript{22}, C. Chames\textsuperscript{23}, B.M. Sibai\textsuperscript{24} and Gaugler-Senden\textsuperscript{25}). They included a restricted patients group in which the hypertensive disorder was diagnosed in second
trimester (e.g. before 28 weeks gestation) and / or diagnosed with an early HELLP syndrome. These four studies are discussed in this review, but were excluded for further calculation of summary estimates because of the restricted patient selection (figure 2).

In the study of van Rijn et al.\textsuperscript{19} primiparous women who delivered under 34 weeks of gestation due to pre-eclampsia were included. All women received low-dose aspirin in their subsequent pregnancy. Six of 120 women experienced a complicated subsequent pregnancy and delivered before 34 weeks. In this study the recurrence risk was 5.0%, (95% CI 1.1% to 8.9%).

Sullivan et al.\textsuperscript{22} studied 122 patients with a HELLP syndrome and their recurrence rate. For a selection of the patients he reports recurrence under 34 weeks. He reported that 11 of 18 women with a previous HELLP syndrome resulting in delivery under 32 weeks, delivered before 32 weeks in their subsequent pregnancy due to a hypertensive disorder (recurrence risk of 61% (95% CI 39% to 84%)).

Visser et al.\textsuperscript{17} described the pregnancy course in 49 women with previous FGR. Of these 49 patients, 43 were diagnosed with pre-eclampsia resulting in delivery between 25 and 34 weeks in the index pregnancy. The subsequent pregnancy outcomes are presented separately for delivery before or after 34 weeks of gestation. The recurrence rate of subsequent delivery before 34 weeks of gestation due to a hypertensive disorder was 14/43 (32%, 95% CI 18% to 46%).

Kupferminc et al.\textsuperscript{20} included a cohort of 24 women who delivered before 34 weeks due to a severe hypertensive complication (pre-eclampsia, FGR or abruptio placentae) in the index pregnancy. All patients were diagnosed with thrombophilia. In the subsequent pregnancies all patients were treated with low-molecular-weight heparin (LMWH) and aspirin. Two of 24 women delivered before 34 weeks in their subsequent pregnancy of which one was diagnosed with FGR and one with pre-eclampsia. (recurrence rate 8.3% (95% CI -2.7% to 19%)).

We included two trials in which the patients were separated in two groups due to the treatment. (Ferrazzani et al.\textsuperscript{18} and Kalk et al.\textsuperscript{21}) (Table 1). Kalk et al.\textsuperscript{21} reported a retrospective multicentre cohort study, in which patients were included with thrombophilia and premature delivery before 34 weeks due to a hypertensive disorder. One group received LWMH and aspirin whereas the other group received aspirin or no medication in the subsequent pregnancy. In total, 58 patients were included of whom 14% had a recurrence (95% CI 4.9% to 23%). In subgroup analysis for treatment groups, the recurrence rate in the group who used LMWH and aspirin (N= 26 and recurrence occurred in 3 patients) was 12% (95% CI -0.7% to 24%). The recurrence rate in the group who used only aspirin or did not use any medication (N= 32 and recurrence occurred in 5 patients) was 16% (95% CI 3.1% to 28%).
Ferrazzini et al.\textsuperscript{18} included patients who delivered in their index pregnancy before 32 weeks of gestation due to a severe pre-eclampsia. Two groups were retrospectively compared in this study. One group used LMWH and aspirin in their subsequent pregnancy and the other group used only aspirin. The recurrence was defined as a delivery before 32 weeks of gestation in the subsequent pregnancy due to a pre-eclampsia or FGR (exact subdivision between pre-eclampsia or FGR was not made). The recurrence risk in the whole group (N=37), recurrence in 8 patients) was 30\% (95\% CI 15\% to 44\%). For the group who received LMWH and aspirin (N=24, recurrence in 3 patients) the risk was 12\% (95\% CI 0.7\% to 26\%). The group who received aspirin alone (N=13, recurrence in 8 patients) had a calculated risk of 62\% (95\% CI 35\% to 88\%).

Chames et al.\textsuperscript{23} reported on a selected population of 48 women who were diagnosed with HELLP syndrome and delivered before 28 weeks of gestation in their index pregnancy. They retrospectively analysed the pregnancy outcome after this severe complication. These women had 62 subsequent pregnancies. 25 Women delivered before 35 weeks of gestation and 33 before 37 weeks of gestation and had a hypertensive disorder. Recurrence rate for N=62, is 40\% (95\% CI 28\% to 53\%).

Sibai et al.\textsuperscript{24} reported on 108 women who had severe pre-eclampsia resulting in deliveries between 18 to 27 weeks of gestation and had 169 subsequent pregnancies, of which 110 were complicated by pre-eclampsia. In 35 pregnancies delivery was before 27 weeks, in 35 pregnancies women delivered between 28 and 36 weeks and in 40 pregnancies delivery was after 37 weeks. The recurrence rate in this study for N=169 was 21\% (95\% CI 15\% to 27\%).

**Table 1.** Included studies with reported number of patients included and outcome of the subsequent pregnancies. (IP, index pregnancy. SP, subsequent pregnancy)

<table>
<thead>
<tr>
<th>First author</th>
<th>Gestational age (weeks) and diagnosis at inclusion</th>
<th>Patients index pregnancy, IP (N)</th>
<th>Patients subsequent pregnancy, SP (N)</th>
<th>Delivery &lt;34 weeks SP (N)</th>
<th>Uncomplicated SP (N)</th>
<th>Missing items</th>
<th>methodological quality</th>
<th>Recurrence rate %, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visser et al.\textsuperscript{17}</td>
<td>25 – 34 FGR</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32% (18% - 46%)</td>
</tr>
<tr>
<td>Ferrazzani et al.\textsuperscript{18} (Patients with aspirin)</td>
<td>≤ 33 Pre-eclampsia or FGR</td>
<td>13</td>
<td></td>
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<tr>
<td>Ferrazzani et al.\textsuperscript{18} (Patients with LMWH and aspirin)</td>
<td>≤ 33 Pre-eclampsia or FGR</td>
<td>24</td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Van Rijn et al.\textsuperscript{19}</td>
<td>≤ 34 Pre-eclampsia</td>
<td>120</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Kupferminc et al.\textsuperscript{20}</td>
<td>23 – 34 Pre-eclampsia or FGR</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.3% (-2.7% -19%)</td>
</tr>
<tr>
<td>Kalk et al.\textsuperscript{21} (LMWH and aspirin)</td>
<td>≤ 34 Pre-eclampsia</td>
<td>26</td>
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<td></td>
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<td></td>
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<tr>
<td>Kalk et al.\textsuperscript{21} (aspirin or no medication)</td>
<td>≤ 34 Pre-eclampsia</td>
<td>32</td>
<td></td>
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<tr>
<td>Sullivan et al.\textsuperscript{22}</td>
<td>≤ 31 HELLP</td>
<td>18</td>
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<td>≤ 28 HELLP</td>
<td>46</td>
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<td>Sibai et al.\textsuperscript{24}</td>
<td>18 – 27 Pre-eclampsia</td>
<td>108</td>
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<tr>
<td>Gaugler et al.\textsuperscript{25}</td>
<td>≤ 24 Pre-eclampsia</td>
<td>17</td>
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<tr>
<td>Sep et al.\textsuperscript{26}</td>
<td>≤ 34 Pre-eclampsia or HELLP</td>
<td>152</td>
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<td>Hernández-Diaz et al.\textsuperscript{27}</td>
<td>≤ 34 Pre-eclampsia</td>
<td>1754</td>
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Gaugler-Senden et al.\textsuperscript{25} reported on 17 women diagnosed with pre-eclampsia and an onset before 24 weeks (all delivered before 30 weeks of pregnancy). They performed a retrospective case-control study to evaluate subsequent pregnancy outcome and the cardiovascular risk profile of women and their partners. These women had 24 subsequent pregnancies of which 12 were complicated by pre-eclampsia and 5 delivered before 34 weeks. Ten women had GH and only two a completely uncomplicated pregnancy. Thus the recurrence risk for this group was 21\% (95\% CI 5\% to 37\% N=24).

Sep et al.\textsuperscript{26} describe a cohort of 186 patients. They included patients who were diagnosed with pre-eclampsia or HELLP before 34 weeks of gestation. The cohort describes 152 patients delivered under 34 weeks in their index pregnancy of whom 11 experienced a recurrence of a delivery under 34 weeks and 45 patients experienced a late onset hypertensive complication. The recurrence rate was 7.24\% (95\% CI 3.1\% to 11.4\% N=152).

Hernández-Diaz et al.\textsuperscript{27} gained information on recurrence of pre-eclampsia from the Swedish Medical Birth Register. They sub-analysed the recurrence of severe pre-eclampsia (severe was defined as delivery before 34 weeks gestational age). From the 1754 patients with severe pre-eclampsia, 119 had a recurrence: giving a recurrence rate of 6.8\% (95\% CI 5.6\% – 8.0\% N=1754).

**Quality assessment**

Table 1 is a composition of the data from the database, some items were not reported in all studies. Methodological quality of the majority of the studies was good. The data in table 1 shows that the difference in the number of patients

<table>
<thead>
<tr>
<th>First author</th>
<th>Gestational age (weeks) and diagnosis at inclusion</th>
<th>Patients index pregnancy, IP (N)</th>
<th>Subsequent Pregnancy, SP (N)</th>
<th>Delivery &lt;34 weeks SP (N)</th>
<th>Uncomplicated SP (N)</th>
<th>Missing items methodological quality</th>
<th>Recurrence rate %, 95% CI</th>
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<td>25 – 34 FGR</td>
<td>43</td>
<td>14</td>
<td>11</td>
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<td>32% (18% - 46%)</td>
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<td>13</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>62% (35%–88%)</td>
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<tr>
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<td>24</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>12% (0.7% - 26%)</td>
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<tr>
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<td>120</td>
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<td>45</td>
<td>0</td>
<td>5% (1.1%–8.9%)</td>
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<td>23 – 34 Pre-eclampsia or FGR</td>
<td>24</td>
<td>2</td>
<td>21</td>
<td>2</td>
<td>8.3% (-2.7%-19%)</td>
<td></td>
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<tr>
<td>Kalk et al.\textsuperscript{21} (LMWH and aspirin)</td>
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<td>26</td>
<td>3</td>
<td>18</td>
<td>2</td>
<td>12% (-0.7%-24%)</td>
<td></td>
</tr>
<tr>
<td>Kalk et al.\textsuperscript{21} (aspirin or no medication)</td>
<td>≤ 34 Pre-eclampsia</td>
<td>32</td>
<td>5</td>
<td>16</td>
<td>2</td>
<td>16% (3.1%-28%)</td>
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<tr>
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<td>≤ 31 HELLP</td>
<td>18</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>61% (39%-84%)</td>
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<tr>
<td>Chames et al.\textsuperscript{23}</td>
<td>≤ 28 HELLP</td>
<td>46</td>
<td>62</td>
<td>25</td>
<td>1</td>
<td>40% (28%-53%)</td>
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<td>108</td>
<td>169</td>
<td>35</td>
<td>1</td>
<td>21% (15%-27%)</td>
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<td>≤ 34 Pre-eclampsia or HELLP</td>
<td>152</td>
<td>1754</td>
<td>119</td>
<td>0</td>
<td>7.2% (3.1%-11.4%)</td>
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<tr>
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<td>1754</td>
<td>119</td>
<td>0</td>
<td>0</td>
<td>6.8% (5.6%-8.0%)</td>
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</table>
included between studies is large. Figure 2 shows the calculated recurrence risk between the seven pooled studies. The pooled data of the seven studies with an unselected patient population, demonstrated a recurrence risk of 7.82% (95% CI 6.7% – 9.0%) for 2188 patients and their subsequent deliveries (Figure 2).

**Figure 2** Risk of recurrence of hypertensive disease leading to delivery < 34 weeks

<table>
<thead>
<tr>
<th>Study</th>
<th>Data</th>
<th>Recurrence of PE/PIH/HELLP leading to delivery &lt; 34 weeks</th>
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<tr>
<td>Visser et al.</td>
<td>'14/49</td>
<td></td>
</tr>
<tr>
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<td>Ferrazzani et al.</td>
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<tr>
<td>Kupferminc et al.</td>
<td>'2/24</td>
<td></td>
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<tr>
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<td>'3/26</td>
<td></td>
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<tr>
<td>Kalk et al.</td>
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<td>Sep et al.</td>
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<tr>
<td>Hernandez et al.</td>
<td>'119/1754</td>
<td></td>
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<tr>
<td><strong>Pooled estimate</strong></td>
<td><strong>'171/2188</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

In this first systematic review, we used the data from eleven studies that provided data to allow calculation of the recurrence rate of a premature delivery before 34 weeks of gestation, due to recurrence of hypertensive disorders. As four studies included a restricted patient population, only seven studies were used for pooling data. The pooled risk of recurrence of hypertension, pre-eclampsia or HELLP syndrome resulting in a delivery before 34 weeks was 7.8% (95% confidence interval 6.7% to 9.0%). As a corollary, there is a more than 90% chance of a delivery after 34 weeks in their subsequent pregnancy. As parents’ anxiety is mainly associated to prematurity of their child, this finding may influence parents’ decision on a future pregnancy in a positive way.

The most important limitation of this review is the lack of uniform criteria in the underlying studies. While some studies define severe by the gestational age at delivery, others define severe by symptomatology (laboratory or height of blood pressure). This results in large differences between studies. The exact gestational age at delivery was not reported in many studies that had to be excluded for that reason. Due to this missing information in the study of
Sullivan et al.\textsuperscript{22} for example, we could only include the 18 patients with a severe, early HELLP syndrome, whereas patients with another hypertensive disorder had to be excluded. A second limitation is the fact that the population size of some studies are small. Selection bias could be the reason that the most small studies had a higher risk estimate than our pooled result.

Another problem in the external generalizability is patient-related. Knowing the outcome of the subsequent pregnancies in patients who did get pregnant cannot as such be a reference for the outcomes of potential pregnancies of women who until now opted not to become pregnant again. How these pregnancies would have developed can only be speculated. Given a possible selection of patients who did and did not become pregnant again may substantially influence results.

For the first time we provided a systematic tool about information on recurrence of a premature delivery before 34 weeks of gestation due to a hypertensive disorder. Several new studies have been published recently. Previously, the publication of Sibai et al. in a highly selected population with a high recurrence rate was the main reference for counselling. Opposed to these previous studies our calculated pooled recurrence rate is generally low. Pooled analysis yields a probably more accurate risk assessment.

More prospective research on this subject, with focus on individual risk prediction is necessary. We did not attempt to obtain the original data from each study, though meta-analysis with individual patient data would be more accurate and would allow multivariable exploration of risk factors and creation of prediction models. If appropriate prediction models can be developed, the obstetrician can adjust the care for each individual patient at high risk of recurrence or low risk of recurrence.
References


Recurrence risk and prediction of a delivery under 34 weeks after a history of a severe hypertensive disorder

Josje Langenveld
Aletta Buttinger
Joris A. van der Post
Hans Wolf
Ben Willem J Mol
Wessel Ganzevoort

Abstract

Objective: The aim of this study was to report outcomes of the subsequent pregnancy after early-onset pre-eclampsia in a first pregnancy (index), and to evaluate the potential risk factors for recurrence of pre-eclampsia and preterm delivery.

Design: We performed a retrospective cohort study of all women who developed early-onset pre-eclampsia (delivery before 34 weeks of gestation) in their first pregnancy between January 1996 and December 2004 in two perinatal centres with regional function. All patients were included consecutively. Information was retrieved on the course of subsequent pregnancies.

Setting: Two tertiary centres with regional function.

Population: Women with a delivery under 34 weeks due to a hypertensive disorder (N=380).

Main outcome measures: We determined the absolute risk of recurrence of an adverse outcome, defined as a hypertensive complication resulting in delivery before 34 weeks of gestation. The available clinical parameters were evaluated as predictors for recurrence using logistic regression analysis.

Results: We identified 380 patients, of whom 46 were lost to follow-up. In total, 123 patients refrained from subsequent pregnancy (79 [64%] from fear of recurrence). Of the 211 patients with a subsequent pregnancy, 36 (17%, 95% CI 12–22%) had a recurrent delivery before 34 weeks of gestation, 30 (14%, 95% CI 9.5–19%) delivered between 34 and 37 weeks of gestation, and 145 (69%, 95% CI 62–75%) delivered later than 37 weeks of gestation. Of this last group, only 67 (32%, 95% CI 25–38%) pregnancies were completely uneventful. Chronic hypertension, maximum diastolic blood pressure, caesarean delivery and level of 24-h proteinuria were independent predictors for an adverse pregnancy outcome.

Conclusions: Women that had early severe pre-eclampsia in their first pregnancy have a 17% risk of recurrence, with a delivery before 34 weeks of gestation. Only 32% had a completely uneventful pregnancy.
Introduction

Hypertensive disorders of pregnancy, including pre-eclampsia and HELLP syndrome (haemolysis, elevated liver enzymes and low platelet count) affect 2–7% of pregnancies. Early-onset hypertensive disorders resulting in a delivery before 34 weeks of gestation constitute approximately 10% of this group. This subgroup is important because they contribute significantly to adverse maternal and perinatal outcomes, and psychosocial sequelae. Gestational age at admission and gestational age at delivery are the key factors in the prediction of adverse infant outcomes. Unfortunately, despite an abundance of early predictive tests for pre-eclampsia, with significant associations, the statistical accuracy of these tests is poor. Having a history of early-onset hypertensive disorders of pregnancy constitutes an increased risk of recurrence.

For parents, the psychosocial impact of a severe hypertensive complication of pregnancy and delivery before 34 weeks of gestation is often huge, and is associated with a high incidence of symptoms of post-traumatic stress disorder and depression, and anxiety of women before and during a subsequent pregnancy. Proper counselling regarding perinatal and maternal risk during future pregnancies is important to help couples in their consideration of a new pregnancy. The decision for future pregnancies largely depends on the information from the obstetrician gynaecologist on the estimated risk of recurrence of a preterm delivery, and the related severe maternal morbidity and serious neonatal morbidity. From a medical point of view, such counselling may influence management during the subsequent pregnancy, especially after early preterm delivery.

A common practical question from patients after these complicated pregnancies, and before engaging in a subsequent pregnancy, is: ‘what are my chances of delivering again early preterm (e.g. before 34 weeks of gestation) as a result of severe hypertensive disorder?’

In the absence of unselected, consecutively included and cohort studies of sufficient power, and in the absence of individual risk prediction models, we performed a cohort study of significant population size, without other selection than by gestational age and hypertension. As patients and doctors are mostly interested in the recurrence of a delivery earlier than 34 weeks, our primary aim was to study the recurrence risk of a delivery before 34 weeks of pregnancy as a result of a hypertensive disorder of pregnancy. Additionally, we identified independent related factors using a multivariate analysis for recurrence of early-onset pre-eclampsia.
Methods

Study population

We retrospectively identified patients from electronic databases from January 1996 to December 2004 in two perinatal centres with a regional function: the Maxima Medical Centre (MMC) in Veldhoven, the Netherlands, and the Academic Medical Centre (AMC) in Amsterdam, the Netherlands. All patients who were diagnosed with hypertension (including patients with chronic hypertension), pre-eclampsia or HELLP syndrome, and delivered before 34 weeks of gestation in the study period were included if they were primiparous with a singleton pregnancy without fetal abnormalities in the first pregnancy. Demographic, medical and obstetric data were extracted from the medical files. Information on subsequent pregnancies was first gained through the medical files. A subsequent pregnancy was defined as an ongoing pregnancy beyond 16 weeks of gestation. If no such information was available in the records of the institution, all individual women were contacted. If the patient reported an ongoing subsequent pregnancy, her gynaecologists and family doctor were contacted for data after informed consent. If the woman did not report a subsequent pregnancy, they were asked if there was a specific reason for not becoming pregnant again. All ethically available public resources were consulted if patients were lost to follow-up.

Primary outcome (adverse pregnancy outcome) among women with a subsequent pregnancy was defined as recurrence of a delivery before 34 weeks of gestation as a result of a hypertensive disorder. Secondary outcomes were delivery between 34 and 37 weeks of gestation, and the incidence of hypertensive disorders and delivery after 37 weeks, and related term disease.

Pre-eclampsia was defined as hypertension (diastolic blood pressure ≥90 mmHg or systolic blood pressure ≥140 mmHg measured on two occasions, 4–5 hours apart) in combination with proteinuria (defined as ≥1 + [0.3 g/l] in a proteinuria dipstick test, a protein/creatinine ratio of ≥30 mg/mmol in a random sample or a urine protein excretion of ≥300 mg per 24 hours) after 20 weeks of gestation. Women without proteinuria were considered to have gestational hypertension. Chronic hypertension is defined as the presence or history of hypertension preconception, or in the first half of pregnancy. HELLP syndrome was defined by haemolysis (elevated lactate dehydrogenase [LDH] levels [≥600 units/l], elevated liver enzymes by levels of aspartate transaminase [ASAT] or alanine transferase [ALAT] ≥ 70 units/l and low platelets <100.000/mm). Small for gestational age (SGA) was defined as a birthweight below the tenth percentile, adjusted for gestational age based on a local reference population. Routine diagnostic work-up in the postpartum period after an early-onset pre-eclampsia or HELLP
includes thrombophilia investigation. This includes factor-V Leiden or prothrombin mutations, protein-C, -S or antithrombin deficiency, or the presence of lupus anticoagulant and anticardiolipine antibodies.

