Perinatal outcome in preterm birth

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ISBN: 978-90-6464-950-9

The studies presented in chapter 7 and 11 of this thesis were supported by a grant of the Dutch Ter Meulen Fund of the Royal Netherlands Academy of Arts and Sciences.

Cover photo: Carla van de Puttelaar

Lay-out: E.O.G. van Vliet en F. van Nispen, *my*-thesis.nl, Bilthoven
Print: GVO Drukkers en Vormgevers, Ede

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Perinatale uitkomst bij vroeggeboorte

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 15 december 2015 des ochtends te 10.30 uur

door

Elvira Odette Geraldine van Vliet
geboren op 25 april 1986 te Hoorn

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Copromotoren:	Dr. M.A. Oudijk Dr. P.G.J. Nikkels

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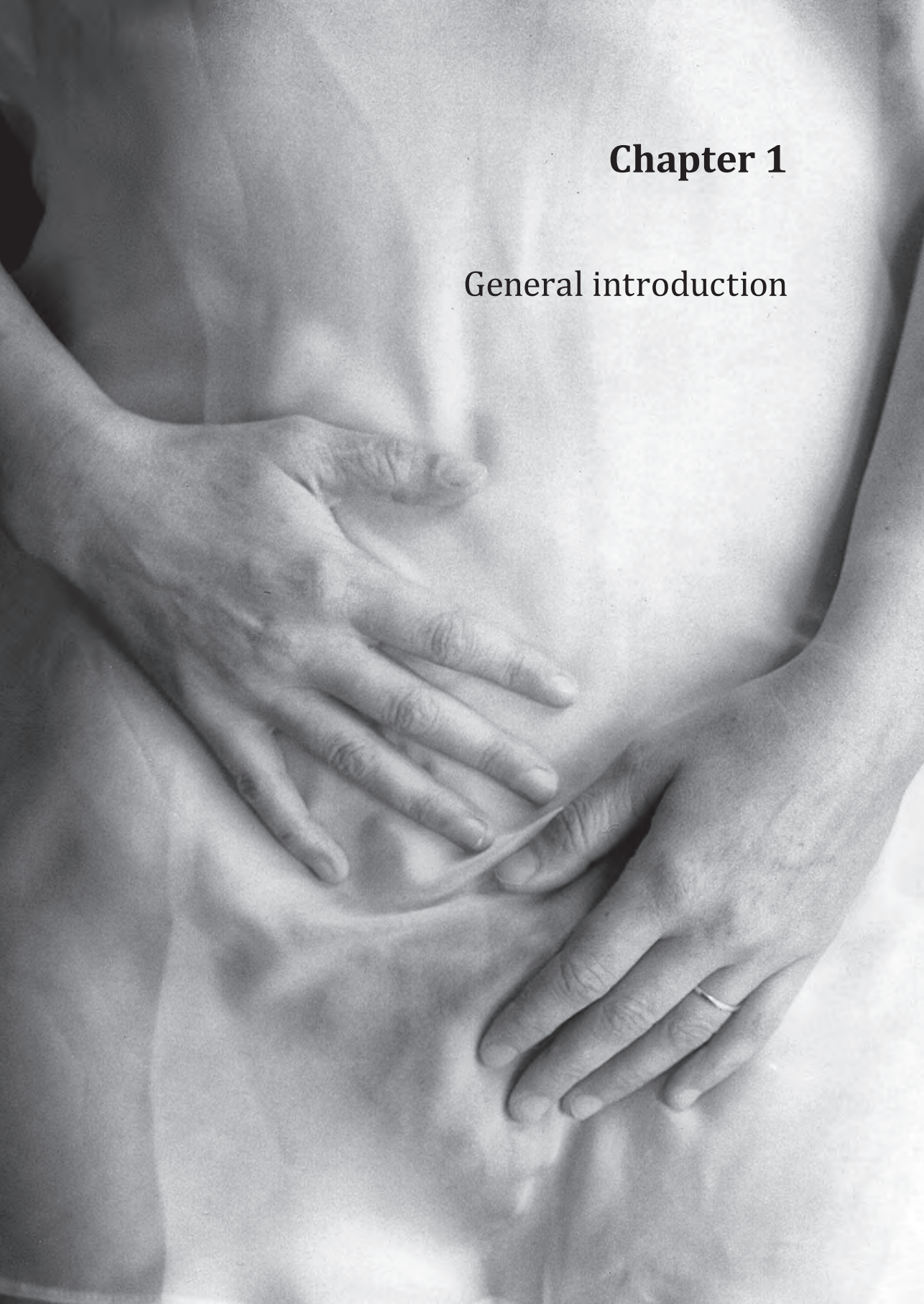


Chapter 1

General introduction

General introduction

Preterm birth, defined as birth before 37 weeks of gestation, is a major cause of perinatal morbidity and mortality worldwide.[1] In Europe, the preterm birth rate is 5.5-11.1%[2] and the incidence is increasing in most countries.[3,4] Preterm infants have an increased risk for neonatal morbidities such as chronic lung disease, intraventricular haemorrhage, perinatal infections and cerebral palsy. Furthermore, preterm infants have more cognitive deficits, motor problems and behaviour problems as compared to their term born peers and these problems persist throughout childhood and adolescence.[5,6] Thus, preterm birth has lifelong consequences and imposes a substantial burden on resources and health services.[7]

The pathway of labour

In both term and preterm birth, labour starts with the common pathway of increased uterine contractility, cervical ripening and dilatation, and rupture of the chorioamniotic membranes.[8] The initiation of labour is triggered by a complex interplay between endocrinological, immunological and mechanical processes.[8,9] Although preterm birth has long been perceived as normal labour that starts too early, preterm birth is now viewed as a complex syndrome caused by multiple pathological processes.[10]

Two groups of preterm birth can be distinguished; spontaneous preterm birth and iatrogenic preterm birth. Spontaneous preterm birth follows spontaneous labour or preterm rupture of the membranes. In iatrogenic preterm birth, pregnancy is prematurely interrupted for maternal or foetal indications.[11] Spontaneous preterm birth is associated with infection. Inflammatory cytokines stimulate prostaglandin release thus contributing to preterm myometrial contractions. Furthermore, cytokines and toxins initiate neutrophil activation, leading to the release of metalloproteases that weaken the membranes and cervix.[12] Approximately 25 to 40 percent of all women who deliver preterm have an intra-uterine infection, although frequently subclinical. The incidence of positive amniotic fluid cultures ranges from 12.8% in women with preterm labour and intact membranes, to 32.4% in women with preterm rupture of the membranes.[13] Other factors that are associated with spontaneous preterm birth are uterine overdistension (mainly in multiple pregnancy or polyhydramnion), cervical disorders (e.g. congenital disorders or traumatic damage after conisation or after cervical dilatation for curettage), hormonal disorders (mainly progesterone deficiency) and placental vascular lesions.[8]

In iatrogenic preterm birth, underlying conditions that prompt the need for medical intervention are maternal pregnancy related complications, foetal complications or pre-existing maternal disease. Pre-eclampsia is the most common indication for iatrogenic delivery (23.3 - 42.5%),[14,15] followed by foetal distress (23.0-26.7%) and intrauterine growth restriction (10-18.9%). Other frequent indications are placental abruption, placenta praevia and foetal congenital malformations.[14] The aetiology of the most common indications for iatrogenic delivery is thought to be ischemic placental

disease caused by a lack of trophoblast invasion of the uteroplacental arteries leading to pre-eclampsia, intrauterine growth restriction or placental abruption.[14]

Placenta pathology and preterm birth

The placenta is the key to foetal development as it plays a critical role in protection of the foetus and exchanging nutrients between mother and child.[16] Both spontaneous and iatrogenic preterm birth are strongly associated with placental dysfunction[16-21], exposing the foetus to an unfavourable intrauterine environment. Thereby placenta pathology might contribute to both preterm delivery and damage to the developing brain.[22] The two most common types of placenta pathology in preterm delivery are maternal vascular underperfusion (i.e. failure of spiral artery remodelling) leading to foetal hypoxia and pathology associated with acute intrauterine infection/inflammation (i.e. chorioamnionitis).[23,24] The association between placental underperfusion and preterm birth is possibly mediated by an interplay between foetal nutritional status, activity of the hypothalamic-pituitary-adrenal axis and increased production of corticotrophin-releasing hormone, inducing the onset of labour.[25] In histological chorioamnionitis, the local release of cytokines possibly stimulates prostaglandin release by foetal membranes and uterine decidua's, both contributing to preterm labour or preterm rupture of the membranes.[22,26] Next to contributing to preterm birth, disorders in placental functioning may also directly affect foetal development and neonatal outcome. Placental underperfusion may lead to foetal hypoxia and thereby trigger glutamate toxicity. The presence of free radicals in combination with a lack of antioxidant enzymes in oligodendrocytes of the preterm foetus may further explain the impact of hypoxia on the premature brain.[27] Placental lesions are found to be related to stillbirth and neonatal illness in the first 24 hours, however associations with long term development remain less clear.[28,29]

Further insight in the different pathophysiological processes underlying preterm birth and the association with neonatal outcome could contribute to a better prediction of outcome and improve selection of infants for early therapeutic interventions.[30] Furthermore, these insights could assist in tailoring preventive strategies for preterm birth. As placental vascular lesions are found to play an important role in the aetiology of both pre-eclampsia and spontaneous preterm birth, antiplatelet agents might be an effective intervention to reduce the risk of these pregnancy complications. Antiplatelet agents have shown to reduce the risk of early-onset pre-eclampsia and preterm birth.[31] However, whether the reduction in preterm birth comprises a reduction in iatrogenic preterm birth (e.g. through lowering the incidence of pre-eclampsia), or also a reduction of spontaneous preterm birth is not known. If antiplatelet agents would reduce the incidence of spontaneous preterm birth, this may be a promising intervention for women at high risk of spontaneous preterm birth.

Management of threatening preterm labour

Current management of threatening preterm labour focusses on the inhibition of contractions. Postponing delivery for at least 48 hours allows the administration of antenatal corticosteroids, aiming to enhance foetal lung maturation and improve neonatal outcome. Furthermore, it allows transportation of the patient to a centre with neonatal intensive care unit facilities. Therefore, the use of tocolytic drugs for 48 hours is common practice in most centres and countries.[32,33] Several types of tocolytic drugs are used as treatment in preterm labour, including β adrenoceptor agonists, cyclooxygenase inhibitors, magnesium sulphate, calcium channel blockers and oxytocin receptor antagonists. While the ultimate goal of treatment is to improve neonatal outcome, many studies on the effectiveness of tocolysis have focused on postponement of delivery as a surrogate outcome. Most studies are therefore underpowered to detect clinically relevant treatment effects. Based on the largest effect on postponement of delivery and the most favourable side effect profile, calcium channel blockers (e.g. nifedipine) and oxytocin antagonists (e.g. atosiban) are currently recommended as drug of first choice.[34] However, there is inconclusive evidence on which of these two tocolytical agents has the most favourable effect on neonatal outcome. Furthermore, as tocolytic drugs intervene at the point where the cascade of preterm birth has already started, the neonatal benefit of this intervention might be small. Therefore, it would be promising to search for treatment options that intervene at an earlier stage in the pathophysiological process of preterm labour.

Aims of the thesis

The aim of this thesis is twofold. First, this thesis aims to provide insight in the nature of the relationship between placenta pathology, brain development and outcome in preterm birth. Second, this thesis aims to evaluate the effectiveness and safety of interventions to prevent preterm birth.

Outline of this thesis

This thesis is divided in two parts. **Part I** of this thesis aims to gain insight into the relationship between placenta pathology, brain development and outcome in preterm birth by aggregating the current evidence in literature and performing three cohort studies. **Chapter 2** presents a meta-analysis on the effect of perinatal infections and neurodevelopmental outcome in very preterm infants. Differential effects of infections on mental development and motor outcomes are explored by aggregating the literature on perinatal infections and neurodevelopmental outcome measured with the Bayley Scales of Infant Development. To disentangle the relationship between placenta pathology and long term neurodevelopmental outcome, we assessed neurodevelopmental outcome at age 2 and 7 years in a cohort of very preterm infants who participated in the Glutamine Enriched Enteral Feeding (GEEF) study.[35] The results of this study are presented in **Chapter 3**. To further explore the association between placenta pathology and brain development, **Chapter 4** presents the results

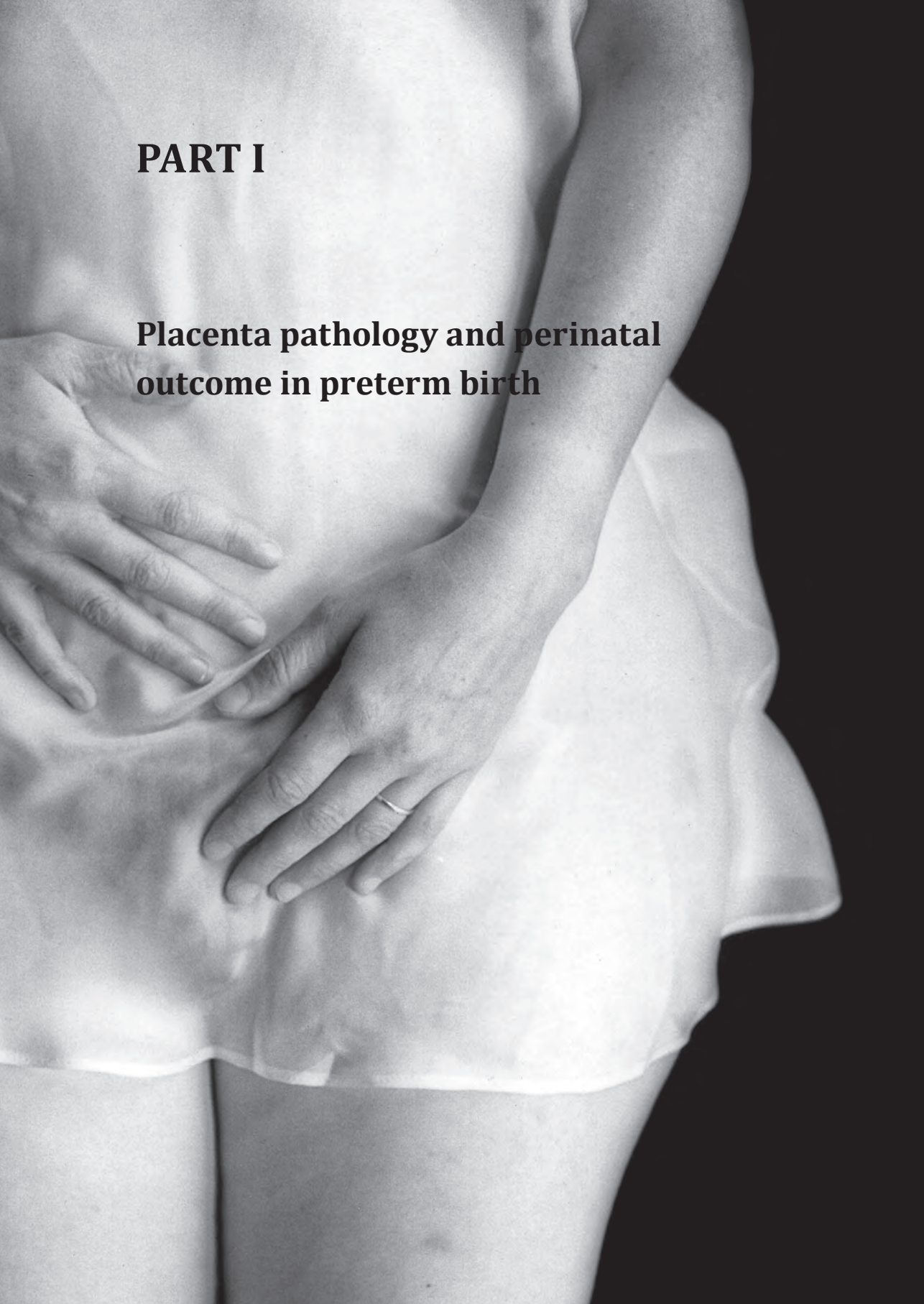
of a cohort study on placenta pathology, cerebral MRI at term equivalent age and neurodevelopmental outcome at age 2, 3.5 and 5 years. The relationship between placenta pathology and the recurrence of preterm birth in a subsequent pregnancy is explored in **Chapter 5**.

Part II of this thesis aims to study the effects of interventions in pregnancy to prevent preterm birth and to optimize outcome in threatened preterm delivery. Firstly, in **Chapter 6** we present an overview of the literature on tocolysis for the treatment of preterm labour. It provides an historical overview and points out the knowledge gaps within the field of research on tocolytics. Previously, the APOSTEL II trial (Assessment of Perinatal Outcome after Specific Tocolysis in Early Labour) assessed the effect of maintenance tocolysis with nifedipine versus placebo in women with threatened preterm birth, and found no effect on neonatal outcome.[36] To further study the possible effect of maintenance tocolysis, an Individual Participant Data meta-analysis (IPD) was performed, including data from all six randomized controlled trials that have been performed on nifedipine maintenance tocolysis. **Chapter 7** presents the results of the meta-analysis of this IPD. **Chapter 8** presents the 2 year follow up of the infants of the APOSTEL II trial to explore the effect of nifedipine maintenance tocolysis on infant development. **Chapter 9** present the study protocol of the APOSTEL III trial, a multicentre randomized controlled trial on the effectiveness and safety of the two most widely used tocolytic drugs in the Netherlands, nifedipine and atosiban. This study was designed within a series of studies to fill the knowledge gaps in the field of tocolytic treatment in preterm labour. Five-hundred and ten women with threatened preterm labour were randomized to treatment with nifedipine or atosiban to assess the effect on adverse perinatal outcome. The results of this trial are presented in **Chapter 10.** As it has been hypothesised that there might be a vascular component in the aetiology of spontaneous preterm birth, the use of antiplatelet agents in pregnancy might provide an effective preventive measure for preterm birth. **Chapter 11** presents the work of an international collaboration with the University of Sydney and the Robinson Research Institute in Adelaide. It presents a secondary analyses of Individual Participant Data of women at risk of pre-eclampsia who were enrolled in randomized controlled trials on antiplatelet agents versus placebo/no treatment. The current analyses study the effect of antiplatelet agents on the incidence of spontaneous preterm birth. The final chapter of this thesis, **Chapter 12**, summarizes the findings of the studies presented in this thesis, discusses the insights of these studies and points out directions of future research towards improving outcomes in pregnancy.

References

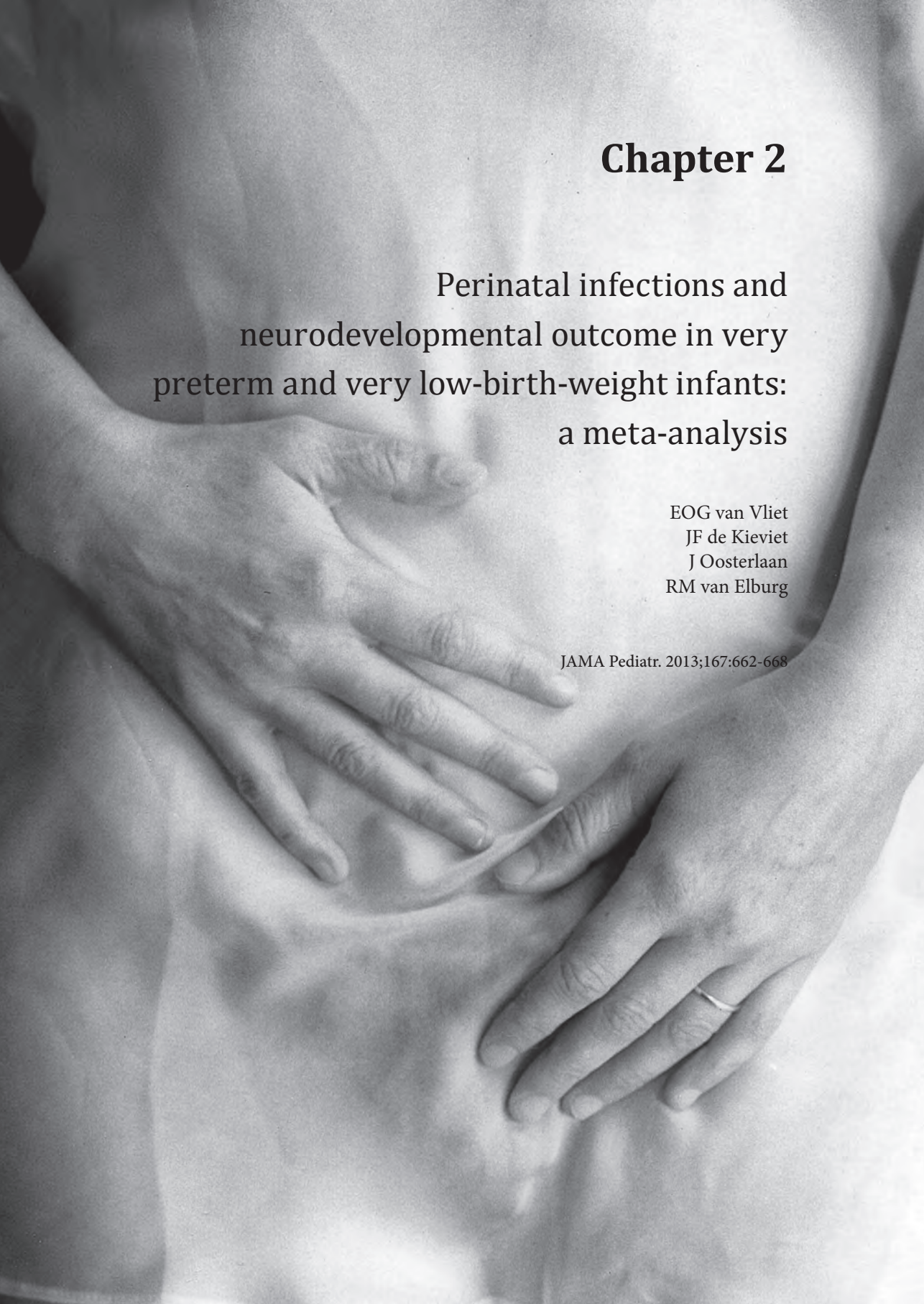
1. Blencowe H., Cousens S., Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012; 379: 2162–72.
2. Zeitlin J, Szamotulska K, Drewniak N, Mohangoo AD, Chalmers J, Sakkeus L, et al. Preterm birth time trends in Europe: a study of 19 countries. BJOG. 2013;120:1356-65.
3. Chang HH, Larson J, Blencow H, Spong CY, Howson CP, Cairns-Smith S, et al. Preventing preterm births: analysis of trends and potential reductions with interventions in 39 countries with very high human development index. Lancet 2013;381:223–34.
4. Goldenberg RL, Culhane JF, Lams JD, Romero R. Epidemiology and causes of preterm birth. The Lancet. 2008;371:75-84.
5. Aarnoudse-Moens CS, Weisglas-Kuperus N, van Goudoever JB, Oosterlaan J. Meta-analysis of neurobehavioral outcomes in very preterm and/or very low birth weight children. Pediatrics. 2009;124:717-28.
6. de Kieviet JF, Piek JP, Aarnoudse-Moens CS, Oosterlaan J. Motor development in very preterm and very low-birth-weight children from birth to adolescence: a meta-analysis. JAMA. 2009; 25;302:2235-42.
7. Petrou S, Eddama O, Mangham L. A structured review of the recent literature on the economic consequences of preterm birth. *Arch Dis Child Fetal Neonatal Ed.* 2010.
8. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, Chaiworapongsa T, Mazor M.The preterm parturition syndrome. BJOG. 2006;113 Suppl 3:17-42.
9. Voltolini C, Torricelli M, Conti N, Vellucci FL, Severi FM, Petraglia F. Understanding spontaneous preterm birth: from underlying mechanisms to predictive and preventive interventions. Reprod Sci. 2013;20:1274-92.
10. Romero R, Dey SK, Fisher SJ. Preterm labour: one syndrome, many causes. Science. 2014 15;345:760-5.
11. Ananth CV, Getahun D, Peltier MR, Salihu HM, Vintzileos AM. Recurrence of spontaneous versus medically indicated preterm birth. Am J Obestet Gynecol 2006;195:643-650.
12. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000;342:1500-7.
13. Romero R, Gómez R, Chaiworapongsa T, Conoscenti G, Kim JC, Kim YM. The role of infection in preterm labour and delivery. Paediatr Perinat Epidemiol. 2001;15 Suppl 2:41-56.
14. Ananth CV, Vintzileos AM. Medically indicated preterm birth: recognizing the importance of the problem. Clin Perinatol. 2008;35:53-67, viii.
15. Meis P, Goldenberg RL, Mercer BM, Iams JD, Moawad AH, Miodovnik M, Menard MK, Caritis SN, Thurnau GR, Bottoms SF, Das A, Robers JM and McNellis D. The preterm prediction study: Risk factors for indicated preterm births. AJOG 1998;178:562–567.
16. Redline RW. Placental pathology: a systematic approach with clinical correlations. Placenta 2008;29(Suppl A):S86-91.
17. Altshuler G. Some placental considerations related to neurodevelopmental and other disorders. J Child Neurol 1993;8:78-94.
18. Amiel-Tison C, Cabrol D, Denver R, Jarreau PH, Papiernik E, Piazza PV. Fetal adaptation to stress. Part I: acceleration of fetal maturation and earlier birth triggered by placental insufficiency in humans. Early Hum Dev 2004;78: 15-27.
19. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. Pediatr Res 1997;42:1-8.
20. Kaukola T, Herva R, Perhomaa M, et al. Population cohort associating chorioamnionitis, cord inflammatory cytokines and neurologic outcome in very preterm, extremely low birth weight infants. Pediatr Res 2006;59:478-83.
21. Mehta R, Nanjundaswamy S, Shen-Schwarz S, Petrova A. Neonatal morbidity and placental pathology. Indian J Pediatr 2006;73:25-8.
22. Ticconi C, Lye SJ. Placenta and fetal membranes in human parturition and preterm delivery—a workshop report. Placenta 2002; 23(Suppl A):S149-52.
23. Beaudet L, Karuri S, Lau J, Magee F, Lee SK, von Dadelszen P. Placental pathology and clinical outcomes in a cohort of infants admitted to a neonatal intensive care unit. J Obstet Gynaecol Can 2007;29:315-23.
24. O'Shea TM, Allred EN, Dammann O, et al. The ELGAN study of the brain and related disorders in extremely low gestational age newborns. Early Hum Dev 2009;85:719-25.

25. Kalantaridou SN, Zoumakis E, Makrigiannakis A, Lavasidis LG, Vrekoussis T, Chrousos GP. Corticotropin-releasing hormone, stress and human reproduction: an update. J Reprod Immunol 2010;85:33-9.
26. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med 2000;342:1500-7.
27. Rezaie P, Dean A. Periventricular leukomalacia, inflammation and white matter lesions within the developing nervous system. Neuropathology 2002;22:106-32.
28. Roescher AM, Hitzert MM, Timmer A, Verhagen EA, Erwich JJ, Bos AF. Placental pathology is associated with illness severity in preterm infants in the first twenty-four hours after birth. Early Hum Dev. 2011;87:315-319.
29. Roescher AM, Timmer A, Erwich JJ, Bos AF. Placental pathology, perinatal death, neonatal outcome, and neurological development: a systematic review. PLoS One. 2014;9:e89419.
30. Latal B. Prediction of neurodevelopmental outcome after preterm birth. Pediatr Neurol 2009;40:413-9.
31. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA; PARIS Collaborative Group. Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. Lancet. 2007;369:1791-8.
32. Keirse MJ. The history of tocolysis. BJOG. 2003;110 Suppl 20:94-97.
33. Hehiri MP, O'Connor HD, Kent EM, Robson MS, Keane DP, Geary MP, Malone FD. Early and late preterm delivery rates- a comparison of differing tocolytic policies in a single urban population. J Matern Fetal Neonatal Med. 2012;25:2234-2236.
34. www.nvog.nl
35. van den Berg A, van Elburg RM, Twisk JW, Fetter WP. Glutamine-enriched enteral nutrition in very low birth weight infants. Design of a double-blind randomised controlled trial. BMC Pediatr. 2004;4:17.
36. Roos C, Spaanderman ME, Schuit E, Bloemenkamp KW, Bolte AC, Cornette J et al. APOSTEL-II Study Group. Effect of maintenance tocolysis with nifedipine in threatened preterm labour on perinatal outcomes: a randomized controlled trial. JAMA. 2013;309:41-47.



PART I

Placenta pathology and perinatal outcome in preterm birth



Chapter 2

Perinatal infections and neurodevelopmental outcome in very preterm and very low-birth-weight infants: a meta-analysis

EOG van Vliet
JF de Kieviet
J Oosterlaan
RM van Elburg

JAMA Pediatr. 2013;167:662-668

Abstract

Importance Perinatal infections are commonly present in preterm and very low-birth-weight (VLWB) infants and might contribute to adverse neurodevelopmental outcome.

Objective To summarize studies evaluating the effect of perinatal infections on neurodevelopmental outcome in very preterm/VLBW infants.

Evidence review On December 12, 2011, we searched Medline, PsycINFO, Embase, and Web of Knowledge for studies on infections and neurodevelopmental outcome. All titles and abstracts were assessed for eligibility by 2 independent reviewers. We also screened the reference lists of identified articles to search for additional eligible studies. Preselected criteria justified inclusion in this meta-analysis: (1) the study included infants born very preterm (≤32 weeks) and/or with VLBW (≤1500 g); (2) the study compared infants with and without perinatal infection; (3) there was follow-up using the Bayley Scales of Infant Development 2nd edition; and (4) results were published in an English-language peer-reviewed journal. The quality of each included study was assessed using the Newcastle-Ottawa Scale.

Findings This meta-analysis includes 18 studies encompassing data on 13.755 very preterm/VLBW infants. Very preterm/VLBW infants with perinatal infections had poorer mental (d= −0.25; P < .001) and motor (d = −0.37; P < .001) development compared with very preterm/VLBW infants without infections. Mental development was most impaired by necrotizing enterocolitis (d= −0.40; P < .001) and meningitis (d= −0.37; P < .001). Motor development was most impaired by necrotizing enterocolitis (d= −0.66; P < .001). Chorioamnionitis did not affect mental (d= −0.05; P = .37) or motor (d= 0.19; P = .08) development.

Conclusion and relevance Postnatal infections have detrimental effects on mental and motor development in very preterm/VLBW infants.

Introduction

Very preterm delivery (≤32 weeks of gestation) and very low birth weight (VLBW) (≤1500 g) are strongly associated with intrauterine infections,[1-3] and the majority of very preterm/VLBW infants develop at least 1 neonatal infection.[4] There is increasing evidence that infections contribute to brain damage, which leads to adverse neurodevelopmental outcome in this at-risk population.[4,5]

Infection and inflammation may contribute to both preterm birth and damage to the developing brain. The local release of inflammatory cytokines, particularly interleukin 1, interleukin 6, and tumor necrosis factor α, could lead to preterm labor through stimulation of prostaglandin release by fetal membranes and uterine deciduas.[6] Furthermore, inflammatory cytokines may directly cause damage to the preterm brain by increasing the permeability of the blood-brain barrier and interfering with normal myelinization by damaging myelin and myelin-producing cells.[7] Very preterm infants with infections are also at increased risk for respiratory and circulatory insufficiency, which further increases the risk for hypoxic and ischemic brain injury. The cytokines that are produced during infections can have a systemic effect causing hypotension and disseminated intravascular coagulation and therefore indirectly contribute to cerebral brain damage.[8] In recent studies, perinatal infections in preterm infants were related to periventricular leukomalacia and echolucent lesions,[8-10] reflections of white matter damage that are in turn associated with poor outcomes in later life.[11]

This meta-analysis quantitatively aggregates the literature on perinatal infections and neurodevelopmental outcome measured with the Bayley Scales of Infant Development 2nd edition (BSID-II). Results may contribute to identifying infants at risk for adverse neurodevelopmental outcome and targeting these vulnerable infants for interventions to improve outcomes.

Methods

Selection of Studies

Medline, PsycINFO, Embase, and Web of Knowledge were searched for studies evaluating the relation between perinatal infections and neurodevelopmental outcome in very preterm and/or VLBW infants. We used the key words "preterm," "prematur*," and "very low birth weight" to select studies involving our target population. To search for studies regarding perinatal infections, we used the key words "infection" and "inflammation" with all possible suffixes (eg, infections, infectious, and inflammatory) and the key words "cytokine*," "cytokinemia," "bacteriemia," and "fungemia" in British and American orthography. In addition, we searched for all common prenatal and neonatal infections, namely urinary tract infection, sepsis, pneumonia, meningitis, necrotizing enterocolitis (NEC), encephalitis, chorioamnionitis, funisitis, candidiasis, villitis, and fetal vasculitis. Neurodevelopmental outcome measurement was searched

for by the key words "Bayley*," "BSID," "bayley scales," "neurodevelopment," "neuropsycholog*," "child development," "executive functioning," "intelligence," "psychomotor," and "aptitude test." We used Medical Subject Headings (MeSH) for Medline, thesaurus terms for PsycINFO, and Emtree terms for Embase. Furthermore, we screened the reference lists of identified articles to search for additional eligible studies. The search was conducted on December 12, 2011.

The following preselected criteria justified inclusion in this meta-analysis: (1) the study included infants born very preterm (≤32 weeks) and/or with VLBW (≤1500 g); (2) the study compared infants with and without perinatal infection; (3) there was follow-up using BSID-II[12]; and (4) results were published in an English-language peer-reviewed journal. We included studies measuring neurodevelopmental outcome with BSID-II.[12] This scale is the most commonly used scale to measure neurodevelopmental outcome.[13] It consists of a Mental Development Index (MDI), Psychomotor Development Index (PDI), and Behavioral Rating Scale. Scores on the BSID-II are normalized and have a mean (SD) of 100 (15). Higher scores indicate better neurodevelopment.

All retrieved titles and abstracts were assessed for eligibility by 2 independent reviewers (E.O.G.vV. and J.F.dK.). Duplicate publications found in more than 1 database were removed. The full-text article was retrieved and evaluated if both reviewers considered the abstract potentially relevant. Disagreements were settled by discussion. For full-text articles meeting all the preselected inclusion criteria, data were extracted on year of study, sample characteristics, type of infection, and BSID-II scores. From studies using the same cohort of participants, the study with the most comprehensive data was included in the meta-analysis.

Quality Assessment

Two of us (E.O.G.vV. and J.F.dK.) independently assessed the quality of each included study using the Newcastle-Ottawa Scale.[14] Scores on this instrument range from 1 to 9, with higher scores indicating higher quality. Each study was assessed on the quality of the selection of participants (4 criteria), comparability of study groups (1 criterion), and outcome assessment (3 criteria). Inconsistencies in ratings were resolved by discussion.

Statistical Analyses

Statistical analyses were conducted using Comprehensive Meta-Analysis version 2.2.[15] We contacted authors for additional data if necessary. When only medians were reported, techniques by Hozo et al[16] were used to estimate means and standard deviations. If means and standard deviations were not reported in the article, odds ratios were calculated using the available data. Effect sizes for both MDI and PDI (Cohen *d*) were determined for each study separately. We calculated mean differences for continuous data. *Q* and I^2 test statistics were conducted to test heterogeneity among the studies' effect sizes. The I^2 value is the percentage of variation in effect sizes among studies due to heterogeneity rather than due to chance. Values of 25%, 50%, and 75%

represent, respectively, low, moderate, and high heterogeneity.[17] The overall effect of infection on both the MDI and PDI subscales was computed by weighting each study's effect size by the study's sample size. We tested if effect sizes were different for studies reporting continuous or dichotomous data. In addition, we tested whether the overall effect of infection was similar for MDI and PDI scores, ie, whether infections affect mental and motor development to a similar extent. If 3 or more studies reported on a particular type of infection, the overall effect size of this type of infection was calculated. Heterogeneity tests were conducted on the overall effect sizes calculated for types of infection to test if all types of infection affect BSID-II scores to a similar extent. The relationship between study quality score and effect size was studied by linear regression analysis.

A major concern in conducting meta-analyses is the possibility of publication bias. We therefore investigated the correlation between sample sizes and effect sizes of each study. Furthermore, the Rosenthal fail-safe *N*[18] was calculated, which measures the necessary number of nonsignificant studies to nullify the overall effect. In addition, we investigated the degree of funnel plot asymmetry using linear regression methods proposed by Egger et al.[19]

The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement was followed whenever appropriate.[20]

We identified 2.573 abstracts using the prespecified search strategy. Data of 18 studies[4,21-37] were pooled for meta-analysis, encompassing data on 13.755 very preterm/VLBW infants. Details on selection of studies and reasons for exclusion are specified in Figure 1. Nine studies reported BSID-II scores on very preterm/VLBW infants with NEC; 8, on infants with sepsis; 4, on infants with meningitis; and 6 studies reported data on infants with chorioamnionitis. No significant association was found between study quality and effect size ($\beta = -0.02$; $P = .26$) or sample size and effect size ($\beta = -0.00001$; $P = .46$). Furthermore, no significant association was found between age at assessment and effect size ($\beta = 0.007$; $P = .42$). There was no difference in effect size between studies reporting continuous outcome measures and studies reporting dichotomous outcome measures ($Q = 0.211$; $P = .65$ for MDI and $Q = 0.737$; $P = .39$ for PDI). None of the studies reported on Behavior Rating Scale scores.

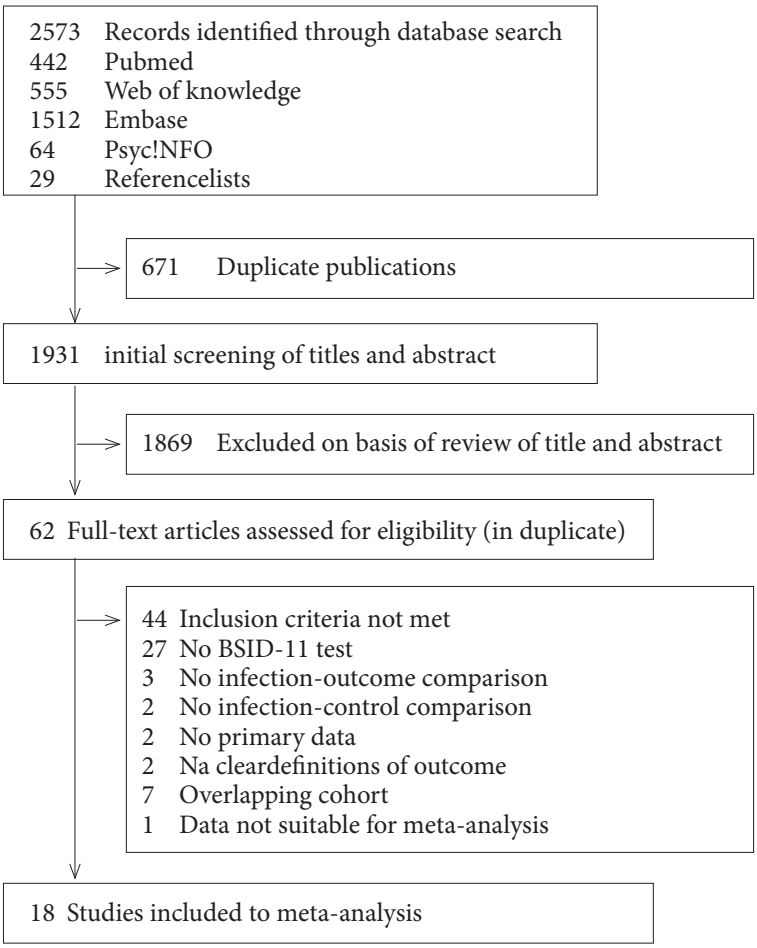


Figure 1. Flow Chart of Study selection

Analysis Including All Types of Infections

Mental Development

The BSID-II MDI scores were reported in 17 studies[4,21,23-37] encompassing 13.649 very preterm/VLBW infants. Seven studies reported data on more than 1 type of infection. Very preterm/VLBW infants with perinatal infections had significantly poorer MDI scores compared with infants without perinatal infections, as indicated by the combined random-effect size of 0.25 (95% CI, 0.14 to 0.36; $P < .001$) (Figure 2). Only random-effect size could be calculated because of heterogeneously distributed data ($Q = 89.05$; $P < .001$).

All but 5 studies[23,24,32,34,39] reported lower MDI scores in infants with perinatal infections. Four studies[24,32,34,39] reported no differences in MDI scores between VLBW/very preterm infants with and without perinatal infections. The study of Fung et al[23] reported nonsignificant higher MDI scores in infants with infection. Interestingly, these results became significant in our meta-analysis, most likely because we used parametric statistics whereas Fung et al used nonparametric statistics. However, the reported effect size for this study was small (−0.34) and excluding the study from our analysis did not change the significance of the overall effect size.

Analyses were based on 12 studies reporting means and standard deviation, 4 studies reporting odds values for MDI scores less than 70, 1 study reporting odds values for MDI scores less than 85, and 1 study reporting odds values for MDI scores less than 55. Excluding the study reporting MDI scores less than 55 did not change the significance of the overall effect size.

The fail-safe *N* for the MDI scores was 435, and the Egger degree of funnel plot asymmetry was not significant ($P = .22$), together indicating no evidence for the presence of any publication bias.

Motor Development

The BSID-II PDI scores were reported in 11 studies.[4,21,22,25- 27,29,30,32,33,39] Meta-analysis of data from these studies encompassing 11.491 very preterm/VLBW infants indicated that infants with perinatal infections had significantly poorer PDI scores compared with infants without perinatal infections ($d = 0.368$; 95% CI, 0.253 to 0.483; $P < .001$) (Figure 3). The data were heterogeneously distributed ($Q = 37.73$; $P < .001$); therefore, a random-effects model was used. Two studies[27,32] reported no significant difference in PDI scores between infants with and without perinatal infections. The fail-save *N* was 342 and the Egger degree of funnel plot asymmetry was not significant ($P = .26$), together indicating that there was no evidence of publication bias. One of the studies reported odds rates for PDI scores less than 55. Excluding this study did not change the significance of the overall effect size.

The overall effect sizes for MDI and PDI scores were significantly different ($Q_1 = 9.038$; $P = .003$), indicating that perinatal infections have a greater impact on PDI than on MDI scores in VLBW/very preterm infants.

Source, Year	Infection	SD in means	SE	Mean Difference	Lower Limit
Benjamin et al, 2006	Candidemia	0.441	0.081	7.90	0.283
Hendson et al, 2011	Chorioamnionitis	0.302	0.090	5.22	0.125
Polam et al, 2005	Chorioamnionitis	0.059	0.152	1.00	- 0.239
Watterberg et al, 2007	Chorioamnionitis	0.053	0.193	1.00	- 0.327
Fung et al, 2003	Chorioamnionitis	- 0.343	0.115	-3.00	-0.568
Kaukola et al, 2006	Chorioamnionitis	0.000	0.276	0.00	-0.541
Bassler et al, 2009	Meningitis	0.895	0.266		0.374
Benjamin et al, 2006	Meningitis	0.508	0.259	9.10	0.001
Stoll et al, 2004	Meningitis	0.259	0.104		0.056
Hack et al, 2000	Meningitis	0.445	0.294		-0.132
Yeh et al, 2004	NEC	0.633	0.323	9.20	-0.000
Bassler et al, 2009	NEC	0.401	0.138		0.131
Salhab et al, 2004	NEC	0.527	0.345	7.00	-0.148
Stoll et al, 2004	NEC	0.259	0.085		0.093
Hack et al, 2000	NEC	0.469	0.378		-0.273
Laughon et al, 2009	NEC	0.485	0.149		0.192
Shah et al, 2008	NEC	0.904	0.350	18.20	0.218
Saldir et al, 2010	NEC	0.924	0.438		0.065
Soraisham et al, 2006	NEC	0.594	0.182	10.20	0.239
Bassler et al, 2009	Sepsis	0.189	0.084		0.024
McKee et al, 2009	Sepsis	-0.263	0.111	-5.77	-0.480
Salhab et al, 2004	Sepsis	0.409	0.480		-0.531
Stoll et al, 2004	Sepsis	0.145	0.066		0.016
Schalpbach et al, 2004	Sepsis	0.174	0.178		-0.174
Hack et al, 2000	Sepsis	-0.221	0.173		-0.559
Shah et al, 2008	Sepsis	0.242	0.151		0.054
Soraisham et al, 2006	Sepsis	0.053	0.465		0.140
		0.250	0.056		0.140

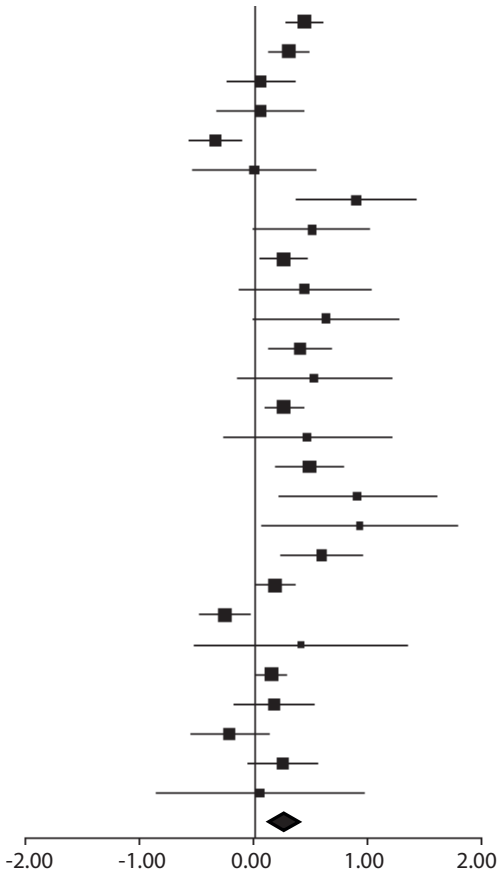
Figure 2. Mental Developmental Index score

Analysis on Different Infections

Nine studies reported MDI scores on NEC[4,25,28-31,33-35]; 4 studies, on meningitis[4,21,34,35]; 8 studies, on sepsis[4,26,29-31,34,35,39]; and 5 studies reported MDI scores on chorioamnionitis.[23,24,27,32,40]

The combined effect size for NEC was 0.40 (95% CI, 0.29 to 0.51; $P < .001$). The combined effect size for meningitis was 0.37 (95% CI, 0.201 to 0.540; $P < .001$). There was no difference in MDI scores between infants with and without sepsis ($d = 0.054$; 95% CI, −0.092 to 0.216; $P = .43$) and between infants with and without chorioamnionitis ($d = 0.054$; 95% CI, −0.063 to 0.170; $P = .37$).

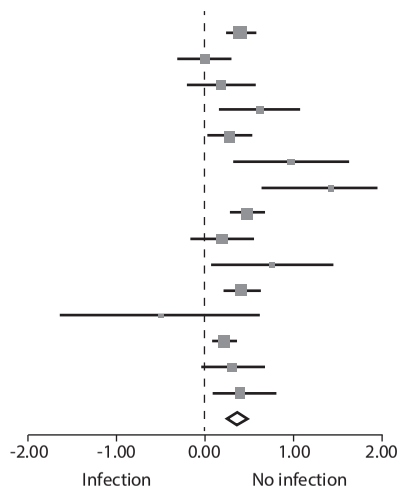
Upper Limit	*z Value*	*P Value*
0.600	5.469	<.001
0.479	3.350	.001
0.257	0.390	.70
0.432	0.272	.79
-0.118	-2.986	.003
0.541	0.000	>.99
1.416	3.367	.001
1.016	1.963	.05
0.462	2.498	.01
1.021	1.512	.13
1.267	1.959	.05
0.671	2.914	.004
1.203	1.530	.13
0.425	3.063	.002
1.210	1.239	.002
0.778	3.244	.001
1.591	2.581	.01
1.783	2.108	.04
0.950	3.274	.001
0.354	2.251	.02
-0.045	-2.370	.02
1.349	0.853	.39
0.274	2.199	.03
0.522	0.977	.33
0.118	-1.278	.20
0.539	1.600	.11
0.360	4.464	<.001
0.360	4.464	<0.001



Combined effect sizes for the 4 types of infections differed significantly ($Q = 29.418$; $P < .001$). The Table depicts the results of post hoc analysis comparing each type of infection. Mental development was mainly affected by NEC and meningitis.

Figure 3. Psychomotor Developmental Index Score

Source, Year	Infection	Statistics for Each Study							Standard Difference in Means and 95% CI
		SD in means	SE	Mean Difference	Lower Limit	Upper Limit	*z* Value	*P* Value	
Benjamin et al, 2006	Candidemia	0,402	0,081	7,30	0,244	0,561	4,988	<,001	
Polam et al, 2005	Chorioamnionitis	0,000	0,152	0,00	-0,298	0,298	0,000	>,99	
Watterberg et al, 2007	Chorioamnionitis	0,200	0,194	4,00	-0,180	0,580	1,031	,30	
Berger et al., 2009	Chorioamnionitis	0,624	0,239		0,156	1,092	2,612	,009	
Stoll et al, 2004	Meningitis	0,293	0,121		0,055	0,530	2,418	,02	
Yeh et al, 2004	NEC	0,895	0,333	13,00	0,333	1,637	2,959	,003	
Salhab et al, 2004	NEC	1,450	0,374	22,00	0,716	2,183	3,874	<0„1	
Stoll et al, 2004	NEC	0,483	0,097		0,292	0,674	4,951	<,001	
Laughon et al, 2009	NEC	0,201	0,184		-0,159	0,561	1,094	,27	
Shah et al, 2008	NEC	0,759	0,349	13,70	0,075	1,443	2,175	,03	
McKee et al, 2009	Sepsis	0,426	0,111	10,08	0,208	0,645	3,822	<,001	
Salhab et al, 2004	Sepsis	-0,505	0,573		-1,629	0,619	-0,881	,38	
Stoll et al, 2004	Sepsis	0,224	0,065		0,097	0,350	3,459	,001	
Schalpbach et al, 2004	Sepsis	0,324	0,180		-0,028	0,676	1,803	,07	
Shah et al, 2008	Sepsis	0,411	0,152		0,113	0,710	2,700	,007	
		0,368	0,059		0,253	0,483	6,281	<,001	



NEC indicates necrotizing enterocolitis.

Table. Post hoc Analysis Comparing Infection Types

Infection	*Q* Value	*Df*	*P* Value
MDI			
NEC vs meningitis	0.095	1	.76
NEC vs sepsis	19.853	1	<.001
NEC vs chorioamnionitis	17.819	1	<0..1
Meningitis vs sepsis	8.717	1	.003
Meningitis vs chorioamnionitis	9.088	1	.003
Sepsis vs chorioamnionitis	0.205	1	.65
PDI			
NEC vs sepsis	5.965	1	.02
NEC vs chorioamnionitis	6.152	1	.01
Sepsis vs chorioamnionitis	0.732	1	.39

Abbreviations: MDI, Mental Development Index; PDI, Psychomotor Development Index; NEC, necrotizing enterocolitis.

Five studies reported PDI scores on NEC[4,25,29,30,33]; 5 studies, on sepsis[4,26,29,30,39]; and 3 studies reported PDI scores on chorioamnionitis.[27,32,36] Only 1 study reported PDI scores on meningitis; therefore, no combined effect size could be calculated for this type of infection.

The combined effect size for NEC was 0.66 (95% CI, 0.313 to 1.013; $P < .001$). The combined effect size for sepsis was 0.308 (95% CI, 0.176 to 0.433; $P < .001$). The combined effect size for chorioamnionitis was small and nonsignificant ($d = 0.186$; 95% CI, −0.024 to 0.396; $P = .08$), indicating that very preterm/VLBW infants with chorioamnionitis do not have significantly lower PDI scores compared with those without chorioamnionitis.

Meta-analytic effect sizes on PDI for the 3 infection types differed significantly ($Q = 8.063$; $P = .02$). The overall effect size of NEC was significantly higher compared with the combined effect size of sepsis and the combined effect size of chorioamnionitis (Table).

Discussion

This meta-analysis provides sound evidence for the presence of impairment in mental and motor development among very preterm/VLBW infants with perinatal infections in comparison with very preterm/VLBW infants without infections, with an average decrease in MDI and PDI scores of 0.25 and 0.37 SD, translating into 3.75 and 5.55 points, respectively. Mental development is mostly impaired by NEC and meningitis, with a decrease of MDI score of 0.40 SD (6 points) and 0.37 SD (5.6 points), respectively, compared with very preterm/VLBW infants without these complications. Motor development is mainly impaired by NEC, with a decrease of PDI score of 0.66 SD (10 points). The reported impact of infections on MDI and PDI scores add up to the well-known detrimental effects of prematurity.

Preterm infants with NEC are often exposed to a suboptimal nutritional condition due to prolonged enteral feed intolerance and dependence on central venous catheters for parenteral nutrition. The need for surgical interventions in NEC may further contribute to poor developmental outcome.[41,42] In meningitis, a high amount of bacteria circulating in the blood is thought to be necessary for the invasion of the central nervous system. As a response to brain invasion, leukocytes release factors that contribute to local vasospasm and vasculitis, contributing to brain damage.[43]

In this meta-analysis, chorioamnionitis had no effect on mental or motor development. Although chorioamnionitis is unequivocally a risk factor for preterm delivery,[8,40] studies assessing the effects of intrauterine infections on neurodevelopmental impairment in very preterm/VLBW infants report contradictory results.[22,24,27,39,40,44,45] There are several possible explanations for these contradictory results. Since chorioamnionitis is often associated with lower gestational age and birth weight, the common correction for gestational age may underestimate the actual contribution of chorioamnionitis to developmental outcome.[46] Furthermore, in a cohort of very preterm infants, the control group of very preterm/VLBW infants without intrauterine infection might be exposed to other, possibly more devastating events leading to preterm birth.[47] This meta-analysis indicates a higher impact of infection on PDI scores than on MDI scores. A possible explanation is that, in particular, white matter development and the white matter myelinization process, essential for corticospinal tract functioning and motor behavior, might be vulnerable to the adverse effects of infections.[48] In addition, the thalamic nucleus, especially the reticular nuclei, and basal ganglia are most commonly involved in periventricular leukomalacia. These areas are important in voluntary motor control and balance.[48] The higher prevalence of periventricular leukomalacia in infants with infections might explain part of the difference in the effect on neurodevelopmental sequelae.

Besides the general associations between infections and neurodevelopmental outcome as described in this meta-analysis, 3 studies conducted additional analysis on type of microorganism causing infections. Schlapbach et al[37] found that gram-positive sepsis was associated with a 4-fold risk of cerebral palsy and a 2-fold risk of neurodevelopmental impairment compared with uninfected infants. Infants with gram-negative sepsis had a somewhat increased risk of neurodevelopmental impairment compared with uninfected infants, but this effect decreased after adjustment for confounders. However, these results should be interpreted with caution since mortality was highest for infants with gram-negative sepsis. Stoll et al[4] found lower MDI and PDI scores among infected infants regardless of pathogen type. Furthermore, Berger et al[22] found higher risk of poor PDI scores in infants with *Ureaplasma* species compared with infants with negative culture results but did not find significant associations between poor PDI scores and isolation of other pathogens.

This meta-analysis has some limitations that should be taken into consideration. Although the BSID-II is the most widely used instrument to assess neurodevelopment, it does have some restrictions. The BSID-II relies on subjective observations and

classifications by examiners for determining mental and motor performance. However, in most studies, the tests were administered by experienced examiners who were blinded for medical history, and the interrater reliability of this test has been extensively studied and was found satisfactory.[49] The BSID-II is found to be poor to moderately predictive for neurodevelopmental outcome at school age.[50,51] Although the validity of standardized mean differences reported in meta-analysis is debatable,[52] it enables the opportunity to aggregate studies reporting outcomes on continuous and dichotomous scales. Additionally, some effect sizes in this meta-analysis were based on a small number of studies and should be interpreted with caution. The considerable heterogeneity found in some analyses indicates variability among the studies. Meta-analytic methods are not without limitations, especially when the source of heterogeneity is unclear or when publication bias is present.[17] Nevertheless, the source of heterogeneity in the outcomes of this meta-analysis was clear: to overcome the limitation of small sample sizes that limits most studies into the effects of infections on the outcomes of preterm infants, we summarized studies on the effect of different types of infections. Furthermore, some heterogeneity in definitions of sepsis and chorioamnionitis was present across studies. All studies defined sepsis by a positive blood culture result. Some studies used additional clinical signs in their definition. For chorioamnionitis, the majority of studies relied on histological evidence, although some exceptions should be noted. Berger et al[22] used positive amnion cultures as a definition of chorioamnionitis, and Fung et al[23] also included infants with exclusively clinical signs of chorioamnionitis. Excluding this study did not change the meta-analytic effect size and its significance. Besides the effect of infection on brain development and neurodevelopmental outcome, prematurity itself is a risk factor for cerebral pathology. For example, intraventricular hemorrhage, periventricular leukomalacia, and diffuse white matter injury are frequently observed in preterm infants. Some studies included in this meta-analysis describe significant associations between infections and brain injuries like intraventricular hemorrhage and white matter disease.[24,27,30] For example, Berger et al[22] found more cystic periventricular leukomalacia in infants with intrauterine *Ureaplasma* infection compared with infants with negative amnion culture findings. Because of small sample size, periventricular leukomalacia was not included in the analysis on the effect of neurodevelopmental outcome in that study. McKee et al[26] found both infection and intraventricular hemorrhage as a predictor for poor neurodevelopmental outcome. However, some other studies did not find significant associations between infection and brain damage[23] or brain damage and mental development.[34]

Recently, Brochu et al[53] found distinctive patterns of neuroinflammatory response on hypoxic-ischemic injury and bacterial endotoxins depending on the stage of brain maturation in rodents. Future studies are warranted to further disentangle the associations between type of infection, brain damage, and neurodevelopmental outcome in very preterm/VLBW infants.

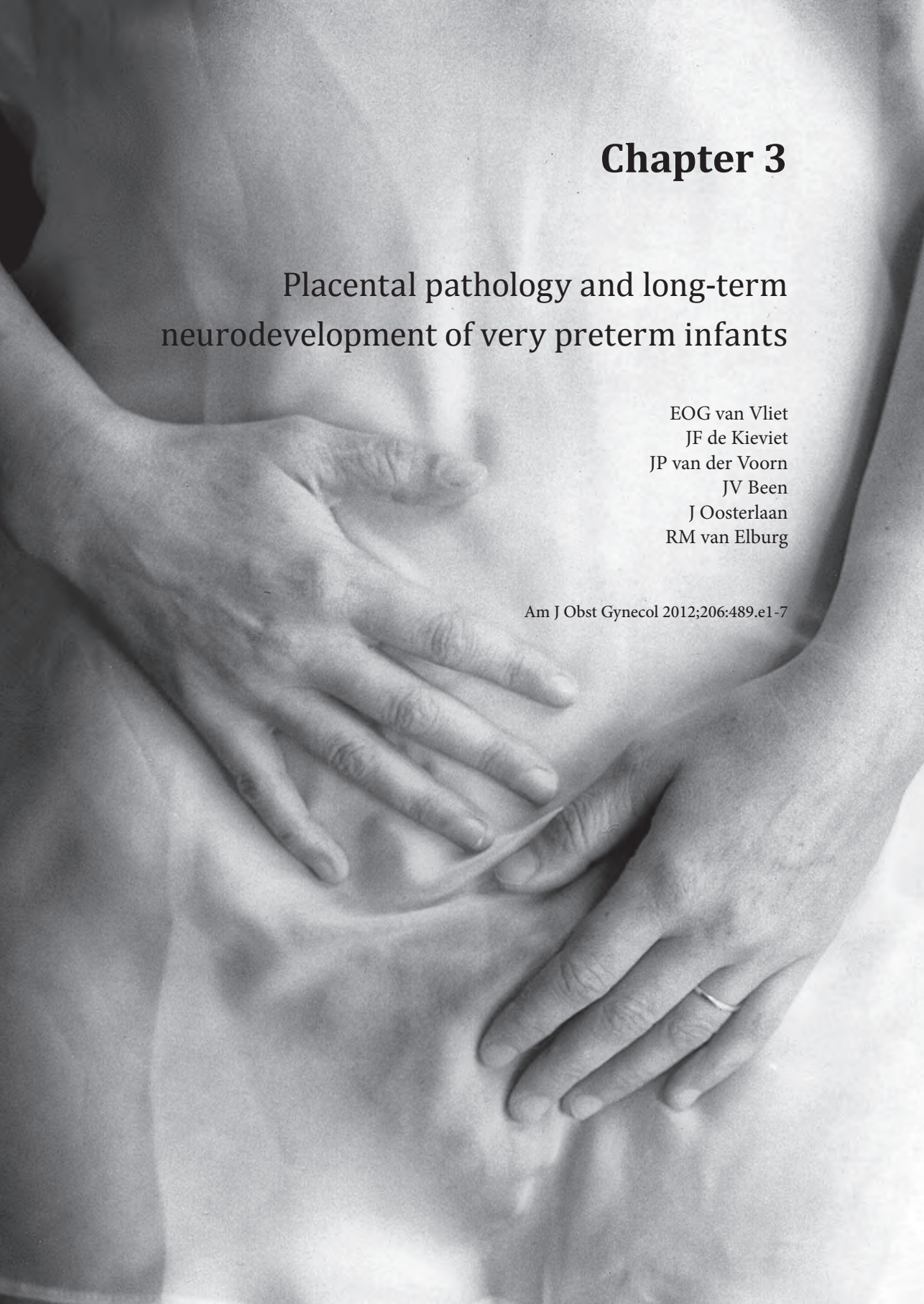
The results of this meta-analysis highlight the additional deteriorating effect of infections on neurodevelopment impairments in very preterm/VLBW infants and stress the clinical importance of the prevention of perinatal infections.

References

1. Viscardi RM, Muhumuza CK, Rodriguez A, et al. Inflammatory markers in intrauterine and fetal blood and cerebrospinal fluid compartments are associated with adverse pulmonary and neurologic outcomes in preterm infants. Pediatr Res. 2004;55(6):1009-1017.
2. Suppiej A, Franzoi M, Vedovato S, Marucco A, Chiarelli S, Zanardo V. Neurodevelopmental outcome in preterm histological chorioamnionitis. Early Hum Dev. 2009;85(3):187-189.
3. Redline RW. Placental pathology: a systematic approach with clinical correlations. Placenta. 2008;29(suppl A):S86-S91.
4. Stoll BJ, Hansen NI, Adams-Chapman I, et al; National Institute of Child Health and Human Development Neonatal Research Network. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. JAMA. 2004;292(19):2357-2365.
5. Edwards AD, Tan S. Perinatal infections, prematurity and brain injury. Curr Opin Pediatr. 2006;18(2):119-124.
6. Ticconi C, Lye SJ. Placenta and fetal membranes in human parturition and preterm delivery: a workshop report. Placenta. 2002;23(suppl A):S149-S152.
7. Rezaie P, Dean A. Periventricular leukomalacia, inflammation and white matter lesions within the developing nervous system. Neuropathology. 2002;22(3):106-132.
8. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. Pediatr Res. 1997;42(1):1-8.
9. Leviton A, Allred EN, Kuban KC, et al. Microbiologic and histologic characteristics of the extremely preterm infant's placenta predict white matter damage and later cerebral palsy: the ELGAN study. Pediatr Res. 2010;67(1):95-101.
10. Silveira RC, Procianoy RS, Dill JC, da Costa CS. Periventricular leukomalacia in very low birth weight preterm neonates with high risk for neonatal sepsis. J Pediatr (Rio J). 2008;84(3):211-216.
11. Adams-Chapman I, Stoll BJ. Neonatal infection and long-term neurodevelopmental outcome in the preterm infant. Curr Opin Infect Dis. 2006;19(3):290-297.
12. Bayley N. Bayley Scales of Infant Development. 2nd ed. San Antonio, TX: Psychological Corporation; 1993.
13. Sattler JM. Assessment of Children: Cognitive Foundations. San Diego, CA: Jerome M. Sattler; 2001.
14. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford_web.ppt. Accessed November 14, 2010.
15. Borenstein M, Rothstein H. Comprehensive Meta-Analysis, a Computer Program for Research Synthesis.2nd ed. Englewood, NJ: Biostat Inc; 1999.
16. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. BMC Med Res Methodol. 2005;5(1):13.
17. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557-560.
18. Rosenthal R. Writing metaanalytic reviews. Psychol Bull. 1995;118(2):183-192.
19. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629-634.
20. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-analyses: the PRISMA Statement. J Clin Epidemiol. 2009;62(10):1006-1012.
21. Benjamin DK Jr, Stoll BJ, Fanaroff AA, et al; National Institute of Child Health and Human Development Neonatal Research Network. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics. 2006;117(1):84-92.
22. Berger A, Witt A, Haiden N, et al. Intrauterine infection with Ureaplasma species is associated with adverse neuromotor outcome at 1 and 2 years adjusted age in preterm infants. J Perinat Med. 2009;37(1):72-78.
23. Fung G, Bawden K, Chow P, Yu V. Chorioamnionitis and outcome in extremely preterm infants. Ann Acad Med Singapore. 2003;32(3):305-310.
24. Kaukola T, Herva R, Perhomaa M, et al. Population cohort associating chorioamnionitis, cord inflammatory cytokines and neurologic outcome in very preterm, extremely low birth weight infants. Pediatr Res. 2006;59(3):478-483.
25. Laughon M, O'Shea MT, Allred EN, et al; ELGAN Study Investigators. Chronic lung disease and developmental delay at 2 years of age in children born before 28 weeks' gestation. Pediatrics. 2009;124(2):637-648.