Statistics

Clinical characteristics were compared between women who refrained from subsequent pregnancies and women who did have a subsequent pregnancy. Primary and secondary outcome are presented as n (%) and the 95% confidence interval (95% CI). We calculated the time interval between the two subsequent deliveries with dates of delivery taken as the reference point. Continuous variables are expressed as means with standard deviations, and are compared with an unpaired Student’s t-test. Categorical variables within the general characteristics were compared with analysis of variance and chi-square tests. P values <0.05 were considered to indicate statistical significance.

Subsequently, we analysed which clinical characteristics (known at the start of the subsequent pregnancy) were related to the primary outcome (recurrence of a delivery before 34 weeks of gestation in the presence of a hypertensive disorder). We used univariable and multivariable logistic regression analysis, with P values of 0.50 and 0.20 for variables for model entry and model stay, respectively. Demographic variables considered as relevant clinical variables were age and body mass index (BMI). Relevant obstetric variables known after the first pregnancy were the maximum systolic and diastolic blood pressures, proteinuria, chronic hypertension, gestational age at time of delivery, caesarean section in the first pregnancy, diagnosis of pre-eclampsia, HELLP and/or SGA in the first pregnancy. The results were expressed as odds ratios and 95% confidence intervals. All data were analysed using spss v16.0 for windows (SPSS Inc. Corp., Chicago, IL, USA).

Results

The study profile is shown in Figure 1. We identified 380 women (MMC n=181; AMC n=199). Of these, 46 (12%) were lost to follow-up, 211 (56%) had a subsequent pregnancy and 123 (32%) did not conceive again. All of these 123 women were contacted and asked for the reason for not conceiving again. Seventy-nine (64%) of the 123 women expressed a fear of recurrence as the main reason. The other 44 women of this group (36%) expressed other reasons for not conceiving again: having a complete family, no partner or subfertility (Figure 1).

To test for selection bias, baseline clinical characteristics of the 380 first pregnancies are summarised in Table 1, subdivided by category: patients with a subsequent pregnancy, loss to follow-up and patients without a subsequent
There were no relevant clinical differences in severity of disease in the first pregnancy between the three groups. There were a few statistically significant but clinically unimportant differences: the group of patients that did not conceive delivered significantly later, were older, had a higher BMI and had more caesarean deliveries.

Table 2 outlines the baseline demographic and clinical characteristics of the 211 subsequent pregnancies, subcategorised based on gestational age at delivery in the subsequent pregnancy.

Adverse pregnancy outcome (recurrence of a delivery before 34 weeks of gestation as a result of a hypertensive disorder) occurred in 36 women (17%, 95% CI 12–22%). Thirty (14%, 95% CI 9.5–19%) of the women delivered between 34 and 37 weeks, and 145 (69%, 95% CI 62–75%) delivered after 37 weeks of gestation. Of this last group that delivered after 37 weeks of gestation, only 67 women (32%, 95% CI 25–38%) had a completely uneventful pregnancy.

Of the women with an adverse pregnancy outcome, nine (25%, 95% CI 11–39%) were diagnosed with HELLP and 23 (64%, 95% CI 48–80%) were diagnosed with pre-eclampsia, compared with four (3%, 95% CI 0.09–5.4%) and 17 (12%, 95% CI 6.5–17%), respectively, who delivered after 37 weeks of gestation. The mean interval time between pregnancies was 31 ± 19 months (SD), which was not different between gestational age groups. The mean gestational age at delivery of the subsequent pregnancy was 37 ± 4 weeks (SD).
In multivariable logistic regression analysis, chronic hypertension, and index pregnancy maximum diastolic blood pressure, 24-h proteinuria and caesarean delivery, were independently correlated with an adverse outcome of a delivery before 34 weeks of gestation as a result of a hypertensive disorder in the subsequent pregnancy. BMI was excluded into the multivariable analysis because of too many missing values (Table 3). Chronic hypertension had a strong correlation

**Table 1**: Baseline clinical characteristics for the first pregnancy (delivery under 34 weeks of gestation due to a hypertensive disorder) of the 380 included patients subdivided by category: with a subsequent pregnancy, lost to follow-up and without a subsequent pregnancy.

<table>
<thead>
<tr>
<th>Demographic characteristic at the first pregnancy</th>
<th>Patients with a subsequent pregnancy (n = 211)</th>
<th>Patients who were lost to follow-up (n = 46)</th>
<th>Patients without a subsequent pregnancy (n = 123)</th>
<th>P values ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at delivery (years)*</td>
<td>28.6 (3.9)</td>
<td>27.3 (5.4)</td>
<td>30.4 (4.5)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Smoking</td>
<td>30 (14%)</td>
<td>4 (9%)</td>
<td>16 (13%)</td>
<td>0.87</td>
</tr>
<tr>
<td>Caucasian</td>
<td>177 (84%)</td>
<td>27 (59%)</td>
<td>103 (84%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Other ethnicity</td>
<td>22 (10%)</td>
<td>10 (22%)</td>
<td>10 (8%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Unknown ethnicity</td>
<td>12 (6%)</td>
<td>9 (20%)</td>
<td>10 (8%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Body mass index (kg/m²) at index pregnancy*</td>
<td>24 (4.4)</td>
<td>24 (5.5)</td>
<td>25 (4.6)</td>
<td>0.049</td>
</tr>
<tr>
<td>Chronic hypertension. (diagnoses complete period)</td>
<td>45 (21%)</td>
<td>9 (20%)</td>
<td>25 (20%)</td>
<td>0.89</td>
</tr>
<tr>
<td>Maximum systolic blood pressure (mmHg*) index pregnancy</td>
<td>174 (20)</td>
<td>176 (20)</td>
<td>175 (24)</td>
<td>0.68</td>
</tr>
<tr>
<td>Maximum diastolic blood pressure (mmHg*) Index pregnancy</td>
<td>111 (12)</td>
<td>114 (13)</td>
<td>110 (12)</td>
<td>0.46</td>
</tr>
<tr>
<td>Thrombophilia tested positive**</td>
<td>31 (15%)</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Index pregnancy characteristics</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery (weeks)*</td>
<td>30 (2)</td>
<td>30 (2)</td>
<td>31 (2)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cesarean Delivery</td>
<td>175 (83%)</td>
<td>42 (91%)</td>
<td>117 (95%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Delivery under 30 weeks</td>
<td>106 (50%)</td>
<td>22 (48%)</td>
<td>51 (42%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Birth weight (grams)*</td>
<td>1070 (379)</td>
<td>1026 (379)</td>
<td>1105 (327)</td>
<td>0.39</td>
</tr>
<tr>
<td>Small for gestational age (&lt;p10)</td>
<td>82 (39%)</td>
<td>26 (39%)</td>
<td>52 (42%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Diagnosis preeclampsia</td>
<td>183 (87%)</td>
<td>37 (80%)</td>
<td>109 (89%)</td>
<td>0.73</td>
</tr>
<tr>
<td>Diagnosis eclampsia</td>
<td>10 (5%)</td>
<td>4 (9%)</td>
<td>4 (3%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Diagnosis HELLP syndrome</td>
<td>122 (58%)</td>
<td>20 (44%)</td>
<td>62 (50%)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*Data are presented as mean (Standard Deviation) or n (%) as appropriate.

**Thrombophilia: factor V Leiden or prothrombin mutations, protein C, S or antithrombin deficiency or the presence of lupus anticoagulant and anticardiolipine antibodies.

*** Comparing patients with a subsequent pregnancy to patients without a subsequent pregnancy P < 0.05 is considered statistically significant.
with the outcome (OR 5.2, 95% CI 0.72–41), and the maximum diastolic blood pressure had an odds ratio of 12.0 per 12 mmHg (95% CI 10–13).

**Discussion**

This large cohort study adds data from 211 patients to the available literature on the course of pregnancies after premature deliveries resulting from hypertensive

<table>
<thead>
<tr>
<th>Baseline maternal characteristics in the subsequent pregnancy</th>
<th>Patients in their subsequent pregnancy subdivided in categories by gestational age in week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients (n = 211)</td>
</tr>
<tr>
<td>Age at delivery index pregnancy (years)*</td>
<td>31.4 (4.0)</td>
</tr>
<tr>
<td>Smoking</td>
<td>19 (9%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>177 (84%)</td>
</tr>
<tr>
<td>Other ethnicity</td>
<td>22 (10%)</td>
</tr>
<tr>
<td>Unknown ethnicity</td>
<td>12 (6%)</td>
</tr>
<tr>
<td>Body mass index (kg/m²) subsequent pregnancy*</td>
<td>25.2 (5.3)</td>
</tr>
<tr>
<td>Chronic hypertension (diagnoses complete period)</td>
<td>45 (21%)</td>
</tr>
<tr>
<td>Thrombophilia**</td>
<td>31 (15%)</td>
</tr>
</tbody>
</table>

**Maternal pregnancy outcome in the subsequent pregnancy**

| Maximum systolic blood pressure (mmHg*) subsequent pregnancy | 145 (23) | 163 (26) | 147 (19) | 138 (19) |
| Maximum diastolic blood pressure (mmHg*) subsequent pregnancy | 94 (13) | 103 (15) | 99 (14) | 90 (11) |
| Diagnosis preeclampsia                                       | 54 (26%) | 23 (64%) | 14 (47%) | 17 (12%) |
| Diagnosis eclampsia                                          | - | - | - | - |
| Diagnosis HELLP syndrome                                     | 18 (9%) | 9 (25%) | 5 (17%) | 4 (3%) |

**Fetal outcome in the subsequent pregnancy**

| Gestational age at delivery (weeks)*                         | 37 (4) | 30 (3) | 36 (1) | 39 (1) |
| Delivery under 30 weeks                                      | 17 (8%) | 17 (47%) | - | - |
| Birth weight (grams)*                                        | 2791 (950) | 1190 (580) | 2466 (568) | 3251 (529) |
| Small for gestational age (<p10)                            | 53 (25%) | 9 (25%) | 6 (20%) | 38 (26%) |

* Data expressed as mean (Standard Deviation) or as n (%) as appropriate

**Thrombophilia: factor V Leiden or prothrombin mutations, protein C, S or antithrombin deficiency or the presence of lupus anticoagulant and antiphospholipid antibodies.
Table 3. Results of the univariable and multivariable analysis for primary endpoint: delivery < 34 weeks due to a hypertensive disorder in the subsequent pregnancy after the first pregnancy. The first index pregnancy was defined as a delivery less than 34 weeks of gestation due to a hypertensive disorder.

<table>
<thead>
<tr>
<th>Demographical characteristics</th>
<th>Number of patients with missing data</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Odds</td>
<td>95% CI</td>
</tr>
<tr>
<td>Maternal age mother (years)</td>
<td>4</td>
<td>1.03</td>
<td>0.94 - 1.13</td>
</tr>
<tr>
<td>Body mass index (per point increase (kg/m²))</td>
<td>113</td>
<td>0.91</td>
<td>0.80 – 1.05</td>
</tr>
<tr>
<td>Chronic hypertension (yes or no)</td>
<td>2</td>
<td>2.2</td>
<td>0.96 – 4.90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Index pregnancy characteristics</th>
<th>Number of patients with missing data</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery (per increment of one day gestational age)</td>
<td>2</td>
<td>0.97</td>
<td>0.95 – 0.97</td>
</tr>
<tr>
<td>RR systolic maximum (per increment of 1 mmHg)</td>
<td>76</td>
<td>1.01</td>
<td>0.99 – 1.03</td>
</tr>
<tr>
<td>RR diastolic maximum (per increment of 1 mmHg)</td>
<td>20</td>
<td>1.05</td>
<td>1.01 – 1.08</td>
</tr>
<tr>
<td>Proteinuria (per increment of 1 gram/24 hours)</td>
<td>56</td>
<td>0.98</td>
<td>0.94 – 1.04</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>2</td>
<td>0.46</td>
<td>0.19 – 1.11</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>3</td>
<td>2.6</td>
<td>0.58 – 11.5</td>
</tr>
<tr>
<td>HELLP syndrome</td>
<td>2</td>
<td>0.64</td>
<td>0.30 – 1.34</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>2</td>
<td>0.79</td>
<td>0.37 – 1.70</td>
</tr>
</tbody>
</table>

disorders of pregnancy. The recurrence rate of a delivery before 34 weeks of gestation in this group was 17%, which fits well within the spectrum of recurrence rates in other studies. Fourteen percent delivered at between 34 and 37 weeks of gestation, and 69% of the women delivered after 37 weeks of gestation. Of this last group only 67 of them (32%) had a completely uneventful pregnancy. Although this 32% might seem a low percentage, the question of interest for the patient and the obstetrician is the recurrence of early preterm delivery. Morbidity of the mother and her child is mainly related to this early gestational age (e.g. under 34 weeks). From this point of view a recurrence rate of 17% is most relevant. The group of women without subsequent pregnancies counts for 32% of the index group, which is in agreement with other studies. Of these women, 64% did not conceive again because of the fear of recurrence. It might be hypothesised beforehand that this group of women had more severe disease in the first pregnancy, but this was not apparent in the data. The statistically significant differences calculated between the groups, such as BMI, age at time of delivery, greater number of caesarean deliveries and an average extra 1 week of gestational age at time of delivery, have no clinical relevance in our opinion. This
finding remains unexplained, therefore, and there may be speculations on the role of baseline psychosocial differences.

The main study for comparison, by Van Rijn et al.,15 also from the Netherlands, reported a lower recurrence rate of 5%. As background populations of both studies are more or less comparable for socio-geographical reasons, the shorter minimum follow-up time of 2 years (compared with our minimum follow-up time of 4 years) may explain the differences. It might be speculated that women who experienced more severe complications (such as maternal disease and early gestational age) take more time to engage in a subsequent pregnancy, and Van Rijn might have missed this group of patients.

A possible interpretation problem may be the confounding factor that women with more severe disease in the first pregnancy are more likely to end up at tertiary university centres. However, this effect is limited, because in principle all women before 32 weeks of gestation are referred to tertiary university centres in the Netherlands, according to the national guidelines. If second pregnancies were not handled in the tertiary care centres, data would be retrieved regardless.

Information about recurrence was until recently fragmentary and outdated, with the most quoted study of Sibai et al.16 dating from 1991. This study was the main reference for counselling, and concerned a highly selected population with a high recurrence rate of pre-eclampsia, of 65%, and a 21% recurrence rate of early pre-eclampsia, compared with the 5% recurrence rate reported by van Rijn et al.15 This variation probably arises as a result of population selection differences, and the small numbers involved. This also holds true for the bigger aspirin and antioxidant prospective prevention trials, such as the CLASP trial and the VIP or INTAPP trials, where secondary and primary prevention are the main concern, and not the specific recurrence rate of a preterm delivery.17-19 The study of Spinnato et al. included an unselected and heterogeneous group of patients, and mentioned a recurrence rate of 11.5% in 338 patients with a history of pre-eclampsia. They did not give the recurrence rate of severe pre-eclampsia resulting in delivery before 34 weeks of gestation.20 Caritis et al. selected a patient population that was at higher risk for developing pre-eclampsia. They included patients with previous pre-eclampsia and mentioned recurrence rates of 17% in the low-dose aspirin group versus 19% in the placebo group.21 These studies assessed the overall recurrence of pre-eclampsia without distinguishing between mild pre-eclampsia and the more clinically relevant category of severe pre-eclampsia. Recently, more specific data on the recurrence rate of an early delivery as a result of a hypertensive disorder became available, and a systematic review was published.14 Eleven studies with a total of 2461 patients (range 18–1754 patients per study) were available. For methodological reasons (heterogeneity), only seven studies (2188 patients) could be included for pooling. The pooled risk of recurrence of
hypertension, pre-eclampsia or HELLP resulting in delivery before 34 weeks was 7.8% (95% CI 6.7–9.0%). Variation between studies was significant, probably because of variation in patient selection and the wide variation in population size. Selection bias could be the reason that the smallest studies had a higher risk estimate than our pooled result, whereas bigger trials like Hernández-Díaz et al. were more comparable with a 6.8% recurrence rate. No data were available on women who decided to refrain from future pregnancies. Translation to clinical everyday practice therefore remains uncertain. The main point of the present study is that it consecutively included all patients who matched the inclusion criteria from 1996 to 2004. The study is well numbered and another strong point is the fact that for the first time data are shown on women who did not become pregnant again. A fourth point of advantage is the high follow-up rate and that there was an adequately long follow-up time. Preconception care becomes increasingly important, and this study adds clinical information for motivated counselling. Because anxiety and fear experienced is related to the preterm delivery, as well as the neonatal and maternal mortality and morbidity, patients want information before their subsequent pregnancy.

With increasing data becoming available, counselling becomes more informed and patients might make better decisions for further pregnancies. However, individual tailored advice and simple prediction models are still unavailable. In this study, we took the first step towards a prediction model based on simple clinical parameters that will be available for each obstetrician. We identified four simple factors in predicting adverse pregnancy outcome. A high diastolic blood pressure at the index pregnancy and chronic hypertension are related to an adverse outcome in the subsequent pregnancy. Chronic hypertension after the first pregnancy was associated with a five-fold increased risk of recurrence. However simple, the model needs elaborate perfection before aiding patient counselling and obstetric care. Given the surprising and not easily explained finding that caesarean section is a protective parameter, the model probably needs adjustments, indicating the limitation of this study. Another limitation is the retrospective character of the study. Factors that may have been interesting for the model might not have been available. Missing values in the multivariable model are another disadvantage arising from the retrospective design. An individual patient data meta-analysis of patients from comparable cohorts is planned. Subsequently, validation in the clinical setting would be the logical next step in the development of such a model.
References

Recurrence risk and prediction of a delivery under 34 weeks


Prediction of recurrence of hypertensive disorders of pregnancy between 34 and 37 weeks of gestation, a retrospective cohort study

Miriam F. van Oostwaard
Josje Langenveld
Rianne Bijloo
Kai Mee Wong
Irma Scholten
Stephanie Loix
Chantal W Hukkelhoven
Yvonne Vergouwe
Dimitri N.M. Papatsonis
Ben Willem J Mol
Wessel Ganjevoort

Submitted
Abstract

Objective: To assess the recurrence risk of late preterm hypertensive disease of pregnancy and to determine whether potential risk factors are predictive.

Design: Retrospective cohort study.

Setting: Three secondary and three tertiary care hospitals in the Netherlands.

Population: We identified women with a hypertensive disorder in the index pregnancy and delivery between 34 and 37 weeks between January 2000 and December 2002.

Methods: Data were extracted from medical files and women were approached for additional information on subsequent pregnancies. Adverse outcome was defined as recurrence of a hypertensive disorder in the next subsequent pregnancy.

Main Outcome Measures: The absolute risk of recurrence and a prediction model containing demographic and clinical factors predictive for adverse outcome.

Results: We identified 425 women who matched the criteria, of whom 351 could be contacted. Of these women, 189 (54%) had had a subsequent pregnancy. Hypertensive disorders recurred in 96 (51%) women, of whom 17 (9%) delivered again before 37 weeks. Chronic hypertension and maternal age were the strongest predictors for recurrence. Women undergoing recurrence had an 9-fold chance of developing chronic hypertension (37% versus 6% RR 8.7).

Conclusions: Women with hypertensive disorders and delivery late preterm have a 50% chance of recurrence, but only a 9% chance of recurrence resulting in delivery before 37 weeks. Women with chronic hypertension are prone to develop recurrence and women with a recurrence more often developed chronic hypertension.
Introduction

Hypertensive disorders of pregnancy, including preeclampsia (PE) and HELLP (Hemolysis, Elevated Liver enzymes and Low Platelets) syndrome affect 2 to 7% of pregnancies. Late preterm onset (e.g. between 34 and 37 week of gestation) represents 5 to 17% of these hypertensive disorders. Hypertensive disorders contribute substantially to both maternal and perinatal outcome worldwide and are the leading cause of maternal death in the Netherlands. Until now, prediction models on recurrence of hypertensive disorders in pregnancy have been unsatisfactory. Hypertensive disorders of pregnancy may have significant psychological impact on patients and their partners and many parents refrain from future pregnancies because of their earlier experiences. Therefore, informed counselling about future pregnancies is important.

For years, recurrence rates of 65% were referenced, based on the most quoted study of Sibai et al in 1991. Recently more studies have been published on recurrence rates of hypertensive disorders. Most studies concentrate on recurrence rates in women who suffered early onset severe hypertensive disorders or on recurrence rates in women who had preeclampsia at term. There are no studies published previously on recurrence of late preterm onset hypertensive disorders. There may be a different underlying pathophysiology mechanism, different risk factors and recurrence risks compared to term or early-onset PE, so data from either group may not be extrapolated to the late preterm gestational ages. For that reason we decided to explore this group, as it comprises a significant quantity in the non-academic setting. Our primary aim was to calculate the absolute risk of recurrence of a hypertensive disorder in the next subsequent pregnancy after delivery between 34 and 37 weeks of gestation due to a hypertensive disorder. Our secondary aim was to identify independent risk factors for recurrence using multivariate analysis and build a prediction model.