26 McKee LA, Fabres J, Howard G, Peralta-Carcelen M, Carlo WA, Ambalavanan N. PaCO2 and neurodevelopment in extremely low birth weight infants. J Pediatr. 2009;155(2):217-221, e1.
27. Polam S, Koons A, Anwar M, Shen-Schwarz S, Hegyi T. Effect of chorioamnionitis on neurodevelopmental outcome in preterm infants. Arch Pediatr Adolesc Med. 2005;159(11):1032-1035.
28. Saldir M, Sarici SU, Bakar EE, Ozcan O. Neurodevelopmental status of preterm newborns at infancy, born at a tertiary care center in Turkey. Am J Perinatol. 2010;27(2):121-128.
29. Salhab WA, Perlman JM, Silver L, Sue Broyles R. Necrotizing enterocolitis and neurodevelopmental outcome in extremely low birth weight infants <1000 g. J Perinatol. 2004;24(9):534-540.
30. Shah DK, Doyle LW, Anderson PJ, et al. Adverse neurodevelopment in preterm infants with postnatal sepsis or necrotizing enterocolitis is mediated by white matter abnormalities on magnetic resonance imaging at term. J Pediatr. 2008;153(2):170-175, e1.
31. Soraisham AS, Amin HJ, Al-Hindi MY, Singhal N, Sauve RS. Does necrotising enterocolitis impact the neurodevelopmental and growth outcomes in preterm infants with birthweight < or =1250 g? J Paediatr Child Health. 2006;42(9):499-504.
32. Watterberg KL, Shaffer ML, Mishefske MJ, et al. Growth and neurodevelopmental outcomes after early low-dose hydrocortisone treatment in extremely low birth weight infants. Pediatrics. 2007;120(1):40-48.
33. Yeh TC, Chang JH, Kao HA, Hsu CH, Hung HY, Peng CC. Necrotizing enterocolitis in infants: clinical outcome and influence on growth and neurodevelopment. J Formos Med Assoc. 2004;103(10):761-766.
34. Hack M, Wilson-Costello D, Friedman H, Taylor GH, Schluchter M, Fanaroff AA. Neurodevelopment and predictors of outcomes of children with birth weights of less than 1000 g: 1992-1995. Arch Pediatr Adolesc Med. 2000;154(7):725-731.
35. Bassler D, Stoll BJ, Schmidt B, et al; Trial of Indomethacin Prophylaxis in Preterms Investigators. Using a count of neonatal morbidities to predict poor outcome in extremely low birth weight infants: added role of neonatal infection. Pediatrics. 2009;123(1):313-318.
36. Hendson L, Russell L, Robertson CMT, et al. Neonatal and neurodevelopmental outcomes of very low birth weight infants with histologic chorioamnionitis. J Pediatr. 2011;158(3):397-402.
37. Schlapbach LJ, Aebischer M, Adams M, et al; Swiss Neonatal Network and Follow-Up Group. Impact of sepsis on neurodevelopmental outcome in a Swiss National Cohort of extremely premature infants. Pediatrics. 2011;128(2):e348-e357.
38. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.
39. Andrews WW, Cliver SP, Biasini F, et al. Early preterm birth: association between in utero exposure to acute inflammation and severe neurodevelopmental disability at 6 years of age. Am J Obstet Gynecol. 2008;198(4):e1, e11.
40. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000;342(20):1500-1507.
41. Rees CM, Pierro A, Eaton S. Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. Arch Dis Child Fetal Neonatal Ed. 2007;92(3):F193-F198.
42. Filan PM, Hunt RW, Anderson PJ, Doyle LW, Inder TE. Neurologic outcomes in very preterm infants undergoing surgery. J Pediatr. 2012;160(3):409-414.
43. Gerber J, Nau R. Mechanisms of injury in bacterial meningitis. Curr Opin Neurol. 2010;23(3):312-318.
44. Dexter SC, Pinar H, Malee MP, Hogan J, Carpenter MW, Vohr BR. Outcome of very low birth weight infants with histopathologic chorioamnionitis. Obstet Gynecol. 2000;96(2):172-177.
45. Mehta R, Nanjundaswamy S, Shen-Schwarz S, Petrova A. Neonatal morbidity and placental pathology. Indian J Pediatr. 2006;73(1):25-28.
46. Hagberg H, Wennerholm UB, Sävman K. Sequelae of chorioamnionitis. Curr Opin Infect Dis. 2002;15(3):301-306.
47. Thomas W, Speer CP. Chorioamnionitis: important risk factor or innocent bystander for neonatal outcome? Neonatology. 2011;99(3):177-187.
48. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol. 2009;8(1):110-124.
49. Harris SR, Megens AM, Backman CL, Hayes VE. Stability of the Bayley II Scales of Infant Development in a sample of low-risk and high-risk infants. Dev Med Child Neurol. 2005;47(12):820-823.
50. Hack M, Taylor HG, Drotar D, et al. Poor predictive validity of the Bayley Scales of Infant Development for cognitive function of extremely low birth weight children at school age. Pediatrics. 2005;116(2):333-341.

51. Potharst ES, Houtzager BA, van Sonderen L, et al. Prediction of cognitive abilities at the age of 5 years using developmental follow-up assessments at the age of 2 and 3 years in very preterm children. Dev Med Child Neurol. 2012;54(3):240-246.
52. Cummings P. Arguments for and against standardized mean differences (effect sizes). Arch Pediatr Adolesc Med. 2011;165(7):592-596.
53. Brochu ME, Girard S, Lavoie K, Sébire G. Developmental regulation of the neuroinflammatory responses to LPS and/or hypoxia-ischemia between preterm and term neonates: an experimental study. J Neuroinflammation. 2011;8(8):55.



Chapter 3

Placental pathology and long-term neurodevelopment of very preterm infants

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Am J Obst Gynecol 2012;206:489.e1-7

Abstract

Objective The objective of the study was to compare neonatal morbidity and long-term neurodevelopmental outcome between very preterm infants with placental underperfusion and very preterm infants with histological chorioamnionitis.
Study design We measured the mental and motor development at age 2 and 7 years in 51 very preterm infants with placental underperfusion and 21 very preterm infants with histological chorioamnionitis.
Results At 2 years, very preterm infants with placental underperfusion had poorer mental development than very preterm infants with histological chorioamnionitis (mean [SD] 90.8 [18.3] vs 104.1 [17.2], adjusted $d = 1.12$, $P < .001$). Motor development was not different between both groups (92.8 [17.2] vs 96.8 [8.7], adjusted $d < 0.52$, $P=.12$). At 7 years, large, although nonsignificant, effects were found for better mental and motor development and fewer behavioral problems in infants with histological chorioamnionitis.
Conclusion Placental pathology contributes to variance in mental development at 2 years and should be taken into account when evaluating neurodevelopmental outcome of very preterm infants.

Introduction

Normal placental functioning is of major importance in fetal development by its critical role in protection of the fetus and exchanging nutrients between mother and child.[1] Very preterm birth (less than 32 weeks of gestation) is strongly associated with pathology of the placenta.[1, 2, 3, 4, 5, 6] Placental dysfunction exposes the fetus to an unfavorable intrauterine environment and may contribute to both preterm delivery and damage to the developing brain.[7]

The 2 most common types of placental pathology in preterm delivery are maternal vascular underperfusion leading to fetal hypoxia (placental underperfusion) and pathology associated with acute bacterial intrauterine infection (histological chorioamnionitis).[8, 9] Other placental lesions associated with preterm birth are chronic villitis, fetal thrombotic vasculopathy, or massive villous fibrin deposition.[10]

The relation between placental underperfusion and preterm birth is possibly mediated by an interplay between fetal nutritional status, activity of the hypothalamic-pituitary-adrenal axis and increased production of corticotrophin-releasing hormone, inducing the onset of labor.[11] In histological chorioamnionitis, the local release of cytokines possibly stimulates prostaglandin release by fetal membranes and uterine deciduas, both contributing to preterm labor.[7,12]

Besides contributing to preterm birth, placental underperfusion and histological chorioamnionitis may also directly affect brain development. Placental underperfusion may lead to fetal hypoxia, which contributes to the breakdown of the blood-brain barrier and triggers glutamate excitotoxicity. Free radicals in combination with a developmental lack of antioxidant enzymes in oligodendrocytes may further explain the impact of hypoxia on the premature brain.[13] Although cerebral blood flow increases in response to fetal hypoxia, this brain-sparing effect is probably insufficient to maintain adequate cerebral oxidative metabolism.[14, 15] In histological chorioamnionitis, inflammatory cytokines may be neurotoxic and may also increase the permeability of the blood-brain barrier. They interfere with normal myelination of the brain directly by damaging myelin and indirectly by causing damage to myelin producing cells.[16, 17] Cytokines may also have a systemic effect causing hypotension and disseminated intravascular coagulation,[3] propelling the development of brain damage.

Neurodevelopmental outcome in very preterm infants varies widely. Better prediction of outcome can improve selection of infants for early therapeutic interventions.[17] Differences in pathological mechanisms in placental underperfusion and histological chorioamnionitis may contribute to variation in neonatal morbidity and outcome. Several studies have addressed the relationship between placental pathology and short-term neurodevelopmental outcome in very preterm infants.[4, 18, 19] However, studies concerning long-term outcome remain scarce.[20] Therefore, the differences in outcome following these 2 etiological causes of preterm delivery remain unclear.

The current study aimed to compare neonatal morbidity and the short-term and long-term neurodevelopmental outcome of very preterm infants with placental underperfusion and very preterm infants with histological chorioamnionitis.

Materials and methods

Sample

The infants of this study are part of a larger cohort of a randomized controlled trial on postnatal glutamine supplementation in very preterm infants (gestational age [GA] <32 weeks or birthweight [BW] <1500 g [range, 24.4–33.3 weeks, n = 102]).[21] To disentangle the effect of placental underperfusion and histological chorioamnionitis, we excluded infants who had both types of pathology (ie, lesions of placental underperfusion and lesions of histological chorioamnionitis [n = 11]). Postnatal glutamine supplementation did not influence placenta pathology and did not influence neurodevelopmental outcome at 2 years.[22] The different types of placental pathology were equally present in both treatment groups (data not shown). The medical ethical review board of our institute approved the study protocol. Written informed consent was obtained from all parents.

Placental histological examination

Placentas were macroscopically examined and preserved in 10% buffered formalin for 24 hours immediately after delivery. A minimum of 2 cassettes containing full-thickness sections from the center of the normal-appearing placental disk were taken. Furthermore, sections were taken from the placental membranes and the placental and fetal ends of the umbilical cord. Placenta dimensions were obtained and the placentas were weighed after the removal of the umbilical cord and membranes. The microscopic examination was undertaken by an experienced perinatal pathologist (J.P.v.d.V.), who was unaware of the clinical outcome of the infants. Histologically, placental underperfusion was scored in the presence of characteristics associated with maternal vascular underperfusion as described by Redline et al[23]: increased syncytial knots, villous agglutination, distal villous hypoplasia, and intervillous fibrin. Grade 1 placental underperfusion was scored when less than 30% of the villous parenchyma showed 2 or more signs of maternal underperfusion, in the presence of no more than 1 nonmarginal villous infarct. Grade 2 placental underperfusion was scored when placental weight was below the 10th percentile for gestation,[24] in the presence of either 2 or more signs of maternal underperfusion in greater than 30% of the villous parenchyma, or multiple nonmarginal villous infarcts.

Histological chorioamnionitis was subdivided into a group with only a maternal response and a group with an additional fetal response. A maternal inflammatory response was defined as the presence of neutrophils in the placental membranes or the

chorionic plate. A fetal inflammatory response was diagnosed when neutrophils were present in the umbilical cord vessel walls or the fetal chorionic plate vessels.

Perinatal characteristics

Perinatal characteristics were recorded in the initial study.[21] In this study, we analyzed perinatal characteristics including maternal age, gravidity, parity, gestational age, BW, BW *z*-scores, head circumference *z*-scores, Apgar score at 5 minutes, and umbilical cord pH. BW and head circumference z-scores were determined according to Usher and McLean. [25]

Mortality and neonatal morbidity

We assessed the incidence of serious neonatal infections as previously described.[21] Furthermore, we assessed the occurrence of intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), Bell stage II or III necrotizing enterocolitis (NEC),[26] retinopathy of prematurity (ROP),[27] bronchopulmonary dysplasia (BPD),[28] age at discharge, and mortality.

Short-term and long-term neurodevelopmental outcome

Short-term neurodevelopmental outcome was assessed at the corrected age of 2 years by an experienced pediatric psychologist using the Bayley Scales of Infant Development II (BSID-II),[29] consisting of Mental Development Index (MDI) and Psychomotor Development Index (PDI).

At 7 years, 4 subtests of the Wechsler Intelligence Scale for Children-III (WISC-III)[30] were used to assess performal IQ (PIQ) and verbal IQ (VIQ). Motor development was measured with the Movement Assessment Battery for Children (MABC),[31] evaluating manual dexterity, ball skills, and balance; higher scores indicate poorer motor performance. Behavioral problems were measured by the parent-rated Children Behavior Checklist[32] and the parent-rated Disruptive Behavior Disorders questionnaire.[33, 34] Higher scores indicate poorer functioning on all subscales.

Statistical analysis

Data were analyzed using analysis of variance for continuous data and logistic regression for dichotomous data. Data that were nonnormally distributed were corrected using a van der Waerden transformation.[35] Effect size Cohen's *d* were calculated. Cohen's *d* is defined by the difference between 2 means divided by the pooled SD for those means, and effect sizes of 0.2, 0.5, and 0.8 are considered a small, medium, and large effect, respectively.

The effect of severity of placental pathology (maternal vs fetal histological chorioamnionitis, grade 1 vs grade 2 placental underperfusion) was determined for outcome measures, which were different between infants with histological chorioamnionitis and infants with placental underperfusion. To account for possible bias in selection because of deceased and very disabled infants in our cohort, we

performed additional logistic regression analysis using a dichotomous outcome measure, with a cutoff point of −1 SD, including deceased and severely disabled infants in the −1 SD group. Stability of neurodevelopmental outcome was evaluated by calculating correlations and the odds ratio between outcome measurements at 2 and 7 years.

To further clarify the relations between placental pathology and long-term neurodevelopment, analyses were performed with and without adjustment for possible confounders. Possible confounders were defined as factors significantly different between both groups and known to be related to outcome. We did not adjust for head circumference *z*-score because of the high correlation with BW *z*-score (r = 0.88). Furthermore, we did not adjust for placental weight because placental weight was part of our definition of placental underperfusion. Therefore, adjusting for placental weight would remove part of the variance associated with placental underperfusion. Adjustments were made using linear or logistic regression analysis. To exclude the possibility that glutamine supplementation influenced the results, we tested the interaction effects between glutamine supplementation, placenta pathology, and outcome. Data were analyzed using SPSS 17.0 (SPSS Inc, Chicago, IL), and a $P < .05$ was considered significant.

Results

Subjects

Twenty-one infants with histological chorioamnionitis and 51 infants with placental underperfusion were included in our analysis. There were no significant interaction effects among glutamine intervention, placental pathology, and outcome (all $P > .15$).

Perinatal characteristics

Infants with histological chorioamnionitis had a lower gestational age than infants with placental underperfusion ($P < .001$, $d = -1.11$) but had higher BW *z*-scores ($d = .79$, $P = .005$) (Table 1). Head circumference *z*-score was higher in infants with histological chorioamnionitis than in infants with placental underperfusion ($d = .85$, $P = .003$). Infants with histological chorioamnionitis were less often born with cesarean delivery (odds ratio [OR], 0.19; 95% confidence interval [CI], 0.06–0.57). Other perinatal characteristics were not different among both groups.

Mortality and neonatal morbidity

Incidence of ROP was higher in infants with histological chorioamnionitis (29%) than in infants with placental underperfusion (7%) (OR, 5.64; $P = .03$; 95% CI, 1.19–26.83) (Table 2). After adjustments for GA, BW *z*-score, and mode of delivery, incidence of ROP was not different between both groups ($P = .75$), indicating that the difference in incidence of ROP is largely explained by differences in these characteristics. The

incidence of mortality, IVH, sepsis, pneumonia, 1 or more infections, and BPD was not different among both groups. Due to the low incidence, meningitis (n = 0), NEC (n = 4), and PVL (n = 4) were not analyzed.

Table 1. Perinatal characteristics

Characteristics	Chorioamnionitis (n = 21)	Placental underperfusion (n = 51)	*P* Value
Birthweight, g[a]	1129.7 (317.8)	111.9 (322.2)	.83
Gestational age, d[a]	193.7 (13.3)	206.7 (9.8)	<.001
Birthweight z-score[a]	0.07 (1.2)	-1.12 (1.78)	.005
Head circumference z-score[a]	0.57 (1.0)	-0.45 (1.35)	.003
Male, n (%)	10 (47.6)	27 (52.9)	.68
Maternal age, y[a]	29.1 (6.3)	30.6 (4.7)	.29
Multiple birth, n (%)	3 (14.3)	10 (19.6)	.64
CRIB score[a]	3.71 (2.9)	3.71 (3.0)	.99
Apgar score at 5 min <7, n (%)	5 (23.8)	15 (29.4)	.63
Umbilical cord pH <7.10, arterial, n (%)	0 (0)	4 (7.8)	.19
Cesarean delivery, n (%)	7 (33.0)	37 (72.6)	.01
Placental weight, [a,b]	285.06 (122.33)	214.98 (86.71)	.02
Pre-eclampsia, eclampsia, HELLP, n (%)	0 (0)	18 (35.29)	.02
Clinical abruption placentae, n (%)	0 (0)	4 (7.8)	.19
Antenatal corticosteroid, n (%)	16 (76.0)	43 (84.3)	.42
Mechanical ventilation, d[a,c]	16.4 (17.8)	9.0 (13.0)	.08
Age at discharge, d[a,c]	84.6 (33.4)	67.9 (35.3)	.10
Social economical status[a]	3.1 (0.8)	3.3 (0.7)	.38

CRIB Clinical Risk Index for Babies; HELLP hemolysis, elevated liver enzymes, and low platelet count.
[a] Data given as mean ± SD; [b]n=16 and n=41; [c]Excluding deceased infants.

Table 2. Mortality and neonatal morbidity

Outcome measure	Chorioamnionitis (n = 21)	Placental underperfusion (n = 51)	OR	*P* value	Adjusted OR[a]	Adjusted *P* value [a]
Mortality, n (%)	4 (19)	5 (10)	2.20	.29	2.35	.41
IVH, n (%)	9 (43)	12 (24)	2.44	.11	1.31	.69
Sepsis, n (%)	10 (48)	32 (63)	0.54	.24	0.36	.13
Pneumonia, n (%)	3 (14)	7 (14)	1.05	.95	1.09	.94
≥1 positive culture, n (%)[b]	12 (57)	35 (69)	0.61	.35	0.25	.05
ROP, stage 1 or greater, n (%)[c]	5 (29)	3 (7)	5.64	.03	1.59	.75
BPD, stage 1 or greater, n (%)	8 (47)	17 (37)	1.57	.43	0.65	.66

BPD bronchopulmonary dysplasia; IVH intraventricular hemorrhage; OR odds ratio; ROP retinopathy of prematurity.
[a]Adjusted for gestational age, birthweight z-score, cesarean delivery; [b]Blood culture or tracheal aspirate; [c] n=18 for chorioamnionitis and n = 47 for placental underperfusion.

Neurodevelopmental outcome at age 2 years

At 2 years, 57 infants (79.2%) were available for follow up (n = 43 for placental underperfusion and n = 14 for histological chorioamnionitis). Nine infants (12.5%) were deceased and 6 (8.3%) were lost to follow-up. Infants with placental underperfusion had significantly lower MDI scores compared with infants with histological chorioamnionitis (d = .75, P = .02) (Table 3). The difference in PDI scores was small (d = .18) and nonsignificant (P = .59). Adjustment for gestational age, BW z-score, and mode of delivery did not change the detrimental effect of placental underperfusion on MDI scores (d = 1.12, P < .01). In the placental underperfusion group (n = 43), infants with grade 2 underperfusion had significantly lower MDI scores than infants with grade 1 underperfusion (84.8 vs 96.6, d = .67, P = .03). In the group of infants with histological chorioamnionitis (n = 14), MDI scores did not differ between infants with only maternal response vs infants with an additional fetal response (108.2 vs 101.9, d = 0.91, P = .53). The proportion of infants that perform less than –1 SD was not different between infants with placental underperfusion and histological chorioamnionitis.

Table 3. Neurodevelopmental outcome at age 2 years

Variable	Chorioamnionitis (n = 14)	Placental underperfusion (n = 43)	*P* value	Cohen's *d*	Adjusted *P* value [a]	Adjusted *d* [a]
MDI score[b]	104.1 (17.2)	90.8 (18.3)	.02	.75	.001	1.12
PDI score[b]	96.8 (8.7)	92.8 (17.2)	.41	.23	.12	.52
	Chorioamnionitis (n = 18)	**Placental underperfusion (n = 48)**	**P value**	**OR**	**Adjusted P value [a]**	**Adjusted OR [a]**
MDI <85 or death[c]	5 (27.7)	20 (41.6)	.30	.54	.19	.32
PDI <85 or death[c]	6 (33.3)	16 (33.3)	.99	1.00	.70	.73

MDI mental developmental index; PDI psychomotor developmental index; OR odds ratio.
[a]Adjusted for gestational age, birthweight z-score, cesarean delivery; [b] Data are given as mean (SD); [c] Including deceased and severely disabled infants, data given as n (%).

Neurodevelopmental outcome at age 7 years

At 7 years, 49 infants (68.1%) participated in the follow-up study (n = 37 for placental underperfusion and n = 12 for histological chorioamnionitis). Two infants (2.8%) were not able to participate because of severe disabilities and 6 (8.3%) were lost to follow-up. At 7 years, mental and motor development or behavioral problems were not different among infants with placental underperfusion and infants with histological chorioamnionitis (Table 4). After adjustment for GA, BW z-score, and mode of delivery, large although nonsignificant effects were found for intelligence: PIQ (d = 0.84, P = .18) and VIQ (d = 0.88, P = .16) as well as for motor performance: manual dexterity (d = –1.37, P = .39), ball skills (d = –2.69, P = .08), and balance (d = –1.24, P = .41). Although neurodevelopmental outcome was not significantly different, infants with placental underperfusion consistently had lower scores throughout all

neurodevelopmental domains. In further analysis comparing infants with grade 1 and grade 2 placental underperfusion, only small effect sizes were found (range, 0.10–0.45, data not shown).

MDI scores at 2 years and WISC scores at 7 years were significantly correlated (r = 0.61, $P < .01$). The proportion of infants performing < -1SD for mental development was not different at age 2 (MDI) compared to age 7 (WISC, OR, 0.70; 95% CI, 0.33–1.48).

Tabel 4. Neurodevelopmental outcome at age 7 years

Outcome measure	Chorioamnionitis (n = 12)	Placental underperfusion (n = 37)	*P* value	Effect size *d*/OR	Adjusted *P* value [a]	Adjusted *d* /OR[a]
Intelligence						
WISC-PIQ (SD)[b]	94.2 (15.5)	89.2 (16.6)	.65	0.31	.18	0.84
WISC-VIQ (SD) [b]	106.7 (18.3)	100.3 (17.6)	.59	0.36	.16	0.88
Motor development						
MABC menual dexterity (SD) [b]	3.5 (3.2)	3.8 (4.0)	.79	-0.08	.39	-1.37
MABC ball skills (SD)[b]	2.8 (2.2)	3.9 (2.7)	.21	-0.45	.08	-2.69
MABC balance (SD) [b]	1.7 (2.4)	2.5 (3.9)	.49	-0.25	.41	-1.24
Behavioral problems						
CBCL internalizing (SD)[b]	4.7 (3.5)	4.7 (4.4)	.96	0.00	.65	0.63
CBCL externalizing (SD)[b]	5.4 (3.8)	6.4 (6.1)	.58	-0.20	.78	-0.09
CBCL attention (SD)[b]	3.7 (2.5)	4.2 (3.1)	.64	-0.18	.32	-1.39
DBD attention (SD)[b]	5.8 (4.9)	4.8 (3.8)	.49	0.23	.76	-0.48
DBD hyperactivity (SD)[b]	4.7 (5.1)	4.6 (3.5)	.95	0.03	.72	-0.58
WISC-PIQ <85 or death, n (%)[c]	8 (50.0)	12 (27.3)	.10	2.67	.29	2.48
WISC-VIQ <85 or death, n (%)[c]	7 (43.8)	12 (31.8)	.39	1.67	.84	1.18
MABC <15th % or death, n (%)[c]	9 (56.3)	25 (56.8)	.97	0.98	.66	0.69

CBCL children behavior checklist; DBD disruptive behavior disorders questionnaire; MABC movement assessment battery for children; OR odds ratio; WISC-PIQ, wechsler intelligence scale for children-performal IQ; WISC-VIQ wechsler intelligence scale for children-verbal IQ.

[a]Adjusted for gestational age, birthweight z-score, cesarean delivery; [b] Data are given as mean (SD); [c] Including deceased and severely disabled infants (n = 16 for chorioamnionitis; n = 44 for placental underperfusion).

Comment

In our study, very preterm infants with placental underperfusion had poorer mental development at 2 years than very preterm infants with histological chorioamnionitis, with a difference in mean MDI score of 1.12 SD. The significant difference in MDI scores between infants with placental underperfusion grade 1 and with grade 2 further

suggest that placental underperfusion is related to mental developmental delay at 2 years of age. At 7 years, large although nonsignificant adjusted effects were found for better mental and motor development and fewer behavioral problems in infants with histological chorioamnionitis.

Infants with placental underperfusion had lower BW, BW *z*-scores, and head circumference *z*-scores. Impaired growth is related to reduction of cerebral cortical gray matter and hippocampal volume, which is associated with impaired cognitive functioning. [36] This may explain the poorer mental development of very preterm infants with placental underperfusion in our study. Differences in etiological pathways between the 2 types of placental pathology may further explain the difference in neurodevelopment. Impaired growth in infants with placental underperfusion may also reflect that infants with placental underperfusion have been chronically exposed to an unfavorable intrauterine environment. Indeed, placental underperfusion is described as a chronic event, with an onset more than 1 week before delivery, whereas histological chorioamnionitis is often a (sub)acute event. [37] Besides prenatal growth, postnatal growth is also an important factor for neurodevelopmental outcome that should be taken into account. [38, 39]

Although the proportion of infants with a mental development less than –1 SD did not change over time (ie, mental developmental disorders do not disappear), there were no significant differences in mental development between both groups at 7 years. However, the large adjusted effect sizes found at 7 years ($d = 0.84$ for PIQ and $d = 0.88$ for VIQ) suggest that our sample may be too small to detect the effects of placental pathology at this age.

No differences in motor development were found at the age of 2 years. This seems to indicate that placental pathology does not interfere with attaining motor milestones as measured with BSID-II. At 7 years, we found no significant differences in motor performance or behavioral problems. However, large effect sizes (d ranging from 1.24 to 2.69) for motor performance, after correction for possible confounders, also suggest that our sample size might have been too small to detect these differences.

The lower GA in infants with histological chorioamnionitis compared with infants with placental underperfusion is in accordance with previous studies.[5, 18] In our study, infants with histological chorioamnionitis had a 5 times higher risk of developing ROP, but after adjustment for baseline differences in GA, BW *z*-score, and mode of delivery, the incidence of ROP was not different. A recent study of Dammann et al [40] provided preliminary evidence for a multihit scenario, in which the effect of chorioamnionitis appears to be overwhelmed by the effect of GA. The results of our study support this hypothesis.

Our study has some limitations that need to be considered. First, the number of infants with placental underperfusion may be an overrepresentation of the incidence in the general very preterm population because this study was conducted in a university medical center specializing in pre-eclampsia, which is associated with placental underperfusion. Multicenter cohort studies would provide more evidence for the relation between placental pathology and neonatal outcome.

Second, the sample size of this study was rather small. Large but nonsignificant effect sizes in our analysis indicate that our statistical tests lacked power to detect these effects. Larger samples would also provide the opportunity to further study the impact of severity of the various placental lesions, helping to further disentangle the etiology of neurodevelopmental impairments in very preterm infants. Studies on outcome in very preterm infants have a relatively high proportion of nonsurviving infants or infants who were too disabled to be tested, thereby interfering with outcome.

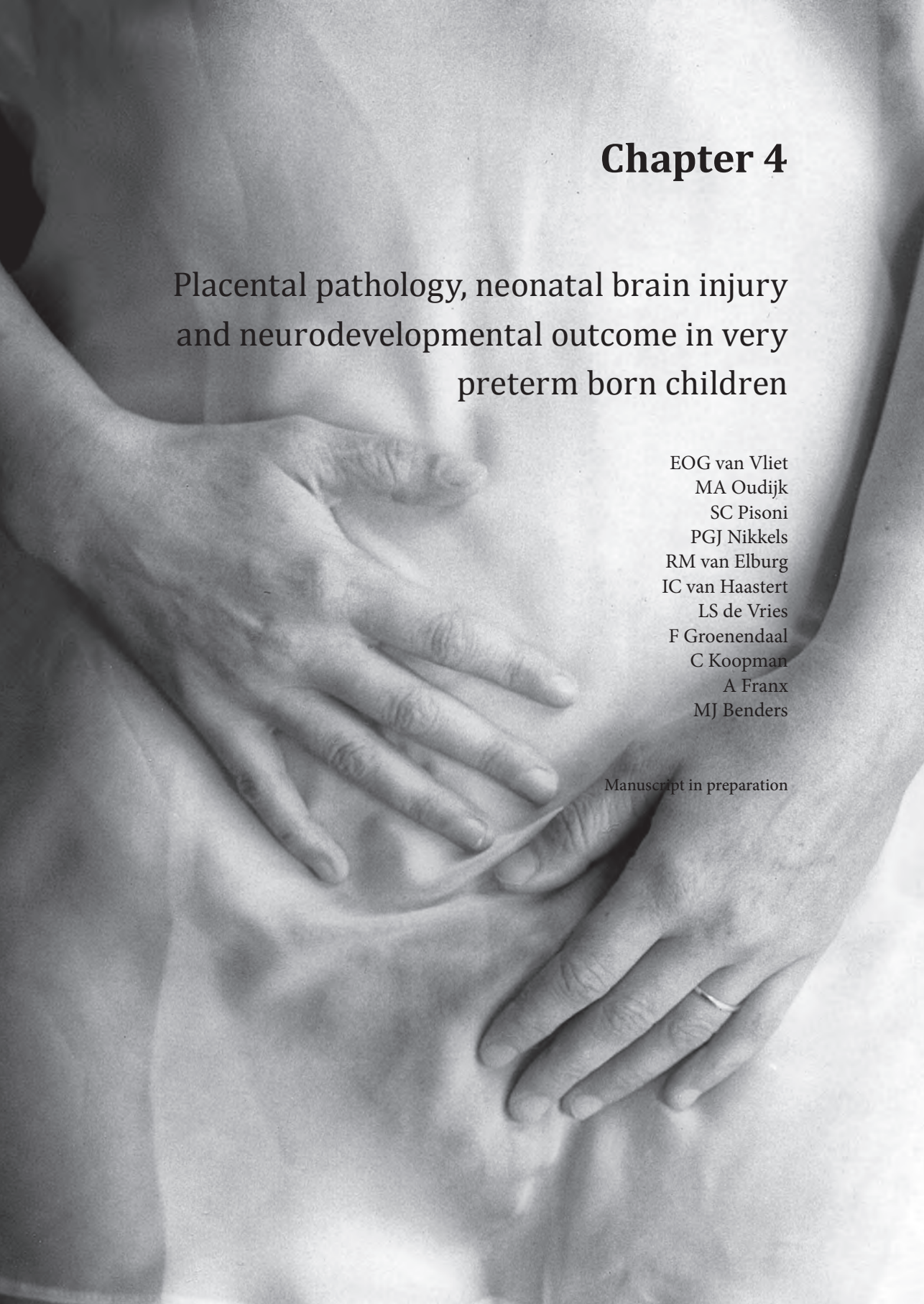
In this study, we also performed analyses on dichotomous outcome measures including nonsurviving infants and very disabled infants. In general, these analyses showed similar results. Furthermore, we focused only on microscopic characteristics of placental dysfunction. Macroscopic abnormalities of the placenta and umbilical cord may also be associated with outcome. For example, hypercoiling of the umbilical cord is associated with histological signs of fetal hypoxia and intrauterine mortality in term infants,[41] but the role of umbilical cord coiling in preterm infants remains unclear. Comprehensive studies assessing macroscopic, histological, and imaging data on placental functioning may elucidate the role of placental functioning in these vulnerable infants.

In conclusion, our study provides evidence that placental pathology contributes to the variance in neonatal characteristics and short-term neurodevelopmental outcome in very preterm infants. Placental pathology should be taken into account when evaluating neurodevelopmental outcome of very preterm infants.

References

1. R.W. Redline Placental pathology: a systematic approach with clinical correlations Placenta, 29 (Suppl A) (2008), pp. S86–S91
2. G. Altshuler Some placental considerations related to neurodevelopmental and other disorders J Child Neurol, 8 (1993), pp. 78–94
3. C. Amiel-Tison, D. Cabrol, R. Denver, P.H. Jarreau, E. Papiernik, P.V. Piazza Fetal adaptation to stress: Part I: acceleration of fetal maturation and earlier birth triggered by placental insufficiency in humans Early Hum Dev, 78 (2004), pp. 15–27
4. O. Dammann, A. Leviton Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. Pediatr Res, 42 (1997), pp. 1–8
5. T. Kaukola, R. Herva, M. Perhomaa, et al. Population cohort associating chorioamnionitis, cord inflammatory cytokines and neurologic outcome in very preterm, extremely low birth weight infants Pediatr Res, 59 (2006), pp. 478–483
6. R. Mehta, S. Nanjundaswamy, S. Shen-Schwarz, A. Petrova Neonatal morbidity and placental pathology Indian J Pediatr, 73 (2006), pp. 25–28
7. C. Ticconi, S.J. Lye Placenta and fetal membranes in human parturition and preterm delivery—a workshop report. Placenta, 23 (Suppl A) (2002), pp. S149–S152
8. L. Beaudet, S. Karuri, J. Lau, F. Magee, S.K. Lee, P. von Dadelszen Placental pathology and clinical outcomes in a cohort of infants admitted to a neonatal intensive care unit J Obstet Gynaecol Can, 29 (2007), pp. 315–323
9. T.M. O'Shea, E.N. Allred, O. Dammann, et al. The ELGAN study of the brain and related disorders in extremely low gestational age newborns. Early Hum Dev, 85 (2009), pp. 719–725
10. O.M. Faye-Petersen. The placenta in preterm birth. J Clin Pathol, 61 (2008), pp. 1261–1275
11. S.N. Kalantaridou, E. Zoumakis, A. Makrigiannakis, L.G. Lavasidis, T. Vrekoussis, G.P. Chrousos. Corticotropin-releasing hormone, stress and human reproduction: an update. J Reprod Immunol, 85 (2010), pp. 33–39.View Record in Scopus
12. R.L. Goldenberg, J.C. Hauth, W.W. Andrews. Intrauterine infection and preterm delivery. N Engl J Med, 342 (2000), pp. 1500–1507
13. P. Rezaie, A. Dean. Periventricular leukomalacia, inflammation and white matter lesions within the developing nervous system. Neuropathology, 22 (2002), pp. 106–132
14. R. Gagnon. Placental insufficiency and its consequences. Eur J Obstet Gynecol Reprod Biol, 110 (Suppl 1) (2003), pp. S99–S107
15. S. Scherjon, J. Briet, H. Oosting, J. Kok. The discrepancy between maturation of visual-evoked potentials and cognitive outcome at five years in very preterm infants with and without hemodynamic signs of fetal brain-sparing. Pediatrics, 105 (2000), pp. 385–391
16. M. Gantert, J.V. Been, A.W. Gavilanes, Y. Garnier, L.J. Zimmermann, B.W. Kramer. Chorioamnionitis: a multiorgan disease of the fetus? J Perinatol, 30 (Suppl) (2010), pp. S21–S30
17. B. Latal Prediction of neurodevelopmental outcome after preterm birth. Pediatr Neurol, 40 (2009), pp. 413–419
18. S. Polam, A. Koons, M. Anwar, S. Shen-Schwarz, T. Hegyi Effect of chorioamnionitis on neurodevelopmental outcome in preterm infants. Arch Pediatr Adolesc Med, 159 (2005), pp. 1032–1035.
19. R.W. Redline, D. Wilson-Costello, M. Hack. Placental and other perinatal risk factors for chronic lung disease in very low birth weight infants. Pediatr Res, 52 (2002), pp. 713–719
20. W.W. Andrews, S.P. Cliver, F. Biasini, et al. Early preterm birth: association between in utero exposure to acute inflammation and severe neurodevelopmental disability at 6 years of age. Am J Obstet Gynecol, 198 (2008), p. 466
21 A. van den Berg, R.M. van Elburg, E.A. Westerbeek, J.W. Twisk, W.P. Fetter. Glutamine-enriched enteral nutrition in very-low-birth-weight infants and effects on feeding tolerance and infectious morbidity: a randomized controlled trial. Am J Clin Nutr, 81 (2005), pp. 1397–1404.
22. A. van Zwol, A. van den Berg, J. Huisman, et al. Neurodevelopmental outcomes of very low-birth-weight infants after enteral glutamine supplementation in the neonatal period. Acta Paediatr, 97 (2008), pp. 562–567.
23. R.W. Redline, T. Boyd, V. Campbell, et al. Maternal vascular underperfusion: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol, 7 (2004), pp. 237–249
24. J.S. Wigglesworth. Textbook of fetal and perinatal pathology. Wiley-Blackwell, London, UK (1998)
25. R. Usher, F. McLean. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr, 74 (1969), pp. 901–910

26. M.J. Bell, J.L. Ternberg, R.D. Feigin, et al. Neonatal necrotizing enterocolitis: Therapeutic decisions based upon clinical staging. Ann Surg, 187 (1978), pp. 1–7
27. An international classification of retinopathy of prematurity: The Committee for the Classification of Retinopathy of Prematurity. Arch Ophthalmol, 102 (1984), pp. 1130–1134
28. A.H. Jobe, E. Bancalari. Bronchopulmonary dysplasia. Am J Respir Crit Care Med, 163 (2001), pp. 1723–1729
29. N. Bayley. Bayley Scales of Infant Development. (2nd ed.)The Psychological Corp, San Antonio, TX (1993)
30. D. Wechsler Manual for the Wechsler Intelligence Scale for Children-Third Edition (WISC-III). The Psychological Corporation, San Antonio, TX (1991)
31. S.E. Henderson, D.A. Sugden. Test of motor proficiency: examiner's manual. The Psychological Corp, Kent (United Kingdom) (1992)
32. F. Verhulst, J. van der Ende, H. Koot. [Manual for CBCL/4-18] (Dutch edition)Erasmus University, Rotterdam (The Netherlands) (1996)
33. J. Oosterlaan, D. Baeyens, A. Scherens, H. Roeyers, J.A. Sergeant. [Manual Children Behavior Checklist, 6-16 years] (Dutch edition)Pearson, Amsterdam (The Netherlands) (2008)
34. W.E. Pelham Jr, E.M. Gnagy, K.E. Greenslade, R. Milich Teacher ratings of DSM-III symptoms for the disruptive behavior disorder. J Am Acad Child Adolesc Psychiatry, 31 (2010), pp. 210–218
35. E.L. Lehmann. Nonparametrics: statistical methods based on ranks. Holden-Day, San Francisco, CA (1975)
36. G.A. Lodygensky, M.L. Seghier, Warfield, et al. Intrauterine growth restriction affects the preterm infant's hippocampus. Pediatr Res, 63 (2008), pp. 438–443
37. R.W. Redline. Disorders of placental circulation and the fetal brain. Clin Perinatol, 36 (2009), pp. 549–559.
38. I. Brandt, E.J. Sticker, M.J. Lentze. Catch-up growth of head circumference of very low birth weight, small for gestational age preterm infants and mental development to adulthood. J Pediatr, 142 (2003), pp. 463–468
39. A.R. Franz, F. Pohlandt, H. Bode, et al. Intrauterine, early neonatal, and postdischarge growth and neurodevelopmental outcome at 5.4 years in extremely preterm infants after intensive neonatal nutritional support. Pediatrics, 123 (2009), pp. e101–e109
40. O. Dammann, M.J. Brinkhaus, D.B. Bartels, et al. Immaturity, perinatal inflammation, and retinopathy of prematurity: a multi-hit hypothesis. Early Hum Dev, 85 (2009), pp. 325–329
41. M.W. de Laat, J.J. van der Meij, G.H. Visser, A. Franx, P.G. Nikkels Hypercoiling of the umbilical cord and placental maturation defect: associated pathology? Pediatr Dev Pathol, 10 (2007), pp. 293–299.



Chapter 4

Placental pathology, neonatal brain injury and neurodevelopmental outcome in very preterm born children

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Abstract

Introduction Brain injury is frequently present in preterm born infants, however the etiology is only partly explained. The placenta might provide valuable insights in the association between pathophysiology and brain injury and adverse outcome following preterm birth. The current study aims to assess placental pathology, patterns of brain injury on neonatal MRI and neurodevelopmental outcome in very preterm born infants using standardized tests.

Methods We examined the placentas of infants born between 24 and 31 weeks of gestation. One hundred and ten infants had a cerebral MRI at term-equivalent age (TEA). We studied the association of placental lesions with white matter injury, intraventricular hemorrhage, cerebellar hemorrhage and abnormal ventricular size. Neurodevelopmental outcome was evaluated at 2, 3.5 and 5.5 years.

Results No associations of placental lesions and brain injury at TEA or neurodevelopmental outcome at age 2 and 3.5 years were found. At age 5.5 years, infants with both vascular and inflammatory lesions have better motor performance as compared to infants with only inflammatory or vascular placental lesions.

Conclusion In this study placental pathology was related to motor performance at age 5.5 years, but not to neonatal brain injury evaluated at TEA using conventional MR imaging.

Introduction

Very preterm born infants are at risk for poor neurodevelopmental outcome.[1-3] Brain injuries, such as periventricular leukomalacia (PVL), intraventricular hemorrhage (IVH) and cerebellar injury are commonly present in very preterm born infants and relate to neurodevelopmental sequelae.[4-6] Perinatal white matter injury (WMI) is found to have a major impact on subsequent microstructural brain development and is associated with a reduction in the volume of total myelinated white matter at term.[7,8] The etiology of brain injury in preterm infants is only partly explained by clinical factors. The placenta might provide additional insight in the cause of brain injury. Disorders in placental functioning, for example placental underperfusion or intrauterine infection/ inflammation, might contribute to preterm birth and thereby place these infants at risk for adverse brain development as ex-utero brain development is found to be different compared to intrauterine brain development, possibly because of the sequelae that follow preterm birth.[9] Besides, placental pathology might directly be related to brain damage by exposing the fetus to an unfavorable intrauterine environment.
Previous studies have found associations between placental pathology and brain injury in preterm born infants.[10-15] Histological inflammatory lesions of the placenta have been related to ventriculomegaly, cerebral palsy,[10] and periventricular leukomalacia (PVL)[11,12] in preterm born infants. Studies on neurodevelopmental outcome in preterm born infants have shown related adverse neurodevelopmental outcome to villous edema,[13] placental villitis[14] and placental underperfusion.[15] Studies on the association between placental pathology, brain injury and long term outcome remain scarce. The aim of this study is to determine the association between placental pathology, patterns of brain injury on neonatal MRI, and neurodevelopmental outcome in very preterm born infants.

Methods

Sample

All infants born in the University Medical Center Utrecht between 24 and 31 weeks of gestation reaching term-equivalent age (TEA) between January 1st 2007 and July 31th 2008 were eligible for inclusion in this study. We excluded infants with major congenital malformations. Cerebral MRI of the brain was acquired at TEA as part of routine clinical care.[16] Cerebral MRI was only performed after obtaining informed parental consent. Histological placental examination is standard care in preterm deliveries in our level III Neonatal Intensive Care Unit.

Neuroimaging

MRI scans were performed on a 3.0 Tesla MR system (Achieva, Philips Medical Systems, Best, The Netherlands) according to a previously described protocol.[16] Vital signs as heart rate, transcutaneous oxygen saturation and respiratory rate were

monitored during scanning. Minimuffs (Natus Medical Incorporated, San Carlos, CA, USA) were used for hearing protection. Scans were performed under supervision of a neonatologist. Two experienced neonatologists independently evaluated the scans (LSdV and MJNLB). A third reader (FG) was consulted in case of disagreement. We evaluated 4 types of MRI abnormalities: (1) moderate to severe WMI; (2) IVH >grade2; (3) cerebellar hemorrhages and (4) abnormal ventricular size.

For WMI, we used a scoring system adapted from Woodward et al..[6] This scoring system assesses white matter signal intensity, size of the subarachnoid space, presence of white matter cysts, size of the ventricles, and thickness of the corpus callosum. All aspects were scored as (1) normal, (2) mildly abnormal, or (3) moderately/severely abnormal. A composite white matter score was calculated as the sum of these subscores (range 5-15) and was applied as an indicator of WMI. A composite score of >9 was considered moderate to severe WMI. IVH >grade 2 was scored if <50% of the ventricular area was involved.[17] Cerebellar hemorrhage was scored according to Kidokoro as 1) unilateral punctate lesion, 2) bilateral punctate lesions, 3) extensive unilateral, 4) extensve bilateral lesions.[18] Abnormal ventricular size was scored according to Woodward as (1) normal without evidence of ventricular dilatation, (2) moderate enlargement resulting in mild rounding of the frontal horns, minimal enlargement of the temporal horns and moderate enlargement of the occipital horns, (3) more global enlargement of a moderate to severe nature including significant enlargement of the frontal, temporal and occipital horn.[6]

Placental histology

All available placentas were macroscopically examined immediately after delivery and preserved in 10% buffered formalin for 24 hours. We weighted placentas without membranes and umbilical cord to determine placental weight percentiles according to Pinar et al..[19] We calculated the umbilical coiling index as the number of complete coils (360˚) divided by the cord length in centimeters. Hypocoiling was defined as an index <0.1 coils/cm, hypercoiling was defined as an index of >0.3 coils/cm. A minimum of 2 Hematoxylin and Eosin stained slides were taken from the umbilical cord at the fetal and placental side. Furthermore, sections were taken from the placental membranes, umbilical cord insertion, decidua and chorionic plate, and additional slides from macroscopical abnormalities. The microscopic examination was undertaken by an experienced perinatal pathologist (PGJN), who was blinded to the clinical diagnosis except for the gestational age (GA) at delivery.

Inflammatory placental lesions consisted of histological chorioamnionitis according to the definition of Redline et al.[20], and included both maternal inflammatory response only as well as placentas displaying an additional fetal response. In short, a maternal inflammatory response was defined as the presence of neutrophils in the placental membranes or chorionic plate, and a fetal inflammatory response (i.e. funisitis) was defined as neutrophils present in the umbilical cord vessel walls with or without involvement of the Wharton's jelly.

Vascular placental lesions included signs of placental underperfusion, ischemic lesions and distal villous hypoplasia. Placental underperfusion was scored in the presence of signs of maternal underperfusion: increased syncytial knots, distal villous hypoplasia, intervillous fibrin deposition and villous agglutination.[21] Placental ischemic lesions were defined as increased hyperchromasia of the trophoblast and increased syncytial knot formation. Distal villous hypoplasia is defined as a decrease in the number and diameter of distal villi, considering GA.[21] Placental histology was divided in four subgroups: 1) placentas without pattern of inflammation/ vascular problems, 2) pattern of inflammation in case of maternal or fetal chorioamnionitis or deciduitis, 3) placental vascular problems: if placenta showed ischemia, insufficiency, distal villous hypoplasia or infarction area and 4) placenta with patterns of both inflammation and vascular problems.

Perinatal characteristics

Perinatal characteristics were derived from chart review. Pregnancy characteristics included: preterm premature rupture of the membranes (PPROM, >24 hours), complete course of antenatal steroids, hypertensive disorders (pre-eclampsia, eclampsia, HELLP syndrome) and mode of delivery. The following neonatal characteristics were recorded: GA, birth weight, birth weight z-scores, gender, Apgar scores <7 at 5 minutes. We assessed the incidence of postnatal mortality (<28 days after birth) and morbidity such as need for mechanical ventilation, patent ductus arteriosus (PDA) with treatment (indomethacin or surgical intervention), chronic lung disease (CLD), culture proven sepsis and necrotizing enterocolitis requiring surgery (NEC).

Neonatal Follow-up

Infants were examined by experienced neonatologists (LdV and, CK), child psychologists, a special educator (ICvH), and a pediatric physiotherapist. At 2 years corrected age, infants were tested with the Bayley Scales of Infant and Toddler Development, third edition (Bayley-III).[22] At 3.5 years of age, neurodevelopmental outcome was tested using the Griffiths Mental Development Scales.[23] The Movement Assessment Battery for Children, second edition (MABC-2)[24] was used to assess motor skills at 5.5 years of age. The Wechsler Preschool and Primary Scale of Intelligence, third edition, Dutch version (WPPSI-III-NL)[25] was used to measure general intellectual functioning, identification of cognitive delay and learning difficulties at age 5.5 years. The WPPSI-III-NL consists of a verbal IQ scale (VIQ), performal IQ scale (PIQ), total IQ scale (TIQ) and a processing speed score.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (Armonk, NY: IBM Corp). Differences in dichotomous outcome measures were analyzed using Chi-square tests. We used Analysis of Variance (ANOVA) to test for differences in continuous outcome measures. We compared the incidence of cerebral lesions between 4 groups: infants

with inflammatory placental lesions, infants with vascular placental lesions, infants with both types of placental lesions, and infants without these placental lesions. Furthermore, we compared neurodevelopmental outcome at age 2, 3.5 and 5.5 years between these four groups. A p-value <0.05 was considered significant.

Results

Of 264 preterm born infants (GA 24-31 weeks), 234 met the inclusion criteria (**Figure 1**). Placentas were available for reexamination in 175 infants (74.8%). Infants with and without placental reexamination did not differ in terms of GA (p=0.58), birth weight (0.93), birth weight <10th percentile (p=0.20), gender (p=0.18), rate of caesarean section (p=0.21), and 5-minute Apgar scores <7 (p=0.55). Placental examination was more often performed in pregnancies complicated by hypertensive disorders (p<0.01). Infants with inflammatory placental lesions had a lower GA compared to infants with placental vascular lesions (27.5 weeks versus 29.1 weeks, p<0.01). Birth weight z-score was significantly lower in infants with placental vascular lesions compared to all other groups (p<0.01). Infants with placental vascular lesions were more often born after cesarean delivery (p=0.03) and after a pregnancy with hypertensive complications (p<0.001) compared to all other groups. The incidence of umbilical cord hypocoiling was lower in the group of infants with placental vascular lesions compared to infants with inflammatory lesions (p<0.001) and both types of lesions (p=0.02). Perinatal mortality was lower in infants with placental vascular lesions compared to infants with inflammatory placental lesions (p<0.001) and no inflammatory/vascular placental lesions (p=0.01), but not after correction for GA at birth (p=0.23). There was no difference in the incidence of need for mechanical ventilation support, persistent ductus arteriosus, chronic lung disease, culture proven sepsis and necrotizing enterocolitis between the four groups (p-values all >0.10).

In 110 of infants with placental examination, MRI of the cerebrum was performed at TEA (mean GA 41.71 weeks SD 1.09 range 39.57 to 45.71 weeks). The association between placental pathology and MRI abnormalities is shown in **Table 1**. No difference between the four placental groups were found for the incidence of moderate/severe WMI (p=0.64), IVH > grade 2 (p=0.45), cerebellar hemorrhage (p=0.24) and abnormal ventricular size (p=0.60).

Figure 1. Flow chart

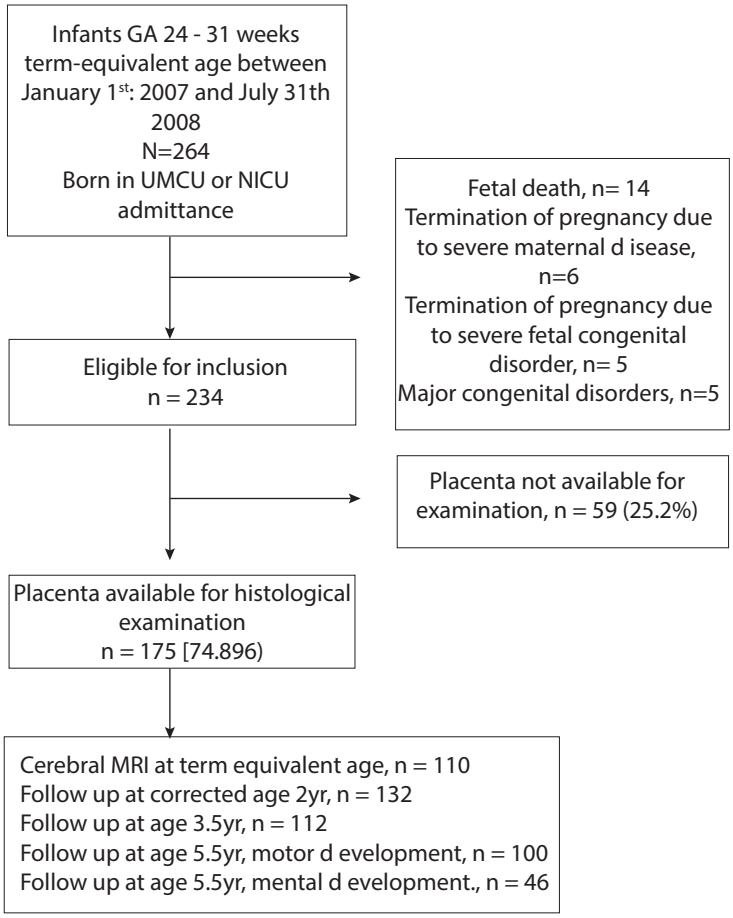


Table 2 shows neurodevelopmental outcome in the four groups. Cognitive and motor development is comparable between the four groups at age 2 and age 3.5 years. At age 5.5 years (**Table 3**), a higher incidence of poor total MABC-II performance was found for infants with inflammatory placental lesions compared to infants with both types of placental lesions (p=0.03), and for infants with vascular placental lesions compared to infants with both types of lesions (p=0.02). For the MABC-II balance performance, a higher incidence of poor performance was found for infants with vascular placental lesions compared to infants with both types of lesions (p=0.01).

Table 1. Baseline characteristics

	No inflammatory/ vascular placental lesions (n= 17)	Inflammatory placental lesions (n=36)	Vascular placental lesions (n=91)	Both types of lesions (n=31)	*P*-value
Baseline					
Gestational age, weeks	28.4 (2.0)	27.5 (2.0)	29.1 (1.5)	28.2 (1.7)	<0.01*
Birth weight, g	1276.5 (421.9)	1067.5 (346.7)	1070.9 (304.0)	1198.8 (332.8)	0.04**
Birth weight z-score	0.67 (0.87)	0.41 (0.68)	-0.30 (0.83)	0.56 (0.68)	<0.01***
Male, n (%)	11 (64.7)	16 (44.4)	52 (57.1)	16 (51.6)	0.47
Apgar score at 5 min <7, n (%)[a]	7 (41.2)	6 (17.1)	10 (11.2)	5 (16.7)	0.03
Placental weight <p10, n (%)[b]	2 (11.8)	4 (11.4)	48 (52.7)	3 (10.3)	<0.01
PPROM, n (%)	2 (11.8)	16 (44.4)	9 (9.9)	19 (61.3)	<0.01
Corticosteroid, n (%)	8 (47.1)	28 (77.8)	73 (80.2)	30 (96.8)	0.01
Pre-eclampsia, eclampsia, HELLP, n (%)	1 (5.9)	0 (0.0)	46 (50.5)	0 (0.0)	<0.01
Cesarean delivery, n (%)	9 (52.9)	8 (22.2)	71 (78.0)	9 (29.0)	<0.01
Umbilical cord hypercoiling, n (%)[c]	0 (0.0)	1 (3.0)	11 (6.6)	1 (3.4)	0.11
Umbilical cord hypocoiling, n (%)[c]	3 (17.6)	10 (30.3)	5 (5.7)	6 (20.7)	0.004
Perinatal morbidity and mortality					
Perinatal mortality, n (%)	4 (23.5)	9 (25.0)	4 (4.4)	4 (12.9)	0.005
Need for mechanical ventilation, n (163) (%)	13 (81.3)	22 (68.8)	54 (62.1)	16 (57.1)	0.38
Persistent ductus arteriosus requiring treatment, n (154) (%)	2 (14.3)	6 (20.7)	25 (29.4)	2 (7.7)	0.11
Chronic lung disease, n (158)(%)	5 (33.3)	11 (36.7)	33 (37.9)	4 (15.4)	0.19
Culture proven sepsis, n (155) (%)	7 (46.7)	14 (48.3)	31 (36.5)	10 (38.5)	0.67
Necrotizing enterocolitis requiring surgery, n (126) (%)	0 (0.0)	2 (8.3)	2 (2.9)	0 (0.0)	0.37

* GA differs significantly between inflammatory lesions and vascular lesions group, using Bonferoni correction (p<0.01)

** No significant differences between any group after Bonferoni correction.

*** BW z-score in the vascular lesion group significantly differs from all other groups (p<0.01)

After correction for GA at birth, the difference in mortality between the groups was non-significant (p=0.23)

[a] Based on n=171 due to missing data. [b] Based on n=172 due to missing data on placental weight. [c] Based on 167 due to missing data on umbilical cord length.

Table 2. Placental pathology and MRI at term-equivalent age.

	No inflammatory/ vascular placental lesions (n=8)	Inflammatory placental lesions (n=24)	Vascular placental lesions (n=59)	Both types of lesions (n=19)	*P*-value
WMI score >1	2 (25.0)	2 (8.3)	7 (11.9)	3 (15.8)	0.64
IVH grade >2	1 (12.5)	1 (4.3)	1 (1.7)	1 (5.6)	0.45
Cerebellar haemorrhage	2 (25.0)	4 (16.7)	9 (15.3)	0 (0.0)	0.24
Abnormal ventricular size	3 (37.5)	15 (62.5)	31 (52.5)	9 (47.4)	0.60

WMI white matter injury; IVH intraventricular haemorrhage

Table 3. Placental pathology and long term outcome.

	No inflammatory/ vascular placental lesions	Inflammatory placental lesions	Vascular placental lesions	Both types of lesions	*P*-value
Outcome at 2 yr CA	n=11	n=24	n=72	n=25	
MDI	105.4 (12.5)	103.1 (10.3)	101.7 (12.1)	106.1 (13.6)	0.40
PDI	109.1 (8.8)	107.5 (9.7)	105.4 (11.6)	112.3 (14.7)	0.09
Outcome at age 3.5yr	n=8	n=23	n=60	n=21	
Griffiths DQ	97.0 (6.6)	96.6 (8.8)	96.9 (6.7)	101.2 (5.9)	0.09
Griffiths locomotor*	39.5 (4.0)	41.9 (3.4)	39.4 (5.4)	42.0 (3.6)	0.05
Griffiths personal social	42.8 (3.1)	45.0 (4.5)	42.7 (4.1)	44.1 (3.0)	0.09
Griffiths language	41.5 (4.0)	42.2 (3.5)	42.0 (4.1)	43.0 (3.1)	0.70
Griffiths eye-hand coordination**	40.8 (3.4)	41.7 (5.2)	40.4 (4.4)	43.2 (4.2)	0.08
Griffiths performance	45.6 (2.9)	45.7 (4.2)	45.6 (3.7)	45.3 (6.9)	0.99
Griffiths practical reasoning	44.0 (2.6)	42.4 (4.0)	43.4 (3.2)	43.8 (2.7)	0.48
Outcome at age 5.5yr					
Mental development	n=3	n=12	n=23	n=8	
Performal IQ	98.7 (11.1)	95.0 (12.5)	92.2 (14.6)	96.5 (12.2)	0.77
Verbal IQ	101.3 (27.0)	93.3 (14.7)	98.2 (17.1)	100.6 (17.1)	0.70
Total IQ	98.3 (19.6)	91.4 (14.0)	95.4 (15.8)	94.3 (11.5)	0.85
Processing speed	94.0 (21.2)	91.6 (6.7)	88.0 (12.6)	88.0 (11.2)	0.76
Motor development	n=7	n=18	n=55	n=20	
MABC manual dexterity <p15	0 (0.0)	7 (38.9)	25 (45.5)	7 (35.0)	0.13
MABC ball skills <p15	2 (28.6)	5 (27.8)	16 (29.1)	2 (10.0)	0.39
MABC balance <p15***	1 (14.3)	4 (22.2)	23 (41.8)	2 (10.0)	0.03
MABC total <p15****	1 (14.3)	10 (52.6)	27 (49.1)	4 (20.0)	0.04

CA corrected age; MDI Mental Development Index; PDI Psychomotor Development Index; GA gestational age;

* After correction for GA p=o.o3, after correction for birthweight z-score non significant

** After correction for GA p=0.01, after correction for birthweight z-score non significant

*** Significantly different between vascular lesion group and both types of lesions group (p=0.01)

**** Significantly different between inflammatory lesions group and both types of lesions group (p=0.03), and between vascular lesion group and both types of lesions group (p=0.02).

All other outcome measures were not different after correction for GA or birth weight z-score.

Discussion

We found that preterm born infants with inflammatory placental lesions had a lower GA compared to infants with vascular placental lesions, but the latter had lower birth weight z-scores. This is in line with previous studies.[15,26,27] Infants with placental vascular lesions had less often hypocoiling of the umbilical cord compared to infants with inflammatory placental lesions or both types of lesions. This association has been described previously by de Laat et al..[28] It has been suggested that in undercoiled umbilical cords the stroma is less turgid and that this associates with less strength of the chorionic and amniotic membranes, thereby increasing the risk of intra-uterine infection.[28] However, it is still under debate whether abnormal umbilical cord coiling is a cause of pathology or a reflection of the sequelae.[29]

The present study found no associations between placental lesions and cerebral MRI abnormalities at TEA. This is in line with a previous study of Chau et al.[30] reporting that WMI was not related to chorioamnionitis, but that postnatal infections did relate to WMI. It could be hypothesized that postnatal events, for example infections and respiratory problems have a larger impact on brain lesions at TEA than intrauterine events, especially in very preterm infants. A meta-analysis on perinatal infections and neurodevelopmental outcome indeed indicated that mainly postnatal infections were associated with adverse outcome in preterm born infants.[31] Furthermore, neonatal complications such as hypotension and respiratory problems might have an effect on outcome that overwhelms the effect of intrauterine events. Other studies suggest a multi-hit scenario, where placental lesions might lower the threshold for neonatal events to cause brain injury.[32] This hypothesis is supported by animal studies showing that growth restricted rat pups produced an increased inflammatory response and subsequent brain injury after injection with lipopolysaccharide compared to non-growth restricted rat pups.[33] Animal studies by Rees and Inder[34] showed that chronic events like placental underperfusion are associated with reduced axonal myelination. It could be that a more advanced quantitative approach of MRI will be able to detect more subtle difference in volumetric measurements or in white matter microstructures, but that conventional MRI does not detect these subtle effects.

This study also found no associations between placental pathology and neurodevelopmental outcome at age 2 and 3.5 years. At age 5.5 years however, infants with only inflammatory placental lesions or only vascular placental lesions had poorer motor performance as compared to infants with both types of placental lesions. Infants with only vascular placental lesions had poorer balance skills in comparison to infants with both types of placental lesions. It might be that the group with both types of placental lesions had less severe inflammatory and vascular lesions. Previous studies have indicated that for example only chorioamnionitis with a fetal vascular response is associated with poor outcome.[13] In our cohort no differences in the incidence of a fetal inflammatory response was found when comparing the inflammatory lesions group to the group with both vascular and inflammatory lesions (all p-values >0.20, data not

shown), however our sample size might have been too small to detect this difference. A lower incidence of ischemic placental lesions was found in the combined group as compared to the group with only vascular placental lesions (12.9% versus 68.1%, $p<0.01$). We only found associations with placental pathology at age 5.5 years but not at earlier ages, suggesting that these infants might have grown into their problems i.e. that motor problems become larger with increasing age. A meta-analysis on motor deficits in preterm infants indeed found a trend towards more motor impairments in older infants,[35] supporting this hypothesis. However, testing motor performance in young infants also involve their attention skills, so poor motor performance might partly reflect attention deficits that are often present in preterm born infants.

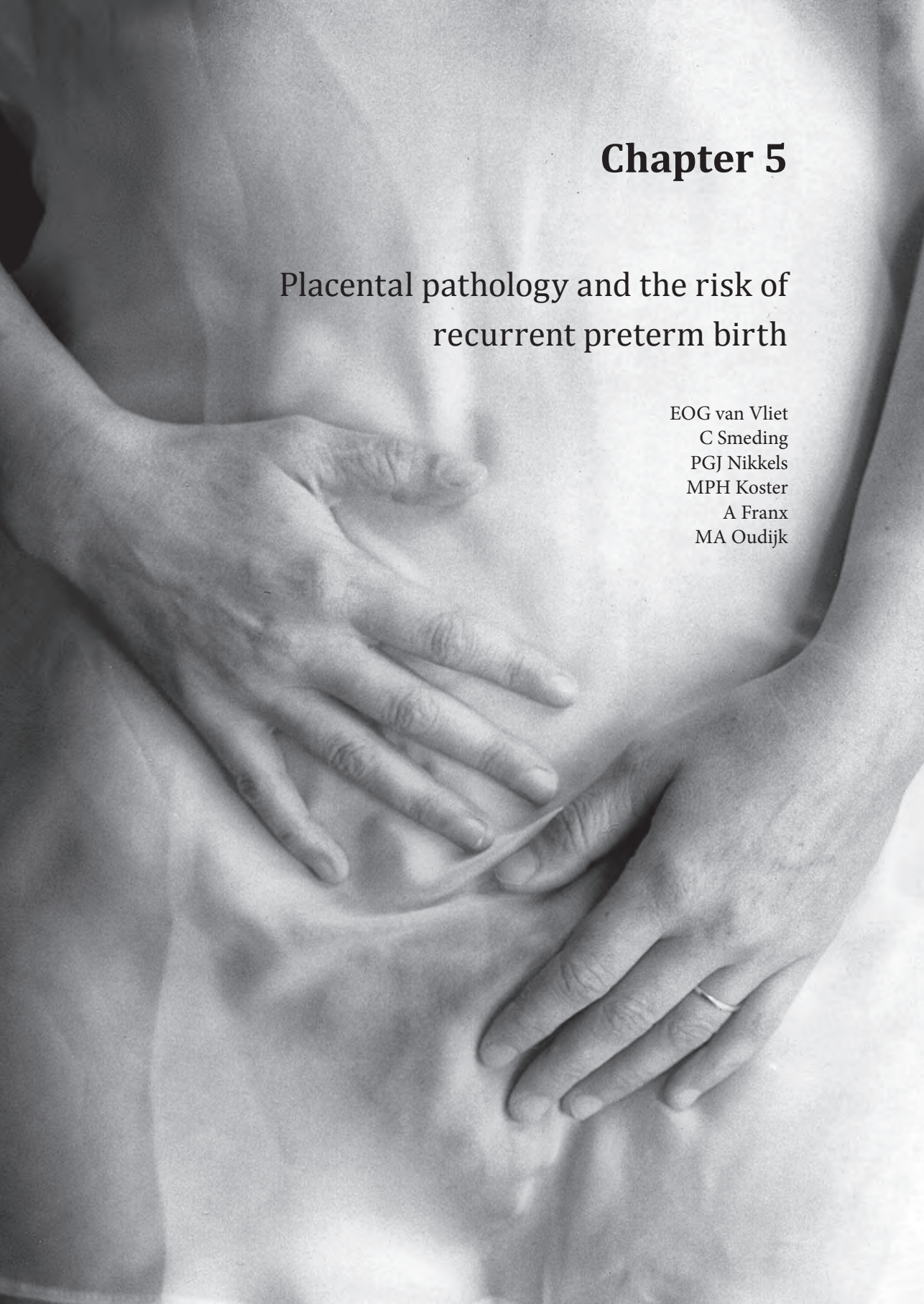
Our study has some limitations that need to be taken into account. First, since the study is retrospective it is susceptible to bias. In placental studies, there is the risk that placentas of relatively uncomplicated preterm births might not be available for placental examination.[36] Indeed, we found that placentas were more often available for examination in pregnancies complicated with hypertensive disorders compared to those without hypertensive disorders, however all other perinatal characteristics were not different between infants with and without available placental pathology. In addition, it is part of routine protocol at the UMCU to examine all placentas of women delivering preterm. Furthermore, there is the risk that drop out in follow-up might have been selective (i.e., parents of infants with normal development were less likely to continue our neonatal follow-up program). The present study involves a detailed examination of the placenta, blinded for perinatal outcome thereby minimizing the risk of bias. The strength of this study is that we were able to combine data of detailed placental examination, clinical characteristics, cerebral MRI and long term neurodevelopmental outcome measured with standardized tests in a relatively large cohort. An interesting direction for future research would be the detection of placental lesions on fetal MRI. A study of Linduska et al.[37] has shown that placental hemorrhages and ischemic lesions could be detected on fetal MRI, providing valuable insights in the functioning of the placenta in pregnancy. Furthermore, studies on transcription factors might provide more insight in the association between the intrauterine environment and postnatal brain development.[38]

In conclusion, our study does not find a significant association of placental pattern of lesions and brain injury at TEA or neurodevelopmental outcome at age 2 and 3.5 years. At age 5.5 years we found a weak correlation indicating better motor performance in infants with both vascular and inflammatory lesions as compared to infants with only inflammatory or only vascular placental lesions.