Materials and Methods

Study population

We retrospectively identified patients from electronic databases in six hospitals in the Netherlands: three secondary care centers (Amphia Hospital, Breda, Deventer Hospital, Deventer and Kennemer Gasthuis, Haarlem) and three tertiary care centers: (Maastricht University Medical Centre, Maastricht, Academic Medical Centre, Amsterdam and Maxima Medical Centre, Veldhoven). We consecutively included patients who delivered between 34 and 37 weeks gestation of their index
pregnancy between January 2000 until December 2002, and were diagnosed with gestational hypertension (GH), preeclampsia (PE), HELLP syndrome and/or delivered a Small for Gestational Age (SGA) child. Women carrying a pregnancy with fetal abnormalities were excluded, but chronic hypertension was not an exclusion criterion. Standard practice for determination of gestational age was ultrasound dating or if not performed a reliable first date of last menstrual cycle. We collected demographic data including: age, body mass index, parity, and cardiovascular risk factors like: smoking, chronic hypertension diagnosed before pregnancy, thrombofilia and family history for cardiovascular disease. Of the index and subsequent pregnancy we collected data on: highest systolic and diastolic blood pressure, use of medication, hospital days and perinatal outcome including gestational age at delivery, birth weight and perinatal death.

Data were extracted from the medical files. Information on subsequent pregnancies was gained first through the medical files. A subsequent pregnancy was defined as an ongoing pregnancy beyond 16 weeks of gestation. If such information was not available in the records of the institution, individual patients were contacted. If the patient reported she had had an ongoing subsequent pregnancy, her gynecologist or family doctor were contacted for data after written informed consent. All ethically available public resources were consulted if patients were lost to follow-up.

The outcome hypertensive disorders of pregnancy is a composite of PE, GH, HELLP syndrome and/or delivery of a SGA child. Preeclampsia was defined as hypertension (diastolic blood pressure ≥ 90 mmHg or systolic blood pressure ≥ 140 mmHg on two occasions, 4-5 hours apart) in combination with proteinuria (defined as 1+ (0,3 g/l) or more on proteinuria dipstick test, a protein/creatinine ratio of 30 mg/mmol or more in a random sample or an urine protein excretion of 300 mg or more per 24 hrs) after 20 weeks of pregnancy. Women with hypertension without proteinuria, or a significant rise in blood pressure if known chronic hypertension, were considered to have GH. Chronic hypertension was defined as the presence or history of preconceptional hypertension or hypertension detected in the first half of pregnancy. Superimposed preeclampsia includes de novo proteinuria, or a sudden increase in proteinuria if already present, in a woman with chronic hypertension. HELLP syndrome was defined by hemolysis (elevated lactate dehydrogenase (LDH) levels (≥ 600 U/L), elevated liver enzymes by levels of aspartate transaminase (ASAT) or alanine transferase (ALAT) ≥ 70 U/L and low platelets < 100,000/mm. SGA was defined as birth weight below the 10th percentile adjusted for gestational age based on a local reference population.
Statistical analysis

We expressed continuous variables as mean with standard deviation or median with range. Differences in baseline characteristics or outcomes between groups were tested with parametric (unpaired t-test) or non-parametric (Mann-Whitney-U test) tests as appropriate. Categorical variables were compared with Chi square tests. P values less than 0.05 were considered to indicate statistical significance. For each of the candidate predictors the odds ratio for the chance of recurrence was calculated. Univariate and multivariate logistic regression analysis was performed with recurrence of hypertensive disease (yes/no) as outcome measure. The Akaike Information Criterion (AIC) was used to select the strongest predictors for recurrence in a backward selection procedure.

Internal validation of the model was assessed with bootstrap procedures to estimate model performance that may be expected for new patients. Models, developed on the own study population, may be over fitted, indicating that high predictions are too high and low predictions are too low. A bootstrap (re-sampling) procedure was performed to assess optimism. One hundred bootstrap samples were drawn with replacement, and models were fitted in each sample. The mean difference in performance between the bootstrap samples and the original data was used to assess optimism. The bootstrap procedure provided a shrinkage factor, with which the regression coefficients of the predictors were multiplied (shrunken) to prevent the model for giving too extreme predictions for women with hypertensive disorders in pregnancy.

Overall performance of the prediction model was assessed with the $R^2$. This performance measure indicates the percentage of the total variation in women with and without recurrence that can be explained by the predictors in the model. The discriminative ability of the model, being the ability of the model to distinguish women with recurrence from women without recurrence, was assessed with the c-statistic. A model with a c-statistic of 0.50 has no discriminative power at all (comparable to a coin flip), and a c-statistic of 1.0 reflects perfect discrimination.

Missing data are often not completely at random, but rather selectively missing. Simply deleting the women with missing values (so-called complete case analysis) would thus result in invalid study results. Imputation of selectively missing values can reduce bias and allows for including all women in the analysis. Therefore, we multiple imputed these missing values 10 times. The imputation models included all the candidate predictors, and also plurality, ethnicity, education, family history of cardiovascular disease, family history of hypertension, family history of diabetes mellitus, family history of preeclampsia, smoking, medical anamnesis, systolic blood pressure, time interval between index pregnancy and second pregnancy and the outcome variable. Analyses were performed in each of the 10 multiple imputed datasets.
imputed data set. Estimates from the 10 data sets were then combined into one overall estimate and variance according to Rubin’s rules.

**Results**

We identified 425 patients who delivered between 34 and 37 weeks due to a hypertensive disorder. Of these 425 women, 79 (19%) were lost to follow-up, 157 (37%) did not become pregnant again and 189 (44%) had a subsequent pregnancy. Of the 157 women who refrained from a subsequent pregnancy, 36 (23%) made known that this was due to perceived risk. The median follow-up time since delivery of the index pregnancy was 8.5 years. The study profile is outlined in Figure 1.

![Figure 1](image-url) Flowchart of consecutively included patients with a history of a delivery between 34 and 37 weeks due to a hypertensive disorder and subsequent pregnancies.

To test for selection bias, we compared baseline characteristics between women with a subsequent pregnancy to women who did not become pregnant or were lost to follow-up. These characteristics are shown in Table 1. Women who conceived again were 3 years younger (29 versus 32 years) and smoked less often (11% versus 20%). They were also more likely to be Caucasian (92% versus 82%), nullipareous at index (89% versus 49%) and more likely to have HELLP syndrome.
in the index pregnancy (31% versus 20%), and less likely to have had a multiple pregnancy (6% versus 28%).

The mean gestational age at delivery in the index pregnancy was 36 weeks (SD = 6 days) and the mean birth weight 2256gr (SD = 525gr). In the subsequent pregnancy, mean gestational age at delivery was 39 weeks (SD 18 days) and mean birth weight 3171gr (SD = 728gr). In the index pregnancy 76 women had a multiple gestation (18%), versus 11 (6%) in the subsequent pregnancy. Perinatal death with various aetiologies occurred in 8 (2%) women in the index pregnancy and in 3 (1%) in the next pregnancy.
In the 189 women with a subsequent pregnancy, 96 women (51%) had recurrence of a hypertensive disorder in the subsequent pregnancy, of whom 17 women (9%) had recurrence of a hypertensive disorder and delivered before 37 weeks of gestation. The subsequent pregnancy was uneventful regarding hypertensive disorders in 93 women (49%).

For the 96 women who had recurrence of a hypertensive disorder in the next pregnancy, we compared the clinical characteristics of the index and subsequent pregnancy. The mean of the highest systolic and diastolic blood pressures were lower (160 versus 148 mmHg, p-value <.001 and 105 versus 96 mmHg, p-value <.001, respectively) in the subsequent pregnancy. In addition, the mean amount of proteinuria was less (3403 versus 808 mg/24h, p-value .006), which diagnosed 47 (49%) women with preeclampsia in the index pregnancy and 22 (23%) in the subsequent pregnancy. The mean duration of admittance at the hospital was shorter (12 versus 6 days, p-value <.001). Furthermore, anticonvulsive medication was used less often: 18 patients (19%) versus 7 (7%), p-value <.019, whereas the use of antihypertensive medication was comparable: 41 patients (43%) versus 31 (32%), p-value 0.100.

For the 189 women with a subsequent pregnancy we compared the recurrence of any of the individual syndromes (Table 2). None of the individual syndromes

### Table 2 Analysis of each individual syndrome on recurrence in the 189 women with a subsequent pregnancy. Percentages at the index sum up to more than 100% because of overlapping of syndromes.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Index pregnancy</th>
<th>Recurrence in the subsequent pregnancy</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small for Gestational Age</td>
<td>74 (39%)</td>
<td>41 (55%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG: 23 (31%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PIH: 21 (28%)</td>
<td>.292</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE: 7 (9%)</td>
<td>.295</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HELLP: 3 (4%)</td>
<td>.571</td>
</tr>
<tr>
<td>Pregnancy induced hypertension</td>
<td>62 (33%)</td>
<td>35 (56%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG: 9 (15%)</td>
<td>.277</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PIH: 27 (44%)</td>
<td>.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE: 3 (5%)</td>
<td>.032</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HELLP: 0 (0%)</td>
<td>.060</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>97 (51%)</td>
<td>47 (48%)</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG: 12 (12%)</td>
<td>.110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PIH: 29 (30%)</td>
<td>.504</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE: 18 (19%)</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HELLP: 6 (6%)</td>
<td>.066</td>
</tr>
<tr>
<td>HELLP syndrome</td>
<td>58 (31%)</td>
<td>27 (47%)</td>
<td>.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SG: 6 (10%)</td>
<td>.084</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PIH: 15 (26%)</td>
<td>.180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE: 12 (21%)</td>
<td>.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HELLP: 6 (10%)</td>
<td>.004</td>
</tr>
</tbody>
</table>
are more related to overall recurrence than the others, but they all seem to have a
tendency to recur in the same type as before.
For women who decided to refrain from a subsequent pregnancy due to perceived
risk, we evaluated pregnancy characteristics to see if they were more at risk than
the entire group. Women who refrained from pregnancy were 2 years older (31
versus 29 years), had had less amount of proteinuria (1303 versus 2653 mg/24h)
and had used antihypertensive medication more often (58% versus 42%) in the
index pregnancy (Table 3).

### Table 3: Characteristics of women who refrained from a subsequent pregnancy due to perceived risk.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Entire group N = 425</th>
<th>No subsequent pregnancy due to perceived risk N = 36</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at delivery (years)</td>
<td>29 ± 4</td>
<td>31 ± 4</td>
<td>.024</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>36 ± 6 days</td>
<td>35 ± 7 days</td>
<td>.184</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>2256 ± 525</td>
<td>2170 ± 513</td>
<td>.680</td>
</tr>
<tr>
<td>Max systolic BP (mmHg)</td>
<td>158 ± 23</td>
<td>163 ± 21</td>
<td>.779</td>
</tr>
<tr>
<td>Max diastolic BP (mmHg)</td>
<td>102 ± 14</td>
<td>108 ± 11</td>
<td>.091</td>
</tr>
<tr>
<td>Max proteinuria (mg/24h)</td>
<td>2653 ± 4267</td>
<td>1303 ± 1901</td>
<td>.007</td>
</tr>
<tr>
<td>HELLP</td>
<td>98 (23%)</td>
<td>13 (36%)</td>
<td>.071</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>15 (4%)</td>
<td>1 (3%)</td>
<td>.632</td>
</tr>
<tr>
<td>Hospital days</td>
<td>12 ± 9</td>
<td>15 ± 11</td>
<td>.607</td>
</tr>
<tr>
<td>Anticonvulsive medication</td>
<td>73 (14%)</td>
<td>10 (28%)</td>
<td>.650</td>
</tr>
<tr>
<td>Antihypertensive medication</td>
<td>178 (42%)</td>
<td>21 (58%)</td>
<td>.028</td>
</tr>
</tbody>
</table>

### Prediction model
The distribution and univariable regression analysis of candidate predictors of women
with and without recurrence is shown in table 4. Gestational age was not assessed
as a predictor, since it was one of the selection criteria. Only the predictors ‘chronic
hypertension before pregnancy’ and ‘maternal age at delivery’ were significantly
related to recurrence. Finally, multivariable analysis of the association between
chronic hypertension and age at delivery and recurrence is also shown in table 4.
The shrinkage factor, estimated with the bootstrapping procedure, was 0.96. Age at
delivery has an effect with an odds ratio of 1.1 per year (95% confidence interval (CI)
1.02 – 1.2). Chronic hypertension demonstrated an odds ratio of 7.9 (95% CI 2.6 - 24)
on the recurrence of hypertensive disorders of pregnancy. The $R^2$ of the model was
0.17 and the c-statistic 0.71 (95% CI 0.64 – 0.78).
All contacted patients were asked if they had developed chronic hypertension at
time of data collection (0.4 - 8.6 years after the last pregnancy). We had in total
missing data on 28 patients. Of 83 women with recurrence, 31 (37%) developed
Table 4 Distribution of and univariate analysis between candidate predictors and recurrence of hypertensive disorders in the subsequent pregnancy

<table>
<thead>
<tr>
<th>Candidate predictor</th>
<th>Recurrence N = 96</th>
<th>No recurrence N = 93</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
<th>% Missing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at delivery (years)</td>
<td>30 ± 4</td>
<td>28 ± 4</td>
<td>1.1</td>
<td>1.03 – 1.2</td>
<td>95% CI</td>
</tr>
<tr>
<td>Body mass index &gt; 30 kg/m²</td>
<td>14 (17%)</td>
<td>7 (9.2%)</td>
<td>1.8</td>
<td>0.69 – 4.4</td>
<td>95% CI</td>
</tr>
<tr>
<td>Chronic hypertension before pregnancy</td>
<td>28 (29%)</td>
<td>4 (4.4%)</td>
<td>9.2</td>
<td>3.1 – 27</td>
<td>95% CI</td>
</tr>
<tr>
<td>Cardiovascular risk factors other than hypertension*</td>
<td>7 (7%)</td>
<td>3 (3%)</td>
<td>1.4</td>
<td>0.27 – 6.9</td>
<td>95% CI</td>
</tr>
<tr>
<td>Characteristics of index pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>82 (85%)</td>
<td>87 (94%)</td>
<td>0.40</td>
<td>0.15 – 1.1</td>
<td>95% CI</td>
</tr>
<tr>
<td>Maximum diastolic blood pressure (mmHg)</td>
<td>104 ± 13</td>
<td>102 ± 15</td>
<td>1.0</td>
<td>0.99 – 1.04</td>
<td>95% CI</td>
</tr>
<tr>
<td>Diagnosis Small for Gestational Age</td>
<td>41 (43%)</td>
<td>34 (37%)</td>
<td>1.3</td>
<td>0.72 – 2.3</td>
<td>95% CI</td>
</tr>
<tr>
<td>Diagnosis HELLP syndrome</td>
<td>24 (25%)</td>
<td>28 (30%)</td>
<td>0.79</td>
<td>0.41 – 1.5</td>
<td>95% CI</td>
</tr>
<tr>
<td>Diagnosis preeclampsia</td>
<td>48 (50%)</td>
<td>51 (55%)</td>
<td>0.82</td>
<td>0.46 – 1.5</td>
<td>95% CI</td>
</tr>
</tbody>
</table>

* Cardiovascular risk factors: diabetes mellitus, manifested cardiovascular disease, thrombophilia and/or kidney disease

chronic hypertension, compared to 5 of 78 (6%) women without recurrence (OR 8.7, 95% CI 3.3 – 23). If they also delivered preterm due to recurrence, the odds ratio to develop chronic hypertension after recurrence was 4.8 (8 of 15 women compared to 28 of the 146 women without recurrence before 37 weeks of gestation; 95% CI 1.7 – 14). Of women who refrained from a subsequent pregnancy 23 of 86 (26%) developed chronic hypertension.

Discussion

This cohort study explores the recurrence risks of hypertensive disorders of pregnancy in the late preterm period. As such it fills a blank in literature. We found that half of the women had a subsequent pregnancy. Hypertensive disorders recurred in 96 (51%) women, of whom 17 (9%) delivered again before 37 weeks. Chronic hypertension and maternal age were statistical significant predictors for recurrence.

In this study, late preterm onset hypertensive disorders are explored for their recurrence rates, which was previously unstudied. Strengths of the present study are the substantial cohort size, the information on women who did not become
pregnant again to account for sources of bias, the adequately long follow-up time and the variability in settings (both academic and non-academic) and type of hypertensive disorder. Although our study is well numbered, it is too small to construct a prediction model on the recurrence of hypertensive disorders in the late preterm period in the subsequent pregnancy. Also, our cohort doesn’t comprise a control group. For that reason, we could not compare the risk of developing hypertensive disorders in a subsequent pregnancy to women with an uncomplicated first pregnancy. The inclusion of normotensive SGA pregnancies is debatable, but appropriate if considered as a continuum of hypertensive disorders of pregnancy. Furthermore, the effect of SGA on recurrence was separately assessed with the multivariate prediction model.

The differences between the women with or without a subsequent pregnancy or who were lost to follow-up are of no clinical importance. If a woman is older, multiparous or has had a multiple pregnancy, it is obviously more likely that she considers her family to be complete and chooses not to engage in a subsequent pregnancy. Ninety-seven (19%) women were lost to follow-up, mostly because they could not be traced after having moved. Twenty-three percent of the women, who refrained from a subsequent pregnancy, indicated this was due to the fear of recurrence. This concurs with other reports of the psychological impact of hypertensive disorders. In our subgroup analysis these women do not seem much more at risk. This suggests room for improvement in counseling.

The recurrence of hypertensive disorders leading to a delivery before 37 weeks, after an index pregnancy with a delivery between 34 to 37 weeks was 9%. While this percentage is relatively low, the overall recurrence rate of hypertensive disorders irrespective of gestational age in the subsequent pregnancy was 51%. If a hypertensive disorder of pregnancy recurred, in general the disorder was milder in the subsequent pregnancy. Mostello et al found an overall recurrence rate of 22% in a subset of the study of women with preeclampsia and a delivery between 33 and 36 weeks in the first pregnancy. Mostello, however, only included women with preeclampsia, which may explain the difference with our study. Two recent population-based studies show recurrence rates (at any gestational age) of 15% and 7% in women with preeclampsia in one previous pregnancy. These studies also show that preterm delivery, cardiovascular risk factors, preexisting renal disease and more preceding pregnancies complicated by the disorder, were associated with a higher chance of recurrence. The recurrence rate was lower in women with multiple gestation in the index pregnancy. Unfortunately, the above mentioned studies only investigated women with (severe) preeclampsia, whereas our cohort
included HELLP syndrome, SGA and GH as well. Ganzevoort et al stated that it is reasonable to regard these diseases as dynamic variations of one syndrome.\textsuperscript{17} Several studies report that HELLP syndrome recurs less often, but SGA recurs in 20 – 24 percent.\textsuperscript{15,18,19} There are almost no data available on recurrence of GH. In our cohort there were 62 patients with GH (alone or in combination with HELLP or SGA). The occurrence of hypertensive disorders in their subsequent pregnancy is 56\% (table 2). GH comprising one third of our cohort might explain the much higher overall recurrence rate in our cohort.

The low recurrence rate in the study of McDonald et al (7\%) can be explained to some extent by the exclusion of women with chronic hypertension before pregnancy.\textsuperscript{16} If the same method would have been used in this cohort, we would have included 156 normotensive women with a subsequent delivery. Recurrence of hypertensive disorders and preterm delivery occurred in 10 patients (6.4\%) and recurrence at any gestational age in 68 (44\%).

The performance and discriminative ability of the prediction model is reasonable, which is in line with several other studies, although inclusion criteria or endpoints do not concur. Sep et al created an overview of prediction models on recurrence of hypertensive disorders.\textsuperscript{6} Almost all studies focus on recurrence of just one disease, and there are only two models that identified chronic hypertension as a predictor.\textsuperscript{20,21} Both studies focus on recurrence of hypertensive disorders after an initial pregnancy with HELLP syndrome. Only one report provides odds ratios: women with chronic hypertension compared to normotensive women have an odds ratio of 1.8 – 3.7 of developing preeclampsia or HELLP, after an initial pregnancy with HELLP syndrome and delivery before 28 weeks.\textsuperscript{21} In our study chronic hypertension shows a greater effect on recurrence. Perhaps chronic hypertension has more effect on recurrence after hypertensive disorders other than HELLP, but it is impossible to compare these outcomes when such different inclusion criteria are used.

**Conclusion**

Preconception care and counselling is a growing aspect of obstetric care. Couples can make a better informed choice about a subsequent pregnancy and if needed, monitoring can be intensified in the subsequent pregnancy. Using the results of this study, individual risk counselling can be improved, but more individually tailored prediction models are needed.
References


Neonatal outcome of pregnancies complicated by hypertensive disorders between 34 and 37 weeks of gestation: a 7-year retrospective analysis of a national registry

Josje Langenveld  
Anita C.J. Ravelli  
Anton H. van Kaam  
David P. van der Ham  
Maria G. van Pampus  
Martina Porath  
Ben Willem J Mol  
Wessel Ganzevoort

Abstract

Objective: To determine the neonatal morbidity in late pre-term infants born from mothers with a hypertensive disorder.

Study Design: Data were obtained from the national Perinatal Registry in the Netherlands on women who delivered between 34+0 and 36+6 weeks with gestational hypertension (n=4316), preeclampsia (n=1864) and normotensive controls (n=20749).