References:

1. Latal B. Prediction of neurodevelopmental outcome after preterm birth. Pediatr Neurol. 2009;40:413–419
2. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol. 2009;8(1):110-124.
3. T.M. O'Shea, E.N. Allred, O. Dammann, et al. The ELGAN study of the brain and related disorders in extremely low gestational age newborns. Early Hum Dev, 85 (2009), pp. 719–725
4. Rezaie P, Dean A. Periventricular leukomalacia, inflammation and white matter lesions within the developing nervous system. Neuropathology. 2002;22(3):106-132.
5. Woodward LJ, Clark CA, Bora S, Inder TE. Neonatal white matter abnormalities an important predictor of neurocognitive outcome for very preterm children. PLoS One. 2012;7:e51879.
6. Woodward LJ, Anderson PJ, Austin NC, Howard K, Inder TE. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. N Engl J Med. 2006;355:685-94.
7. Inder TE, Huppi PS, Warfield S, Kikinis R, Zientara GP, Barnes PD, Jolesz F, Volpe JJ. Periventricular white matter injury in the premature infant is followed by reduced cerebral cortical gray matter volume at term. Ann Neurol. 1999;46:755-60.
8. Huppi PS, Inder TE. Magnetic resonance techniques in the evaluation of the perinatal brain: recent advances and future directions. Semin Neonatol. 2001;2:195-210.
9. Haldipur P, Bharti U, Alberti C, Sarkar C, Gulati G, Iyengar S, Gressens P, Mani S. Preterm delivery disrupts the developmental program of the cerebellum. PLoS One. 2011;6:e23449.
10. Leviton A, Allred EN, Kuban KC, Hecht JL, Onderdonk AB, O'shea TM, Paneth N. Microbiologic and histologic characteristics of the extremely preterm infant's placenta predict white matter damage and later cerebral palsy. the ELGAN study. Pediatr Res. 2010 Jan;67(1):95-101.
11. Wu YW, Colford JM. Chorioamnionitis as a risk factor for cerebral palsy: A meta-analysis. JAMA. 2000;284(11):1417-24.
12. Maleki Z, Bailis AJ, Argani CH, Askin FB, Graham EM. Periventricular leukomalacia and placental histopathologic abnormalities. Obstet Gynecol. 2009;114:1115-20.
13. Redline RW, Minich N, Taylor HG, Hack M. Placental lesions as predictors of cerebral palsy and abnormal neurocognitive function at school age in extremely low birth weight infants (<1 kg). Pediatr Dev Pathol. 2007;10:282-92.
14. Torrance HL, Bloemen MC, Mulder EJ, Nikkels PG, Derks JB, de Vries LS, Visser GH. Predictors of outcome at 2 years of age after early intrauterine growth restriction. Ultrasound Obstet Gynecol. 2010;36:171-7.
15. van Vliet EO, de Kieviet JF, van der Voorn JP, Been JV, Oosterlaan J, van Elburg RM. Placental pathology and long-term neurodevelopment of very preterm infants. Am J Obstet Gynecol. 2012;206:489.e1-7.
16. van Kooij BJ, van Pul C, Benders MJ, van Haastert IC, de Vries LS, Groenendaal F. Fiber tracking at term displays gender differences regarding cognitive and motor outcome at 2 years of age in preterm infants. Pediatr Res. 2011;70:626-32.
17. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr. 1978;92:529-34.
18. Kidokoro H, Neil JJ, Inder TE. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. AJNR Am J Neuroradiol. 2013;34:2208-14.
19. Pinar H, Sung CJ, Oyer CE, Singer DB. Reference values for singleton and twin placental weights. Pediatr Pathol Lab Med. 1996;16:901-7.
20. Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C; Society for Pediatric Pathology, Perinatal Section, Amniotic Fluid Infection Nosology Committee. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2003;6:435-48.
21. Redline RW, Ariel I, Baergen RN, Desa DJ, Kraus FT, Roberts DJ, Sander CM. Fetal vascular obstructive lesions: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2004;7:443-52.
22. Bayley Scales of Infant and Toddler Development, third edition. San Antonio, TX, USA: Harcourt Assessment, Inc., 2006.
23. Griffiths R. The Abilities of Young Children. A Comprehensive System of Mental Measurement for the First Eight Years of Life. London: The Test Agency Ltd, 1984
24. Movement Assessment Battery for Children, second edition (Movement-ABC-2). London, UK: Pearson Education, Inc., 2007.
25. Wechsler, D.. WPPSI-III-NL Nederlandstalige bewerking: Afname- en scoringshandleiding [Dutch version of the WPPSI-III-NL: Administration and scoring manual]. Amsterdam, The Netherlands: Pearson Assessment and Information BV. 2009.

26. Polam S, Koons A, Anwar M, Shen-Schwarz S, Hegyl T. Effect of chorioamnionitis on neurodevelopmental outcome in preterm infants. Arch Pediatr Adolesc Med 2005;159:1032-5.
27. Kaukola T, Herva R, Perhomaa M, Pääkkö E, Kingsmore S, Vainionpää L, Hallman M. Population cohort associating chorioamnionitis, cord inflammatory cytokines and neurologic outcome in very preterm, extremely low birth weight infants. Pediatr Res 2006;59:478-83.
28. de Laat MW, van Alderen ED, Franx A, Visser GH, Bots ML, Nikkels PG. The umbilical coiling index in complicated pregnancy. Eur J Obstet Gynecol Reprod Biol. 2007;130:66-72.
29. de Laat MW, Franx A, van Alderen ED, Nikkels PG, Visser GH. The umbilical coiling index, a review of the literature. J Matern Fetal Neonatal Med. 2005;17:93-100.
30. Chau V, McFadden DE, Poskitt KJ, Miller SP. Chorioamnionitis in the pathogenesis of brain injury in preterm infants. Clin Perinatol. 2014;41:83-103.
31. van Vliet EO, de Kieviet JF, Oosterlaan J, van Elburg RM. Perinatal infections and neurodevelopmental outcome in very preterm and very low-birth-weight infants: a meta-analysis. JAMA Pediatr. 2013;167:662-8.
32. Redline RW, Wilson-Costello D, Borawski E, Fanaroff AA, Hack M. Placental lesions associated with neurologic impairment and cerebral palsy in very low-birth-weight infants. Arch Pathol Lab Med. 1998;122:1091-8.
33. Campbell LR, Pang Y, Ojeda NB, Zheng B, Rhodes PG, Alexander BT. Intracerebral lipopolysaccharide induces neuroinflammatory change and augmented brain injury in growth-restricted neonatal rats. Pediatr Res. 2012;71:645-52.
34. Rees S, Inder T. Fetal and neonatal origins of altered brain development. Early Hum Dev. 2005;81:753-61.
35. de Kieviet JF, Piek JP, Aarnoudse-Moens CS, Oosterlaan J. Motor development in very preterm and very low-birth-weight children from birth to adolescence: a meta-analysis. JAMA. 2009;302:2235-42.
36. Nelson KB, Blair E. The placenta and neurologic and psychiatric outcomes in the child: study design matters. Placenta. 2011;32:623-5.
37. Linduska N, Dekan S, Messerschmidt A, Kasprian G, Brugger PC, Chalubinski K, Weber M, Prayer D. Placental pathologies in fetal MRI with pathohistological correlation. Placenta. 2009;30:555-9.
38. Torres-Espínola FJ, Altmäe S, Segura MT, Jerez A, Anjos T, Chisaguano M, Carmen López-Sabater M, Entrala C, Alvarez JC, Agil A, Florido J,Catena A, Pérez-García M[11], Campoy C. Maternal PPARG Pro12Ala polymorphism is associated with infant's neurodevelopmental outcomes at 18months of age. Early Hum Dev. 2015;91:457-62.



Chapter 5

Placental pathology and the risk of recurrent preterm birth

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Abstract

Objective To examine whether placental pathology in preterm birth is associated with recurrent preterm birth, and to describe the incidence of placental pathology within different gestational age groups.
Study Design This retrospective cohort study was conducted in a tertiary care center in cooperation with all referral centers and midwifery practices in the central part of the Netherlands. We included one hundred fourteen women with a spontaneous preterm delivery with placental histologic examination and a subsequent delivery. Placental pathology was classified into chorioamnionitis, funisitis, increased maturation, chronic villitis, placental infarction and macro pathological abnormalities of the placenta. Variables considered as possible confounders included maternal age, ethnicity, smoking, diabetes and gestational age at the index delivery. We compared placental pathology between women with a recurrent preterm birth and women with a subsequent term delivery.
Results We compared 72 term deliveries in subsequent pregnancies with 42 recurrent preterm births. Fewer women who delivered at term in their subsequent pregnancy had funisitis at placental pathological examination in the index pregnancy (p=0.02). In a multivariable analysis funisitis and infarction were inversely associated with recurrent preterm birth (OR 0.26 95%CI 0.09-0.77; OR 0.32 95% CI 0.12-0.92).
Conclusion With funisitis and placental infarction present in the index pregnancy, the risk of recurrent preterm delivery was slightly lower.

Introduction

Preterm birth, defined as delivery before 37 weeks of gestation, is the leading cause of perinatal morbidity and mortality in the Western world.[1] In Europe the preterm birth rate is 5.5-11.1%[2] and the incidence is increasing.[3,4] Preterm birth can be distinguished in spontaneous preterm birth and iatrogenic preterm birth. Preterm birth is considered spontaneous when it follows spontaneous labor or preterm rupture of membranes. In iatrogenic preterm birth, pregnancy is prematurely interrupted for maternal or fetal indication. Preterm birth is associated with a high risk of recurrence.[5] Spontaneous preterm birth accounts for two third of all preterm births in industrialized countries. Known risk factors for spontaneous preterm birth are primiparity, multiple pregnancy, smoking and ethnicity.[6-8] Although the majority of women with a preterm delivery will subsequently deliver at term[8], the most consistently reported risk factor for preterm birth is a previous preterm delivery.[5,9,10] There is a close association between the mean gestational age at delivery between two pregnancies. Among women with a second delivery between 20 to 31 weeks, the pregnancy is preceded by a preterm delivery in 29.4% of white women and 37.8% of black women. Second deliveries occurring between 32 to 36 weeks are preceded by a previous preterm birth in 19.9% for white women and 25.9% among black women.[8]

Estimation of the risk for recurrent spontaneous preterm birth in individual women is important and might support targeted preventive strategies in subsequent pregnancies. In preterm birth, abnormalities in the placenta are frequently found and differences in placental pathology may underlie differences in perinatal and neurodevelopmental outcome.[11,12] Few previous studies addressed the relation between placental pathology and recurrent preterm birth. One study found acute inflammatory placental lesions as a significant risk factor for recurrent SPTB. Women with inflammatory placental lesions in their first preterm pregnancy were also more likely to have these lesions in their next delivery regardless of whether the next delivery was at term or preterm. A recent study of Hackney et al.[13] found that funisitis was associated with recurrent preterm birth before 34 weeks of gestation.[13] Placental pathology in spontaneous preterm birth might provide insight in the underlying etiology, and thereby might assist to differentiate between women with and without a risk of recurrent preterm birth.[13]

In this study we aimed to assess the association between placental lesions found in preterm pregnancies and the risk of preterm birth in a subsequent pregnancy. Second, we aimed to describe the patterns of placental pathology within different gestational age groups.

Materials and methods

This study was exempted from approval of the review board after advice by the Local Research Ethics Committee of the University Medical Centre Utrecht (UMCU). The study was conducted at the UMCU in collaboration with all referral centers and midwifery practices in the central part of the Netherlands.

This retrospective population based cohort study includes all women who delivered preterm (20-37 weeks of gestation) in our tertiary care center between January 2006 and December 2008. We excluded women with 1) multiple gestations 2) fetal abnormalities 3) indicated preterm birth on maternal or fetal indication. We examined all subsequent deliveries of these women until October 2012 by chart review at our institution, and contacting all affiliated hospitals and midwifery practices within our region.

We collected data on the index pregnancy including maternal age, parity, ethnicity, type of conception (i.e. spontaneous versus assisted conception), smoking, diabetes, and start of delivery (preterm contractions versus rupture of the membranes). Neonatal data of the index pregnancy included gestational age at delivery, gender, birth weight and still birth. To examine the incidence of placental lesions according to gestational age, we divided our study cohort in three groups, namely infants born after 20-23^{+6}, 24-31^{+6} and 32-36^{+6} weeks.

Placenta histology

Details on placenta histology and macropathology were retrieved from the pathology record. According to our local protocol, two sections of the umbilical cord, at the fetal and placental side, a membrane roll, one sample of the umbilical cord insertion, and two slides of normal placental parenchyma, including both decidua and chorionic plate, were collected and stained with standard haematoxylin and eosin. Additional samples were taken from macroscopically abnormal parenchyma. Placentas were weighed

after the removal of the umbilical cord and membranes. All placentas were analyzed by an experienced perinatal pathologist (PGJN) who was blinded for outcome of the subsequent pregnancy.

Chorioamnionitis was diagnosed as the presence of neutrophilic granulocytes in the chorionic plate or the extra placental membranes. Funisitis was defined as the presence of neutrophils in the wall of the umbilical vein or arteries. Chronic villitis was diagnosed as an infiltrate of lymphocytes and macrophages in groups of at least five placental villi. Infarction was diagnosed when groups of necrotic villi are present. Shock villi were diagnosed as presence of intravillous hemorrhage in groups of at least 5 villi. Placenta circumvallate, a macroscopic diagnosis, was diagnosed as an insertion of the membranes away from the peripheral edge due to a folding or rolling of the chorion on itself. Placental weight was scored <10^{th} percentile according to the reference cures of Wigglesworth.[14]

Statistical analysis

Statistical analyses were performed with IBM SPSS 20. Baseline characteristics were compared using Fisher exact tests for categorical data and analysis of variance (ANOVA) for continuous data. We compared the incidence of placental pathology per gestational age group using Fisher exact tests with a Bonferroni correction for multiple testing. We included variables in the final logistic regression model if the factor had a p-value of <0.15 in the univariate analysis. For the multivariate analyses, a two tailed P value <0.05 was considered statistically significant.

Results

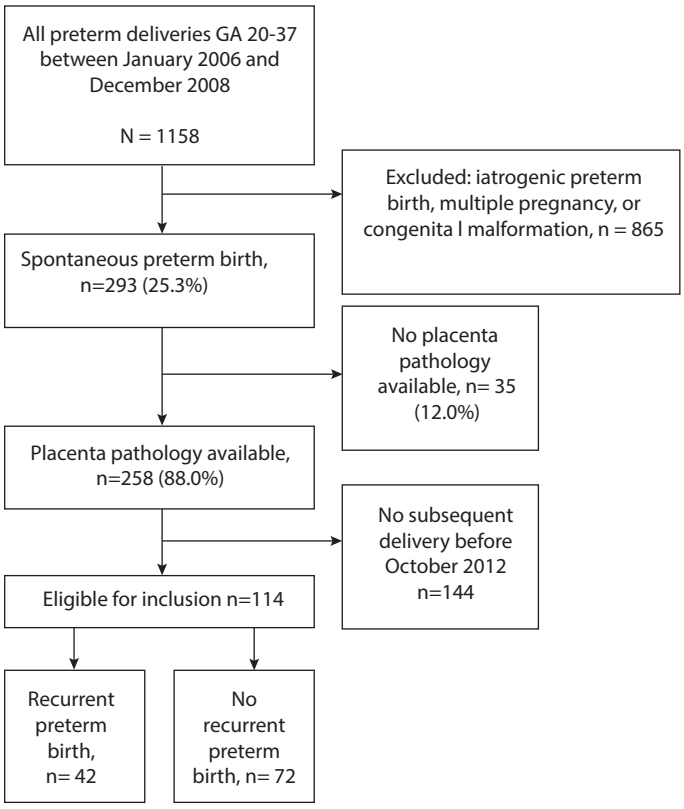
A total of 5593 women delivered in our institution within the study period, of which 1158 (20.7%) delivered preterm. We excluded women with indicated preterm birth, multiple pregnancy and fetal congenital malformations (n=865). Two hundred ninety-three women (25.3%) had a spontaneous, singleton preterm delivery. Placenta pathology was available for 258 (88.0%) of those women. We included 114 women who had a subsequent delivery before October 2012. Of these women, 42 (36.8%) experienced recurrent preterm birth and 72 (63.2) had a subsequent term delivery (figure 1). Obstetric and neonatal characteristics of the index pregnancy did not differ between woman with and without recurrent preterm birth (table 1).

Table 1. Characteristics of index pregnancy

	Subsequent term delivery (n= 72)	**Recurrent preterm delivery (n=42)**	***P* value**
Maternal age, years	29.8 (4.7)	29.6 (4.6)	0.84
Etnicity (caucasian)	60 (83.3%)	35 (83.3%)	1.00
Smoking	3 (15.0%)[1]	6 (17.6%)[1]	1.00
Diabetes	3 (4.2%)	5 (11.9%)	0.14
Spontaneous pregnancy	67 (93.0%)	37 (88.1%)	0.28
Start delivery; contractions [2]	37 (51.4%)	27 (64.3%)	0.24
Gestational age, weeks	30.0 (4.5)	30.1 (4.0)	0.89
Gestational age groups			0.94
- Group 1; 20-24 weeks	6 (8.3%)	3 (7.1%)	
- Group 2; 24-32 weeks	44 (61.1%)	27 (64.3%)	
- Group 3; 32-37 weeks	22 (30.6%)	12 (28.6%)	
Primiparous	52 (72.2%)	32 (76.2%)	0.83
Still birth	5 (6.9%)	0 (0.0%)	0.16
Gender , male	39 (54.2%)	24 (57.1%)	0.85
Birth weight (grams)	1634.6 (831.7)	1678.1 (789.6)	0.78
Mean gestational age at subsequent delivery, weeks	38.9 (1.3)	33.7 (3.2)	

Data presented as n (%) or mean (SD)[1] Based on n=54 due to missing data. [2]All other deliveries started with rupture of the membranes, indicated deliveries were excluded from our study.

Figure 1. Flow chart



The frequency of placental pathology in the index pregnancy in different gestational age groups (20^{+0}-23^{+6}, 24^{+0}-31^{+6}, 32^{+0}-36^{+6} weeks) is presented in table 2. Chorioamnionitis and funisitis were most frequently present in preterm births between 20^{+0}-31^{+6} weeks of gestation. Increased maturation of the placental villi is most frequently seen in births between 24^{+0} – 31^{+6} weeks of gestation. Placental infarction, placental weight <p10 and the absence of histological lesions were mostly seen in births between 32^{+0}-36^{+6} weeks of gestation.

Of all women who delivered preterm, 19 (16.6%) had no histological lesions of the placenta.

Table 2. Placental pathology according to gestational age at index pregnancy

Pathology placenta	GA 20-23+6 weeks (n=9)	GA 24-31+6 weeks (n=71)	GA 32-36+6 weeks (n=34)	*P* value
Histology				
Chorioamnionitis	8 (88.9)[a]	43 (60.6)[a]	6 (17.6)	<0.01
Funisitis	5 (55.6)[a]	23 (32.4)[a]	0 (0.0)	<0.01
Increased maturation	2 (22.2)	32 (45.1)[a,b]	7 (20.6)	0.03
Severely increased maturation	1 (11.1)	4 (5.7)	1 (2.9)	0.60
Chronic villitis	0 (0.0)	2 (2.8)	4 (11.8)	0.12
Infarction	1 (11.1)	13 (18.3) [a]	14 (41.2)	0.02
No histological lesions	1 (11.1) [a,b]	13 (18.3) [a]	20 (58.8)	<0.01
Macropathology				
Placenta circumvallate	1 (11.1)	5 (7.0)	0 (0.0)	0.28
Placental weight <p10	1 (11.1)	10 (14.3) [a]	13 (38.2)	0.01

Data presented as n (%)

[a]significantly different from GA 32-36+6, p <0.05

[b]no longer statistically significant after Bonferroni correction for multiple testing.

Table 3 shows the differences in placental pathology between women with recurrent preterm birth and women with subsequent term deliveries. Compared to women who delivered at term in their subsequent delivery, women who had recurrent preterm birth had less often funisitis in the index pregnancy (p=0.02). The significance of this association did not change after adjustment for gestational age. Of all women with recurrent preterm birth, 11.9% of the placentas showed signs of funisitis, compared to 31.9% of the placentas in the group of women with a subsequent term delivery (OR 0.29; 95% CI 0.10-0.83). Other placental characteristics were not associated with recurrent preterm birth in univariate analysis. Chorioamnionitis significantly related to funisitis (p<0.01), other placental pathologies were not significantly associated with each other.

Maternal diabetes, funisitis and infarction (all p-values <0.15) were included in multivariable analyses (table 4). In multivariable analysis, funisitis and infarction were negative predictors of recurrent preterm birth (OR 0.26 95%CI 0.09-0.77; OR 0.32 95% CI 0.12-0.92). This prediction model had an area under the curve (AUC) of 0.66 (95% CI 0.56-0.76), indicating that this model is only a moderately predictive for recurrent preterm birth.

Table 3. Placenta pathology in index pregnancy

	Subsequent term delivery (n= 72)	Recurrent preterm delivery (n=42)	*P* value
Histology			
Chorioamnionitis	36 (50%)	21 (50%)	1.00
Funisitis	23 (31.9%)	5 (11.9%)	0.02
Increased maturation	24 (33.3%)	17 (40.5%)	0.54
Severely increased maturation	4 (5.6%)	2 (4.9%)	1.00
Infarction	21 (29.2%)	7 (16.7%)	0.18
Chronic villitis	3 (4.2%)	3 (7.1%)	0.67
No histological lesions	23 (31.9%)	11 (26.2%)	0.67
Infarction	21 (29.2%)	7 (16.7%)	0.18
Macropathology			
Placenta circumvallate	4 (5.6%)	2 (4.8%)	1.00
Placental weight <p10	15 (20.8%)	9 (22.0%)	1.00

Table 4. **Risk for recurrent preterm delivery**

	O.R. (95%CI) univariate	*P* value	O.R. (95% CI) multivariate	*P* value
Pregnancy characteristics				
Maternal age; mean	0.99 (0.91-1.08)	0.84		
Etnicity (caucasian)	1.00 (0.36-2.78)	1.00		
Smoking	0.82 (0.18-3.73)	0.80		
Diabetes	3.11 (0.70-13.74)	0.14	3.34 (0.67-16.54)	0.14
Spontaneous pregnancy	0.55 (0.15-2.03)	0.37		
Start delivery (contractions)	1.70 (0.78-3.72)	0.18		
Gestational age first delivery(days)	1.00 (0.99-1.01)	0.89		
Primiparous	0.81 (0.34-1.95)	0.64		
Still birth	0 in recurrent preterm birth	1.00		
Gender (male)	0.89 (0.41-1.90)	0.76		
Birth weight	1.00 (1.00-1.00)	0.78		
Placenta histology				
Chorioamnionitis	1.00 (0.47-2.14)	1.00		
Funisitis	0.29 (0.10-0.83)	0.02	0.26 (0.09-0.77)	0.02
Increased maturation	1.36 (0.62-2.99)	0.44		
Severely increased maturation	0.87 (0.15-4.98)	0.88		
Infarction	0.49 (0.19-1.27)	0.14	0.32 (0.12-0.92)	0.03
Chronic villitis	1.77 (0.34-9.19)	0.50		
No histological lesions	1.32 (0.57-3.09)	0.52		
Placenta macropathology				
Placenta circumvallate	0.85 (0.15-4.85)	0.86		
Placental weight <p10	1.07 (0.42-2.72)	0.89		

Comment

In our study funisitis was inversely correlated with recurrent preterm birth, i.e. women with funisitis in their index preterm birth tend to have a lower risk on preterm birth in their subsequent pregnancy as compared to women without funisitis. In our multivariable analysis, funisitis and placental infarction are inversely predictive for recurrent preterm birth.

There are some limitations that need to be taken into account. We excluded women who did not have available placental examination. Although it is part of our local preterm birth protocol to have the placenta analyzed histologically, the protocol is less strictly followed in case of near term birth. As a result, with progressing of gestation fewer placentas were send for histological examination which could have led to selection bias. However, the overall rate of available placental histology of 88.0% is rather high compared to other studies on this subject. For example, the study of Himes et al. performed histological examination of the placenta in 65% of the women.[2] A second limitation is that we only had data for subsequent births within our region until October 2012, therefore we necessarily excluded women whose second pregnancy occurred after the study period or outside our region. However, within our region we were able to contact all hospitals and midwifery practices to ensure that the data we have from women within our region are comprehensive. This is the first study on placental pathology in recurrent preterm birth that was performed in a multicenter setting. Thirdly, due to the retrospective design of this study the placentas were not assessed by a single pathologist blinded to clinical data. However, the pathologist was unaware of the outcome of the second pregnancy. Furthermore, the use of 17α-hydroxyprogesterone might alter the risk on recurrent preterm birth but unfortunately data on the use of this intervention are lacking in the current study. In future studies it would be recommended to examine the relation between the effectiveness of 17α-hydroxyprogesterone in relation to placental pathology.

The results of our study are not in line with previous publications about placental pathology and the risk of recurrent preterm birth. Himes et al.[6] found that acute inflammatory lesions were associated with recurrent spontaneous preterm birth (OR 2.2, $p<0.05$) before 37 weeks of gestation. The study of Hackney et al.[13] ($n=131$) found a higher risk of recurrent preterm birth before 34 weeks of gestation (OR 3.38, $p=0.016$) associated with funisitis, though this association did not remain statistically significant after correction for gestational age. Two studies analyzed the relation between placental lesions in preterm birth and the history of preterm birth.[15,16] Ghindi et al.[15] found a higher incidence of placental lesions associated with acute and chronic inflammation among women with a history of preterm birth, whereas Goldenberg et al.[16] did not found a relation between placental histology and previous preterm birth.

Both studies on recurrent preterm delivery[6,13] included women with their index and subsequent delivery in the same tertiary care university hospital which may have resulted in bias towards a higher risk population. For the current study, we contacted

all referral centers and midwifery practices in the central part of the Netherlands to include women who had a subsequent pregnancy. Indeed, we found a somewhat lower rate of recurrent preterm birth in our cohort, probably better reflecting the risk of the unselected population. We found a recurrence rate of 36.8%, whereas Himes found a recurrence rate of 41.8% for spontaneous preterm birth. Hackney reported a recurrence rate of 39.7%. However, these differences are rather small and therefore unlikely to explain the differences in results. In the study of Hackney[13] the association between funisitis and recurrent preterm birth was not significant after correction for gestational age. Funisitis is more frequently present in the most early preterm births, and very early preterm births tend to have a higher recurrence rate.[8] The association found in the analyses without adjustment for gestational age may reflect the effect of other factors than the presence of funisitis. In our logistic regression model there was no correlation between gestational age and recurrent preterm birth. This is probably due to the small number of women in our very early preterm birth group. Differences in demographic factors might further explain the difference between our study and previous results. The study of Himes[6] and Hackney[13] report a higher percentage of African American women (25.4% and 25% respectively), compared to the rate of 16% non-Caucasian women in our study . Himes found a significantly higher percentage of African American women among women with a recurrent preterm birth.[6] It could be hypothesised that etiological pathways in preterm birth differ between ethnicities and therefore the difference in findings between our study and previous studies might be driven by differences in patient population. Furthermore, the study of Himes[6] included women from 2001 – 2006. Different clinical management in the prevention of recurrent preterm birth, for example the use of 17α-hydroxyprogesterone, may further explain the differences with previous studies.

A possible explanation for the inverse correlation between funisitis and recurrent preterm birth could be that the factors leading to infection and inflammation do not persist in the uterus but resolve during the inter pregnancy interval. Indeed, a previous study has shown that the recurrence of preterm birth is inversely related to pregnancy interval, also after adjustment for other risk factors for recurrent preterm birth.[17,18] Another study showed that the recurrence rate of PPROM, associated with infection and inflammation, was higher in women with a short inter pregnancy interval.[19] The authors of that study hypothesized that a shorter pregnancy interval may not allow sufficient time for recovery from inflammation of a previous pregnancy. Most probably, other factors that are persistently existent account for the recurrence of preterm birth. Furthermore, interventions to prevent recurrent preterm birth might have altered the incidence of recurrence. For example, treatment with clindamycin in early pregnancy in women with bacterial vaginosis is found to reduce the incidence of preterm birth and to increase gestational age at birth[20]. Screening for bacterial vaginosis using the Nugent score[21] is part of our regional protocol 'prevention of recurrent preterm birth'. Since funisitis is associated with infection[22], it might be that this preventive measure is mostly effective in women who had funisitis in their previous preterm pregnancy

and that the incidence of recurrent preterm birth is therefore reduced in this group. Besides, women at risk of preterm birth receive 17α-hydroxyprogesterone injections according to our regional protocol. The mechanism of 17α-hydroxyprogesterone is not completely understood, but may have an immune-modulatory effect both systemically and at the maternal-fetal interface[23]. Interestingly, we found a relation between recurrent preterm birth and funisitis, but not for chorioamnionitis. In our cohort, funisitis was always accompanied by chorioamnionitis. Funisitis is only occasionally present without chorioamnionitis[24], and is regarded as a fetal response to maternal inflammation. Chorioamnionitis accompanied by funisitis is associated with a higher proportion of positive bacteriological cultures compared to placentas with only chorioamnionitis[22]. It could be hypothesized that mainly inflammatory lesions associated with intrauterine infection contribute to recurrent preterm birth.

We found that inflammatory lesions are most often seen in women with very early preterm birth. This is in line with previous studies showing a gradual decrease in the prevalence of chorioamnionitis with advancing gestational age.[25-28] The association between inflammatory lesions and very early preterm birth has not been explained satisfactorily. One possible explanation could be that the expanding membranes seal the endometrial cavity around mid-pregnancy thereby inhibiting –as long as the membranes are intact- microorganisms to ascend to the uterus. A second hypothesis is that only the maturing fetal immune system is able to generate hormonal or cytokine response necessary to initiate labor as a response to intrauterine inflammation.[1]

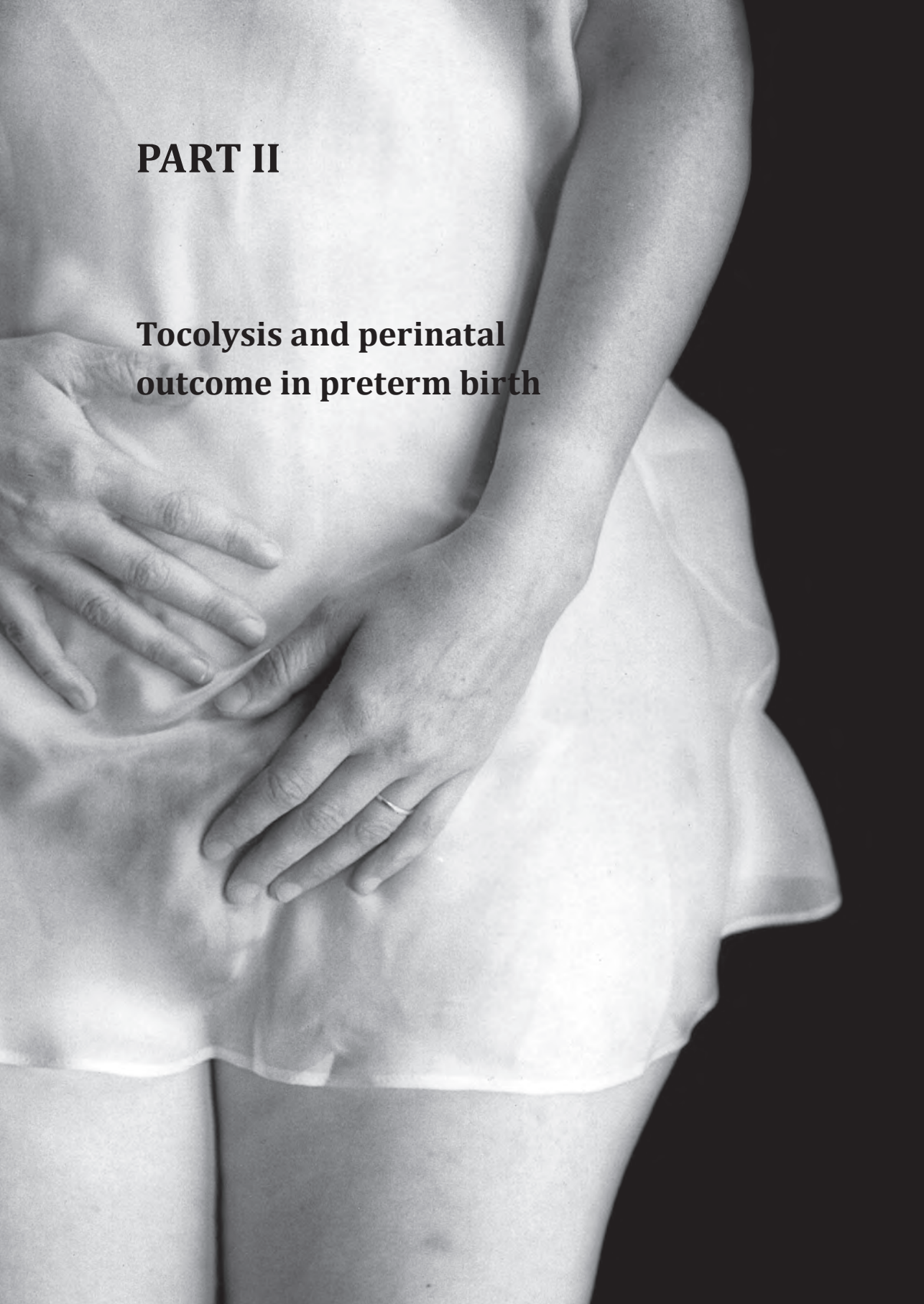
Increased maturation of the placental villi was most often seen in the gestational age group between 24^{+0} and 31^{+6} weeks of gestation. This is also in line with previous studies.[25,26] A possible explanation could be that fetal growth is accelerating around this time[29] thereby placing the fetus at risk of insufficient nutrient supply. An interplay between a poor fetal nutritional status, activity of the hypothalamic pituitary-adrenal axis and increased corticotrophin-releasing hormone production might result in the onset of preterm labour.[30] Placental infarction and placental weight <p10 were mostly seen in births between 32-36^{+6} weeks of gestation. Besides, in this gestational age group the absence of histological lesions was more common compared to placentas of earlier preterm births. This is also in accordance with previous studies[25,26].

This study reconfirms the association of placental pathology and spontaneous preterm birth and provides somewhat more insight in the pathophysiological processes of spontaneous preterm birth. However the associations are rather small making it unlikely that they will be of benefit for the development of targeted interventions for individual women at high risk of recurrence.

References

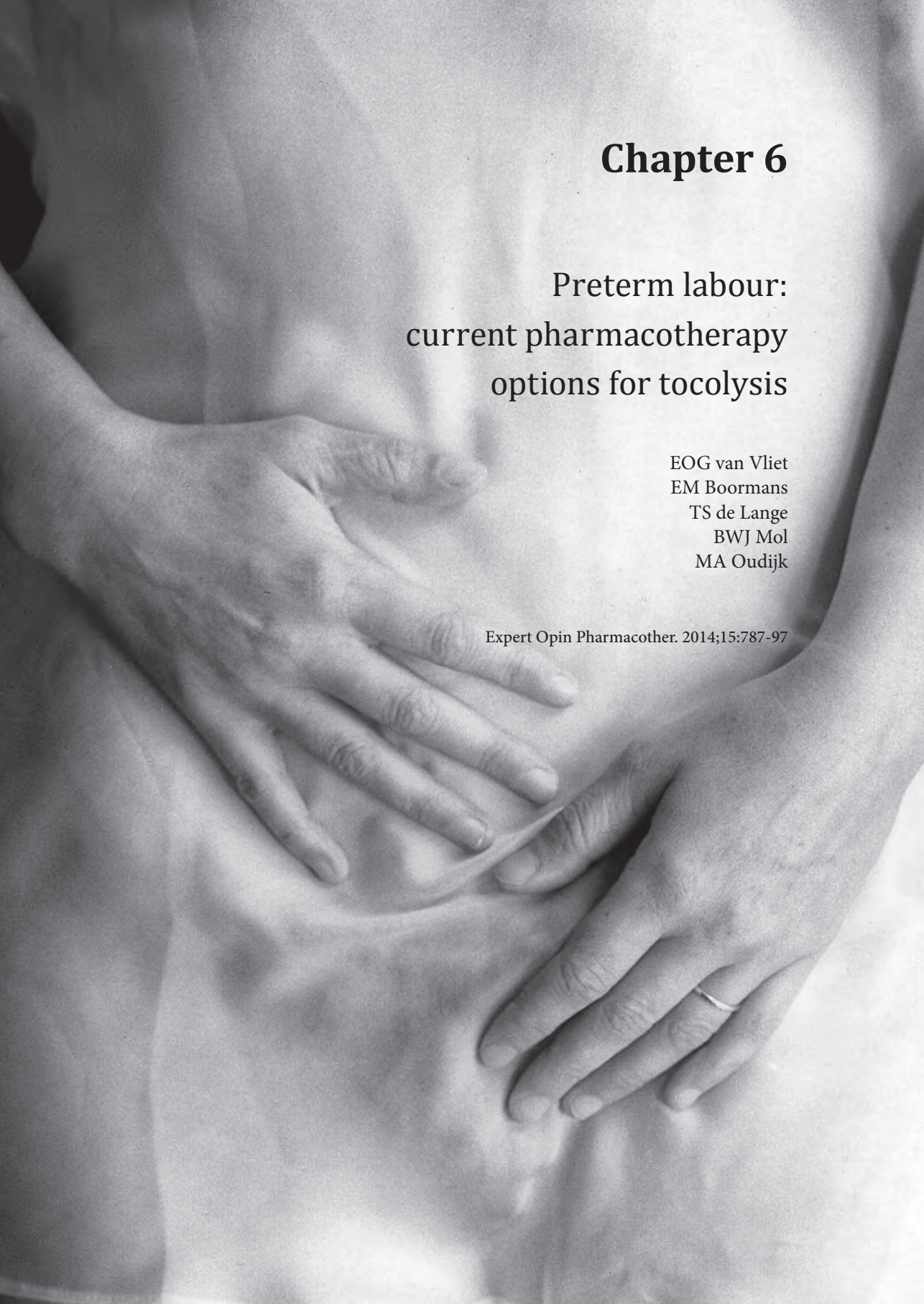
1. Goldenberg RL, Culhane JF, Lams JD, Romero R. Epidemiology and causes of preterm birth. The Lancet. 2008;371:75-84.
2. Zeitlin J, Szamotulska K, Drewniak N, Mohangoo AD, Chalmers J, Sakkeus L, et al.Preterm birth time trends in Europe: a study of 19 countries. BJOG. 2013;120(11):1356-65.
3. Chang HH, Larson J, Blencow H, Spong CY, Howson CP, Cairns-Smith S, et al. Preventing preterm births: analysis of trends and potential reductions with interventions in 39 countries with very high human development index. Lancet 2013;381:223–34.
4. Blencowe H., Cousens S., Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012; 379: 2162–72.
5. Ananth CV, Getahun D, Peltier MR, Salihu HM, Vintzileos AM. Recurrence of spontaneous versus medically indicated preterm birth. Am J Obestet Gynecol 2006; 195: 643-50
6. Himes KP, Hyagriv SN. Risk of recurrent preterm birth and placental pathology. Obstetrics & Gynecology. 2008;112(1):12-126.
7. Ananth CV, Peltier MR, Getahun D, Kirby RS, Vintzileos AM. Primiparity: an 'intermediate' risk group for spontaneous and medically indicated preterm birth. J Matern Fetal Neonatal Med. 2007 Aug;20(8):605-11.
8. Adams MM, Elam-Evans LD, Wilson HG, Gilbertz DA. Rates of and factors associated with recurrence of preterm delivery. JAMA. 2000;283(12):1591-6.
9. Mercer BM, Goldenberg RL, Das A, Moawad AH, Iams JD, Meis PJ, et al. The preterm prediction study: a clinical risk assessment system. Am J Obstet Gynecol. 1996;174(6):1885-93.
10. Bloom SL, Yost NP, McIntire DD, Leveno KJ. Recurrence of preterm birth in singleton and twin pregnancies.Obstet Gynecol. 2001;98(3):379-85.
11. van Vliet EO, de Kieviet JF, van der Voorn JP, Been JV, Oosterlaan J, van Elburg RM. Am J Obstet Gynecol. 2012;206(6):489.e1-7.
12. Roescher AM, Timmer A, Erwich JJ, Bos AF. Placental pathology, perinatal death, neonatal outcome, and neurological development: a systematic review. PLoS One. 2014;25:9(2):e89419.
13. Hackney DN, Tirumala R., Salamone LJ, Miller RK, Katzman PJ. Do placental histologic findings of chorion-decidual hemorrhage or inflammation in spontaneous preterm birth influence outcomes in the subsequent pregnancy? Placenta 2014;35:58e63.
14. Wigglesworth JS. Textbook of fetal and perinatal pathology. London, UK: Wiley-Blackwell; 1998.
15. Ghindi A and Salafia CM. Histologic placental lesions in women with recurrent preterm delivery. Acta Obstet Gynecol Scand 2005;84:547–550.
16. Goldenberg L, Andrews WW, Faye-Petersen O, Cliver S, Goepfert AR, Hauth JC. The Alabama Preterm Birth Project: Placental histology in recurrent spontaneous and indicated preterm birth. Am J Obstet Gynecol. 2006;195:792–6.
17. DeFranco EA, Stamilio DM, Boslaugh SE, Gross GA, Muglia LJ. A short interpregnancy interval is a risk factor for preterm birth and its recurrence. Am J Obstet Gynecol. 2007;197(3):264.e1-6.
18. DeFranco EA, Ehrlich S, Muglia LJ. Influence of interpregnancy interval on birth timing. BJOG 2014 Jun 4.
19. Getahun D, Strickland D, Ananth CV, Fassett MJ, Sacks DA, Kirby RS, et al. Recurrence of preterm premature rupture of membranes in relation to interval between pregnancies. Am J Obstet Gynecol. 2010;202(6):570.e1-6.
20. Lamont RF, Nhan-Chang CL, Sobel JD, Workowski K, Conde-Agudelo A, Romero R. Treatment of abnormal vaginal flora in early pregnancy with clindamycin for the prevention of spontaneous preterm birth: a systematic review and metaanalysis. Am J Obstet Gynecol. 2011;205:177-190.
21. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation.J Clin Microbiol. 1991;29:297-301.
22. Queiros da Mota V, Prodhom G, Yan P, Hohlfheld P, Greub G, Rouleau C. Correlation between placental bacterial culture results and histological chorioamnionitis: a prospective study on 376 placentas. J Clin Pathol. 2013;66:243-248.
23. MacIntyre DA, Sykes L, Teoh TG, Bennett PR. Prevention of preterm labour via the modulation of inflammatory pathways. J Matern Fetal Neonatal Med. 2012;25 Suppl 1:17-20.
24. Katzman PJ, Metlay LA. Fetal inflammatory response is often present at early stages of intra-amniotic infection, and its distribution along cord is variable. Pediatr Dev Pathol. 2010;13:265-72.
25. Redline RW. Placental pathology: a systematic approach with clinical correlations. Placenta. 2008;29 Suppl A:S86-91.

26. Lee J, Kim JS, Park JW, Park CW, Park JS, Jun JK, Yoon BH. Chronic chorioamnionitis is the most common placental lesion in late preterm birth. Placenta 2013;34:681-689.
27. Stanek J Comparison of placental pathology in preterm, late-preterm, near-term, and term births. Am J Obstet Gynecol 2014;210:234.e1-6.
28. Goldenberg RLG, Hought JC and Andrews WW. Intrauterine infection and preterm delivery. NEJM 2000;342(20):1500-7.
29. Peleg D, Kennedy CM, Hunter SK. Intrauterine growth restriction: identification and management. Am Fam Physician. 1998;58(2):453-60, 466-7.
30. Kalantaridou SN, Zoumakis E, Makrigiannakis A, Lavasidis LG, Vrekoussis T, Chrousos GP. Corticotropin-releasing hormone, stress and human reproduction: an update. J Reprod Immunol 2010;85:33-9.



PART II

Tocolysis and perinatal outcome in preterm birth



Chapter 6

Preterm labour: current pharmacotherapy options for tocolysis

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Expert Opin Pharmacother. 2014;15:787-97

Abstract

Introduction In the developed world, preterm birth is in quantity and in severity the most important issue in obstetric care. Adverse neonatal outcome is strongly related to gestational age at delivery. Since the pathophysiological mechanism of preterm birth is not yet completely unravelled, the development of successful preventive strategies is hampered. When preterm labour is actually threatening, current pharmacological therapies focus on inhibition of preterm contractions. This allows for transportation of the mother to a centre with a neonatal intensive care unit and administration of corticosteroids to enhance fetal lung maturation. Globally, however, large practice variation exists.

Areas covered The aim of this review is to provide an overview of current pharmacological therapies for preterm labour.

Expert opinion For the use of initial tocolysis, the use of atosiban or nifedipine for 48 hours is recommended based on the largest effectiveness and most favourable side effect profile. However since data that convincingly indicate the beneficial effect of tocolytics on neonatal outcome are lacking, it might well be that tocolytics are ineffective. The role of progesterone in treatment of acute tocolysis is limited, but it might play a role in the prevention of preterm labour or as sensitizer for other tocolytic agents.

1. Introduction

Preterm birth is defined as birth before 37 completed weeks' of gestation and can either present by rupture of the membranes or by frequent uterine contractions leading to changes in the cervix. Preterm birth is the leading cause of neonatal morbidity and mortality worldwide. Almost 75% of perinatal deaths occur in infants born before 37 weeks' gestation.[1] The prevalence of adverse neonatal outcome is strongly related to gestational age at delivery and declines from 77% at 24-27 weeks to less than 2% at 34 weeks and more.[2] Consequently, preterm birth is associated with a large burden of disease, high costs for medical care, special education, and institutionalized care for disabled infants.[3]

It has been suggested that preterm labour can be regarded as a syndrome initiated by multiple mechanisms, including infection or inflammation, uteroplacental ischemia or haemorrhage, uterine overdistension, stress, and immunologically mediated processes.[4] However, whether spontaneous preterm labour is the consequence of premature activation of the normal labour process or the consequence of a pathological insult that sets of the progression from uterine quiescence to overt labour is currently not clear, hampering our ability to develop successful preventive measures.[5]

Current interventions in threatening preterm labour therefore focus on the inhibition of preterm contractions, at the endpoint of the cascade of the pathophysiological process. The treatment of women at risk for preterm delivery consists of administration of tocolytic drugs for 48 hours to allow for administration of corticosteroids to enhance fetal lung maturation and transport the patient to a centre with neonatal intensive care (NICU) facilities in case gestational age is less than 32 weeks. The aim of this review is to provide an overview of the current insight in tocolysis in threatened preterm labour.

2. Current pharmacotherapy for preterm labour

Perinatal death and morbidity are not only strongly related to early gestational age but also to whether or not antenatal steroids are administrated[6] and whether a preterm infant is transferred to a tertiary care centre in- or ex-utero. Tocolytics can delay preterm delivery , and they are therefore given for 48 hours to allow maximal effect of maternal parenteral steroid administration and transportation of the mother to a centre with NICU facilities.
Several types of tocolytic drugs are commonly used as treatment in preterm labour. The most commonly used drugs include beta-adrenoceptor agonists (ritodrine), calcium channel blockers (nifedipine), oxytocin receptor antagonist (atosiban), prostaglandin inhibitors (indomethacin) and magnesium sulphate . The ideal tocolytic drug should be efficient in postponing preterm labour, have a favourable safety profile in both

mother and fetus, and should be able to result in a reduction in neonatal morbidity and mortality.

No consensus exists in daily obstetrical care on the type of tocolytic therapy and as a consequence, strong practice variation exists internationally. This review will focus on the evidence on efficacy of tocolytic therapy by reviewing the effect on prolongation of gestation, improvement of neonatal outcome and safety of these drugs.

2.1 Beta-adrenoceptor agonists

Beta-adrenoceptor agonists such as ritodrine, isoxsuprine and terbutaline cause myometrial relaxation by activating adenyl cyclase to form cyclic adenosine monophosphate (cAMP). The increased cellular levels of cAMP decrease myosin light-chain kinase activity, both by phosphorylation of the myosin light-chain kinase itself, and by reducing intracellular calcium through increasing calcium uptake by sarcoplasmic reticulum.[7] These types of drugs have been used for a long time in preterm labour.

2.1.1 Efficacy

A Cochrane review shows that beta-adrenoceptor agonists are effective in delaying delivery for 48 hours (RR 0.63; 95% CI 0.53 to 0.75) as compared to placebo.[8] However, the use of beta-adrenoceptor agonists itself has not been associated with an improvement in neonatal morbidity and no benefit was demonstrated for beta-adrenoceptor agonists on perinatal death (RR 0.84; 95% CI 0.46 to 1.55) or neonatal death (RR 1.00; 95% CI 0.48 to 2.09).[8] It is important to notice that no tocolytic studies have been powered by a sufficient sample size to demonstrate an effect on neonatal outcome. In a Cochrane review on the use of beta-adrenoreceptor agonists or oxytocin receptor agonists for inhibiting preterm labour no superiority was demonstrated in terms of tocolytic efficacy or infant outcomes.[9]

A network meta-analysis[10] included 60 trials on beta mimetics and indirectly calculated its efficacy compared to placebo. This network analysis concludes that beta-adrenoceptor agonists were effective in delaying delivery with 48 hours (OR 2.41; 95% CI 1.27 to 4.55), but did not significantly reduce the rate of RDS (OR 0.85; 95% CI 0.50 to 1.45). The network meta-analysis also indicated that the chance that beta-adrenoceptor agonists are the most effective tocolytic therapy was very low.[10]

2.1.2 Side effects

For maternal side effects, the Cochrane review showed that compared to placebo beta-adrenoceptor antagonist more frequently leads to palpitations (RR 10.11; 95% CI 6.56 to 15.58), tachycardia (RR 4.08; 95% CI 1.55 to 10.73) chest pain (RR 11.29, 95% CI 3.81 to 33.46), dyspnoea (RR 3.86; 95% CI 2.21 to 6.77) headache (RR 4.07 95% CI 2.60 to 6.35) and tremor (RR 10.74, 95% CI 6.20 to 18.59).[8] The network meta-analysis[10]

reports that beta-adrenoceptor agonists are associated with the most maternal side effects compared to placebo (Odds ratio 22.68, 95%CI 7.51 to 73.67).
A prospective cohort study [11] registering serious maternal and neonatal side effects of tocolytics showed a relatively high incidence of serious adverse drug effects of 1.7% for beta-adrenoceptor agonists.. The relative risk for an adverse drug reaction was 3.8 for beta adrenoceptor agonist (95% CI 1.6 to 9.2) compared to other tocolytical agents.[11]

In evaluating the optimal tocolytic drug for clinical practice, it is important to take the costs of side effects into account. A cost decision analysis comparing indomethacin, magnesium sulphate, nifedipine and terbutaline indicates that terbutaline is the most expensive tocolyticum because of the high cost of monitoring and treating adverse events.[12] These side effects make beta-adrenoceptor agonists, despite their effectiveness, not the tocolytic therapy of first choice and its use in preterm labour has been largely abandoned.

2.2 Calcium channel blockers

Calcium channel blockers such as nifedipine and nicardipine are non-specific smooth muscle relaxants, predominantly used for the treatment of hypertension in adults. They exert their tocolytic effect by preventing the influx of extracellular calcium ions into the myometrial cell. The most widely used and studied calcium channel blocker is nifedipine that belongs to the dihydropiridine group.

2.2.1 Efficacy

There are no large randomized trials directly comparing the efficacy of calcium channel blockers to placebo to prevent preterm labour. Therefore the absolute efficacy is unclear. A Cochrane systematic review of randomised trials showed that when compared with any other tocolytic agents -mainly betamimetics- calcium channel blockers significantly reduced the number of women giving birth within seven days of receiving treatment and prior to 34 weeks' gestation. More importantly, calcium channel blockers resulted in a significantly improved neonatal outcome. When compared with any other tocolytic agent, the use of calcium channel blockers resulted in an increase in gestational age at birth (weighted mean difference 0.70 weeks; 95% CI 0.19 to 1.20), and a reduction in neonatal respiratory distress syndrome (RDS) (RR 0.63; 95% CI 0.46 to 0.88), necrotising enterocolitis (RR 0.21; 95% CI 0.05 to 0.96) and intraventricular haemorrhage (IVH) (RR 0.59; 95% CI 0.36 to 0.98). The accompanying Numbers Needed to Treat were 14 (95% CI 8 to 50) to prevent one case of RDS and 13 for intraventricular haemorrhage (95% CI 7 to 100).[13] This meta-analysis has been criticized because the conclusion that nifedipine is able to delay delivery beyond 7 days was based on 4 studies, 3 of which showed no significant benefit and the fourth study was conducted by one of the authors of the Cochrane review.[14] Lamont et al. have raised concerns about the quality of studies evaluating nifedipine.[15]

The previously mentioned network meta-analysis[10] including 29 trials on calcium channel blockers indicated a benefit from calcium channel blockers in the reduction of delivery rates within 48 hours (OR 2.78; 95% CI 1.26 to 8.61), but not on neonatal RDS (OR 0.71 95% CI 0.37 to 1.43) and neonatal mortality (OR 0.39 95% CI 0.09 to 1.49). This meta-analysis concluded that calcium channel blockers, together with prostaglandin inhibitors, had the highest probability of being the best therapy for preterm delivery on the basis of four outcomes; delivery delayed by 48 hours, neonatal mortality, neonatal respiratory distress syndrome, and maternal side effects. For the two neonatal outcomes, calcium channel blockers also had the highest probability of being the best class.[10] One should keep in mind that the included studies on calcium channel blockers were of poor quality and there was a lack of heterogeneity of the reported outcomes.

A recent randomized controlled trial comparing nifedipine and atosiban showed that atosiban was found to be superior to nifedipine in terms of the proportion of women who did not give birth and who did not require an alternate tocolytic agent within 48 hours from the initiation of therapy (OR 2.26; 95% CI 1.10 to 4.63). In addition, atosiban was better tolerated by women compared with nifedipine. Nifedipine however was associated with a longer postponement of delivery and more importantly, a trend towards an improved neonatal outcome.[16] Nifedipine is found to be as effective as the prostaglandin synthetase inhibitor indomethacin in postponing delivery.[17] Besides, no differences were found in the incidence of respiratory distress syndrome, patent ductus arteriosus, sepsis, necrotizing enterocolitis, intraventricular haemorrhage and periventricular leukomalacia between nifedipine and indomethacin.[18] Significantly more patients treated with nifedipine had hypotension and maternal tachycardia compared to patients using indomethacin, whereas ductus constriction and oligohydramnion was more frequently observed among women receiving indomethacin.[17]

2.2.2 Side effects

Calcium channel blockers are associated with maternal side effects. Most adverse drug reactions reported in women treated with nifedipine were related to the effects of the drug on blood pressure, such as severe hypotension. Other described side effects are headache, flushing, nausea, tachycardia and vomiting.[19] Compared with atosiban, nifedipine is associated with a higher incidence of adverse drug reactions (RR 12, 95% C.I. 1.9-69).[11] Severe hypotension and fetal death after tocolysis with nifedipine has been described in a case report[20], however, a large prospective cohort study registering serious maternal and neonatal side effects of tocolytics showed an incidence of less than 0.5% for hypotension leading to discontinuation of treatment. No fetal deaths as a result of side effects were reported in this study. Women with multiple pregnancies were not at increased risk. Neither mothers nor children suffered permanent damage.[11] Follow up of children after in-utero exposure to nifedipine showed no adverse effects at 9-12 years of age.[22] Calcium channel blockers

are found to be effective and relatively safe. Monitoring of blood pressure during tocolysis with nifedipine is recommended.

2.3 Oxytocin receptor antagonist

Oxytocin receptor antagonists block oxytocin receptors in the myometrium, preventing a rise in intracellular calcium and thereby relaxing the myometrium. The mixed oxytocin/vasopressin (V_{1a}) receptor antagonist atosiban is specifically developed for inhibition of preterm labour.

2.3.1 Efficacy

A Cochrane review did not show superiority of atosiban over beta-mimetics or placebo in terms of tocolytic effectiveness[9] The review showed comparable effectiveness to beta-adrenoceptor agonists in the number of women giving birth within 48 hours (RR 0.98; 95% CI 0.68 to 1.14) and birth within 7 days (RR 0.91; 95% CI 0.69 to 1.20). Compared with beta-adrenoreceptor agonists, atosiban did not reduce important perinatal outcomes such as respiratory distress syndrome, and admission to neonatal intensive care. Perinatal outcome for atosiban compared with placebo was also similar except for mean birth weight (weighted mean difference -138.31 grams; 95% CI -248.76 to -27.86) and maternal side-effects requiring cessation of treatment which favoured the placebo. It should be noted that the results comparing atosiban to placebo are based on only 2 studies.

An indirect comparison between nifedipine and atosiban (nifedipine versus β-agonists and β-agonists versus atosiban) indicated a benefit for nifedipine, with a reduction of respiratory distress syndrome (OR 0.55; 95% CI 0.32 to 0.97) and an increase in the delay of delivery for more than 48 hours, although the latter was not statistically significant (OR 1.20; 95% CI 0.73 to 1.95) [23]. Currently, we are performing a large randomized trial comparing atosiban and nifedipine in patients with preterm labour < 34 weeks within our consortium for women's health and reproductivity studies (Dutch Trial Register NTR 2947)0[24]. The recent network meta-analysis indicated that atosiban was effective in delaying delivery with 48 hours (OR 2.02; 95% CI 1.10 to 3.80), but did not reduce the rate of RDS (OR 0.89; 95% CI 0.55 to 1.37) or perinatal mortality (OR 0.62; 95% CI 0.16 to 2.35). The network meta-analysis also indicated that the chance that atosiban would be the most effective tocolytic drug was very low.[10] One study compared standard timing of administration of atosiban to early administration of atosiban (i.e. in presence of only contractions or dilatation). The study indicated that in the early treatment group there was a larger proportion of women who remained undelivered without requiring alternative tocolytics for 48 hours.[25]

2.3.2 Side effects

Atosiban has a superior maternal and fetal adverse effects profile compared to other tocolytics. The prospective cohort study on adverse drug reactions to tocolytic treatment

for preterm labour observed no serious adverse drug reaction after treatment with a single course of atosiban (575 patients). Relative risk for adverse events was 0.07 (95% CI 0.01 to 0.4). In this study, indometacin and atosiban were the only tocolytic drugs not associated with serious adverse reactions.[11] Recently, one case report was published on the use of atosiban in a multiple pregnancy where pulmonary oedema occurred.[26] Compared to beta-adrenergic agonist, atosiban has fewer maternal cardiovascular side effects.[27] The Cochrane review on atosiban for inhibiting preterm labour showed that compared with beta-adrenoceptor agonists atosiban was associated with a significant reduction in maternal drug reactions requiring cessation of treatment (RR 0.04; 95% CI 0.02 to 0.11). Long-term outcomes up to two years of age were described in one placebo controlled trial. There were no differences between infants of mothers who received atosiban or placebo. However, only 55% of the infants who were originally included in the study were assessed for Bayley II Mental and Motor Development Index and neurological examination at two years.[28] An analysis of the economic importance of side-effects indicates that atosiban is cost-saving compared to betamimetics in the treatment of preterm labour, owing to its superior safety profile.[29]

2.3.3 Developments in the field of oxytocin receptor antagonists

Next to atosiban, there are several new oxytocin receptor antagonist under study. The competitive oxytocin receptor antagonist barusiban has a high affinity for the human oxytocin receptor, and has shown to inhibit myometrium contractions in in-vitro term and preterm myometrium strips.[30] In clinical studies, barusiban was well tolerated and found to be safe for mother, fetus and infant. However, barusiban has not proven to be more effective than placebo in ceasing preterm contractions.[31] It remains uncertain if the lack of clinical effectiveness of barusiban can be contributed to the oxytocin receptor selectivity. Presumably, in atosiban the antagonizing effect on the vasopressin receptor contributes to the tocolytic activity. Indeed, the orally administered relcovaptan, a V_{1a} receptor antagonist, has shown some tocolytic effect. A small study involving 19 pregnant women with preterm contractions between 31 and 37 weeks of gestation found a significant reduction in the frequency of contractions amongst women receiving relcovaptan compared to women treated with a placebo. However, there could have been an effect on the oxytocin receptor and the relative importance of the oxytocin receptor and V_{1a} receptor cannot be fully delineated.[33] In conclusion, there are potentially effective drugs in development in the field of oxytocin antagonist, and atosiban has found to be safe and effective in postponing delivery. However, to date no studies have been powered to detect effects on neonatal outcome.

2.4 Prostaglandin synthetase inhibitors

Prostaglandins induce uterine contractions by enhancing myometrial gap junction formation and increasing intracellular calcium concentration. Prostaglandins are produced by cyclo-oxygenase (COX), an enzyme that increases the level of prostaglandins. There are two distinctive forms of COX, namely COX-1 and COX-2.

COX-2 is especially associated with myometrial contractility. Prostaglandin synthetase inhibitors decrease the prostaglandin production thereby interfere with an important pathway of labour. In addition, as intrauterine inflammation and infection play an important role in preterm labour, the anti-inflammatory action of prostaglandins may be one of the reasons why prostaglandin inhibitors may be effective in delaying delivery.

2.4.1 Efficacy

A Cochrane review[33] included 13 trails encompassing 713 women with preterm labour (<35 weeks of gestation). Ten of the 13 trials used indomethacin, other trials used nimesulide, sulindac, ketorolac and rofecoxib. Compared to placebo, COX inhibitors resulted in a higher gestational age and birth weight, and a reduction in the number of deliveries before 37 weeks of gestation. No differences were found in perinatal mortality and morbidity. However, the results should be interpreted with caution, since only 3 trials encompassing 106 women compared COX inhibitors to placebo. Besides, two of these 3 trials included women up to 35 weeks of gestation, where the incidence of perinatal morbidity and mortality is low. Comparing non selective COX inhibitors and selective COX 2 inhibitors, no difference was found in perinatal mortality, respiratory distress syndrome, premature closure of the ductus, pulmonary hypertension of the newborn, intraventricular haemorrhage or neonatal renal failure. The recent network meta-analysis[10] indicated that tocolysis with COX inhibitors was effective in delaying delivery with 48 hours (OR 5.39, 95% CI 2.14 to 12.34), but did not affect the rate of RDS (RR 0.87; 95% CI 0.40 to 1.75) or neonatal mortality (OR 0.62; 95% CI 0.04 to 4.63). The network meta-analysis indicated that prostaglandin inhibitors, together with calcium channel blockers, have the highest probability of being the best therapy for preterm delivery.[10]

2.4.2 Side effects

COX inhibitors, such as indomethacin and rofecoxib freely cross the placenta, and therefore interfere with prostaglandin homeostatis of the fetus. Side effects associated with the use of COX inhibitors have been reported, such as oligohydramnios, antenatal constriction of the ductus arteriosus and renal failure. Amongst the 403 women receiving COX inhibitors included in the Cochrane review, there was only one case of antenatal closure of the ductus arteriosus, and no increase in the incidence of patent ductus arteriosus postnatally compared with placebo. Based on these data, the role of COX inhibitors for women in preterm labour warrants further attention.[33]

2.5 Magnesium sulphate (MgSO4)

The exact mechanism of magnesium as a tocolytic agent, either for initial use or as maintenance therapy, is only partially understood. Magnesium decreases the frequency of depolarisation of smooth muscle by modulating calcium uptake, binding and distribution in smooth muscle cells. The net result is inhibition of uterine contractions.

2.5.1 Efficacy as a tocolytic drug

A Cochrane review on the use of magnesium sulphate as initial tocolytic treatment included over 2000 women in 23 trials.[34] Only nine studies had high quality for concealment of allocation. No benefit was found for magnesium sulphate on the risk of giving birth preterm (<37 weeks) , very preterm (<34 weeks), or within 48 hours from start of treatment. The risk of neonatal death was higher for infants exposed to magnesium sulphate (RR 2.82; 95% CI 1.20 to 6.62, 7 trials, 727 infants). However, this is based on analysis of 7 studies from which only one study reported two fetal deaths in the magnesium sulphate group. Six other studies reported no fetal deaths. The authors conclude that there was no clear reason for this finding. The higher death rate was reported in a subgroup where the maintenance dose of magnesium sulphate was high, and the total number of deaths was low. Magnesium sulphate caused less side effects compared to beta-adrenoceptor agonists, although compared with other tocolytics magnesium sulphate is more likely to cause maternal side effects leading to discontinuation of medication.[34] Based on the lack of efficacy, magnesium sulphate is not recommended as a tocolytic agent for women with preterm labour.

A recent network meta-analysis indicated that tocolysis with magnesium sulphate was effective in delaying delivery with 48 hours (OR 2.76; 95% CI 1.58 to 4.94), but did not affect perinatal mortality (OR 0.97; 95% CI 0.29 to 3.29) or RDS (OR 0.99; 95% CI 0.58 to 1.71). The network meta-analysis also indicated that the chance that magnesium sulphate would be the most effective tocolytic was very low.[10] A randomised controlled trial comparing magnesium sulphate to nifedipine and indomethacin indicated that there was no difference in tocolytic effectiveness between these drugs.[17] Although magnesium sulphate is a widely used tocolytic agent, there is no convincing evidence that magnesium sulphate is more safe and effective compared to nifedipine or atosiban. Therefore, magnesium sulphate is not recommended as a tocolytic drug of first choice.

2.5.2 Magnesium sulphate to enhance neonatal outcome

Magnesium sulphate may however have a positive effect on neonatal outcome. A Cochrane review included five trials (6145 babies) and showed that antenatal administration of magnesium sulphate reduced the risk of cerebral palsy (RR 0.68; 95% CI 0.54 to 0.87) and reduced the rate of substantial gross motor dysfunction in early childhood (RR 0.61; 95% CI 0.44 to 0.85).[35] No effect on paediatric mortality was seen (RR 1.04; 95% CI 0.92 to 1.17). Overall, apart from cerebral palsy and substantial gross motor dysfunction there were no significant differences found in the risk of other neurological impairments (developmental delay or intellectual impairment, blindness, deafness) or major neurological disabilities. Magnesium sulphate administration leads to minor maternal side effects compared to placebo. The number of women needed to be treated to prevent one case of cerebral palsy was 63 (95% CI 43 to 155). Further studies are warranted to determine the optimal regime.[36] Caution is advised since symptomatic hypocalcaemia has been reported after treatment with magnesium sulphate, especially in combination with nifedipine.[37] In conclusion, magnesium

suphate might be recommended for neuroprotection of the fetus but not as a tocolytic agent. Therefore the use of magnesium sulphate in preterm labour should be accompanied by tocolytic drugs, and caution is advised when combining these agents.

2.6 Progestagens

Progestagens such as progesterone and 17-alpha-hydroxyprogesterone (17-P) are thought to act by suppressing smooth muscle activity in the uterus and the inhibition of the formation of gap junctions between myometrial cells.[38,39] Studies on 17-P and vaginal progesterone as tocolytic treatment, maintenance and preventive therapy have been performed.