Results: Children from preeclamptic mothers had an increased risk for NICU admission compared to children from normotensive mothers (odds ratio of 2.0, 95% CI 1.8– 2.2). A cesarean delivery and decreasing gestational age were independent risk factors for neonatal respiratory morbidity. Gestational hypertension or preeclampsia reduced the risk of respiratory distress syndrome compared to the control group (odds ratios of 0.81 (95% CI 0.64 – 1.0) and 0.69 (95% CI 0.49 – 0.96), respectively).

Conclusions: Neonatal morbidity in the late pre-term period is considerable. Hypertensive disorders appear to protect for neonatal respiratory morbidity, but higher rates of cesarean section diminish this protective effect.
Introduction

The majority of hypertensive disorders of pregnancy (gestational hypertension (GH), preeclampsia (PE)) present at term or late pre-term, only 10% occurs before 32 weeks.¹ Hypertension in pregnancy is associated with severe complications such as eclampsia, placental abruption, HELLP syndrome, pre-term delivery or even fetal or maternal death.¹-³ The probability of adverse perinatal outcome increases with lower gestational age.⁴,⁵

The only causal treatment for hypertensive disorders of pregnancy is to deliver the baby. There is a general consensus that in pregnancies complicated by early preeclampsia (e.g. under 32 weeks gestational age), temporizing management with close monitoring of mother and fetus is justified and prolongation of pregnancy can be achieved without irreversible maternal morbidity and with improved neonatal outcome.⁶,⁷ In contrast, in women with mild GH or PE at term, induction of labour resulted in a decrease of progression to severe disease or complications as well as a decreased number of cesarean sections compared to temporizing management.⁸ Moreover, induction of labour showed a trend to a better neonatal outcome.

Until now, only a few studies have focused on the management of women with hypertensive disorders between 34⁺⁰ and 36⁺⁶ weeks of gestational age. The NICE guideline “hypertensive disorders during pregnancy” refers in the 2010 consensus statement to the issue of mild or moderate preeclampsia between 34 and 36 weeks of gestation in terms of a “grey zone” at which the optimal timing of birth is not clear.⁹ Babies born late pre-term (e.g. 34⁺⁰ – 36⁺⁶ weeks gestational age) account for more than 70% of the pre-term deliveries (< 37 weeks).¹⁰ There are reports that these late pre-term children have significantly more morbidity than babies born at term.¹¹-¹⁷ However, it is unknown if this finding also applies to infants born from mothers with a hypertensive disorder, because in most studies these women were excluded from analysis. This also makes it difficult to determine the optimal obstetric management for these patients. To improve our understanding on the neonatal outcome of this specific population, and its causative factors, we analysed the data on neonatal morbidity in infants born from mothers with a hypertensive disorder between 34 and 37 weeks’ gestation from the Dutch National Registry, compared to morbidity rates of children born between 34 and 37 weeks’ gestation from normotensive mothers.
Materials and methods

Study population

Data were obtained from the Netherlands Perinatal Registry (PRN-registry) between January 2000 and December 2006. Since 2000 all gestation/delivery records (National Delivery Record - LVR), both home deliveries (LVR-1 registry) and hospital deliveries (LVR-2 registry), are combined with neonatal admission records (National Neonatal Register, LNR) into a national perinatal register (PRN). Méray and Tromp et al. have extensively described the technical approach and subsequent validation of the probabilistic linkage of these three anonymous population based registries of the midwives, obstetricians and neonatalogists. The LVR-1 and LVR-2 register have a 96% national coverage on all births (approximately 180,000 deliveries per year at > 16 completed weeks of gestation in The Netherlands). The LNR-registry has a 68% coverage of all hospitals in the Netherlands of whom the 10 perinatal centers have a coverage of 100% and the other hospitals 58%.

For the present study we included all women who delivered between 34 +0 – 36 +6 weeks of gestation. Exclusion criteria were; chronic hypertension, multiple pregnancies, non-cephalic presentation, congenital malformations, mothers diagnosed with AIDS, diabetes or drug use (drugs, not cannabis) and more than 24 hours rupture of membranes (in the Netherlands an expectant monitoring management is often followed for this latter group in the late pre-term period). These exclusion criteria were selected, because these specific conditions themselves are related to neonatal morbidities. If neonatal follow-up was not available, data were excluded. From the available data, cases were selected where the mothers were diagnosed with gestational hypertension (GH group) or preeclampsia (PE group). The other patients were the normotensive control group (pre-term delivery without a reason).

GH was defined as de novo hypertension, occurring after 20 weeks gestational age. Hypertension is defined as a diastolic blood pressure of ≥ 90 mmHg or a systolic blood pressure of ≥ 140 mmHg. PE was defined as de novo hypertension after 20 weeks gestational week and proteinuria (≥ 300 mg/day or a spot urine protein/creatinine ratio ≥ 30 mg/mmol). Patients could only be included based on their diastolic blood pressure and on the amount of proteinuria. The systolic blood pressure is not an item in this national database. This means that we might have missed a small group of patients that didn’t reach a diastolic blood pressure ≥ 90 mmHg during their entire pregnancy. According to the national guidelines, calculation of gestational age was based on the first day of the last menstrual
period and verified by a routinely performed first-trimester ultrasound in all patients.\textsuperscript{22}

Maternal baseline characteristics recorded were maternal age, ethnicity and parity. Variables recorded were gestational age at delivery, birth weight, small for gestational age (SGA - defined as birth weight below the 10\textsuperscript{th} percentile adjusted for gestational age based on a local reference population), 5 minute Apgar score < 7.0. Poor neonatal outcome included admission to the neonatal intensive care unit (NICU), metabolic and gastro-intestinal morbidity subdivided in hypoglycemia (defined as glucose serum or plasma level < 2.5 mmol/l), hyperbilirubinemia (indirect hyperbilirubinemie defined as above the phototherapy level, direct hyperbilirubinemia defined as > 10\% of the total serum bilirubine) or any stage of necrotizing enterocolitis (NEC).\textsuperscript{23} Respiratory problems were subdivided in the need for oxygen therapy more than 24 hours, any grade of infant respiratory distress syndrome (RDS: based on radiographic thorax findings according to Giedeon gr I-IV), bronchopulmonary disease (BPD: defined as more than 28 days oxygen therapy or oxygen therapy after 36 weeks postmenstrual) transient tachypnoe of the newborn (TTN: based on the typical clinical picture (respiratory support which is rapidly weaned within the first 24 hours of life) and chest radiograph (high lung volume)).\textsuperscript{24} Neurologic morbidity was subdivided into intracranial hemorrhage including intraventricular hemorrhage (IVH: defined according to Papile (grade I; hemorrhage subependymal - germinal matrix, grade II; intraventricular hemorrhage with normal ventricle size, grade III; intraventricular hemorrhage with ventricular dilation and grade IV; parenchymal hemorrhage), cerebral ischemia subdivided in any grade of periventricular leucomalacia (PVL: based on ultrasound images or MRI) or ischemia other than PVL, any stage of hypoxic ischemic encephalopathy (HIE) or convulsions.\textsuperscript{25,26} For this study only the first admission data after birth were used.

**Analysis**

The study population was categorised in 3 groups; GH, PE and the normotensive control group. In later analysis the 3 study groups were further subdivided by 3 groups of gestational age (GA) per week: delivered at 34\textsuperscript{+0} – 34\textsuperscript{+6} GA; 35\textsuperscript{+0} – 35\textsuperscript{+6} and 36\textsuperscript{+0} – 36\textsuperscript{+6} GA.

Data are presented as N (%) and in mean (± SD), as appropriate. We used Chi-Square to test for categoric data and Students T-test or one-way ANOVA (in case of > 2 variables) for continuous variables. Two-sided probability of <.05 was considered statistically significant. Data are expressed in odds ratio’s (OR) and 95\% Confidence Interval (CI). A multivariable logistic regression analysis was performed to examine respiratory morbidity outcomes across maternal subgroups (GH, PE and control) controlling for gestational age in 3 weeks groups (34, 35
and 36 weeks) and for onset or mode of delivery (in 7 groups). We checked for two possible interaction effects; first we tested for interaction between the study group (GH, PE and control) and gestational age in weeks and second between the study group and mode of delivery. Reference groups were the groups with the lowest risk. In addition we adjusted in the multivariable analysis for parity, maternal ethnicity and small for gestational age. Data of the multivariable analysis are expressed in odds ratio’s with 95% Confidence Intervals (CI). All analyses were performed using SAS software (Version 9.2; SAS Institute, Carry NC).

Results

From January 1st 2000 until December 31st 2006 a total of 1,246,440 singleton pregnancies were identified in the PRN database. After application of our in-and exclusion criteria 26,929 deliveries were the study population: 4,316 (16%) women in the GH group; 1,864 (7%) in the PE group; 20,749 (77%) in the control group. Differences in onset of labour between groups are shown in table 1. Both induction of labour and primary cesarean section occurred more frequent in the PE group compared to the GH group, with odds ratio’s of 2.8 (95% CI 2.5 to 3.2) and 2.1 (95% CI 1.9 to 2.4) respectively. Compared to the control group, the risk of induction of labour and primary cesarean section was increased even more strongly OR 16 (95% CI 15 to 18) and 7.4 (95% CI 6.6 to 8.2).

Baseline maternal and neonatal characteristics of all groups are outlined in table 2. All data were significantly different among the three groups. Birth weight was

<table>
<thead>
<tr>
<th>Table 1: Onset of labour</th>
</tr>
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<tbody>
<tr>
<td>Onset of labour</td>
</tr>
<tr>
<td>Gestational age in weeks</td>
</tr>
<tr>
<td>Proportion of deliveries</td>
</tr>
<tr>
<td>Spontaneous start of labour</td>
</tr>
<tr>
<td>Induction of labour</td>
</tr>
<tr>
<td>Primary cesarean</td>
</tr>
</tbody>
</table>

Table 1: Onset of labour between 34+0 and 36+6 weeks of gestational age in normotensive control mothers, hypertensive (GH) and preeclamptic (PE) mothers. Data are presented as No. (%) of deliveries. Spontaneous start of labour includes spontaneous start of contractions and / or spontaneous rupture of membranes. Induction of labour includes the use of prostaglandins and / or oxytocine and / or artificial rupture of membranes.
significantly different between groups: 2686 ± 425, 2482 ± 510 and 2248 ± 461 grams in the control, GH and PE mothers respectively, P<0.001. SGA was significantly more diagnosed in the PE group (27%) than in the GH group (18%; OR 1.7, 95% CI 1.5 to 1.9) and the control group (5.3%, OR of 6.7, 95% CI 5.9 to 7.5). More children in the PE group were admitted to the NICU compared to the GH and control group; OR 1.6 (95% CI 1.4 to 1.9) and RR 2.0 (95% CI 1.8 to 2.2) respectively.

Table 3 presents the neonatal outcome for each gestational age group in the normotensive control, GH and PE group.

The incidences of hypoglycemia, hyperbilirubinemia and NEC were not significantly different between the PE and GH group. However, there was a small but significant difference between the PE and control group for hypoglycemia OR 1.5 (95% CI 1.3 to 1.7) and for NEC OR 5.0 (95% CI 2.1 to 11). Throughout the groups there was a gestational age effect for hyperbilirubinemia and NEC (decreases with advancing gestational age), but not for hypoglycemia.

The incidence of ≥ 24 hours oxygen therapy was significantly different between the groups with an OR of 1.9 (95% CI 1.6 to 2.2) between the PE and the control group and an OR of 1.6 (95% CI 1.3 to 1.9) between the PE and the GH group. The incidence of RDS was not significantly different between the three groups. The PE group experienced more often TTN compared to the control OR 1.5 (95% CI 1.2 to 1.9) and to the GH group OR 1.5 (95% CI 1.1 to 1.9). The incidence of BPD was very low in all three groups. The risk for respiratory morbidity was inversely related to the gestational age.
The overall incidence of neurologic morbidity was low. The incidences of HIE, cerebral ischemia (not PVL), PVL and convulsions were not significantly different between all groups. In the PE group there was an increased risk for intracranial hemorrhage compared to the control group OR 2.2 (95% CI 1.0 to 4.8). Compared to the GH group, incidences were not significantly different. The gestational effect was also present in the neurologic morbidity (decreasing with advancing gestational age).

For the multivariable logistic regression analysis interaction between all independent variables were checked. There were no interaction effects between groups. The incidences of the neurologic and metabolic/gastro-intestinal morbidities were very low and therefore not calculated. The multivariable analysis showed an increased risk on respiratory morbidity with decreasing gestational age (Table 4). With the control group as a reference, the odds on a respiratory morbidity decreased in case of any hypertensive disorder. The adjusted odds ratio’s (OR) for RDS were 0.81 (95% CI 0.64 – 1.0) and 0.69 (95% CI 0.49 – 0.96) in the GH and PE group, respectively. For TTN the odds ratios were 0.81 (95% CI 0.67 – 0.98) and 0.91 (95% CI 0.70 – 1.2) in the GH and PE group respectively. The adjusted OR for ≥ 24 hours oxygen therapy were 0.87 (95% CI 0.76 – 0.99) and 1.0 (95% CI 0.84 – 1.2) in the GH and PE group respectively. Cesarean delivery was consistently associated with higher odds on a respiratory morbidity.

Table 2: Maternal and neonatal baseline characteristics of the cohort per category normotensive (control), hypertensive (GH) and preeclamptic (PE) mothers. NICU: neonatal intensive care unit. Small for Gestational Age was defined as birth weight below the 10th percentile adjusted for gestational age based on a local reference population.

<table>
<thead>
<tr>
<th>Maternal and neonatal characteristics*</th>
<th>Control</th>
<th>GH</th>
<th>PE</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deliveries*</td>
<td>20749 (77)</td>
<td>4316 (16)</td>
<td>1864 (6.9)</td>
<td>26929 (100)</td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)**</td>
<td>35.8 (0.78)</td>
<td>35.7 (0.79)</td>
<td>35.5 (0.84)</td>
<td>35.7 (0.79)</td>
<td></td>
</tr>
</tbody>
</table>

** Maternal characteristic

| Maternal age at delivery (years)** | 29.8 (5.0) | 30.6 (4.8) | 29.9 (5.1) | 29.9 (5.0) | <.0001 |
| Caucasian ethnicity*               | 17430 (84) | 3874 (90) | 1503 (81)  | 22807 (85) | <.0001 |
| Primiparous*                       | 11690 (56) | 2853 (66) | 1303 (70)  | 15846 (59) | <.0001 |

** Maternal characteristic

| Birth weight (grams)**              | 2686 (425) | 2482 (510) | 2248 (461) | 2623 (460) | < 0.0001 |
| Small for Gestational Age           | 1099 (5.3) | 776 (18)   | 508 (27)   | 2383 (8.9) | <.0001 |
| 5 minute Apgar score < 7*           | 735 (3.5)  | 197 (4.6)  | 82 (4.4)   | 1014 (3.8) | 0.002   |
| NICU admission ≥ 24 hours*          | 2666 (13)  | 684 (16)   | 419 (22)   | 3769 (14)  | <.0001 |

*Data are presented as No. (%) of deliveries

** Date are presented as mean ± standard deviation

The overall incidence of neurologic morbidity was low. The incidences of HIE, cerebral ischemia (not PVL), PVL and convulsions were not significantly different between all groups. In the PE group there was an increased risk for intracranial hemorrhage compared to the control group OR 2.2 (95% CI 1.0 to 4.8). Compared to the GH group, incidences were not significantly different. The gestational effect was also present in the neurologic morbidity (decreasing with advancing gestational age).

For the multivariable logistic regression analysis interaction between all independent variables were checked. There were no interaction effects between groups. The incidences of the neurologic and metabolic/gastro-intestinal morbidities were very low and therefore not calculated. The multivariable analysis showed an increased risk on respiratory morbidity with decreasing gestational age (Table 4). With the control group as a reference, the odds on a respiratory morbidity decreased in case of any hypertensive disorder. The adjusted odds ratio’s (OR) for RDS were 0.81 (95% CI 0.64 – 1.0) and 0.69 (95% CI 0.49 – 0.96) in the GH and PE group, respectively. For TTN the odds ratios were 0.81 (95% CI 0.67 – 0.98) and 0.91 (95% CI 0.70 – 1.2) in the GH and PE group respectively. The adjusted OR for ≥ 24 hours oxygen therapy were 0.87 (95% CI 0.76 – 0.99) and 1.0 (95% CI 0.84 – 1.2) in the GH and PE group respectively. Cesarean delivery was consistently associated with higher odds on a respiratory morbidity.
Comment

In this large retrospective analysis we analyzed the morbidity on late pre-term neonates born between 34+0 and 36+6 weeks of gestation due to a hypertensive disorder of the mother, compared to normotensive controls. Children from mothers with PE or GH are more often SGA, experience more morbidity and have higher NICU admission rates compared to the neonates born from normotensive mothers. The incidences of gastro-intestinal and neurologic morbidity were very low (below 1%) for all the three groups at all gestational ages.

Neonatal morbidity increased significantly within all three groups when gestational age was decreasing. As gestational age was approaching the term period, respiratory morbidity decreased significantly (below 1%). The multivariable analysis confirmed lower gestational age as an independent risk factor for respiratory morbidity. This result is in concordance with the recent publication of Hutcheon et al. reporting on neonatal morbidity between 36 and 41 weeks of gestational age in women with gestational hypertension.27

From literature we know that the incidence on neonatal respiratory morbidity is increased if mode of delivery is by cesarean delivery compared to a vaginal delivery.28,29,30 Also in our study the multivariable analysis indicates a primary cesarean delivery as an independent risk factor for respiratory morbidity. Consequently, the most logical explanation for the lower odds on respiratory morbidity in the multivariable analysis for the GH and PE group (compared to the not significant different incidences between the three group in table 3), is the correction for mode of delivery in the multivariable analysis (thus correcting for an independent factor that increases the risk on respiratory morbidity). The rate of primary cesarean section was much higher in the GH and PE group, compared to the control group. This is a potential source of bias, because cesarean section is probably associated with clinical severity of the hypertensive disorders of pregnancy.

The strength of this study is the size of the cohort, including patients over a long but recent period (2000 until 2006). Data are derived from a reliable and validated population-based data system. It includes almost all deliveries in the country and is therefore a good reflection of current clinical decision making.

Our study also has some limitations. First, the reliability of our data depends on the preciseness of registration of obstetricians and pediatrics. The size of the database minimizes this influence. Second, missing neonatal follow-up in the PRN excluded almost 30% of the cohort. We assume that the incidence of the neonatal morbidity in these hospitals does not differ from those who do have a registered neonatal follow-up. Both type of hospitals are equally distributed over the country, use the same national guidelines and are financed similarly. Some
The lack of information on administered

<table>
<thead>
<tr>
<th>Table 3: Neonatal morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neonatal outcome</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Gestational age</strong></td>
</tr>
<tr>
<td>Proportion of deliveries</td>
</tr>
<tr>
<td>(16)</td>
</tr>
<tr>
<td><strong>Metabolic / Gastro-intestinal morbidity</strong></td>
</tr>
<tr>
<td>Hypoglycemia</td>
</tr>
<tr>
<td>(7.4)</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
</tr>
<tr>
<td>(29)</td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
</tr>
<tr>
<td>(0.15)</td>
</tr>
<tr>
<td><strong>Respiratory morbidity</strong></td>
</tr>
<tr>
<td>Oxygen therapy ≥ 24 hours</td>
</tr>
<tr>
<td>(16)</td>
</tr>
<tr>
<td>Respiratory Distress Syndrome</td>
</tr>
<tr>
<td>(7.7)</td>
</tr>
<tr>
<td>BronchoPulmonary Disease</td>
</tr>
<tr>
<td>(0.03)</td>
</tr>
<tr>
<td>Transient Tachypnoe of Newborn</td>
</tr>
<tr>
<td>(5.4)</td>
</tr>
<tr>
<td><strong>Neurologic morbidity</strong></td>
</tr>
<tr>
<td>Intracranial Hemorrhage</td>
</tr>
<tr>
<td>(0.59)</td>
</tr>
<tr>
<td>Hypoxic Ischemic Encephalopathy</td>
</tr>
<tr>
<td>(0.46)</td>
</tr>
<tr>
<td>Cerebral Ischemia (not PVL)</td>
</tr>
<tr>
<td>(0.28)</td>
</tr>
<tr>
<td>PeriVentricular Leucomalacia</td>
</tr>
<tr>
<td>(0.37)</td>
</tr>
<tr>
<td>Convulsions</td>
</tr>
<tr>
<td>(0.40)</td>
</tr>
</tbody>
</table>

Table 3: Neonatal outcome of normotensive (control), hypertensive (GH) and preeclamptic (PE) mother between 34+0 and 36+6 weeks of gestation. Data are presented as No. (%). PVL: periventricular leucomalacie

data of interest were not available (systolic blood pressure and the administration of corticosteroids). The lack of the systolic blood pressure may have resulted in missing a small group of patients that did not reach a diastolic blood pressure ≥ 90 mmHg after 20 weeks gestational age. These patients might have been included in the control group. As this is a small group of patients we don’t think this will significantly influence the results. The lack of information on administered
<table>
<thead>
<tr>
<th>Gestational Age</th>
<th>GH</th>
<th>PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>34+0-6</td>
<td>1243</td>
<td>741</td>
</tr>
<tr>
<td>(29)</td>
<td>(17)</td>
<td></td>
</tr>
<tr>
<td>35+0-6</td>
<td>2332</td>
<td>4316</td>
</tr>
<tr>
<td>(54)</td>
<td>(100)</td>
<td></td>
</tr>
<tr>
<td>36+0-6</td>
<td>4316</td>
<td>1864</td>
</tr>
<tr>
<td>(100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1864</td>
<td></td>
</tr>
<tr>
<td>(100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of deliveries</td>
<td>741</td>
<td>20749</td>
</tr>
<tr>
<td>(100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic/Gastro-intestinal morbidity</td>
<td>1243</td>
<td>741</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>504</td>
<td>84</td>
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<tr>
<td>(8.9)</td>
<td>(11)</td>
<td></td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>1209</td>
<td>181</td>
</tr>
<tr>
<td>(21)</td>
<td>(24)</td>
<td></td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td>917</td>
<td>118</td>
</tr>
<tr>
<td>(7.8)</td>
<td>(57)</td>
<td></td>
</tr>
<tr>
<td>Respiratory morbidity</td>
<td>240</td>
<td>84</td>
</tr>
<tr>
<td>Oxygen therapy ≥ 24 hours</td>
<td>450</td>
<td>1243</td>
</tr>
<tr>
<td>(8.9)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Respiratory Distress Syndrome</td>
<td>141</td>
<td>50</td>
</tr>
<tr>
<td>(2.5)</td>
<td>(6.8)</td>
<td></td>
</tr>
<tr>
<td>BronchoPulmonary Disease</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>(0.04)</td>
<td>(0.24)</td>
<td></td>
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<tr>
<td>Transient Tachypnoe of Newborn</td>
<td>297</td>
<td>118</td>
</tr>
<tr>
<td>(2.5)</td>
<td>(5.3)</td>
<td></td>
</tr>
<tr>
<td>Neurologic morbidity</td>
<td>12</td>
<td>57</td>
</tr>
<tr>
<td>Intracranial Hemorrhage</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>(0.08)</td>
<td>(1.2)</td>
<td></td>
</tr>
<tr>
<td>Hypoxic Ischemic Encephalopathy</td>
<td>33</td>
<td>9</td>
</tr>
<tr>
<td>(0.26)</td>
<td>(3.2)</td>
<td></td>
</tr>
<tr>
<td>Cerebral Ischemia (not PVL)</td>
<td>7</td>
<td>53</td>
</tr>
<tr>
<td>(0.09)</td>
<td>(1.6)</td>
<td></td>
</tr>
<tr>
<td>PeriVentricular Leucomalacia</td>
<td>2</td>
<td>31</td>
</tr>
<tr>
<td>(0.03)</td>
<td>(1.0)</td>
<td></td>
</tr>
<tr>
<td>Convulsions</td>
<td>234</td>
<td>56</td>
</tr>
<tr>
<td>(4.1)</td>
<td>(4.5)</td>
<td></td>
</tr>
</tbody>
</table>
| Corticosteroids is another omission in the dataset. Standard care in the Netherlands is not to give corticosteroids after 34 weeks of gestation, so the amount of patients that received corticosteroids (before 34 weeks) is probably a very small group. It might be speculated that patients who are admitted to the hospital before 34 weeks, but who delivered after 34 weeks did get corticosteroids. Furthermore the...
Table 4. Multivariable Logistic Regression Respiratory Morbidity across subgroups

<table>
<thead>
<tr>
<th></th>
<th>Gestational age (per week)</th>
<th>Maternal group</th>
<th>Onset and mode of delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-36</td>
<td>-35</td>
<td>- Spontaneous start → spontaneous delivery</td>
</tr>
<tr>
<td></td>
<td>-35</td>
<td></td>
<td>- Spontaneous start → assisted vaginal delivery</td>
</tr>
<tr>
<td></td>
<td>-34</td>
<td></td>
<td>- Spontaneous start → secondary cesarean</td>
</tr>
<tr>
<td></td>
<td>-36</td>
<td></td>
<td>- Induced labour → spontaneous delivery</td>
</tr>
<tr>
<td></td>
<td>-35</td>
<td></td>
<td>- Induced labour → assisted vaginal delivery</td>
</tr>
<tr>
<td></td>
<td>-34</td>
<td></td>
<td>- Induced labour → secondary cesarean</td>
</tr>
<tr>
<td></td>
<td>-36</td>
<td></td>
<td>- Primary cesarean</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adjusted Odds Ratio (95% confidence interval)*</th>
<th>RDS</th>
<th>Transient tachypnoea of Newborn</th>
<th>≥ 24 hours oxygen therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDS Transient tachypnoea of Newborn ≥ 24 hours oxygen therapy</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Gestational age (per week)</td>
<td>3.6 (2.8 – 4.6)</td>
<td>1.6 (1.4 – 1.9)</td>
<td>2.4 (2.1 – 2.7)</td>
</tr>
<tr>
<td>Maternal group</td>
<td>11 (8.4 – 13)</td>
<td>2.0 (1.7 – 2.4)</td>
<td>4.7 (4.2 – 5.3)</td>
</tr>
<tr>
<td>Onset and mode of delivery</td>
<td>0.81 (0.64 – 1.0)</td>
<td>0.81 (0.67 – 0.98)</td>
<td>0.87 (0.76 – 0.99)</td>
</tr>
<tr>
<td>- Normotensive control group</td>
<td>0.69 (0.49 – 0.96)</td>
<td>0.91 (0.70 – 1.2)</td>
<td>1.0 (0.84 – 1.2)</td>
</tr>
<tr>
<td>- Hypertensive mothers</td>
<td>0.97 (0.67 – 1.4)</td>
<td>1.3 (1.0 – 1.7)</td>
<td>1.3 (1.0 – 1.5)</td>
</tr>
<tr>
<td>- Preeclamptic mothers</td>
<td>1.9 (1.3 – 2.8)</td>
<td>2.4 (1.8 – 3.1)</td>
<td>3.0 (2.5 – 3.7)</td>
</tr>
<tr>
<td>- Induced labour → spontaneous delivery</td>
<td>1.5 (1.0 – 2.1)</td>
<td>1.1 (0.79 – 1.5)</td>
<td>1.1 (0.91 – 1.4)</td>
</tr>
<tr>
<td>- Induced labour → assisted vaginal delivery</td>
<td>1.4 (0.49 – 3.7)</td>
<td>1.8 (1.0 – 3.3)</td>
<td>1.1 (0.64 – 1.9)</td>
</tr>
<tr>
<td>- Induced labour → secondary cesarean</td>
<td>1.9 (1.1 – 3.3)</td>
<td>2.8 (2.0 – 4.0)</td>
<td>2.5 (1.9 – 3.2)</td>
</tr>
<tr>
<td>- Primary cesarean</td>
<td>3.1 (2.5 – 3.8)</td>
<td>3.1 (2.6 – 3.7)</td>
<td>3.6 (3.2 – 4.1)</td>
</tr>
</tbody>
</table>

*We adjusted for parity, ethnicity and small for gestational age.

RDS: respiratory distress syndrome. Spontaneous start of labour includes spontaneous start of contractions and / or spontaneous rupture of membranes. Induction of labour includes the use of prostaglandins and / or oxytocine and / or artificial rupture of membranes.

A protective effect of hypertensive disorders on respiratory morbidity was present through all gestational ages.

In what perspective should we see this potential protective mechanism of a hypertensive disorder on neonatal respiratory morbidity? Conflicting results on the true effect of hypertensive disorders on neonatal respiratory morbidity are reported. Studies that did not correct for mode of delivery in their analysis, report a higher risk on a neonatal respiratory disorder when hypertensive disorders are present. However the studies that corrected for mode of delivery did not show a higher risk on neonatal respiratory disorders or even a protective effect. This latter result is supported by our study.

As mode of delivery seems to be an important influencing factor on the outcome, one could speculate that in case of a hypertensive disorder of the mother in the late pre-term period, induction of labour is preferable before deterioration of the maternal condition occurs and a primary cesarean section is the only solution for fast improvement of the maternal condition. Choosing the right moment for delivery though, remains a difficult task for the obstetrician. With increasing...
gestational age neonatal morbidity will decrease, but with increasing risk for maternal deterioration. In this perspective, the superior strategy is a randomised controlled trial comparing induction of labour to temporizing management. A lesser alternative could be a prospective closed cohort study.

In conclusion, neonatal morbidity in babies born from mothers with hypertensive disorders is still considerable in the late pre-term period, which is predominantly driven by gestational age and mode of delivery. Very interestingly, in our study the presence of a hypertensive disorder per se seems to protect for neonatal respiratory morbidity. However this protective effect is diminished by the higher cesarean rate in this group even resulting in higher incidences of respiratory morbidity in case of a hypertensive disorder.
References


Induction of labour versus expectant monitoring for gestational hypertension or mild pre-eclampsia between 34 and 37 weeks’ gestation (HYPITAT-II): a multicentre, open-label randomised controlled trial

Josje Langenveld
Kim Broekhuijsen
Gert-Jan van Baaren
Maria G. van Pampus
Anton H. van Kaam
Henk Groen
Martina Porath
Martijn A. Oudijk
Kitty W. Bloemenkamp

Christanne J. de Groot
Erik van Beek
Marloes E. van Huizen
Herman P. Oosterbaan
Christine Willekes
Ella J. Wijnen-Duverkok
Maureen T.M. Franssen
Denise A.M. Perquin
Jan M.J. Sporken

Mallory D. Woiski
Henk A. Bremer
Dimitri N.M. Papatsonis
Jozien T.J. Brons
Mesure Kaplan
Bas W.A. Nij Bijvanck.
Ben Willem J Mol

Abstract

Background: Gestational hypertension (GH) and pre-eclampsia (PE) can result in severe complications such as eclampsia, placental abruption, syndrome of Hemolysis, Elevated Liver enzymes and Low Platelets (HELLP) and ultimately even neonatal or maternal death. We recently showed that in women with GH or mild PE at term induction of labour reduces both high risk situations for mothers as well as the caesarean section rate. In view of this knowledge, one can raise the question whether women with severe hypertension, pre-eclampsia or deterioration chronic hypertension between 34 and 37 weeks of gestation should be delivered or monitored expectantly. Induction of labour might prevent maternal complications. However, induction of labour in late pre-term pregnancy might increase neonatal morbidity and mortality compared with delivery at term.

Methods/Design: Pregnant women with severe gestational hypertension, mild pre-eclampsia or deteriorating chronic hypertension at a gestational age between 34+0 and 36+6 weeks will be asked to participate in a multi-centre randomised controlled trial. Women will be randomised to either induction of labour or expectant monitoring. In the expectant monitoring arm, women will be induced only when the maternal or fetal condition deteriorates or at 37+0 weeks of gestation. The primary outcome measure is a composite endpoint of maternal mortality, severe maternal complications (eclampsia, HELLP syndrome, pulmonary oedema and thromboembolic disease) and progression to severe pre-eclampsia. Secondary outcomes measures are respiratory distress syndrome (RDS), neonatal morbidity and mortality, caesarean section and vaginal instrumental delivery rates, maternal quality of life and costs. Analysis will be intention to treat. The power calculation is based on an expectant reduction of the maternal composite endpoint from 5% to 1% for an expected increase in neonatal RDS from 1% at 37 weeks to 10% at 34 weeks. This implies that 680 women have to be randomised.

Discussion: This trial will provide insight as to whether in women with hypertensive disorders late pre-term, induction of labour is an effective treatment to prevent severe maternal complications without compromising the neonatal morbidity.
Background

Hypertensive disorders (including gestational hypertension (GH) and pre-eclampsia (PE)) complicate 10% of all pregnancies and can result in severe complications for the mother such as eclampsia, placental abruption, pre-term delivery, the syndrome of Hemolysis, Elevated Liver enzymes and Low Platelets (HELLP) and ultimately even neonatal and maternal death [1]. Hypertensive disorders in pregnancy are the largest cause of maternal mortality in the Netherlands [2,3]. Unfortunately, maternal mortality in the Netherlands has increased over the past decade, with pre-eclampsia remaining the number one cause of maternal death [4].

The majority of the cases of GH and PE declare itself after 32 weeks. As the origins of hypertensive disorders are probably related to faulty implantation of the placenta early in pregnancy, the only causal treatment of the disease is immediate delivery. Delivery will result in removal of the placenta, which will result in disappearance of the signs and symptoms of GH or PE within a few days. The timing of induction of labour has been debated, as induction was thought to increase the number of caesarean sections and in case of pre-term delivery, might compromise neonatal outcome. Our recent HYPITAT-I study provided us evidence that in women with GH and PE above a gestational age of 37 weeks, induction of labour not only resulted in a decrease of progression to severe pre-eclampsia or maternal complications, but unexpected also in a decrease in the number of caesarean sections (19% versus 14%, RR 0.75, 95% CI 0.55 – 1.04) [5]. Moreover, induction of labour showed a trend to a better neonatal outcome, a better maternal quality of life and a reduction in costs [6,7]. However for women with GH, PE or deteriorating chronic hypertension in the late pre-term period (e.g. 34, 35 and 36 weeks’ gestation), there remains uncertainty on the best policy. The situation in these patients is different, as apart from maternal morbidity, the condition of the child is at state. Late pre-term born children experience significantly more morbidity, compared to children born at term. Unfortunately, children born from mothers with a hypertensive disorder were mostly excluded from studies [8-10]. Thus, for women with hypertensive related complications of pregnancy between 34 and 37 weeks, the issue on whether these women should be monitored expectantly or not, remains an obstetric dilemma. In addition, the NICE guideline “hypertensive disorders during pregnancy” refers in the consensus statement to the issue of mild or moderate pre-eclampsia between 34 and 36 weeks of gestation in terms of a “grey zone” at which the optimal timing of birth is not clear and more research is necessary for this period [11].

In view of the relatively poor outcome of women with hypertensive pregnancy complications in The Netherlands as compared to abroad and in view of the
neonatal morbidity in case of a late pre-term birth, there is a need for critical evaluation of the management of hypertensive diseases during pregnancy in the late pre-term period in the Netherlands. With lack of good clinical evidence on the subject and the resulting practice variation, we think that additional data comparing immediate delivery and expectant monitoring in patients with hypertensive disease late pre-term are urgently needed. We therefore propose a nationwide randomised clinical trial and an economic cost analysis as well as quality of life analysis on the subject. We aim to provide data on the balance between cost (both financial and in terms of neonatal morbidity) and effects (prevention of maternal complications).

Methods and design

Aims
The aim of this study is to investigate whether planned induction of labour compared to expectant monitoring in women with gestational hypertension, mild pre-eclampsia or deteriorating chronic hypertension at gestational age of 34 – 37 weeks of pregnancy, will reduce maternal morbidity and / or increase neonatal morbidity. We hypothesize that induction of labour will reduce maternal morbidity and mortality, but to the costs of increased neonatal morbidity. The study will also provide insight on whether induction of labour in women with GH, PE or deteriorating chronic hypertension in the late pre-term period will influence the quality of life and costs compared to expectant monitoring. Long term follow-up of the neonates and the mothers will be formulated.

Participant / Eligibility Criteria
Patients 18 years of age or older are eligible if they have gestational hypertension, mild pre-eclampsia or deteriorating chronic hypertension at a gestational age between 34+0 and 36+6 weeks of pregnancy. The diagnosis of GH is made in case the diastolic blood pressure is equal to or above 100 mmHg at two occasions at least six hours apart in a woman who was normotensive until at least 20 weeks of gestation. The diagnosis of mild PE is made in case the diastolic blood pressure is above 90 mmHg at two occasions at least six hours apart in a woman who was normotensive until at least 20 weeks of gestation and if proteinuria exists (> 300 mg total protein in a 24 hour urine collection or > 30 in a spot urine protein:creatinine ratio). The diagnose of chronic hypertension is made in case the diastolic blood pressure is equal to or above 90 mmHg at two occasions at least six hours apart, diagnosed before 20 weeks of gestation. Women with chronic
hypertension are eligible in case there is need for additional medication or in case superimposed PE is diagnosed between 34+0 and 36+6 weeks’ gestation. Patients with singleton or multiple pregnancies are eligible, independent of the position of the fetus (i.e. cephalic or breech). Neither diabetes mellitus, nor small for gestational age nor a history of caesarean section are exclusion criteria. Randomisation only occurs after informed consent.

Exclusion criteria are a diastolic blood pressure equal to/greater than 110 mmHg despite medication, systolic blood pressure equal to/greater than 170 mmHg despite medication, proteinuria equal to/greater than 5 g/L, eclampsia, HELLP syndrome, pulmonary oedema or cyanosis, oliguria less than 500 ml in 24 hours, renal disease, heart disease, HIV-positive, non-reassuring fetal heart rate, zero-flow or reverse flow in the umbilical artery, fetal abnormalities including abnormal karyotype, severe pre-eclamptic complaints such as frontal headache or ruptured membranes.

**Procedures, recruitment, randomisation and collection of baseline data**

Eligible women will be identified by the local research coordinator and/or the staff of participating hospitals. After counselling and reading the patient information form, patients will be asked for written consent. We will provide patient information in Dutch as well as several other languages (Turkish, French, Spanish and English), in order to facilitate inclusion of patients from cultural minorities. After informed consent, patient data will be entered in a web-based database, which will also facilitate randomisation.

Prior to randomisation, we will perform a digital examination to establish a Bishop score and we will measure cervical length. Women will be randomly allocated to either delivery within 24 hours (experimental arm) or expectant monitoring (control arm) until 37 weeks of gestational age. The study will be an open label study, as it is impossible to blind the health care workers and patients involved for the strategy to which the woman is allocated. Randomisation will be performed centrally with the use of a permuted-block design, stratified for recruiting centre. Randomisation will be 1:1 for intervention and expectant monitoring management. Although it will not be possible to prevent all cross-overs, both strategies will be performed according to strict criteria, as mentioned below.

At study entry baseline demographic, past obstetric and medical history will be recorded into the web-based Case Report Form (CRF) that is accessible through a closed part of a central website. Details of delivery, maternal and neonatal assessments during pregnancy or post-partum are recorded in the CRF. The collected data will be coded and processed with adequate precautions to ensure patient confidentiality.
A subset of 200 women will receive quality of life questionnaires prior to randomisation, one day after randomisation and subsequently after 1 week, 2 weeks, 6 weeks, 3 months and 6 months. Since the gestational age at delivery will differ between the two groups, we will ask the participating women also to complete questionnaires at 1 day and 2 days after delivery. A pain scale will be added to those questionnaires. We hypothesize differences on the emotional and anxiety scales prior to delivery and differences in physical scales after delivery (due to differences in instrumental delivery rates). The questionnaires will contain the Medical Outcome Study 36-Item Short Form Health Survey (SF-36), the European Quality of Life 6 dimensions 3 levels (EuroQoL 6D3L) with subsequent general health Visual Analogue Scale (VAS), the Hospital Anxiety and Depression scale (HADS), and the Symptom Check List (SCL-90), all validated in Dutch and English.

**Interventions**

*Immediately delivery*

In the intervention group labour will be induced within 24 hours after randomisation. Induction of labour consists of amniotomy and augmentation of labour by administration of oxytocine. In case of an unfavourable cervix induction of labour will be preceded according to the local protocol. Prostaglandins will not be administered to women with a history of caesarean section. Cervical dilatation in these women will be achieved by a cervical Foley catheter followed by amniotomy and augmentation of labour with oxytocine. In case the patient gives no consent for vaginal delivery (for example breech presentation or history of two caesarean sections) the patient will be delivered by caesarean section within 24 hours after randomisation. After delivery, all patients in the intervention group will be monitored clinically.

*Expectant monitoring until 37 weeks*

In the expectant monitoring group, patients will be monitored until the onset of spontaneous delivery. If onset of labour has not occurred at 37+0 weeks gestation, labour will be induced. Monitoring will consist of assessment of fetal movements as reported by the mother, as well as electronic fetal heart rate monitoring at least twice a week. Maternal evaluation consists primarily of frequent evaluation of blood pressure measurement and screening of urine for protein using a dipstick or protein/creatinin ratio twice a week (24 hour urine collection for protein in case of positive screening). Blood tests (platelet count, liver enzymes and renal function) will be performed in case of abnormal maternal blood pressure and/or proteinuria. In the expectant monitoring group, intervention is recommended in case the fetal or maternal condition does not justify expectant monitoring anymore. These
criteria are similar to the exclusion criteria of the trial. All patients in the expectant monitoring group will be monitored clinically until after delivery.

**Follow-up of women and infants**

All details of delivery, maternal and neonatal assessments and admission during pregnancy are recorded in the CRF that is accessible through the website. Mortality and morbidity will be specified for the mother and the child until date of discharge from hospital and six weeks postpartum.

We plan long term follow-up at the age of 2 years with the Ages & Stages Questionnaires (ASQ) and the Child Behavioral Checklist (CBCL). By means of e-mails generated by our web-based registration system, our research nurses will be notified when a child approaches the corrected age of two years. After this alert, the research nurse will approach the parents of the child by phone and announce the sending of the questionnaire. In case the parents do not return the questionnaire, a reminder will be sent. We use the validated Dutch translation of the ASQ, covering the age-range 4-60 months and the validated Dutch translation of the CBCL 1½ - 5 years [12]. The ASQ is developed as a developmental screener and has been validated to pick up children at risk of a developmental disability. The CBCL (Child Behavioral Checklist) evaluates maladaptive behavioral and emotional problems in children reported by parents. It assesses internalizing (i.e., anxious, depressive, and overcontrolled) and externalizing (i.e., aggressive, hyperactive, noncompliant, and undercontrolled) behaviors. We will compare the total mean scores and T-scores between study arms and in addition we will compare the number of children scoring at 2 standard deviations below the mean of the Dutch reference group. A plan for long term follow-up of the mothers on their cardiovascular risk in the future is in preparation [13].