A review by Jayasoorya and Lamont[40] provides a comprehensive overview of the literature on progesterone and preterm birth. An early meta-analysis on prophylactic progesterone therapy in women at risk for preterm birth indicates that progesterone reduces the occurrence of preterm birth.[41]

2.6.1 Efficacy

Vaginal progesterone has a potential tocolytic effect by suppression of the prostaglandin cascade. A randomised controlled trial[42] comparing vaginal progesterone to no treatment in women with preterm labour showed that mean gestational age and mean time of postponing delivery were significantly higher in patients who used vaginal progesterone. Infants in the progesterone group compared to the no treatment group had a higher mean birth weight (2950.6 ± 420.3 vs. 2628.0± 385.1, P<0.001) and less NICU admissions (8.3% versus 23.6%; P < 0.001). However, it should be noted that patients also received magnesium sulphate in both groups.

It has been suggested that progestagens may not prevent preterm birth, but sensitize the uterus to tocolytic drugs.[40] The combination of high-dosage progesterone and β agonist has shown a synergistic effect by decreasing the need for high concentrations of β agonist, thereby leading to a reduction of side effects.[43] Two other studies with limited numbers showed that progesterone, when used with another tocolytic agent (atosiban or ritodrine), results in a reduction of preterm birth at less than 37 weeks of gestation.[44,45]

In-vitro studies with isolated myometrium strips indicate that natural progesterone increases the relaxant effect of ritodrine by reducing 50% of the maximal response, amplitude, and frequency of myometrial contraction.[46] A small trial including 70 patients suggested an additional benefit of vaginal progesterone as maintenance therapy in patients with preterm labour after arrest of uterine activity.[47] However, a recent larger randomized trial including 184 patients failed to confirm these results using intramuscular injections with 17P.[48]

The results should prompt further studies to investigate the possible role of progesterone in the sensitization of the uterus to other tocolytic drugs.

2.6.2 Progestagens in the prevention of preterm birth

The effect of progestagens as preventive treatment has been demonstrated in randomized clinical trials; progestagens reduced preterm birth in women with a previous preterm birth by 50%.[49,50] Besides, one of these trials found a decreased incidence of intraventricular haemorrhage in the progesterone treatment group.[49] A recent randomised controlled trial studied the effect of vaginal progesterone gel in 465 women with a short cervix in the mid trimester. Vaginal progesterone was associated with a 45% reduction of preterm birth before 28, 33 and 35 weeks of gestation. Besides, vaginal progesterone was associated with a significant reduction of respiratory distress rate and a composite outcome of neonatal morbidity and mortality.[51] In 2010, a Cochrane systematic review confirmed this effectiveness of progesterone in the prevention of preterm birth (risk ratio of 0.80; 95% CI 0.70 to 0.92).[52] Moreover, a meta-analysis indicated that in women with a short cervix who are known to have an increased risk for preterm birth, progesterone generated lower rates of delivery <33 weeks (12.4% vs. 22.0%; RR 0.58; 95% CI 0.42 to 0.80), lower rates of respiratory distress syndrome (6.1% vs. 12.5%; RR 0.48, (5% CI 0.30 to 0.76) and lower composite neonatal morbidity and mortality rates (9.7% vs. 17.3%; RR 0.57, 95% CI 0.40 to 0.81).[53] An individual patient data (6608 children) meta-analysis of our group on nine trials in multiple pregnancies using progesterone shows no effect of progestagens on adverse neonatal outcome in twin pregnancies (RR 1.05; 95% CI 0.91 to 1.2). However, this meta-analyses detected a potential effect on the preterm birth rate among women with a cervical length below 25mm in the second trimester, although numbers are too small to draw definite conclusions (41% vs. 61%, RR 0.67; 95% CI 0.40 to 1.1).[54]

2.6.3 Side effects

Some potential side effects need to be taken in consideration. Although masculinisation of the genital tract in female fetuses was feared in the past no such side effect has been found after thorough follow-up even when 17-α-hydroxyprogesterone caproate was administered in early pregnancy. The same applies for the incidence of hypospadias in male infants. No difference was observed between exposed and non-exposed infants.[55-57] Besides, a recent study showed no effect of progesterone injections in twin pregnancies on femur length, head and abdominal circumference and birth weight.[58]

In conclusion, the role of progesterone in treatment of acute tocolysis as primary or add-on therapy is limited. Progesterone might play a role in the prevention of preterm labour, or as sensitizer for other tocolytic agents.

3 Expert opinion

Preterm birth is the most common cause of neonatal morbidity and mortality worldwide. Prevention and treatment of preterm birth is therefore an important goal of obstetrical care. However, the pathophysiological mechanisms of preterm birth are partially unclear thereby hampering the development of successful preventive therapies and leading to large practice variation for treatment of women with threatened preterm labour.

3.1 Recommendations for current practice

To optimize outcome in threatened preterm labour, tocolysis is recommended for a period of 48 hours to allow for administration of corticosteroids to enhance fetal lung maturation and transport the patient to a centre with NICU facilities. For the use of acute tocolysis, atosiban or nifedipine are preferred based on the largest effectiveness in terms of delay of delivery, perinatal outcomes and most favourable side effect profile. Future studies should compare the safety and effectiveness of nifedipine and atosiban. Based on the knowledge summarized above, we currently perform a randomised comparison between nifedipine and atosiban (Dutch Trial Register NTR 2947) with neonatal outcome as primary outcome.[59] In the future, the most effective treatment from this study will be compared to prostaglandin synthetase inhibitors. Another potential important field of study is the on-going development of a new oxytocin receptor antagonist that might be more effective and safe than atosiban.

Beta-adrenoreceptor agonists are less effective than nifedipine and have more maternal side effects compared to nifedipine and atosiban and are abandoned from clinical practice. The literature on prostaglandin synthetase inhibitors is scarce, but the recent network meta-analysis from Haas et al.[11] indicates a potentially strong beneficial effect. Fetal side effects remain a concern with prostaglandin synthetase inhibitors and should be part of future trials.

Although magnesium sulphate is not recommended as a tocolytic drug, there may be a role for magnesium sulphate as neuroprotectant in the preterm infant. Therefore it is recommended as an additional drug to the treatment strategy in preterm labour consisting of tocolytic drugs and corticosteroids. The role of progesterone in treatment of acute tocolysis as primary therapy is limited at this moment since evidence is available from low quality and dated studies. It has been established that preventive progestagen treatment in women with a previous preterm birth and in women with a short cervical length at 16-22 weeks gestational age leads to a reduction in preterm birth.[49,50,52-54]

3.1 Maintenance tocolysis

Maintenance therapy, i.e. tocolytic therapy after acute initial preterm labour is arrested, is not recommended as there is no positive effect on neonatal outcome. A large randomised placebo controlled study has shown that maintenance tocolytic

therapy with nifedipine does not improve neonatal outcome.[60] A randomised placebo control trial indicates that patient who respond to early intravenous treatment with atosiban have a longer interval before the recurrence of labour when treated with atosiban maintenance therapy compared to placebo (median number of days: 32.6 vs. 27.6, p=0.2). However, no difference was found in the incidence of respiratory distress syndrome, intraventricular haemorrhage, patent ductus arteriosus and necrotizing enterocolitis.[61] A Cochrane review on maintenance therapy with magnesium sulfate included four trials, of which three trials had a high risk of bias and none included long-term follow-up of neonates.[62] The trials did not demonstrate any differences between magnesium maintenance therapy and placebo or other treatments in the incidence of preterm birth or perinatal mortality. Magnesium sulphate was associated with less tachycardia and palpitations compared to alternative tocolytics –mainly betamimetics-. The incidence of diarrhoea was higher in magnesium sulphate compared to other tocolytical agents. Maintenance therapy with micronized natural progesterone was associated with a longer mean latency period and a higher gestational age. However, also in this study no positive effects on neonatal outcome were found.[63] Possibly, these studies were too small to test the effect of tocolysis on neonatal outcome and further studies are recommended.

3.2 Prophylactic tocolysis in PPROM

In preterm premature rupture of the membranes, preterm delivery is frequently imminent. Prophylactic tocolytic therapy, i.e. tocolysis before the onset of regular contractions, may prevent preterm labour. Prophylactic tocolysis in women with PPROM remains subject of debate. Three early trials have demonstrated a marginal benefit of prophylactic tocolysis in short term prolongation of the latency period.[64] None of the studies reported improvement of neonatal outcome. A recent trial compared the use of prophylactic indomethacin versus placebo in women with PPROM.[65] Due to a slow inclusion rate this study was prematurely concluded. Based on the analysis of 47 patients, no differences were found in latency of labour or neonatal outcome. A retrospective analysis of 99 cases of PPROM[66] compared two different treatment strategies. The incidence of pathological chorioamnionitis and funisitis was significantly higher in the group were tocolytic therapy was continued until clinical chorioamnionitis was diagnosed. However, this retrospective study does not provide conclusive evidence to indicate whether prophylactic tocolysis is safe and beneficial. At the moment, we are performing a large randomised trial to compare nifedipine with placebo in women with PPROM without contractions (Dutch Trial Register NTR 3363).[67]

3.3 Prevention of preterm birth- beyond pharmacotherapy

Although this report focuses on pharmacological treatment, an important insight in the use of a cervical pessary in the prevention of preterm delivery should be pointed out. The PECEP trial showed that cervical pessary in women with short cervical length

reduced preterm birth before 34 weeks (6% versus 27%).[68] A recent study on the effectiveness of pessary in twin pregnancies indicated no treatment effect of pessary in the group of women with a twin pregnancy as a whole.[69] In the pre-specified subgroup of women with a cervix less than the 25th percentile (< 38 mm), the pessary group significantly reduced preterm delivery rates (11% vs. 25%, RR 0.44; 95% CI 0.20 to 0.98 for delivery less than 32 weeks) and reduced poor neonatal outcome rate as compared to no intervention (7% vs. 30%, RR 0.23; 95%CI 0.09 to 0.60). The use of pessary as a preventive measure in high risk patients such as patients with a short cervix or multiple pregnancies requires further investigation.

3.4 Recommendations for future studies

Insight in the underlying mechanism contributing to preterm birth might give direction to further research. Preterm birth starting with premature rupture of the membranes (PPROM) is associated with inflammation and might react different to tocolytic treatment compared to threatened preterm labour without PPROM. Future research should be directed toward therapies tailored to the specific underlying causes of preterm labour. Furthermore, studies on fetal fibronectin as a predictor for the occurrence of preterm birth in women with threatened preterm labour may enable physicians to target high risk patients and avoid unnecessary treatment.[70] Tocolytic treatment in selected high risk patients might alter safety and effectiveness profiles of tocolytic drugs.

Preterm birth is an important health care problem, however there is still much uncertainty about the effectiveness of medical treatment in preterm labour. In particular, there are great uncertainties on the effectiveness of tocolytic therapy to improve neonatal outcome. Postponing delivery enables the administration of corticosteroids, which has unequivocally been demonstrated to reduce neonatal morbidity and mortality.[71] However, tocolytic drugs could have unfavourable side effects that might abolish the beneficial effect of corticosteroids. The ultimate goal of tocolytic therapy is improvement of neonatal outcome, not primarily prolonging gestational age. Current studies frequently lack power to detect differences in neonatal outcome. In view of the absence of data that convincingly indicate the beneficial effect of tocolytics on neonatal outcome, it might well be that tocolytics are ineffective. Future studies should be powered to detect effects –either positive or negative- on neonatal outcome. With regard to the different outcome measures, it is important that there will be consensus on the outcome measures to be used to enhance comparability of results. Economic analysis should be taken into account the costs of side effects. Besides, long term follow-up is valuable to determine the best tocolytic therapy in women with preterm labour. On the basis of the results of studies on neonatal outcome, the effectiveness of the existing therapy could be improved.

References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371:75-84.
2. Landelijke Neonatale Registratie 2003. Dutch Neonatal Database 2002. Prismant 2002.
3. Petrou S, Eddama O, Mangham L. A structured review of the recent literature on the economic consequences of preterm birth. Arch Dis Child Fetal Neonatal Ed 2011;96:225-232.
4. Romero R, Espinoza J, Kusanovic JP, et al. The preterm parturition syndrome. BJOG 2006;113:17-42.
5. de Laat MW, Pieper PG, Oudijk MA, et al. The clinical and molecular relations between idiopathic preterm labor and maternal congenital heart defects. Reprod Sci 2013;20:190-201.
6. Roberts D, Dalziel Z. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev. 2006;CD004454.
7. Huszar G, Roberts JM. Biochemistry and pharmacology of the myometrium and labor: regulation at the cellular and molecular levels. Am J Obstet Gynecol 1982;142:225-37.
8. Anotayanonth S, Subhedar NV, Garner P, et al. Betamimetics for inhibiting preterm labour. Cochrane Database Syst Rev 2004;CD004352.
9. Papatsonis D, Flenady V, Cole S, Liley H. Oxytocin receptor antagonists for inhibiting preterm labour. Cochrane Database Syst Rev 2005;CD004452.
10. Haas DM, Caldwell DM, Kirkpatrick P, et al. Tocolytic therapy for preterm delivery: systematic review and network meta-analysis. BMJ 2012;345:e6226.
11. de Heus R, Mol BW, Erwich JJ, et al. Adverse drug reactions to tocolytic treatment for preterm labour: prospective cohort study. BMJ 2009;338:b744
12. Hayes E, Moroz L, Pizzi L, Baxter J. A cost decision analysis of 4 tocolytic drugs. Am J Obstet Gynecol. 2007;197:383.e1-6.
13. King JF, Flenady VJ, Papatsonis DN, et al. Calcium channel blockers for inhibiting preterm labour. Cochrane Database Syst Rev 2003;CD002255.
14. Lyndrup J, Lamont RF. The choice of a tocolytic for the treatment of preterm labor: a critical evaluation of nifedipine versus atosiban. Expert Opin Investig Drugs. 2007;16:843-53.
15. Lamont RF, Khan KS, Beattie B et al. The quality of nifedipine studies used to assess tocolytic efficacy: a systematic review. J Perinat Med. 2005;33:287-95.
16. Salim R, Garmi G, Nachum Z, et al. Nifedipine compared with atosiban for treating preterm labor: a randomized controlled trial. Obstet Gynecol 2012;120:1323-31.
17. Klauser CK, Briery CM, Martin RW et al. A comparison of three tocolytics for preterm labor: a randomized clinical trial. J Matern Fetal Neonatal Med. 2013 Oct 11.
18. Klauser CK, Briery CM, Keiser SD et al. Effect of antenatal tocolysis on neonatal outcomes. J Matern Fetal Neonatal Med. 2012;25:2778-81.
19. Khan K, Zamora J, Lamont RF et al. Safety concerns for the use of calcium channel blockers in pregnancy for the treatment of spontaneous preterm labour and hypertension: a systematic review and meta-regression analysis. J Matern Fetal Neonatal Med. 2010;23:1030-1038.
20. van Veen AJ, Pelinck MJ, van Pampus MG, Erwich JJ. Severe hypotension and fetal death due to tocolysis with nifedipine. BJOG. 2005;112:509-510.
21. Papatsonis DN, Carbonne B, Dekker GA et al. Severe hypotension and fetal death due to tocolysis with nifedipine. BJOG. 2005;112:1582-1583.
22. Houtzager BA, Hogendoorn SM, Papatsonis DN, et al. Long-term follow up of children exposed in utero to nifedipine or ritodrine for the management of preterm labour. BJOG 2006;113:324-331.
23. Coomarasamy A, Knox EM, Gee H, et al. Effectiveness of nifedipine versus atosiban for tocolysis in preterm labour: a meta-analysis with an indirect comparison of randomised trials. BJOG 2003;110:1045-1049.
24. www.studies-obsgyn.nl
25. Husslein P, Roura L, Dudenhausen J et al. Clinical practice evaluation of atosiban in preterm labour management in six European countries. BJOG 2006;113:105–110.
26. Seinen LH, Simons SO, van der Drift MA et al. Maternal pulmonary oedema due to the use of atosiban in cases of multiple gestation. Ned Tijdschr Geneeskd. 2013;157:A5316.
27. The Worldwide Atosiban versus Beta-agonists Study Group Participants. Effectiveness and safety of the oxytocin antagonist atosiban versus beta-adrenergic agonists in the treatment of preterm labour. BJOG 2001;108:133–142.
28. Romero R, Sibai BM, Sanchez-Ramos L, et al. An oxytocin receptor antagonist (atosiban) in the treatment of preterm labor: a randomized, double-blind, placebo-controlled trial with tocolytic rescue. Am J Obstet Gynecol 2000;182:1173-1183.

29. Wex J, Abou-Setta AM, Clerici G, Di Renzo GC. Atosiban versus betamimetics in the treatment of preterm labour in Italy: clinical and economic importance of side-effects. Eur J Obstet Gynecol Reprod Biol. 2011;157:128-35.
30. Pierzynski P, Lemancewicz A, Reinheimer T et al. Inhibitory effect of barusiban and atosiban on oxytocin-induced contractions of myometrium from preterm and term pregnant women. J Soc Gynecol Investig. 2004;11:384-7.
31. Thornton S, Goodwin TM, Greisen G et al. The effect of barusiban, a selective oxytocin antagonist, in threatened preterm labor at late gestational age: a randomized, double-blind, placebo-controlled trial. Am J Obstet Gynecol. 2009;200:627.e1-10.
32. Steinwall M, Bossmar T, Brouard R et al. The effect of relcovaptan (SR 49059), an orally active vasopressin V1a receptor antagonist, on uterine contractions in preterm labor. Gynecol Endocrinol. 2005;20:104-109.
33. King J, Flenady V, Cole S, Thornton S. Cyclo-oxygenase (COX) inhibitors for treating preterm labour. Cochrane Database Syst Rev. 2005;18:CD001992.
34. Crowther CA, Hiller JE, Doyle LW. Magnesium sulphate for preventing preterm birth in threatened preterm labour. Cochrane Database Syst Rev 2002;CD001060.
35. Doyle LW, Crowther CA, Middleton P, et al. Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus. Cochrane Database Syst Rev 2009;CD004661.
36. Bain E, Middleton P, Crowther CA. Different magnesium sulphate regimens for neuroprotection of the fetus for women at risk of preterm birth. Cochrane Database Syst Rev. 2012;15:CD009302.
37. Koontz SL, Friedman SA, Schwartz ML. Symptomatic hypocalcemia after tocolytic therapy with magnesium sulfate and nifedipine. Am J Obstet Gynecol. 2004;190:1773-1776.
38. Garfield RE, Kannan MS, Daniel EE. Gap junction formation in myometrium: control by estrogens, progesterone, and prostaglandins. Am J Physiol. 1980;238:C81-89.
39. Hendrix EM, Myatt L, Sellers S. et al. Steroid hormone regulation of rat myometrial gap junction formation: effects on cx43 levels and trafficking. Biol Reprod. 1995;52:547-560.
40. Jayasooriya GS, Lamont RF. The use of progesterone and other progestational agents to prevent spontaneous preterm labour and preterm birth. Expert Opin Pharmacother. 2009;10:1007-1016.
41. Keirse MJ. Progestogen administration in pregnancy may prevent preterm delivery. Br J Obstet Gynaecol. 1990;97:149-154.
42. Saleh Gargari S, Habibolahi M, Zonobi Z. Outcome of vaginal progesterone as a tocolytic agent: randomized clinical trial. ISRN Obstet Gynecol. 2012;2012:607906.
43. Di Renzo GC, Rosati A, Mattei A et al. The changing role of progesterone in preterm labour. BJOG. 2005;112:57-60.
44. Facchinetti F, Paganelli S, Comitini G, et al. Cervical length changes during preterm cervical ripening: effects of 17-alpha-hydroxyprogesterone caproate. Am J Obstet Gynecol 2007;196:453-454.
45. Noblot G, Audra P, Dargent D, et al. The use of micronized progesterone in the treatment of menace of preterm delivery. Eur J Obstet Gynecol Reprod Biol 1991;40:203-209
46. Chanrachakul B, Broughton Pipkin F, Warren AY et al. Progesterone enhances the tocolytic effect of ritodrine in isolated pregnant human myometrium. Am J Obstet Gynecol. 2005;192:458-463.
47. Borna S, Sahabi N. Progesterone for maintenance tocolytic therapy after threatened preterm labour: a randomised controlled trial. Aust N Z J Obstet Gynaecol 2008;48:58-63.
48. Rozenberg P, Chauveaud A, Deruelle P, et al. Prevention of preterm delivery after successful tocolysis in preterm labor by 17 alpha-hydroxyprogesterone caproate: a randomized controlled trial. Am J Obstet Gynecol 2012;206:206-209.
49. da Fonseca EB, Bittar RE, Carvalho MH, Zugaib M. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: a randomized placebo-controlled double-blind study. Am J Obstet Gynecol 2003;188:419-424.
50. Meis PJ, Klebanoff M, Thom E, et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. N Engl J Med 2003;348:2379-2385.
51. Hassan SS, Romero R, Vidyadhari D et al. Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: a multicenter, randomized, double-blind, placebo-controlled trial.Ultrasound Obstet Gynecol. 2011;38:18-31.
52. Su LL, Samuel M, Chong YS. Progestational agents for treating threatened or established preterm labour. Cochrane Database Syst Rev 2010;CD006770.
53. Romero R, Nicolaides K, Conde-Agudelo A et al. Vaginal progesterone in women with an asymptomatic sonographic short cervix in the midtrimester decreases preterm delivery and neonatal morbidity: a systematic review and metaanalysis of individual patient data. Am J Obstet Gynecol. 2012;206:124.e1-19.

54. Schuit E, Stock S, Groenwold RH, et al. Progestogens to prevent preterm birth in twin pregnancies: an individual participant data meta-analysis of randomized trials. BMC Pregnancy Childbirth 2012;12:13.
55. Resseguie LJ, Hick JF, Bruen JA, et al. Congenital malformations among offspring exposed in utero to progestins, Olmsted County, Minnesota, 1936-1974. Fertil Steril 1985;43:514-519.
56. Yovich JL, Turner SR, Draper R. Medroxyprogesterone acetate therapy in early pregnancy has no apparent fetal effects. Teratology 1988;38:135-144.
57. Katz Z, Lancet M, Skornik J, et al. Teratogenicity of progestogens given during the first trimester of pregnancy. Obstet Gynecol 1985;65:775-780.
58. Mulder EJ, Versteegh EM, Bloemenkamp KW et al. Does 17-α-hydroxyprogesterone caproate affect fetal biometry and birth weight in twin pregnancy? Ultrasound Obstet Gynecol. 2013;42:329-34.
59. Assessment of Perinatal Outcome by use of Specific Tocolytics in Early Labour (APOSTEL III) - NTR 2947.
60. Roos C, Spaanderman ME, Schuit E, et al. Effect of maintenance tocolysis with nifedipine in threatened preterm labor on perinatal outcomes: a randomized controlled trial. JAMA 2013;309:41-47.
61. Valenzuela GJ, Sanchez-Ramos L, Romero R et al. Maintenance treatment of preterm labor with the oxytocin antagonist atosiban. The Atosiban PTL-098 Study Group. Am J Obstet Gynecol. 2000;182:1184-1190.
62. Han S, Crowther CA, Moore V. Magnesium sulphate for preventing preterm birth after threatened preterm labour. Cochrane Database Syst Rev 2013; CD000940.
63. Arikan I, Barut A, Harma M, Harma IM. Effect of progesterone as a tocolytic and in maintenance therapy during preterm labor. Gynecol Obstet Invest. 2011;72:269-273.
64. Aagaard-Tillery KM, Nuthalapaty FS, Ramsey PS, Ramin KD. Preterm premature rupture of membranes: perspectives surrounding controversies in management. Am J Perinatol. 2005;22:287-297.
65. Ehsanipoor RM, Shrivastava VK, Lee RM et al. A randomized, double-masked trial of prophylactic indomethacin tocolysis versus placebo in women with premature rupture of membranes. Am J Perinatol. 2011 Jun;28:473-478.
66. Shinjo A, Otsuki K, Sawada M et al. Retrospective cohort study: a comparison of two different management strategies in patients with preterm premature rupture of membranes. Arch Gynecol Obstet. 2012;286:337-345.
67. Assessment of Perinatal Outcome by use of Specific Tocolytics in Early Labour (APOSTEL VI) - NTR 3363
68. Goya M, Pratcorona L, Merced C, et al. Cervical pessary in pregnant women with a short cervix (PECEP): an open-label randomised controlled trial. Lancet 2012;379:1800-1806.
69. Liem S, Schuit E, Hegeman M. et al. Cervical pessaries for prevention of preterm birth in women with a multiple pregnancy (ProTWIN): a multicentre, open-label randomised controlled trial. Lancet. 2013;382:1341-1349.
70. Vis JY, Wilms FF, Oudijk MA et al. Cost-effectiveness of fibronectin testing in a triage in women with threatened preterm labor: alleviation of pregnancy outcome by suspending tocolysis in early labor (APOSTEL-I trial). BMC Pregnancy Childbirth. 2009;9:38.
71. Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev. 2006;19:CD004454.

Chapter 7

Effectiveness of maintenance tocolysis with nifedipine in the reduction of adverse perinatal outcome in imminent preterm labor: an individual participant data meta-analysis

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Abstract

Objective To evaluate the effectiveness of maintenance tocolytic therapy with oral nifedipine on the reduction of adverse neonatal outcomes and prolongation of pregnancy by performing an individual patient data meta-analysis (IPDMA).
Data resources We searched PubMed, Embase and Cochrane databases for randomized controlled trials of maintenance tocolysis therapy with nifedipine in preterm labor.
Study eligibility criteria We selected trials including pregnant women between 24 and 36 $^{6/7}$ weeks of gestation with imminent preterm labor who had not delivered after 48 hours of initial tocolysis and compared maintenance nifedipine tocolysis with placebo or no treatment.
Study appraisal and synthesis methods Studies were reviewed for eligibility by 2 authors. Investigators of identified trials were asked to share their IPD. The primary outcome was perinatal mortality. Secondary outcome measures were IVH, NEC, IRDS, prolongation of pregnancy, gestational age at delivery, birth weight, neonatal intensive care unit admission and days on ventilation support. Pre-specified subgroup analyses were performed.
Results Six randomized controlled trials were found to be eligible and all agreed to participate in this IPDMA. Three trials compared maintenance nifedipine tocolysis to no treatment, and three compared nifedipine to placebo. In total, data of 787 patients (390 allocated to nifedipine and 397 allocated placebo/no treatment) were analyzed. Baseline demographics and clinical characteristics between studied groups were comparable. There was no difference between the nifedipine and control group for the incidence of perinatal death, (RR 1.2; 95%CI 0.44-3.4), NEC (RR 1.2; 95% CI 0.52-2.6), RDS (RR. 1.00; 95% CI 0.50-2.0) and IVH ≥ grade 2 (RR 0.66; 95% CI 0.14-3.19 95%CI). Prolongation of pregnancy was similar in both groups (HR 0.86; 95% CI 0.71-1.03). No differences were found for the other secondary outcomes. Within a subgroup of singleton pregnancies, nifedipine was associated with a longer prolongation of pregnancy when compared to placebo / no treatment (median difference 5 days; HR 0.80; 95% CI 0.66-0.99), but not with a lower incidence of perinatal mortality.
Conclusion According to the best available evidence, nifedipine maintenance tocolysis is not associated with improved perinatal outcome. Therefore maintenance tocolysis with nifedipine is not recommended for routine practice.

Introduction

Preterm birth, defined as birth before 37 weeks of gestation, has an incidence between 5% and 11%, and the rates are increasing.[1,2] Preterm birth is the leading cause of neonatal mortality and morbidity worldwide. In developed countries, 75% of perinatal deaths and more than half of childhood neurological morbidities are caused by preterm birth.[3]Besides the high burden of disease for child and family, preterm birth results in high costs for medical care, health insurance, social support systems and special education.[4,5]

To improve outcome, women with imminent preterm labor receive tocolytic therapy and antenatal corticosteroids in most perinatal care centers. The goal of initial tocolysis is to delay delivery for at least 48 hours, as this allows antenatal corticosteroid treatment to improve fetal lung maturation and maternal transfer to a tertiary health care center with a neonatal intensive care unit (NICU).[6]

Whether continued tocolysis after 48 hours further improves neonatal outcome is subject to debate. Because of its oral administration and minimal side effects the calcium antagonist nifedipine could be a potential tocolytic for maintenance therapy.[7] Recently, an aggregated data meta-analysis on nifedipine maintenance tocolysis was published.[8] This study found a prolongation of pregnancy in women treated with nifedipine maintenance tocolysis (mean difference (MD) 5.35 days; 95% CI 0.49 to 10.21), based on 4 trials. No improvements of maternal or infant outcomes were found. Where meta-analyses use aggregated data from published articles and therefore have difficulties pooling data of included studies due to different outcome and subgroup definitions, an individual patient data meta-analysis (IPDMA) uses individual level data to overcome these problems. Individual data can be merged into one database, which allows analysis of specific subgroups with a possible higher risk of adverse outcome.[9-11] Moreover, performing an IPDMA avoids bias caused by selective reporting, since all collected data is included and it provides standardization of in- and exclusion criteria across studies.[9] By performing an IPDMA more reliable conclusions on the effect of maintenance nifedipine tocolysis are provided.

Objective

We performed an Individual Participant Data Meta-Analysis including randomized clinical trials on nifedipine maintenance tocolysis compared to placebo / no treatment in women with preterm labor. This study aims to provide clinicians with the best available evidence on the effectiveness of nifedipine maintenance tocolysis.

Methods

Sources and search strategy

This IPDMA was conducted according to a prospectively prepared protocol (available upon request) following the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines for meta-analysis of randomized controlled trials.[12]

We searched PubMed, Embase and Cochrane databases for trials of maintenance tocolysis therapy with nifedipine in preterm labor. We used the key words "preterm", "labor" and "nifedipine". The complete list of search terms is presented in the Appendix. Furthermore, we searched clinicaltrial.gov and controlled-trials.com for ongoing or unpublished studies. The last search was conducted on September 28th 2014.

Eligibility criteria and study selection

The following preselected criteria justified inclusion in this IPD analysis: (1) the study included women between 24 $^{0/7}$ and 36 $^{6/7}$ weeks of gestation with threatened preterm labor; (2) in a randomized controlled trial comparing maintenance tocolysis by oral nifedipine with placebo or no treatment; (3) the intervention was started after an initial course of tocolytics and a completed course of corticosteroids.

Corresponding authors of eligible studies were approached and asked to participate in the IPDMA and to send their original study database. The original databases were pooled into one database and assessed for quality (e.g. discrepancies between published and shared data). In cases where study quality was not clear from the published article or trial protocol, the authors were contacted for clarification. The risk of bias of the included studies was assessed independently by two reviewers (G.D. and E.v.V.) and by the authors of the original study using the risk of bias tool developed by the Cochrane Collaboration.[13] This includes assessment of adequate sequence generation, allocation concealment, blinding of participants and personnel and blinding for outcome measures, incomplete outcome data and other sources of bias.[13] Selective reporting of outcome was not considered as a relevant bias in this IPDMA, since original databases are used instead of reported outcomes.

Outcomes

The primary outcome was perinatal death, defined as intra-uterine fetal death and death of the neonate before discharge. Neonatal morbidity was evaluated as a secondary outcome, including intraventricular hemorrhage (IVH) ≥ grade II, necrotizing enterocolitis (NEC) and infant respiratory distress syndrome (IRDS). We created a composite score of perinatal death and these morbidities. Other secondary outcomes were prolongation of pregnancy, gestational age at delivery, birth weight, length of neonatal intensive care unit (NICU) admission and days on ventilation support. Prolongation of pregnancy was calculated as the number of days between date of randomization and date of delivery.

Subgroups

Pre-established subgroup analyses were performed to assess the effect of maintained nifedipine treatment within the following groups (1) intact or ruptured membranes at study enrollment, (2) singleton or multiple gestation, (3) cervical length <25mm at study enrollment and (4) gestational age at randomization (classified in 24 $^{0/7}$-25 $^{6/7}$ weeks, 26 $^{0/7}$ – 27 $^{6/7}$ weeks, 28 $^{0/7}$ – 29 $^{6/7}$ weeks, 30 $^{0/7}$ -31 $^{6/7}$ weeks, 32 $^{0/7}$ – 33 $^{6/7}$ weeks, 34 $^{0/7}$ – 36 $^{6/7}$ weeks). Subgroup analyses were performed for the primary outcomes and prolongation of pregnancy only.

Statistical Analyses

Analyses were conducted on an intention to treat basis. Data of baseline characteristics were collected and comparability between the two study groups was assessed within each study and in the complete dataset. Continuous variables were presented as means with standard deviations (SD) or as medians or geometric means with interquartile ranges (IQR) as appropriate. Dichotomous or categorical variables were presented as the number and percentage of the study specific or total study population. For binomial outcomes, we used a mixed model with a log link, thus resulting in risk ratios (RR) with 95% confidence interval (CI). These models included a random intercept to account for differences in prevalence between studies, and a random slope to account for differences in treatment effect between studies. In the analysis on child level, we incorporated a compound symmetric residual error variance to account for clustering of children within one mother.[14] Continuous outcomes were analyzed using a linear mixed model to compare means or linear quantile mixed models to compare medians. Time-to-delivery analysis was performed with Cox proportional hazards regression analysis, resulting in a hazard ratio (HR) with 95% CI, in which dependency between data originating from the same study was taken into account by including a study identifier as strata.

Heterogeneity across trials was assessed using the I^2 measure and the values were interpreted as follows: 0% indicates no observed heterogeneity; 25%, 50%, and 75% indicate low, moderate, and high heterogeneity, respectively.[15] We calculated the Number Needed to Treat (NNT) for all significant associations.

Subgroup effects were investigated using an interaction term between the subgroup and the treatment in the regression model. When the interaction was found to be significant ($p < 0.10$), we performed a stratified analysis to investigate the effect of nifedipine treatment versus control treatment in different strata of the subgroups. Statistical analyses were performed using R software, Version 2.15.2 (The R Foundation for Statistical Computing, 2012), SAS software, Version 9.2 (SAS Institute Inc., 2011) and SPSS software, Version 20 (IBM SPSS Statistics, 2011).