**Outcome measures**

*Primary outcome measure*  
The primary outcome measure will be a composite endpoint of maternal mortality, maternal complications (eclampsia, HELLP syndrome, pulmonary oedema, thromboembolic disease) and progression to severe pre-eclampsia. Eclampsia is defined as severe gestational hypertension or pre-eclampsia resulting in maternal seizures [14]. HELLP syndrome is defined as a complication of severe pre-eclampsia involving Hemolysis, Elevated Liver functions, and Low Platelets [14,15]. Thromboembolic disease is defined as deep-vein thrombosis, pulmonary embolism or both. Patients will be examined for deep-vein thrombosis by duplex Doppler if thrombosis is suspected from clinical examination. A diagnosis of pulmonary embolism will be confirmed by pulmonary angiography, computed
tomography, magnetic resonance imaging or a ventilation-perfusion lung scan
[16-18].

Progression to severe pre-eclampsia is defined as diastolic blood pressure equal/
greater than 110 mmHg despite medication, systolic blood pressure equal/greater
than 170 mmHg despite medication and/or proteinuria equal to/greater than 5 g/L.
The neonatal primary outcome will be respiratory distress syndrome (RDS). Diagnosis of RDS requires signs of respiratory distress, consistent radiologic features, and oxygen therapy with a fraction of inspired oxygen (FIO2) of 0.40 or
greater for at least 24 hours or until death [19].

**Secondary outcome measures**

Secondary maternal outcomes will be caesarean section rate, instrumental vaginal
delivery rate, maternal quality of life and costs. Secondary neonatal outcomes
will be transient tachypnoe of the newborn, hypoglycaemia, newborn sepsis,
confirmed seizures, necrotizing enterocolitis, hypoxic–ischemic encephalopathy,
cardiopulmonary resuscitation or ventilator support within 24 hours after birth,
umbilical-cord-blood arterial pH below 7.0, a 5-minute Apgar score of 3 or below,
admission to the neonatal intensive care unit (NICU) or neonatal death [19]. Transient
tachypnoe of the newborn is defined by the presence of tachypnoe within hours
after birth and typical radiologic findings. The diagnosis of necrotizing enterocolitis
requires confirmation by radiologic findings, surgery, or autopsy. The diagnosis of
hypoglycaemia requires a serum or plasma glucose level of less than 35 mg per
decilitre (1.9 mmol per litre) and treatment with intravenous glucose. Newborn
sepsis includes both suspected infections (with clinical findings suggesting
infection) and proved infections (as confirmed in a subgroup of neonates with
positive cultures of blood, cerebrospinal fluid, or urine obtained by catheterization
or suprapubic aspiration; cardiovascular collapse; or an unequivocal radiograph
confirming infection in a neonate with clinical sepsis).

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**Statistical issues**

**Data analysis**

Data will be analysed on an intention-to-treat basis. Analysis of data includes comparison of maternal baseline characteristics at randomisation, maternal morbidity and mortality until hospital discharge and 6 weeks post-partum, neonatal morbidity and mortality until hospital discharge, method of delivery, type of hospital care and days of maternal and neonatal hospital stay. Differences between groups with normally distributed data will be tested using the Student’s \( t \) test and for data with a skewed distribution, a non-parametric Mann-Whitney U test will be used. Categorical data will be analysed with \( \chi^2 \) statistics. Calculation of the percentages will be based on the number of valid observations. Treatment effect will be presented as relative risk (RR) with 95% CIs, and where appropriate as absolute risk reduction with 95% CIs and number needed to treat. A p-value of less than 0.05 indicates statistically significance. To assess treatment effects between different categories of patients in the trial we will perform subgroup analysis: per gestational age of the fetus per week (34+0 – 34+6, 35+0 – 35+6 and 36+0 – 37+0), parity (women with versus women without a previous vaginal delivery), hypertensive related disease (gestational hypertension versus pre-eclampsia), the use of corticosteroids (administered prior to delivery versus no use), mode of delivery (vaginal delivery versus caesarean section), caesarean delivery in history (history of a caesarean delivery versus no history of caesarean delivery) and the Bishop score (<2, 2-6 and >6).

**Sample size**

We hypothesize that immediate delivery can reduce maternal complications from 5% to 1%. Using a two sided test (alpha error 5%, beta error 20%) and assuming a 10% rate lost to follow-up and protocol violations, we aim to recruit 680 patients. This sample size is also large enough to assess whether there will be relevant differences in the primary neonatal outcome (incidence of RDS around 10% at 34 weeks versus 1-3% at 37 weeks).
Economic analysis

We plan an economic analysis alongside the clinical trial. This analysis will be performed from the societal perspective, meaning that we include both medical and non-medical costs to examine the economic impact of both strategies on the whole society.

Within the economic evaluation, we will compare the costs and effects of immediate delivery and expectant monitoring. The key question in the economic evaluation is to assess whether the expected increase of cost of maternal care in case of expectant monitoring will be higher than the expected increase of cost of neonatal care in case of immediate delivery.

The process of care is distinguished into three cost stages (antenatal stage, delivery/childbirth, postnatal stage) and three cost categories (direct medical costs [all health care sector costs], direct non-medical costs [costs outside the health care sector that are affected by health status or health care] and indirect costs of the pregnant woman and her partner [costs of sick leave].

For each stage and each cost category, costs are measured as the volumes of resources used multiplied with appropriate valuation (cost-per-unit estimates, fees, national reference prices). Cost volumes in the antenatal stage consist of admissions of the mother, maternal monitoring with various laboratory tests and fetal monitoring. In this stage, direct non-medical and indirect costs may be generated if role patterns or household routines shift. Costs generated during delivery are dominated by the course of childbirth and type of delivery. Resource utilization in the postnatal stage consist of maternal and neonatal health care during the hospital admission (maternal ward, NICU/neonatology admissions), and primary care following discharge. If neonatal health at discharge is suboptimal, further direct medical, direct non-medical and indirect costs may occur. Hence, for these infants, resource use of infants and/or parents is measured up till the corrected child age of 24 months.

Serious adverse events

Serious adverse events will be reported to an independent data safety monitoring committee. A formal interim analysis is not planned.

Ethical consideration

This study has been approved by the ethics committee of the Academic medical centre Amsterdam (ref.no MEC 08/244)
Confidentially and data security

Initials of participants as well as a local patient number are recorded in the electronic database. Linking names with patients’ numbers can only be done in the local clinics. Each participating clinic receives a login name and password to gain access to the web-secured database. The access is restricted to the database of the clinic to which the password and login name belongs. Full access to the entire database is restricted to some members of the research staff.

Discussion

Gestational hypertension (including chronic hypertension) and pre-eclampsia are important hypertensive disorders during pregnancy which are associated with increased maternal and neonatal morbidity and mortality. There is no consensus on how to manage these hypertensive disorders between 34 and 37 weeks gestation. Induction of labour will prevent maternal complications, but might also increase the neonatal morbidity due to the pre-term birth. This trial will provide evidence as to whether or not induction of labour in women with a hypertensive disorder in the late pre-term period is an effective treatment to prevent severe maternal complication, without compromising the neonatal morbidity.
References


Part III

Summary and general discussion
Summary and discussion
Summary

This thesis explores questions concerning hypertensive disorders during pregnancy and covers three main questions: first, what are the long-term health consequences for neonates born after a pregnancy with a compromised uterine environment? Second, what are the rates of recurrence of a hypertensive disorder in subsequent pregnancies? And third, what is the best management strategy, when hypertensive disorders arise in the late pre-term period (between 34 and 37 weeks’ gestation)?

The presentation of the studies in this thesis follows the timeline of the execution of the studies. The basis of this thesis was laid in 2004 - 2005 at the University of Texas Medical Branch, with a “basic science” program on vascular function, exploring the underlying pathophysiological mechanism of the association between an impaired uterine environment and the long-term health consequences for the offspring. On return in the Netherlands, studies were initiated on recurrence rates. In 2008 preparation started for the multicentre trial, HYPITAT-II. After development of the protocol, the case record form, gaining ethical approval and applying for a Grant, this study had its start in 2009.

Part I: Fundamental research

Stimuli or insults to the fetus during the critical period of intrauterine development lead to “fetal programming” and produce adaptive changes in fetal anatomy, physiology, and metabolism that have long-term consequences. For example, being born small for gestational age (a common complication of preeclampsia) is related to cardiovascular disease later in life. This theory is known as the “fetal origin of adult disease”.

In chapter 2 and 3 mice models with a compromised vascular system and endothelial dysfunction are used to study the pathophysiological mechanism of long-term health consequences after preeclampsia for offspring. These murine models have an impaired utero-placental perfusion and offspring of these animals experience a compromised uterine environment resembling the condition of placental insufficiency that is thought to be essential in early-onset preeclampsia. Chapter 2 presents a study using transgenic mice lacking endothelial nitric oxide synthase (eNOS). In vasculature eNOS is the main isoform responsible for the production of nitric oxide. Nitric oxide is a potent vasodilator. Defects in the nitric oxide pathway have been implicated in various vascular pathologic states that include hypertension and intra-uterine growth restriction. Offspring of eNOS-knockout mice are growth restricted and have abnormal vascular function...
later in life, compared with the genetically identical offspring of wild-type mice. This indicates a fetal programming effect of the abnormal uterine environment. To evaluate the role of parity in the modulation of the fetal programming of the fetal growth and vascular responses oligoparous (0-2 previous pregnancies) and multiparous (5-9 previous pregnancies) mice were used. Nitric oxide synthase knockout (−/−KO) female mice were bred with nitric oxide synthase−/−KO and wild type (+/+WT) male mice to produce nitric oxide synthase−/−KO and maternally derived heterozygous (+/−Mat) litters. Nitric oxide synthase knockout and nitric oxide synthase maternal litters that were born to oligoparous mothers had significant growth lag compared with corresponding multiparous litters. The optimal diameter of the carotid artery from the offspring (a measure of vascular tensile properties and resistance) was decreased in oligoparous compared with multiparous female offspring. Vasorelaxation was abolished and the contractile response of the carotid artery was increased in oligoparous, but not multiparous, female offspring ($P < .05$). These data support the role of abnormal uterine environment in the fetal programming of postnatal growth and vascular function in later life. Successive pregnancies may lead to maternal uterine adaptations that bypass the lack of a functional nitric oxide synthase, which leads to improvement in postnatal growth and vascular function in the offspring. Given the reported effect of parity on the risk of preeclampsia, similar mechanisms may be operative in human pregnancy.

In chapter 3 transgenic mice are used lacking a functional low-density lipoprotein receptor (LDLR). In the process of arteriosclerosis, lipoprotein particles (especially low-density lipoprotein (LDL)) enter the arterial wall and undergo various modifications, such as oxidation. Oxidation of LDL has been proposed as an important mechanism in the pathogenesis of the arteriosclerotic process. The LDL-receptor (LDLR) is involved in the clearing of lipoproteins from the circulation, and its lack leads to hypercholesterolemia and arteriosclerosis. LDLR−/−KO mice fed a normal diet develop moderate fatty streak lesions and intima thickening with foam cells and smooth muscle cell infiltration. In this chapter the role of maternal hypercholesterolemia in fetal programming of adult vascular function is examined. Homozygous LDLR knockout mice (LDLR−/−KO) and their wild-type controls (LDLR+/+WT) were cross-bred to produce 4 litter groups: LDLR−/−KO, maternally derived heterozygous (LDLR±Mat), paternally derived heterozygous (LDLR±Pat) and LDLR+/+WT offspring. In vitro experiments using the carotid artery of the offspring at adult age showed increased contractile responses in the LDLR−/−KO and LDLR±Mat male offspring compared to the LDLR±Pat and LDLR+/+WT offspring. The contractile responses in female mice were only significantly increased in the LDLR−/−KO offspring. Despite being genomically similar, heterozygous offspring that developed in a hypercholesterolemic maternal environment
Chapter 4 summarized the accuracy of various tests performed to predict the onset of preeclampsia and the effectiveness of preventative treatment. Tests to predict preeclampsia include clinical history, examination findings, laboratory and hemodynamic tests. In general, tests in early pregnancy for predicting later development of preeclampsia have a better specificity than sensitivity, as BMI (Body Mass Index) >34, alpha-fetoprotein, fibronectin and uterine artery Doppler (bilateral notching) all have specificities above 90%. Only uterine artery Doppler resistance index and combinations of indices have a sensitivity of over 60%. None of the tests are sufficiently accurate to recommend routine use in clinical practice. The various treatment options to prevent preeclampsia include pharmacological agents, dietary supplementation and lifestyle modification. Antiplatelet agents, primarily low-dose aspirin reduce the risk of preeclampsia by 10% (RR 0.90, 95% CI 0.84 to 0.97). Calcium only effectively prevents preeclampsia in high risk groups (RR 0.22; 95% CI 0.12 – 0.42) and in the group with low nutritional calcium intake (RR 0.36; 95% CI 0.20 -0.65). Pharmacological agents like low molecular weight heparin, progesterone, nitric oxide donors, anti-hypertensives and diuretics are not effective in preventing preeclampsia. Dietary supplements like magnesium, anti-oxidants, marine oils and folic acid do not reduce the incidence of preeclampsia. There is a paucity of evidence to demonstrate that lifestyle interventions like rest, exercise and reduced dietary salt intake prevent preeclampsia.

Chapter 5, 6 and 7 focus on recurrence rates of a hypertensive disorder during pregnancy after a history of a pre-term delivery due to a hypertensive disorder. The psychosocial impact of a severe hypertensive complication of pregnancy and pre-term delivery is often huge and associated with a high incidence of symptoms of post-traumatic stress disorder, depression and anxiety. The psychosocial condition of the mother after delivery is mainly associated with the gestational age at diagnosis. Evidence on the recurrence risk of hypertensive disorders is important for several reasons. From the patient perspective, the decision for future pregnancies will partly depend on this knowledge. From a medical perspective, such knowledge may influence management during the subsequent pregnancy.
Chapter 5 presents a systematic review on existing cohort studies on recurrence rates of a hypertensive disorder and pre-term delivery after a history of delivery under 34 weeks gestational age due to a hypertensive disorder. We searched Medline, Embase, and the Cochrane Library for articles published until September 2009. Recurrence rates of premature deliveries due to hypertensive disorders were calculated for each study separately. Pooled data were calculated. The search retrieved 36 relevant articles, of which 11 fulfilled the inclusion criteria. These 11 studies reported on 2,377 patients (range 18 to 1,754 patients per study), who had 2,461 deliveries. Most studies were excluded because of missing data on the exact gestational age of delivery in the subsequent pregnancy. The lack of uniform criteria for severe hypertensive disorders is another omission. Of these 11 studies, four were excluded for further calculations as they included a restricted patient population of very early onset of disease (e.g. before 28 weeks’ gestation) implicating a selection bias, leaving seven studies for further calculations. The pooled risk of a delivery before 34 weeks due to recurrence of hypertension, preeclampsia, or HELLP was 7.8% (95% confidence interval 6.7 to 9.0%). In conclusion, there is a more than 90% chance of a delivery after 34 weeks in patients’ subsequent pregnancy. As parents’ anxiety is mainly associated to the gestational age at diagnose, this finding may influence parents’ decision on a future pregnancy in a positive way.

The systematic review showed a lack of good quality cohort studies, as many studies report on a selected non-consecutive series of patients with limited numbers. Consequently, reported recurrence rates range from as high as 65% to the lowest of 5%.11,12

Chapter 6 presents a retrospective cohort study of significant population size, without other selection than by gestational age and hypertension. The primary aim was to determine the absolute risk of recurrence of an adverse outcome, defined as a hypertensive complications resulting in a delivery under 34 weeks’ gestation. Additionally, independent related factors were identified using a multivariate analysis for recurrence of early-onset preeclampsia. All women who developed early-onset preeclampsia (delivery <34 weeks gestation) in their first pregnancy between January 1996 and December 2004 were included, in two perinatal centers with regional function: the Academic Medical Centre Amsterdam (AMC) and the Maxima Medical Centre Veldhoven (MMC). Patients were included consecutively. Information was retrieved on the course of subsequent pregnancies. 380 Patients were identified, of whom 46 were lost to follow-up. 123 Patients refrained from subsequent pregnancy (79 (64%) due to fear of recurrence). Of the 211 patients with a subsequent pregnancy, 36 (17%, 95% CI 12% to 22%) had a recurrent delivery under 34 weeks, 30 (14%, 95% CI 9.5% to 19%) between 34 and 37 weeks and 145 (69%, 95% CI 62% to 75%) above 37 weeks of gestation. Of this last group,
only 67 (32%, 95% CI 25% to 38%) pregnancies were completely uneventful. A high diastolic blood pressure at the index pregnancy and chronic hypertension are related with an adverse outcome in the subsequent pregnancy. Chronic hypertension after the first pregnancy was associated with a 5 fold increased risk of recurrence (odds ratio 5.2, 95% CI 0.72-41). In conclusion, women with early severe preeclampsia in their first pregnancy have a 17% risk of recurrence of a delivery before 34 weeks and having chronic hypertension is an important risk factor for recurrence. Only 32% however had a completely uneventful pregnancy. Although this 32% might seem a low percentage, the question of interest for the patient and the obstetrician is recurrence of early pre-term delivery. Morbidity of the mother and her child is mainly related to this early gestational age (e.g. under 34 weeks). In that point of view a recurrence rate of 17% is most relevant.

In chapter 7 the recurrence risk of hypertensive disease in pregnancy between 34 and 37 weeks' gestation is assessed, in a retrospective cohort study in six hospitals in the Netherlands. There are no studies published previously on recurrence of late pre-term onset of hypertensive disorders in pregnancy (e.g. between 34 and 37 weeks gestational age). There may be different underlying pathophysiological mechanisms and risk factors for early-onset preeclampsia versus term preeclampsia. Therefore recurrence risks from either early-onset or term preeclampsia may not be extrapolated to the late pre-term gestational age group. Meanwhile this group comprises a significant quantity in the non-academic setting. The primary aim was to determine the absolute risk of recurrence and assess whether cardiovascular risk factors were predictive. 425 Women were identified, of whom 351 could be contacted. In the 189 women with a subsequent pregnancy, 94 women (50%, 95% CI 43% to 57%) had recurrence of a hypertensive disorder in the subsequent pregnancy, of whom 17 women (9.0%, 95% CI 4.9% to 13%) had recurrence of a hypertensive disorder and delivered before 37 weeks of gestation. The subsequent pregnancy was uneventful in 95 women (50%). Chronic hypertension and maternal age were statistical significant predictors for recurrence. In conclusion, women with hypertensive disorders and delivery late pre-term have only a 9% chance of recurrence resulting in delivery before 37 weeks. While this percentage is relatively low, the overall recurrence rate of hypertensive disorders irrespective of gestational age in the subsequent pregnancy was 51%.

A summary of the results of chapter 6, 7 and 8 is presented in table 1.
Chapter 8 and 9 focus on questions regarding management of hypertensive disorders in the late pre-term period. Until now, only a few studies have focused on the management of women with hypertensive disorders between 34 +0 and 36 +6 weeks of gestational age. The NICE guideline “hypertensive disorders during pregnancy” refers in the 2010 consensus statement to the issue of mild or moderate preeclampsia between 34 and 36 weeks of gestation in terms of a “grey zone” at which the optimal timing of birth is not clear. Although the HYPITAT trial showed that in women with mild GH or PE above a gestational age of 37 weeks induction of labour is preferable, there remains uncertainty on the best policy in the late pre-term period (34-37 weeks). The situation in these patients is different from term age because, apart from maternal morbidity, the potential neonatal consequences for premature delivery are also in the equation.

To improve the understanding on the neonatal outcome of this specific population, and its causative factors, data on neonatal morbidity in infants born from mothers with a hypertensive disorder between 34 and 37 weeks’ gestation were analysed in chapter 8. Data were obtained from the Netherlands Perinatal Registry (PRN-registry) between January 2000 and December 2006. All women who delivered between 34 +0 – 36 +6 weeks of gestation with gestational hypertension (n=4316), preeclampsia (n=1864) and normotensive controls (n=20749) were included. Compared to the control group, the risk of induction of labour and primary cesarean section in the PE group was strongly increased odds ratio (OR) 16 (95% CI 15 to 18) and 7.4 (95% CI 6.6 to 8.2) respectively. Children from PE mothers were more often small for gestational age (27%) than in the GH group (18%; OR 1.7, 95% CI 1.5 to 1.9) and the control group (5.3%, OR of 6.7, 95% CI 5.9 to 7.5). More children in the PE group were admitted to the high care or NICU compared to the GH and control group; OR 1.6 (95% CI 1.4 to 1.9) and OR 2.0 (95% CI 1.8 to 2.2) respectively. In the multivariable logistic regression analysis a

<table>
<thead>
<tr>
<th>Study design (chapter thesis)</th>
<th>Delivery index pregnancy (weeks)</th>
<th>Patients included (N)</th>
<th>Recurrence rates of a hypertensive disorder in the subsequent pregnancy*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Delivery &lt; 34 weeks</td>
</tr>
<tr>
<td>Systematic review (chapter 5)</td>
<td>&lt; 34</td>
<td>2188</td>
<td>7.8% (6.7 – 9.0)</td>
</tr>
<tr>
<td>Cohort study (chapter 6)</td>
<td>&lt; 34</td>
<td>211</td>
<td>17% (12 – 22)</td>
</tr>
<tr>
<td>Cohort study (chapter 7)</td>
<td>34 – 37</td>
<td>189</td>
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</tr>
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</table>

*Data are expressed as % (95% confidence intervals)
cesarean delivery and decreasing gestational age were independent risk factors for neonatal respiratory morbidity. Gestational hypertension or preeclampsia reduced the risk for respiratory distress syndrome compared to the control group (odds ratios of 0.81 (95% CI 0.64 – 1.0) and 0.69 (95% CI 0.49 – 0.96), respectively). In conclusion, neonatal morbidity in babies born from mothers with hypertensive disorders is still considerable in the late pre-term period, which is predominantly driven by gestational age and mode of delivery. Very interestingly, in our study the presence of a hypertensive disorder per se seems to protect for neonatal respiratory morbidity. However this protective effect is diminished by the higher cesarean rate in this group thus resulting in higher incidences of respiratory morbidity in hypertensive disorders.

As mode of delivery seems to be an important influencing factor on the neonatal outcome, one could speculate that in case of a hypertensive disorder of the mother in the late pre-term period, induction of labour is preferable before deterioration of the maternal condition occurs and a primary cesarean section is the only solution for fast improvement of the maternal condition. Choosing the right moment for delivery though, remains a difficult task for the obstetrician as with increasing gestational age the neonatal morbidity might decrease. In this perspective, the superior strategy is a randomised controlled trial comparing induction of labour to temporizing management.

**Chapter 9** provides a detailed description of the study protocol of the currently running HYPITAT-II study (Hypertension and Preeclampsia Intervention Trial in the Almost Term patients): a multicentre randomised controlled trial. The aim of this study is to investigate whether planned induction of labour compared to temporizing management in women with gestational hypertension, mild pre-eclampsia or deteriorating chronic hypertension at gestational age of 34 – 37 weeks of pregnancy, reduces maternal morbidity and / or increases neonatal morbidity. Women are randomly allocated to either delivery within 24 hours (experimental arm) or temporizing management (control arm) until 37 weeks of gestational age. The primary outcome measure is a composite endpoint of maternal mortality, maternal complications (eclampsia, HELLP syndrome, pulmonary oedema, thromboembolic disease) and progression to severe pre-eclampsia. The neonatal primary outcome is respiratory distress syndrome (RDS). Data will be analysed on an intention-to-treat basis. We aim to recruit 680 patients to show a reduction of maternal complications from 5% to 1%. The trial started recruitment in May 2009 within the structure of the Dutch consortium for studies in Obstetrics, Fertility & Gynaecology (URL: http://www.studies-obsgyn.nl/hypitat2). 49 Hospitals in the Netherlands participate in the study indicating the experienced national sense of urgency for such a trial. The expected end date of recruitment will be December 2012.
Clinical implications

In this thesis absolute recurrence rates of hypertensive disorder in pregnancy after a pre-term delivery due to a hypertensive disorder were calculated. Existing information about recurrence was until recently fragmentary and outdated, with the most quoted study of Sibai et al, dating from 1991. These data will help the obstetrician in counselling patients in considering a new pregnancy and adjust management to it in a subsequent pregnancy. The recurrence rates of a pre-term delivery were lower than expected. In women with a history of an early-onset hypertensive disorder, 17% had a recurrence of a delivery before 34 weeks due to a hypertensive disorder. In women with a history of a late pre-term delivery (e.g. between 34 and 37 weeks’ gestation), 9% had a recurrence of delivery before 37 weeks due to a hypertensive disorder. However the rate of a completely uncomplicated subsequent pregnancy was relatively low for both the early-onset group as for the late pre-term group, indicating these groups as high-risk patients (table 1). Prediction modelling indicated chronic hypertension as the strongest independent risk factor for recurrence.

This thesis also deals with management of hypertensive disorders in the late pre-term period. The neonatal morbidity is still considerable in this period. Decreasing gestational age and primary cesarean rate are independent risk factors for neonatal respiratory morbidity. Hypertensive disorders per se seem to protect for neonatal respiratory morbidity. Induction of labour may prevent maternal deterioration and a primary cesarean section, however the exact moment of induction is still debated as increasing gestational age might reduce neonatal morbidity. The HYPITAT-II study will bring the evidence concerning this management dilemma.

The implementation of the HYPITAT-II study might influence obstetric management regarding hypertensive disorders in the late pre-term period (e.g. between 34 and 37 gestational age). The HYPITAT-I study already had its influences during the trial. The number of cases of eclampsia reduced more strongly in the centres that participated in the trial. Moreover, after the trial the interventionist approach was implemented in the Netherlands. Results of this study are adapted shortly after publication in national and international guidelines. In continuation of this study the HYPITAT-II study may result in a shift from a temporizing management approach to an interventionist approach in the late pre-term period. Over ten years we might look back at “ten years after the HYPITAT-II trial” and hopefully conclude that although the induction of labour rate in this period has increased, the number of cesarean section have not, the maternal morbidity and mortality due to hypertensive disorders in the late pre-term period are reduced and neonatal morbidity did not change.
Future research

Regarding the clinical implications stated above on management of hypertensive disorders in the late pre-term period, the results of the HYPITAT-II trial will provide more insight into the best management strategy for this specific population. Results of this study will be translated into national and international guidelines. Only few follow-up studies have been performed after obstetric intervention trials, leaving long-term consequences for this specific group still unknown. With evidence available (chapter 8) for short-term neonatal morbidity after a delivery in the late pre-term period due to a hypertensive disorder, we also plan long-term neonatal follow-up at 2 year and 5 year old infants. The main short-term morbidities in the late pre-term period are hypoglycemia, hyperbilirubinemia and respiratory distress syndrome. All of these morbidities have the potential to cause long-term neurodevelopmental sequelae. The results of this follow-up study will influence the interpretation of the results of the HYPITAT-II trial. Since the last two decades, abundant evidence is appearing of the long-term health consequences of adverse pregnancy outcome. Pregnancy events like hypertension in pregnancy, pre-term birth, stillbirth, fetal growth restriction and gestational diabetes are associated with long-term maternal morbidity. The child itself, born from the hypertensive pregnancy, might also be at increased risk for long-term health consequences in the light of the Barker hypothesis (chapter 2 and 3). Pregnancy represents a unique opportunity to identify women and children who may be at increased risk for chronic diseases later in life. A huge field for preventive medicine rises up. Most of this evidence is based on retrospective case control studies. To provide more insight into an individuals’ vascular risk profile following a pregnancy complicated by a hypertensive disorder in the late pre-term period a maternal follow-up study is planned: HyRAS-II (Hypitat Risk Assessment Study-II). The aim of this study is to screen women from the HYPITAT-II trial on risk factors for cardio-vascular disease, two years after their complicated pregnancy and to estimate the 10-year cardiovascular event risk in these women using validated prediction algorithms, in order to establish the proportion that is likely to benefit from preventive interventions, according to widely accepted guidelines. Another implication of prediction models based on individual patient data is planned on recurrence rates, so more individual tailored prediction models can be developed. Preconception care and counseling might become the most important therapeutic tool the obstetrician holds in his or her hand. A healthy mother will provide a healthy environment for herself, her child and maybe even for her grandchild.
Nederlandse samenvatting

Dit proefschrift behandelt drie hoofdvragen die gaan over de gevolgen van hoge bloeddruk in de zwangerschap. Op de eerste plaats vragen wij ons af welke lange termijn gevolgen er zijn voor een neonaat die geboren is uit een gecompromitteerde uteriene omgeving? Ten tweede behandelen we de herhalingsrisico’s van een hypertensieve aandoening in een vervolgzwangerschap. Op de derde plaats wordt gekeken naar de beste behandelstrategie als een hypertensieve aandoening optreedt in de laat pre-term periode (34-37 weken amenorroeduur).

De volgorde van presentatie van de studies in dit proefschrift volgde de tijdslijn van uitvoering van de studies. De basis voor dit proefschrift werd gelegd in 2004-2005, met een fundamenteel wetenschappelijk programma op de University of Texas Medical Branch in Galveston, Verenigde Staten. Hier werd het onderliggende pathofysiologische mechanisme onderzocht met betrekking tot de associatie tussen een gecompromitteerde uteriene omgeving en de gezondheidsrisico’s voor de nakomelingen op latere leeftijd. Bij terugkomst in Nederland, werden de studies naar herhaalrisico’s geïnitieerd. In 2008 werd begonnen met de voorbereidingen van een multicentre trial onder het acroniem HYPITAT-II (Hypertension or Preeclampsia Intervention Trial in the Almost Term patient). De studie volgde de HYPITAT trial (Hypertension or Preeclampsia Intervention Trial At Term) op, nadat uit die studie bleek dat inleiden van de baring effectief was bij vrouwen met zwangerschapshypertensie of milde preeclampsie à terme. HYPITAT II is van start gegaan in 2009, na ontwikkeling van het protocol, case report formulier, het verkrijgen van toestemming van de medisch ethische commissie en het aanvragen van subsidie.

Deel I: fundamenteel onderzoek

Tijdens de kritische periode van de intra-uteriene ontwikkeling van de foetus leiden stimuli of insulten ten op zichte van de foetus tot “foetale programmering”. Dit resulteert in aanpassingen in de foetale anatomie, fysiologie en metabolisme, welke consequenties hebben tot op latere leeftijd. Zo is bijvoorbeeld een te laag geboortegewicht voor de termijn (foetale groei retardatie, wat een bekende complicatie is van preeclampsie), geassocieerd met een hogere kans op een cardiovasculaire aandoening op latere leeftijd. Deze theorie staat bekend als de “fetal origin of adult disease”.

In hoofdstuk 2 en 3 zijn muismodellen met een gecompromitteerd vasculair systeem en endotheelcel disfunctie gebruikt, om het pathofysiologische mechanisme van de lange termijn consequenties van preeclampsie voor...
nakomelingen te bestuderen. Deze diermodellen hebben een gestoorde utero-placentaire perfusie. Nakomelingen van deze dieren ontwikkelen zich in een gecompromitteerde uteriene omgeving. Deze situatie geeft de conditie weer zoals die gedacht wordt te zijn in preeclampsie.\(^3\)

In **hoofdstuk 2** zijn genetisch gemanipuleerde muizen gebruikt die geen “endothelial nitric oxide synthase (eNOS)” hebben. Het enzym eNOS is essentieel voor de productie “nitric oxide” (koolstofmonoxide) in het vasculaire systeem. Koolstofmonoxide is een sterke vasodilatierende stof. Verschillende pathologische condities, zoals hypertensie en foetale groei retardatie worden geassocieerd met een defect in dit stikstofmonoxide traject.\(^4\)

Nakomelingen van eNOS-knockout muizen zijn ten opzichte van genetische identieke nakomelingen van wild-type muizen, groeibeperkt en hebben abnormale vasculaire eigenschappen.\(^3\) Deze waarneming impliceert een foetaal programmering effect als gevolg van de abnormale uteriene omgeving. Om de rol van pariteit te onderzoeken in de modulatie van het foetaal programmeren van foetale groei en vasculaire eigenschappen, is er gebruik gemaakt van oligopara (0-2 eerdere zwangerschappen) en multipara (5-9 eerdere zwangerschappen) muizen. Vrouwelijke stikstofmonoxide knock-out \((-/-KO)\) muizen werden gebroed met eNOS\(^{-/-KO}\) muizen en met wild type \((+/+WT)\) mannelijke muizen. Op deze manier werden er eNOS\(^{-/-KO}\) en maternale heterozygote \((+/−Mat)\) nakomelingen geboren. eNOS\(^{-/-KO}\) en eNOS\(^{+/−Mat}\) nakomelingen geboren uit de oligopara moeders waren significant lager in geboorte gewicht ten opzichte van de nakomelingen geboren uit de multipara moeders. De optimale diameter van de arteria carotis van de nakomelingen (een maat voor vasculaire eigenschappen en weerstand), was afgenomen in de oligopara nakomelingen ten opzichte van de multipara nakomelingen. De arteria carotis toonde een afgenomen relaxatie en een toegenomen contractie in de oligopara nakomelingen ten opzichten van de multipara nakomelingen \((P < .05)\). Deze data ondersteunen de rol van een abnormale uteriene omgeving in het foetaal programmeren voor postnatale groei en vasculaire eigenschappen op latere leeftijd. Mogelijk leiden meerdere zwangerschappen tot maternale uteriene aanpassingen die het gebrek aan een werkzaam eNOS omzeilen. Deze aanpassing leidt tot een betere postnatale groei en vasculaire eigenschappen van de nakomelingen. Gezien de rol van pariteit en het risico op preeclampsie, zouden vergelijkbare mechanismen werkzaam kunnen zijn in de menselijke zwangerschap.

In **hoofdstuk 3** zijn genetisch gemanipuleerde muizen gebruikt die geen werkzame “low-density lipoprotein receptor (LDLR)” hebben. Tijdens het proces van arteriosklerose, gaan lipoproteïne deeltjes (voornamelijk “low-density lipoprotein” (LDL)) de wand van arterie binnen en ondergaan meerdere veranderingen waaronder oxidatie. Het oxideren van LDL wordt gezien als een belangrijk mechanisme in
De pathogenese van het arteriosclerotisch proces. De LDL-receptor (LDLR) is betrokken bij het verwijderen van lipoproteïne uit de circulatie. Een gebrek aan deze receptor leidt tot hypercholesterolemie en arteriosclerose. LDLR-/-KO muizen die een normaal dieet hebben, ontwikkelen al plaques en verdikkingen van de intima van de vaatwand met “schuimcel” en gladde spiercel infiltratie. Wij onderzochten de rol van hypercholesterolemie van de moeder in het foetaal programmeren van de vasculaire functie op volwassen leeftijd. Homozygote LDLR knock-out muizen (LDLR-/-KO) en hun wild-type controles (LDLR+/+WT) werden met elkaar gebroed om vier groepen van nakomelingen te produceren: LDLR-/-KO, maternale heterozygote (LDLR=Mat), paternale heterozygote (LDLR=Pat) en LDLR+/+WT nakomelingen. In vitro experimenten met de arteria carotis van de nakomelingen op volwassen leeftijd, vertoonde een toegenomen contractie in de LDLR-/-KO en de LDLR=Mat mannelijke nakomelingen. In de vrouwelijke nakomelingen was alleen de contractie in de LDLR-/-KO muizen significant verhoogd. Ondanks dat de heterozygote nakomelingen genetisch identiek zijn, toonden de muizen, die geboren waren uit de uterine omgeving met een hypercholesterol status (LDLR=Mat), een abnormale vasculaire respons op latere leeftijd ten opzichte van de heterozygote nakomelingen die geboren zijn uit een normale uterine omgeving (LDLR=Pat). Hypercholesterolemie en arteriosclerose van de moeder hebben in dit model dus lange termijn consequenties voor de nakomelingen.

**Deel II: Klinisch onderzoek**

In **hoofdstuk 4** wordt de accuraatheid van verschillende voorspellende testen op het ontstaan van preeclampsie en de effectiviteit van de preventie ervan samengevat. Testen om preeclampsie te voorspellen betreffen de klinische anamnese, gegevens verkregen door lichamelijk onderzoek, laboratorium onderzoeken en hemodynamische testen. Over het algemeen hebben voorspellende testen in de vroege zwangerschap een betere specificiteit dan sensitiviteit. BMI (Body Mass Index) > 34, alpha-foeto proteïne, fibronectine en de Doppler van de arteria uterina hebben allemaal een specificiteit boven de 90%. Alleen de weerstand index (resistance index) van de Doppler van de arteria uterina heeft een sensitiviteit boven de 60%. Geen enkele test is voldoende accuraat om aan te bevelen in de dagelijkse praktijk.

Om preeclampsie te voorkomen zijn er verschillend behandel opties; farmacologische producten, voedingssupplementen en het aanpassen van levensstijl. Antagonisten van bloedplaatjes, voornamelijk lage dosering van aspirine, reduceren het risico op preeclampsie met 10% (Relatief Risico (RR) 0.90,
95% betrouwbaarheid interval (BI) 0.84 tot 0.97). Calcium voorkomt preeclampsie alleen in vrouwen met een hoog risico (RR 0.22; 95% BI 0.12 tot 0.42) en in de groep vrouwen met een lage intake van calcium (RR 0.36; 95% BI 0.20 tot 0.65).\(^8\) Farmacologische producten zoals laag molecular heparine, progesteron, donoren van stikstofmonoxide, antihypertensiva en diuretica zijn niet effectief in de preventie van preeclampsie. Voedingssupplementen zoals magnesium, anti-oxidanten, visolie en foliumzuur reduceren de incidentie van preeclampsie niet. Er is te weinig bewijs om aan te tonen dat interventies in levensstijl (zoals rust, bewegen of verminderde zoutname) preeclampsie voorkomt.

**Hoofdstuk 5, 6 en 7** richten zich op de herhalingsrisico’s van een hypertensieve aandoening in de zwangerschap, na een eerdere vroegeboorte als gevolg van een hypertensieve aandoening. Een ernstige hypertensieve aandoening met een vroegeboorte heeft vaak een enorme psychosociale invloed en is geassocieerd met een hoge incidentie van symptomen passend bij posttraumatische stressstoornis, depressie en angst.\(^9\) De psychosociale conditie van de moeder na een premature bevalling als gevolg van een hypertensieve aandoening is met name geassocieerd met de amenorrooeduur op het moment dat de diagnose werd gesteld.\(^10\) Kennis over herhalingsrisico’s is belangrijk vanwege meerdere redenen. Vanuit het perspectief van de patiënt zal de beslissing over een eventuele volgzwangerschap mede afhangen van dit herhaalrisico. Vanuit medisch perspectief zal deze kennis invloed hebben op de te verlenen zorg in een volgende zwangerschap.

**Hoofdstuk 5** presenteert een systematisch review van gepubliceerde cohort studies over herhalingsrisico’s op een hypertensieve aandoening in de zwangerschap na een eerdere bevalling onder de 34 weken als gevolg van een hypertensieve aandoening. Hiervoor werden datasets van medische publicaties op relevante artikelen gescreeën tot en met september 2009 (Medline, Embase en de Cochrane Library). Per studie werd het herhaalrisico op een premature bevalling als gevolg van de hypertensieve aandoening berekend, waarna in de gepoolde data herhalingskansen werden berekend. De zoekopdracht leverde 36 relevante artikelen op, waarvan er 11 voldeden aan de inclusie criteria. Deze 11 studies omvatten 2,377 patiënten (range van 18 tot 1,754 patiënten per studie) en 2,461 bevallingen. Doordat de exacte zwangerschapsduur ten tijde van de bevalling in de vervolgzwangerschap veelal niet gerapporteerd werd, moesten de meeste studies geëxcludeerd worden. Een andere omissie is het gebrek aan uniforme criteria voor ernstige hypertensie. Van de 11 studies werden alsnog 4 studies geëxcludeerd, omdat een zeer strikte patiëntenpopulatie was geselecteerd. In deze patiëntenpopulaties werd de diagnose van de aandoening vóór de 28e week gesteld, wat een selectie bias introduceert. Het gepoolde herhalingsrisico op een
Samenvatting en discussie

bevalling voor de 34e week als gevolg van hypertensie, preeclampsie of HELLP was 7.8% (95% BI 6.7 tot 9.0%). Concluderend is in een vervolgzwangerschap de kans groter dan 90% op een bevalling na de 34e week. Gezien het feit dat angst van ouders voornamelijk geassocieerd is met de amenorroeduur ten tijde van de diagnose, zullen deze resultaten een positieve invloed kunnen hebben op de beslissing over een eventuele vervolgzwangerschap.

Tijdens het uitvoeren van deze systematische review constateerden wij het gebrek aan kwalitatief goede cohort studies. Veel studies includeerden niet opeenvolgende casus, patiëntenpopulaties waren sterk geselecteerd en waren vaak klein in aantallen. Als gevolg hiervan variëren herhaalrisico's van 65% tot 5%.

In hoofdstuk 6 hebben we een retrospectieve cohort studie uitgevoerd, waarbij de selectie van patiënten slechts bestond uit amenorroeduur ten tijde van de bevalling (<34 weken) en het hebben van een hypertensieve aandoening. Ons primaire doel was het bepalen van het absolute herhaalrisico op een negatieve uitkomst, gedefinieerd als een bevalling onder de 34 weken amenorroeduur ten gevolge van een hypertensieve aandoening. Met behulp van een multivariabele analyse werden onafhankelijke factoren geïdentificeerd die gerelateerd zijn aan deze negatieve uitkomst. Alle vrouwen die bevallen waren van hun eerste kind onder de 34 weken als gevolg van aan hypertensieve aandoening, in de periode van januari 1996 tot en met december 2004 werden geïncludeerd. Deze patiënten bevielen in twee perinatale centra met een regionale functie; het Academisch Medisch Centrum (AMC) te Amsterdam of het Máxima Medisch Centrum (MMC) te Veldhoven. Alle patiënten werden opeenvolgend geïncludeerd en de informatie over hun vervolgzwangerschap werd opgevraagd. Er werden 380 patiënten geselecteerd, waarvan er 46 lost to follow-up waren en 123 patiënten hadden geen vervolgzwangerschap (79 (64%) in verband met angst voor herhaling). Van de 211 patiënten met een vervolgzwangerschap hadden 36 patiënten (17%, 95% BI 12% tot 22%) wederom een bevalling onder de 34 weken, 30 patiënten (14%, 95% BI 9.5% tot 19%) een bevalling tussen de 34 en 37 weken en 145 patiënten (69%, 95% BI 62% tot 75%) een bevalling boven de 37e week. Van deze laatste groep hadden slechts 67 patiënten (32%, 95% BI 25% tot 38%) een volledig ongecompliceerde zwangerschap. Een hoge diastolische bloeddruk in de indexzwangerschap en het hebben van chronische hypertensie waren beide geassocieerd met een bevalling onder de 34 weken in de vervolgzwangerschap. Het hebben van chronische hypertensie na de index zwangerschap gaf een 5 maal hoger risico op herhaling (odds ratio 5.2, 95% BI 0.72 tot 41). Concluderend hebben vrouwen na een ernstige vroege preeclampsie in hun eerste zwangerschap een herhaalrisico op een bevalling voor de 34e week van 17%. Het hebben van chronische hypertensie is een belangrijke risicofactor voor herhaling. Slechts 32%
had een volledig ongecompliceerde zwangerschap. Ondanks dat dit percentage van 32% laag lijkt, blijft de belangrijkste vraag voor ouders en de obstetricus de kans op herhaling van een ernstige vroeggeboorte, aangezien maternale en neonatale morbiditeit voornamelijk geassocieerd zijn met deze vroeggeboorte. In dat kader is een herhalingsrisico van 17% het meest relevant.

In hoofdstuk 7 worden herhaalrisico op een hypertensieve aandoening tussen de 34 en 37 weken berekend met behulp van een retrospectieve cohort studie in 6 Nederlandse ziekenhuizen. Er zijn geen eerder studies gepubliceerd met betrekking tot herhaalrisico’s van een hypertensieve aandoening in de laat pre-terme periode (tussen de 34 en 37 weken zwangerschapsduur). Mogelijk liggen er verschillende pathofysiologische mechanismen ten grondslag aan vroege preeclampsie versus preeclampsie à terme. Herhaalrisico’s van vroege preeclampsie, noch van preeclampsie à terme mogen daarom niet zomaar geëxtrapoleerd worden naar de laat pre-terme groep. Echter, de laat pre-terme groep is wel een significante proportie van de non-academische setting. Het primaire doel van deze studie was het berekenen van het absolute herhalingsrisico voor deze specifieke groep en beoordeelden of cardiovasculaire risicofactoren voorspellend waren voor herhaling. Er werden 425 vrouwen geselecteerd, waarvan er 351 gecontacteerd konden worden. Van de 189 vrouwen met een vervolgzwangerschap hadden 94 vrouwen (50%, 95% BI 43% tot 57%) een hypertensieve aandoening in de vervolgzwangerschap, waarvan 17 vrouwen (9.0%, 95% BI 4.9% tot 13%) een herhaling van een hypertensieve aandoening en een bevalling voor de 37e week hadden. De vervolgzwangerschap verliep in 95 vrouwen (50%) ongecompliceerd. Chronische hypertensie en leeftijd van de moeder waren beide onafhankelijke voorspellers op herhaling van een hypertensieve aandoening. Concluderend kunnen we zeggen dat vrouwen die bevallen zijn tussen de 34e en 37e week als gevolg van een hypertensieve aandoening, een herhalingsrisico hierop hebben van 9%. Hoewel dit percentage laag is, blijft het herhalingsrisico op een hypertensieve aandoening in de vervolgzwangerschap ongeacht de termijn met 51% hoog.

Een samenvatting van de resultaten van hoofdstuk 6, 7 en 8 worden weergegeven in tabel 1.

Hoofdstuk 8 en 9 richt zich op vraagstukken betreffende management van hypertensieve aandoeningen in de laat pre-terme periode (34⁺0 tot 36⁺6 weken amenorroeduur). De recente “NICE” richtlijn “hypertensive disorders during pregnancy”, verwijst naar deze specifieke periode als een “grijze zone”. Het optimale moment van bevalling in deze periode, in geval van milde of matige preeclampsie, is niet duidelijk. Het gegeven dat de HYPITAT trial aantoonde dat na 37 weken inleiding van de baring de voorkeur geniet, kan dit niet zomaar worden vertaald naar de laat pre-terme periode. Naast de maternale morbiditeit,
zijn er ook potentiële consequenties voor de neonaat als gevolg van de premature geboorte tussen de 34 en 37 weken. In hoofdstuk 8 is de neonatale morbiditeit geanalyseerd van kinderen geboren tussen de 34 en 37 weken zwangerschapsduur, waarbij de zwangerschap gecompliceerd werd door hypertensie of preeclampsie. De data over de periode januari 2000 tot en met december 2006, werden verkregen uit de Perinatale Registratie Nederland (PRN). Alle vrouwen met zwangerschapshypertensie (GH, n=4316) of preeclampsie (PE, n=1864) en normotensieve controle vrouwen (n=20749), bevallen tussen de 34+0 – 36 +6 weken amenorroeduur, werden geïncludeerd. Het risico op een inleiding of een primaire sectio Caesarea was significant verhoogd in de PE groep vergeleken met de controle groep, odds ratio (OR) 16 (95% betrouwbaarheid interval (BI) 15 tot 18) en 7.4 (95% BI 6.6 tot 8.2) respectievelijk. Kinderen van PE moeders waren vaker groeibepaald (27%), ten opzichte van kinderen van de GH groep (18%, OR 1.7, 95% BI 1.5 tot 1.9) en van de controle groep (5.3%, OR 6.7 95% BI 5.9 tot 7.5). Er werden meer kinderen uit de PE groep opgenomen op de NICU ten opzichte van kinderen uit de GH en controle groep; OR 1.6 (95% BI 1.4 tot 1.9) en OR 2.0 (95% BI 1.8 tot 2.2) respectievelijk. Een sectio Caesarea en afnemende zwangerschapsduur waren beide onafhankelijke risicofactoren voor neonatale respiratoire morbiditeit in het multivariabele logistische regressie model. Zowel zwangerschapshypertensie als preeclampsie op zichzelf reduceerde het risico op neonatale "respiratoire distress syndroom” (OR 0.81 (95% BI 0.64 tot 1.0) en 0.69 (95% BI 0.49 tot 0.96), respectievelijk). Concluderend is de neonatale morbiditeit van kinderen geboren in de laat pre-term periode van moeders met hypertensie of preeclampsie niet verwaarloosbaar. Deze morbiditeit wordt voornamelijk bepaald door de amenoroeduur ten tijde van de bevalling en door de wijze van bevallen. Een interessante bevinding van onze studies

Tabel 1: Samenvatting van herhaalrisico’s op een hypertensieve aandoening in de zwangerschap, na een eerdere premature bevalling als gevolg van een hypertensieve aandoening.

<table>
<thead>
<tr>
<th>Studie design (hoofdstuk proefschrift)</th>
<th>Index bevalling (weken)</th>
<th>Aantal patiënten (N)</th>
<th>Herhaalrisico op een hypertensieve aandoening in de vervolgzwangerschap*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systematisch review (hoofdstuk 5)</td>
<td>&lt; 34</td>
<td>2188</td>
<td>7.8% (6.7 – 9.0)</td>
</tr>
<tr>
<td>Cohort studie (hoofdstuk 6)</td>
<td>&lt; 34</td>
<td>211</td>
<td>17% (12 – 22)</td>
</tr>
<tr>
<td>Cohort studie (hoofdstuk 7)</td>
<td>34 – 37</td>
<td>189</td>
<td>9% (4.1 – 13)</td>
</tr>
</tbody>
</table>

*Data worden weergegeven in % (95% betrouwbaarheid interval)
is het beschermend effect van de hypertensieve aandoening van de moeder op de neonatale respiratoire morbiditeit. Echter dit beschermend effect verdwijnt als gevolg van het hoger sectio percentage in deze groep, waardoor de uiteindelijke incidentie van neonatale respiratoire morbiditeit in deze groep hoger is. De wijze van bevallen blijkt een belangrijke invloed te hebben op de neonatale morbiditeit. We zouden kunnen speculeren dat het inleiden van de baring in geval van een hypertensieve aandoening in de laat pre-term periode de voorkeur geniet, voordat de conditie van de moeder ontspoort en een keizersnede de enige oplossing lijkt voor een snelle verbetering van de maternale conditie. Toch blijft het kiezen van het juiste moment van bevalling een moeilijke taak voor obstetrici, aangezien een toename van de amenorroeduur mogelijk leidt tot een afname van de neonatale morbiditeit. In dit perspectief lijkt een gerandomiseerde gecontroleerde trial een optimale strategie om inleiden van de baring te vergelijken met afwachtend monitoren. **Hoofdstuk 9** is een gedetailleerde beschrijving van het studie protocol van de HYPITAT-II studie (Hypertension and Preeclampsia Intervention Trial in the Almost Term patient): een multicentre gerandomiseerde gecontroleerde trial. Het doel van deze studie is om bij vrouwen tussen 34 en 37 weken amenorroeduur met zwangerschapshypertensie of preeclampsie te onderzoeken of inleiden van de baring versus afwachtend monitoren leidt tot een afname van de maternale morbiditeit en / of een toename van de neonatale morbiditeit. Vrouwen worden at random toegewezen tot inleiden van baring binnen 24 uur (experimentele arm) of tot afwachtend monitoren (controle arm) tot 37 weken. De primaire uitkomstmaat is een samengesteld eindpunt van maternale mortaliteit, maternale complicaties (eclampsie, HELLP syndroom, pulmonaal oedeem, thrombo-embolische aandoeningen) en progressie naar een ernstige preeclampsie. De primaire neonatale uitkomstmaat is respiratoire distress syndroom (RDS). Data worden op basis van intention-to-treat geanalyseerd. Er zullen 680 patiënten worden geïncludeerd, om een afname van maternale complicaties aan te tonen van 5% naar 1%. Deze trial is van start gegaan in mei 2009, binnen het verloskundige consortium Nederland (URL: http://www.studies-obsgyn.nl/hypitat2). 49 Ziekenhuizen in Nederland nemen deel aan deze studie. Dit hoge deelname getal geeft aan dat er grote behoefte in Nederland is voor deze trial. De verwachte einddatum van inclusie is december 2012.

**Klinische implicaties**

Dit proefschrift richt zich op de absolute herhaalkansen van een hypertensieve aandoening in de zwangerschap, na een eerder doorgemaakte premature bevalling als gevolg van een hypertensieve aandoening. Bestaande informatie
De gepresenteerde nieuwe data bieden obstetrici hulp in de counseling van patiënten die een volgende zwangerschap overwegen en zij kunnen de bijbehorende begeleiding van deze vervolgzwangerschap hierop aanpassen. De herhaalkansen op een premature bevalling waren lager dan verwacht. Vrouwen met een vroeggeboorte onder de 34 weken in de anamnese hadden een herhaalkans op wederom een vroeggeboorte voor de 34e week van 17%. Vrouwen met een geboorte in de laat pre-terme periode in de anamnese (34 en 37 weken amenorroeduur) hadden een herhaalkans op een bevalling voor de 37e week als gevolg van een hypertensieve aandoening, van 9%. Echter het percentage vrouwen met een compleet ongecompliceerde vervolgzwangerschap was relatief laag in beide groepen. Deze groep vrouwen moet dus nog steeds als hoogrisico patiënten gezien worden (tabel 1). In de predictie modellen bleek chronische hypertensie de sterkste onafhankelijke risicofactor op herhaling van een premature bevalling als gevolg van een hypertensieve aandoening.

Dit proefschrift richt zich ook op de obstetrische behandeling van een hypertensieve aandoening in de laat pre-terme periode (34 – 37 weken amenorroeduur). De neonatale morbiditeit in deze periode is nog steeds aanzienlijk. Afnemende zwangerschapsduur en een primaire sectio Caesarea zijn onafhankelijke risicofactoren op neonatale respiratoire morbiditeit. De hypertensieve aandoeningen op zichzelf, lijken juist beschermend tegen neonatale respiratoire morbiditeit. Het inleiden van de baring kan mogelijk verslechtering van de maternale conditie en een primaire sectio Caesarea voorkomen. Echter het exacte moment van bevalling blijft een discussiepunt, aangezien aan de andere kant toenemende zwangerschapsduur mogelijk leidt tot een afname van de neonatale morbiditeit. De HYPITAT-II studie zal ons meer informatie verschaffen ten aanzien van dit dilemma.

De implementatie van de HYPITAT-II studie zelf, kan al invloed hebben op het obstetrische handelen met betrekking tot hypertensieve aandoeningen in de laat pre-terme periode. De HYPITAT-I studie had haar invloed al ten tijde van de uitvoering van de trial. Het aantal eclamptische insulten nam sterker af in ziekenhuizen die in de studie participeerden dan in ziekenhuizen die niet deelnamen. Daarnaast werd na het afronden van de studie, de interventie aanpak geïmplementeerd in Nederland. De resultaten van deze studie werden snel na publicatie in nationale en internationale richtlijnen aangenomen. In het verlengde van de HYPITAT-I trial, kan de HYPITAT-II trial leiden tot een verschuiving van een afwachtend monitoren beleid, naar een interventie beleid in de laat pre-terme periode. Over 10 jaar kijken we mogelijk terug op “tien jaar na de HYPITAT-II trial” en kunnen we hopelijk concluderen dat ondanks het aantal inleidingen in deze periode toegenomen zijn,
het aantal sectio Caesarea niet zijn toegenomen, de maternale morbiditeit als gevolg van een hypertensieve aandoening in deze periode is afgenomen en dat de neonatale morbiditeit niet veranderd is.

Toekomstig onderzoek

Gezien de klinische implicaties van de HYPITAT-II trial, zullen de resultaten van deze studie meer inzicht verschaffen in de beste behandelstrategie van hypertensieve aandoeningen in de laat pre-term periode. Resultaten van deze studie zullen vertaald worden naar nationale en internationale richtlijnen.

Er zijn slechts weinig follow-up studies uitgevoerd na obstetrische interventie trials, waardoor de lange termijn consequenties voor deze specifieke groepen onbekend zijn. Aangezien er bewijs is voor neonatale morbiditeit op de korte termijn (hoofdstuk 8) als gevolg van een geboorte in de laat pre-term periode door een hypertensieve aandoening van de moeder, plannen we ook lange termijn follow-up op 2 en 5 jarige leeftijd van deze kinderen. De belangrijkste neonatale morbiditeit op de korte termijn zijn hypoglycemie, hyperbilirubinemie en respiratoire problemen. Deze aandoeningen hebben allemaal de potentie om lange termijn gevolgen te hebben voor de (neurologische) ontwikkeling. De resultaten van deze follow-up studie zullen invloed hebben op de interpretatie van de resultaten van de HYPITAT-II trial.

De laatste twee decennia is er veel gepubliceerd over de gezondheidsrisico’s op de lange termijn na een complicatie in de zwangerschap. Gebeurtenissen tijdens de zwangerschap zoals hypertensie, premature bevalling, intra-uteriene vruchtdood, foetale groei retardatie en zwangerschapsdiabetes, zijn geassocieerd met maternale morbiditeit op de lange termijn. Het kind dat geboren is uit die zwangerschap met hypertensie, heeft zelf ook mogelijk een verhoogd risico op gezondheidsproblemen op latere leeftijd. Deze associatie staat bekend als de “Barker hypothese” (hoofdstuk 2 en 3). De zwangerschap is een uniek moment om vrouwen en kinderen te identificeren die mogelijk een verhoogd risico hebben op chronische ziekten op latere leeftijd. Een enorm onderzoeksgebied komt hiermee ten tonele met betrekking tot preventieve geneeskunde. Het gros van bewijs voor deze associatie is afkomstig van retrospectieve case controle studies. Om meer inzicht te krijgen in een individueel vasculair profiel, na een zwangerschap die gecompliceerd werd door een hypertensieve aandoening, is er een maternale follow-up studie gepland: HyRAS-II (Hypitat Risk Assessment Study-II). Het doel van deze studie is om 2 jaar na deelname aan de HYPITAT-II studie, vrouwen te screenen op risicofactoren voor cardiovasculaire aandoening. Daarnaast wordt er met behulp van gevalidateerde predictie algoritmes een schatting gemaakt van
het 10-jarig risico op een cardiovasculaire gebeurtenis, om op deze manier de groep vrouwen te identificeren die mogelijk baat heeft bij preventieve interventies gebaseerd op bestaande richtlijnen.

Naast predictie van de lange termijn gezondheid van moeder en kind zullen er ook predictie modellen ontwikkeld worden gebaseerd op individuele patiënt data, ten behoeve van herhaalrisico’s van hypertensieve aandoeningen. Op deze manier kunnen meer individueel aangepaste predictie modellen ontwikkeld worden.

Preconceptie geneeskunde en counseling worden mogelijk de meest potentiële behandelopties die een obstetricus kan bieden.
References


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Samenvatting en discussie


Abbreviations
Publications
Dankwoord
Curriculum Vitae
Abbreviations

ACR  Albumin Creatinin Ratio
AFP  Alpha-Fetoprotein
AMC  Academic Medical Centre
ALAT Alanine-aminotransferase
ASAT Aspartate-aminotransferase
ASQ  Ages and Stages Questionnaires
AUC  Area Under Curve
BMI  Body Mass Index
BP   Blood Pressure
BPD  Bronchopulmonary Disease
CBCL Child Behavioral Checklist
CI   Confidence Interval
CRF  Case Report Form
FGR  Fetal Growth Restriction
FN   Fibronectin
GA   Gestational Age
GH   Gestational Hypertension
HADs Hospital Anxiety and Depression scale
HCG  Human Chorionic Gonadotropin
HELLP Hemolysis Elevated Liver enzymes Low Platelets
HIE  Hypoxic Ischemic Encephalopathy
HTA  Health Technology Assessment
HYPITAT-I HYpertension and Pre-eclampsia Intervention Trial At Term
HYPITAT-II HYpertension and Pre-eclampsia Intervention Trial in the Almost Term Patient
HyRAS Hypertension Risk Assessment Study
IVH  Intraventricular Hemorrhage
KO   Knock-Out
LDH  Lactate Dehydrogenase
LDL  Low Density Lipoprotein
LDLR Low Density Lipoprotein Receptor
LVR  National Delivery Record
LR   Likelihood Ratio
LMWH Low Molecular Weight Heparin
Log  Logarithm
MMC  Maxima Medical Centre
NEC  Necrotizing Enterocolitis
NICE National Institute for Health and Clinical Excellence
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>NICU</td>
<td>Neonatal Intensive Care Unit</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric Oxide Synthase</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PCR</td>
<td>Protein Creatinine Ratio</td>
</tr>
<tr>
<td>PE</td>
<td>Preeclampsia</td>
</tr>
<tr>
<td>PIH</td>
<td>Pregnancy Induced Hypertension</td>
</tr>
<tr>
<td>PIGF</td>
<td>Placental Growth Factor</td>
</tr>
<tr>
<td>PRN</td>
<td>Perinatal Registry Netherlands</td>
</tr>
<tr>
<td>PVL</td>
<td>Periventricular Leucomalacia</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised Controlled Trial</td>
</tr>
<tr>
<td>RDS</td>
<td>Respiratory Distress Syndrome</td>
</tr>
<tr>
<td>RR</td>
<td>Relative Risk</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</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>TTN</td>
<td>Transient Tachypnoe of Newborn</td>
</tr>
<tr>
<td>UTMB</td>
<td>University of Texas Medical Branch</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analogue Scale</td>
</tr>
<tr>
<td>WT</td>
<td>Wild-Type</td>
</tr>
</tbody>
</table>
Publications


Dankwoord

Als eerste wil ik alle patiënten bedanken die deelgenomen hebben aan de onderzoeken in dit proefschrift. Dankzij deze vrouwen zijn we meer te weten gekomen over herhaalrisico’s van vroeggeboortes door hypertensieve aandoeningen, waardoor we beter kunnen counselen. Ook zullen we dankzij hen te weten komen wat het beste verloskundig beleid is voor een hypertensieve aandoening tussen de 34 en 37 weken zwangerschapsduur.

In het bijzonder wil ik mijn promoteres Prof. B.M.J. Mol en G.R. Saade bedanken. It is not a coincidence that both of you met. Both of you are great persons with the enormous ability to inspire, to create opportunities for young colleagues and most of all to oversee the greater goal above individual profits.

Beste Ben Willem, na 2 AIOS jaren in het MMC Veldhoven wist je mij over te halen om klinisch onderzoek te gaan doen. Ik zei ‘ja’ onder één voorwaarde: “dat ik goed begeleid zou worden.” 5 Jaar later is het boekje af, naast mijn fulltime werk als AIOS. Ik geloof dat die begeleiding wel gelukt is. Ik wil je bedanken voor je enorme inspiratievermogen, maar vooral voor je tomeloze inzet om de verloskundige zorg in Nederland naar een hoger niveau te brengen. Ga zo door, geef niet op (ook al heeft dat soms een generatie nodig!).

Dear George, thank you for offering me the opportunity to work in your laboratory. It might have been the most important period for my career. At my first oral presentation at SMFM you told me: “Just have fun up there, enjoy the moment” and I did. Thank you for these words, the warm welcome at the lab and of course the introduction in Lebanese food together with your family.

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Curriculum Vitae


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