Results

Study selection

The literature search resulted in 439 studies of which 21 were considered potentially eligible. A search on Clinicaltrials.gov resulted in two more suitable trials; however, one of these had not yet started with inclusions and the second had been terminated for unknown reasons. Details on study selection and reasons for exclusion are specified in Figure 1. Six eligible trials remained after the extensive search.[16-21] Details of the search strategy are available upon request. Research groups of these trials were approached and they all agreed to participate in the IPDMA. Their original databases were used to construct the IPDMA dataset.

Study characteristics

We included 6 trials encompassing data of 390 women treated with nifedipine maintenance tocolysis and 397 women with placebo or no treatment. Three trials evaluated maintenance nifedipine treatment against no treatment[17,19,20] and three trials evaluated maintenance nifedipine treatment against placebo.[16,18,21] The sample size ranged from 64[21] to 406[16] women. *Carr et al.* [17], *Lyell et al.*[18], *Uma et al.*[19], *Sayin et al.*[20] and *Parry et al.*[21] primarily assessed for prolongation of pregnancy, whereas *Roos et al.*[16] used a composite of adverse neonatal outcomes as primary outcome measure. The study of *Roos et al.*[16] included women with and without ruptured membranes, while all other trials excluded women with ruptured membranes at first examination. *Uma et al.* and *Parry et al.* [19,21] excluded multiple gestation pregnancies, while the other trials included both singleton and multiple gestation pregnancies. Data concerning the second twin could not be retrieved for the study of *Carr et al.*.[17] The trial of *Parry et al.* [21]only included women with a positive fetal fibronectin test.

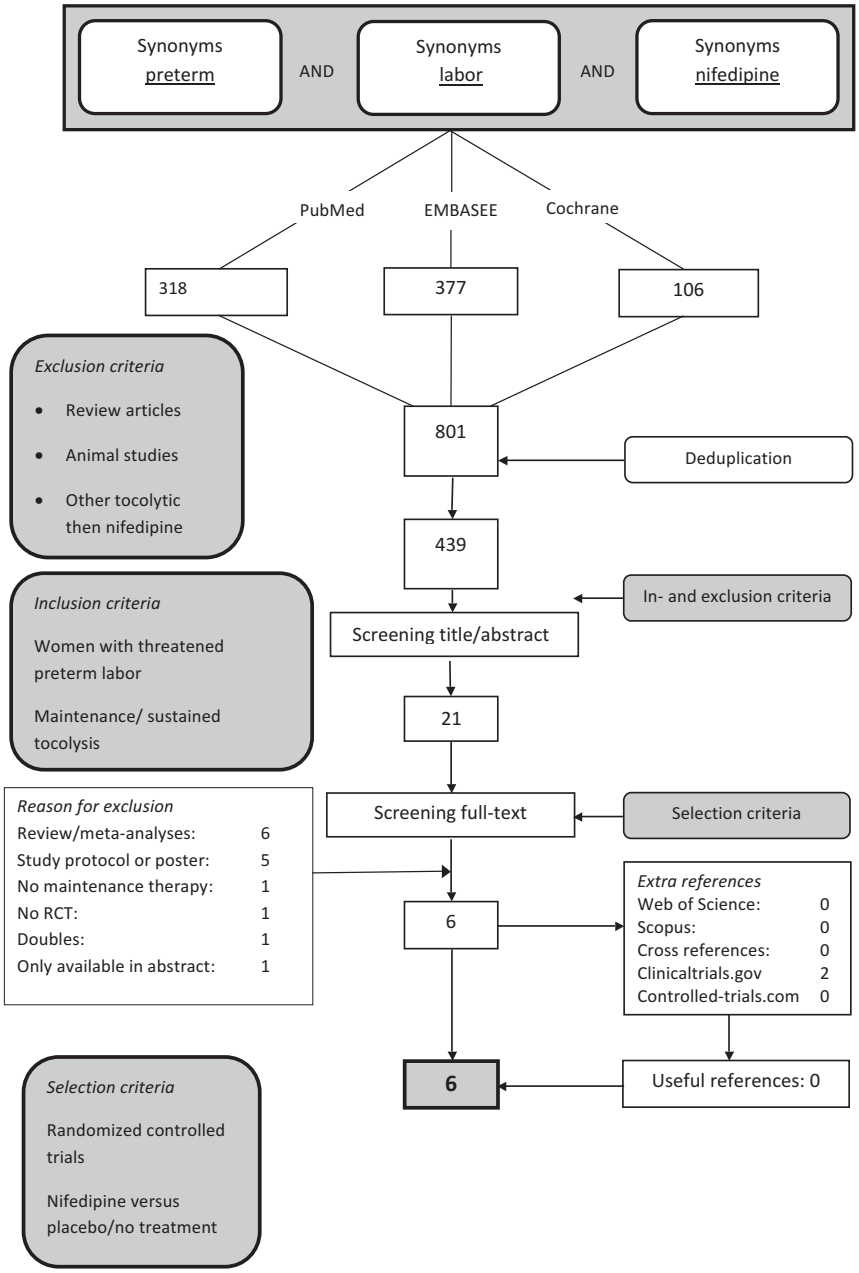
Risk of bias within studies

Overall, the risk of bias was considered moderate according to the reviewer's and author's judgment (Appendix). The risk of performance bias and detection bias were scored high for the studies of *Carr et al.*, *Sayin et al.* and *Uma et al.* since these trials were not placebo controlled. Within the trial of *Sayin et al.* randomization was performed sequentially by physicians instead of computerized randomization.

Heterogeneity across studies

There was a moderate heterogeneity for gestational age at delivery (I^2 49%), prolongation in pregnancy (I^2 51%) and duration of ventilation support (I^2 57%). For IRDS, there was low heterogeneity across studies (I^2 34%). All other outcome measures were homogeneously distributed across studies.

Figure 1. Flow Chart.



Search conducted on September 28th 2014

Table 1. Baseline Demographics and Clinical Characteristics IPDMA.

	IPDMA	
Characteristics	**Nifedipine (n=390)**	**Placebo/ no treatment (n=397)**
Age, mean (SD) years	28.7(5.8)	28.8 (5.7)
Race, n (%)		
Caucasian	244 (63)	236 (60)
Asian	57 (15)	58 (15)
African	30 (8)	35 (9)
Other	50 (13)	60 (15)
Nulliparous, n (%)	201 (52)	178 (45)
Prior preterm birth, n (%)	59 (24)	80 (31)
Gestational age at study entry, mean (SD) weeks	30.1 (2.6)	29.9 (2.4)
Multifetal gestation, n (%)	59 (15)	73 (18)
pPROM at study entry,	55 (14)	53 (13)
Vaginal examination at study entry, median (IQR)		
Dilatation (cm)	1.0 (0.0-2.0)	1.0 (0.0-2.0)
Effacement (%)	50 (50 –75)	50 (50-75)
Length (mm)	23 (15 -35)	20 (15-30)

Abbreviations: SD, standard deviation; IQR, interquartile range; NA, not available; pPROM, premature preterm rupture of membranes.

Results of IPD (synthesis of studies)

Baseline characteristics for the maintenance nifedipine treatment group and control group were comparable, both separately per study and overall (Table 1).

Maternal and perinatal outcomes for the individual trials and in the overall IPD are presented in eTable 4 and Table 2. The incidence of perinatal death was 2% in the nifedipine group and 1% in the control group (RR 1.2; 95%CI 0.44-3.4; Table 2). No differences were found between the nifedipine and control group for the incidence of IVH ≥ grade 2 (1% vs. 2%; RR 0.66; 95% CI 0.14-3.19 95%CI), NEC (2% vs. 2%; RR 1.2; 95% CI 0.52-2.6), IRDS (15% vs. 15; RR. 1.00; 95% CI 0.50-2.0), nor in the incidence of the composite of poor neonatal outcome (18% vs. 18%; RR 0.96; 95% CI 0.59-1.56). Prolongation of pregnancy was similar in both groups with a median prolongation of 33 days (IQR 13-55) in the nifedipine group and 30 days (IQR 7.8-55) in the control group (HR 0.86; 95% CI 0.71-1.03). The Kaplan Meier curve (Figure 2) demonstrated a log-rank test with *p*-value of 0.11 indicating no difference in prolongation of pregnancy between both groups. Furthermore no differences were found between the two groups for gestational age at delivery, birth weight, NICU admission rates and length of admission in days and duration of ventilation support (Table 2).

Table 2. Maternal and perinatal outcomes for IPD meta analysis

	IPDMA			
Characteristics	**Nifedipine (n=390)**	**Placebo/ notreatment (n=397)**	**Statistical analysis**	
Maternal Outcomes			**HR(95%CI)**	**i^2 [%]**
Gestational age at delivery, median (IQR) weeks	36.0 (32.6-38.0)	35.4 (32.0-37.9)	0.87 (0.72-1.04)	49
Prolongation of pregnancy, median (IQR) days	33 (13-55)	30 (7.8-55)	0.86 (0.71-1.03)	51
Perinatal Outcomes[a]	**(n=449)**	**(n=446)**	**Mean difference (95% CI)**	
Birth weight, mean (SD), grams	2398 (827)	2371 (817)	35 (-8.3; 79)	0
			RR (95% CI)	
Perinatal death, n (%)	7 (2)	6 (1)	1.2 (0.44-3.4)	0
IVH, n (%)[b]	5 (1)	9 (2)	0.66 (0.14-3.19)	0
NEC, n (%)[b]	8 (2)	7 (2)	1.2 (0.52-2.6)	0
IRDS, n (%)[b]	52 (15)	54 (15)	1.0 (0.5-2.0)	34
Pooroutcome, n (%)[b,c]	64 (18)	63 (18)	0.96 (0.59-1.56)	
NICUadmission, n (%)[d]	157 (37)	157 (38)	0.94 (0.82-1.06)	0
Ventilationsupport, n (%)[e]	89 (33)	85 (30)	1.1 (0.80-1.38)	57
			Median difference (95%CI)	
NICU admission length, geometric mean (IQR) days[d]	10 (5-23)	10 (5-24)	-0.03 (-3.5; 3.4)	0
Ventilation support length, median (IQR), days[e]	3 (2-7)	3 (2-6)	-0.02 (-1.9; 1.8)	0

Abbreviations: IQR, interquartile range; SD, standard deviation; HR, HazardRatio; RR, risk ratio; CI, confidence interval; NA, notavailable; IVH, intraventricular hemorrhage ≥ grade II; NEC, necrotizing enterocolitis; IRDS, infant respiratory distress syndrome; NICU, neonatal intensive care unit.

[a] Data are calculated on neonatal level, in case of data from Carr et al. this was only the first twin.

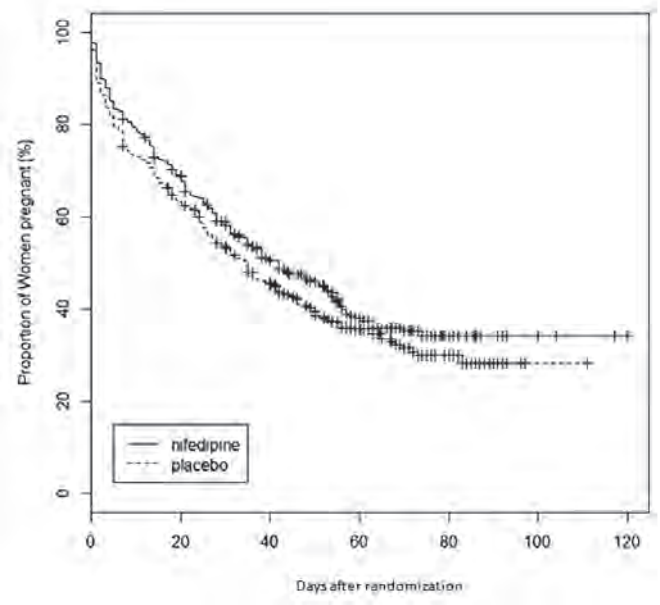
[b] Variable contains no data from the trials of Uma et al. and Parry et al.

[c] Composite of perinatal death, IVH, NEC and IRDS.

[d] NICU admission length contains no data of the trial of Sayin et al. Percentages are calculated from total amount of neonates that were admitted to a NICU

[e] Ventilation support contains only data from the trials of Roos et al.,Carr et al. and Lyell et al.

Figure 2. Prolongation of pregnancy Maintenance nifedipine tocolysis compared with control group.



Data are based on Kaplan-Meier analysis. Hazard ratio of prolongation of pregnancy 0.85 (95% CI 0.71-1.02).

Subgroup analysis

Nifedipine had a significant interaction ($p < 0.10$) with the following subgroups (Table 3): (1) multiple gestation pregnancy for the outcomes perinatal death (p-value 0.050) and prolongation of pregnancy (p-value 0.09), and (2) cervical length ≤ 25 mm for perinatal death (p-value 0.045). Stratification based on whether the pregnancy was singleton or multiple gestational pregnancy showed that in singleton pregnancies nifedipine was associated with a longer prolongation of pregnancy when compared to placebo / no treatment (median of 35 days IQR 14-56 vs. 30 IQR 8.0-57; HR 0.80; 95% CI 0.66-0.99). Stratified subgroup analysis for perinatal death according to cervical length indicated no benefit of nifedipine over placebo / no treatment in women with cervical length < 25 mm or ≥ 25 mm. No interactioneffect of nifedipine was found for the subgroups intact or ruptured membranes at study entry, or gestational age at study entry.

Table 3. Subgroup analysis

Subgroup		Outcome	Nifedipine	Placebo / no treatment	RR (95% CI)	*p*-value for interaction
Multiple gestation	Yes	Perinatal death, n (%)	3/107	1/125	3.7 (0.76-17.6)	0.05
		Prolongation of pregnancy, median (IQR) days	25 (8.5-41)	30 (7.0-46)	1.13 (0.77-1.66)[a]	0.09
	No	Perinatal death, n (%)	4/244	5/240	0.78 (0.21-2.87)	-
		Prolongation of pregnancy, median (IQR) days	35 (14-56)	30 (8.0-57)	0.80 (0.66-0.99)	-
Cervical length < 25 mm	Yes	Perinatal death, n (%)	3/114	1/126	NC	0.045
	No	Perinatal death, n (%)	2/84	2/76	0.82 (0.20-3.3)	-

Abbreviations: NC: not calculated due to very large confidence interval; pPROM, premature preterm rupture of membranes; IQR, interquartile range. [a] Hazard Ratio (95% Confidence Interval)

Comment

This IPDMA on six randomized clinical trials encompassing data on 787 women with imminent preterm labor and 922 infants showed that nifedipine maintenance tocolysis does not improve adverse neonatal outcome or prolongation of pregnancy when compared with placebo/no treatment. In a subgroup analysis of singleton pregnancies, nifedipine maintenance tocolysis was associated with a longer prolongation of pregnancy when compared to placebo/no treatment (median days (IQR) 35 (14-56) vs. 30 (8.0-57); HR 0.80).

Strengths and limitations

There are several limitations that need consideration. First, because not all studies evaluated neonatal morbidities and incidence of severe morbidities is rather low, there remains a somewhat limited power to detect differences in IVH, NEC and IRDS. Initially, a composite score of adverse perinatal outcome was chosen as outcome of primary interest, consisting of perinatal death and neonatal morbidity (IVH, NEC and IRDS). However, since 2 trials[19,21] did not report data on neonatal morbidity, we decided to take perinatal death as the primary outcome measure. Information concerning need and length of ventilation support was not available from the trials of *Uma et al, Sayin et al.,* and *Parry et al.*.[19-21] Unfortunately, for some trials it was impossible to retrospectively collect information on these missing outcomes because the trials had been conducted too long ago. The researchers of the trial of *Carr et al.* could not retrieve data of both neonates in case of twin pregnancies, except for perinatal death. Despite the large number of patients analyzed, subgroup analysis could not be calculated for every outcome due to low numbers. These low numbers are

explained by the low prevalence of neonatal morbidities. In addition, due to different exclusion criteria and outcome measures within the original trials, subgroup analyses could not always be based on all trials.
This IPDMA includes all trials, according to our current knowledge, that assessed the effects of maintenance nifedipine tocolysis compared to a control group on perinatal death, prolongation of pregnancy and adverse neonatal outcome. Research groups that have not yet published their data were willing to participate. Gathering all data for an IPDMA is generally time consuming and can often be difficult because not all identified research groups are willing to share their data. By analyzing individual patient data, we overcame the problem of selective reporting. For example, our analysis on perinatal mortality was based on 6 studies encompassing 922 infants, while the aggregated data analysis of Gaunekar[8] was based on 2 studies encompassing 133 infants, and the analysis on prolongation of pregnancy was based on 4 studies on 175 women, while we were able to analyze data on all 787 women. A second strength of our IPDMA is that it enables to overcome the problem of differences in definitions of outcome measures. Due to differences across studies in for example the definition of IVH, the Cochrane review only provides aggregated data on IVH vs. no IVH, while we were able to conduct analysis on the frequency of IVH ≥ grade 2, which is a clinically more relevant outcome measure. Therefore, we feel that our IPDMA has a high additive value and provides clinicians with the best available evidence on nifedipine maintenance tocolysis.

Comparison of the findings with previous studies.
This meta-analysis is the first that evaluated all available evidence of maintenance nifedipine treatment in imminent preterm labor compared with placebo or no treatment using individual patient data. Recently, a Cochrane meta-analysis of *Gaunekar et al.* for maintenance therapy with calcium channel blockers was published.[8] The six trials included in their meta-analysis were the same as the ones included in this IPDMA, though their results were based on aggregated data retrieved from the articles or abstracts, while our results are based on IPD. *Gaunekar et al.* reported an increased mean prolongation of pregnancy in favor of the nifedipine group (+5.35 days; 95% CI 0.49-10.21), based on four trials that included a total of 275 women.[8] The largest trial by *Roos et al.*[16] (406 participants) was not included since prolongation of pregnancy was reported as median and interquartile range instead of a mean with SD. Using IPD we were able to include data from all six trials and analyses showed there was no statistically significant prolongation of pregnancy with nifedipine treatment.
There are many outcomes in the Cochrane meta-analysis of Gaunekar based on only 1,2 or 3 trials, due to differences in reporting or limited information in case of published abstracts. Since we had IPD available from all trials, even unpublished studies, the information on which our results are based is more comprehensive and therefore more reliable. Especially for neonatal outcomes and subgroups not reported in the primary trials, the IPDMA is a valuable addition to the Cochrane meta-analysis.

Moreover, the availability of IPD allowed for uniform cut-off values over trials, e.g. when performing subgroup analysis, solving the problem of having different cut-off values when meta-analyzing aggregated data.

Clinical implications

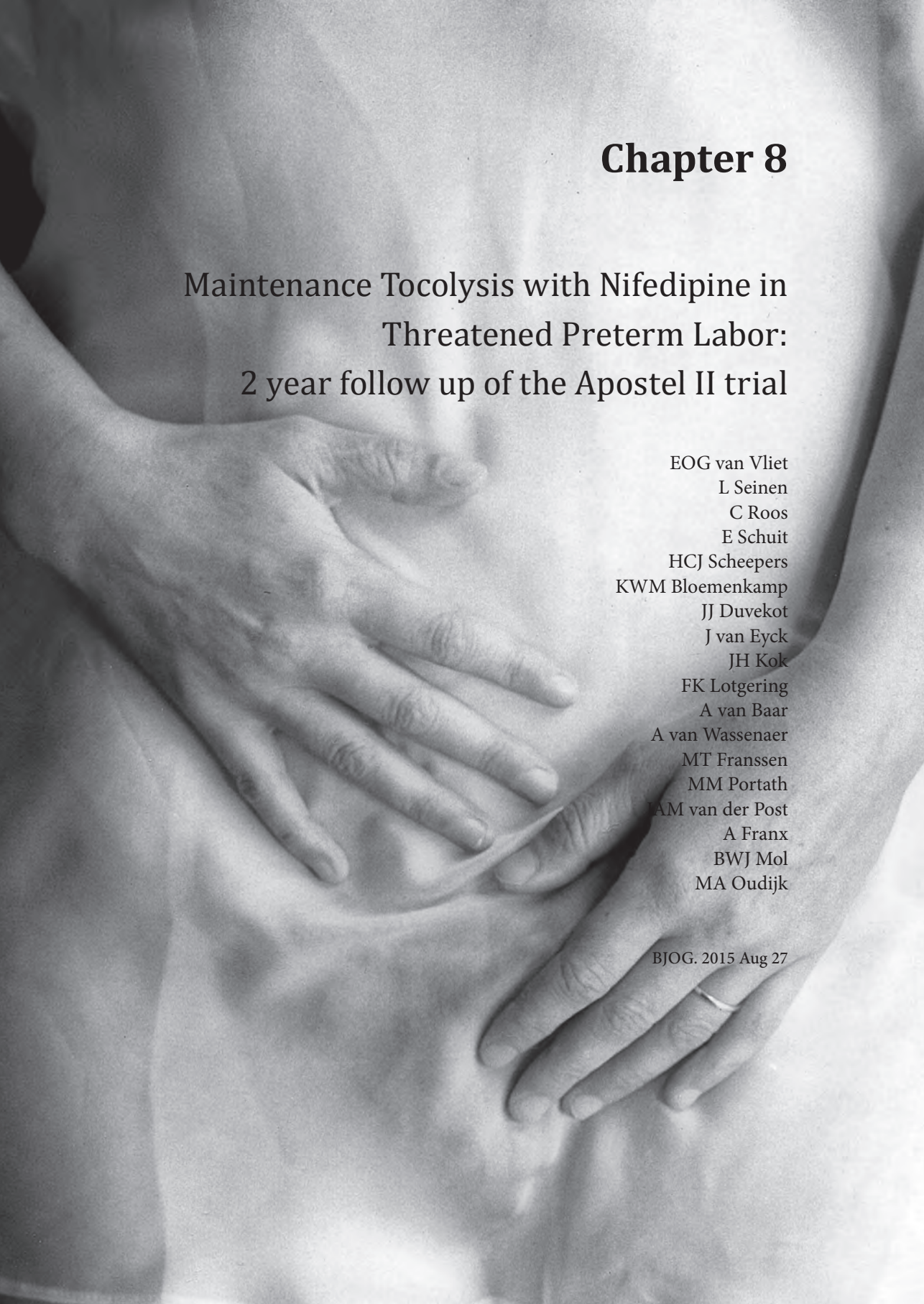
We established that overall, substantiated with the current best available evidence, maintenance tocolysis with nifedipine after the first 48 hours has no additional value for the reduction of perinatal mortality, adverse neonatal outcome or prolongation of pregnancy. In a subgroup of women with singleton pregnancies we found that nifedipine maintenance tocolysis prolongs pregnancy with a median difference of 5 days compared to placebo/no treatment. This might be a promising finding, although it is important to realize that the ultimate goal of treatment is reducing the incidence of neonatal morbidities and mortality. It is questionable whether postponement of delivery with 5 days will be of clinical relevance, especially when antenatal corticosteroids have already been provided. Furthermore, it could be questioned whether prolongation of pregnancy in threatened preterm birth will optimize outcome at all. It has been argued that prolonging pregnancy in threatened preterm birth might even be harmful.[22]

Although nifedipine is known to less likely cause maternal side effects when compared with other tocolytics such as beta-mimetics or magnesium sulfate, some case reports found associations with serious maternal side effects like pulmonary edema, hypoxia, myocardial infarction, atrial fibrillation and severe maternal hypotension.[23-25] Besides, since nifedipine crosses the placenta easily, [26,27] maintenance tocolysis exposes the fetus to medication with unknown effects on fetal development. Since we found no effect of maintenance tocolysis in the reduction of perinatal morbidity and mortality, we feel that nifedipine maintenance tocolysis should not be used in routine practice.

References

1. Flood K and Malone FD. Prevention of preterm birth. Semin Fetal Neonatal Med 2012;17:58-63.
2. Blencowe H, Cousens S, Oestergaard MZ et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: A systematic analysis and implications. Lancet 2012;379:2162-72.
3. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371:75-84.
4. Kusters CD, van der Pal SM, van Steenbrugge GJ, den Ouden LS, Kollee LA. The impact of a premature birth on the family; consequences are experienced even after 19 years. Ned Tijdschr Geneeskd 2013;157:A5449.
5. Westrupp EM, Lucas N, Mensah FK, Gold L, Wake M, Nicholson JM. Community-based healthcare costs for children born low birthweight, preterm and/or small for gestational age: Data from the longitudinal study of australian children. Child Care Health 2014;40:259-66.
6. Roberts D and Dalziel Stuart R. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev. 2006;19:CD004454.
7. Kim A and Shim J-. Emerging tocolytics for maintenance therapy of preterm labour: Oxytocin antagonists and calcium channel blockers. BJOG 2006;113:113-5.
8. Gaunekar NN and Crowther CA. Maintenance therapy with calcium channel blockers for preventing preterm birth after threatened preterm labour. Cochrane Database Syst Rev 2004;CD004071.
9. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: Rationale, conduct, and reporting. BMJ 2010;340:c221.
10. Simmonds MC, Higgins JP, Stewart LA, Tierney JF, Clarke MJ, Thompson SG. Meta-analysis of individual patient data from randomized trials: A review of methods used in practice. Clin Trials 2005;2:209-17.
11. Thompson SG and Higgins JP. Treating individuals 4: Can meta-analysis help target interventions at individuals most likely to benefit? Lancet 2005;365:341-6.
12. Liberati A, Altman DG, Tetzlaff J et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. BMJ 2009;339:b2700.
13. Higgins JP, Altman DG, Gotzsche PC et al. The cochrane collaboration's tool for assessing risk of bias in randomised trials. BMD 2011;343:d5928.
14. Gates S and Brocklehurst P. How should randomised trials including multiple pregnancies be analysed? BJOG 2004;111:213-9.
15. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557-560.
16. Roos C, Spaanderman MEA, Schuit E et al. Effect of maintenance tocolysis with nifedipine in threatened preterm labor on perinatal outcomes: A randomized controlled trial. JAMA 2013;309:41-7.
17. Carr DB, Clark AL, Kernek K, Spinnato JA. Maintenance oral nifedipine for preterm labor: A randomized clinical trial. Am J Obstet Gynecol 1999;181:822-7.
18. Lyell DJ, Pullen KM, Mannan J et al. Maintenance nifedipine tocolysis compared with placebo: A randomized controlled trial. Obstet Gynecol 2008;112:1221-6.
19. Uma M, Ixora KA, Nor Azlin MI, Mahdy ZA. Maintenance nifedipine for tocolysis in preterm labour: A prospective randomised controlled trial. BJOG 2012;119:35-6.
20. Sayin NC, Varol FG, Balkanli-Kaplan P, Sayin M. Oral nifedipine maintenance therapy after acute intravenous tocolysis in preterm labor. J Perinat Med 2004;32:220-4.
21. Parry E, Roos C, Stone P, Hayward L, Mol BW, McCowan L. The NIFTY study: A multicentre randomised double-blind placebo-controlled trial of nifedipine maintenance tocolysis in fetal fibronectin-positive women in threatened preterm labour. Aust N Z J Obstet Gynaecol 2014;54:231-6
22. Alfirevic Z. Tocolytics: do they actually work? BMJ 2012;345:e6531
23. Abramovici A, Cantu J, Jenkins SM. Tocolytic therapy for acute preterm labor. Obstet Gynecol Clin North Am 2012;39:77-87.
24. Jaju PB and Dhabadi VB. Nifedipine versus ritodrine for suppression of preterm labor and analysis of side effects. J Obstet Gynaecol India 2011;61:534-7.
25. Pryde PG, Janeczek S, Mittendorf R. Risk-benefit effects of tocolytic therapy. Expert Opin Drug Saf. 2004;3:639-54.
26. Silberschmidt AL, Ku WN, Velten HN, Juon AM, Zimmermann R, von Mandacha U. (2008) Nifedipine concentration in maternal and umbilical cord blood after nifedipine gastrointestinal therapeutic system for tocolysis. *BJOG.* 115(4):480-485.

27. Ferguson JE, Schutz T, Pershe R, Stevenson DK, Blaschke T. (1989) Nifedipine pharmacokinetics during preterm labour tocolysis. *Am J Obstet Gynecol.* 161:1485-1490.



Chapter 8

Maintenance Tocolysis with Nifedipine in Threatened Preterm Labor: 2 year follow up of the Apostel II trial

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BJOG. 2015 Aug 27

Abstract

Objective To evaluate long-term effects of maintenance tocolysis with nifedipine on neurodevelopmental outcome of the infant.

Design, Setting and Population Follow-up of infants of women who participated in a multicentre randomized controlled trial on maintenance tocolysis with nifedipine versus placebo.

Methods Two years after the APOSTEL II trial on maintenance tocolysis with nifedipine versus placebo, we asked participants to complete the Ages and Stages Questionnaire.

Main Outcome measures Infant development was measured on 5 domains. Developmental delay was defined as a score of a score of <-1 standard deviation in one or more developmental domains. We performed exploratory subgroup analysis in women with preterm prolonged rupture of the membranes, and in women with a cervical length <10 mm at study entry.

Results Of the 276 women eligible for follow-up, 135 (52.5%) returned the questionnaire, encompassing data of 170 infants. At 2 years of age, infants of women with nifedipine maintenance tocolysis compared to placebo had a higher overall incidence of fine motor problems (22.2% vs. 7.6%, OR 3.43, 95%-CI 1.29-9.14, p=0.01), and a lower incidence of poor problem solving (21.1% vs. 29.1%, OR 0.27, 95%-CI 0.08-0.95, p=0.04).

Conclusions This follow-up study revealed no clear benefit of nifedipine maintenance tocolysis at age 2. As short term adverse perinatal outcome was not reduced in the original APOSTEL II trial, we conclude that maintenance tocolysis does not appear beneficial at this time.

Introduction

Early preterm birth is a major health care problem. In very premature infants it may cause long-term physical and developmental impairment and has a substantial impact on parents and families.[1,2] Perinatal morbidity and mortality is strongly inversely related to gestational age.[3]

In threatened preterm labour before 34 weeks of gestation, antenatal corticosteroids enhance foetal lung maturation and thereby improve outcome in these infants.[4] To allow optimal effect of maternal steroid administration, the simultaneous use of tocolytic drugs for 48 hours is common practice in most perinatal centres. Since perinatal morbidity and mortality is strongly related to gestational age[3], further postponement of delivery through maintenance tocolysis was long thought to improve neonatal outcome. Yet, recent randomized controlled trials failed to show any effect of nifedipine maintenance tocolysis on prolongation of pregnancy and perinatal outcome.[5-8] Despite the lack of effect on short term outcome, nifedipine maintenance tocolysis might have an effect on long-term infant outcome. Nifedipine crosses the placenta easily,[9,10] thereby exposing the foetus to medication with unknown effects on foetal brain development. Previous studies have shown a decline in uterine artery and middle cerebral artery flow[11] and resistance[12] after nifedipine tocolysis, while other studies did not find this effect.[13] In vitro studies have shown a potential neuroprotective effect of nifedipine.[14,15] In the light of potential effects on foetal brain development, it is important to determine the effect of nifedipine maintenance tocolysis on long-term infant outcome.

The APOSTEL-II (Assessment of Perinatal Outcome with Sustained Tocolysis in Early Labour) trial[7] included women with threatened preterm labour between $26^{+0/7}$ weeks and $32^{+2/7}$ weeks of gestation who had not delivered after 48 hours of tocolysis and a completed course of corticosteroids. Women were randomly assigned to maintenance tocolysis with nifedipine or placebo, for a maximum of 12 days. The objective of the current study was to determine the extent to which maintenance tocolysis with nifedipine is associated with long-term neurodevelopmental outcome, assessed at 2 years of age in infants of women who participated in the APOSTEL II trial. We analyzed neurodevelopmental outcome for the group as a whole, and for subgroups with and without preterm prolonged rupture of the membranes (PPROM), and with and without a cervical length <10 mm at study entry.

Methods

The APOSTEL II trial is a double-blind placebo controlled trial and has been performed in all 10 perinatal centres and one large teaching hospital in The Netherlands. The study protocol and the main results of the APOSTEL II trial have been published elsewhere.[7,16] Four hundred and six patients participated in the APOSTEL II trial

between June 2008 and February 2010. Patients with threatened preterm labour and a gestational age between $26^{+0/7}$ and $32^{+2/7}$ weeks who had not delivered after a complete 48-hour course of tocolytics and corticosteroids, were allocated to maintenance tocolysis with nifedipine (n= 201) or placebo (n= 205). Study medication was 20 mg of nifedipine slow-release tablets every 6 hours, or placebo tablets. The APOSTEL II trial included women with singleton and multiple pregnancies with and without ruptured membranes.

The intention to follow-up the children was described in the study protocol.[16] The follow-up study was approved by the institutional review board of all participating centres as part of the APOSTEL II trial.

Participants

The follow-up of the APOSTEL II study started in 2009 and sought information from infants born from the 406 women who were randomized in the APOSTEL II trial. Figure 1 shows that after exclusion of 130 women (not eligible because infants died, not traceable or infants older than 24 months at start of follow-up), follow-up data were available on 170 infants of 145 women, a response rate of 52.5% (145/276 women). One infant was excluded from analysis because the infant had West syndrome. Parents were contacted by mail, and in case of no response trained research midwifes sought contact by phone. Data on demographic and clinical characteristics were collected as described in the original trial.[16]

Follow-up measures

Parents were contacted and asked to fill out the Dutch translation of the parent-rated Ages and Stages Questionnaire (ASQ) at the corrected age of 24 months. The ASQ consists of 5 subscales, each with 6 items, on communication, gross motor performance, fine motor performance, problem solving and personal-social functioning. Parents indicate if their child showed the behaviour as described in an item, or not, or sometimes/a little. Poor outcome on a subscale was defined as a score lower than one standard deviation below the mean of the norm group.[17] Developmental delay was defined as a score of <-1 standard deviation (SD) in one or more developmental domains. A score <-1 SD points out that this infant is at risk of poor outcome, and warrants targeted interventions and close follow-up of the infant. The ASQ is a valuable screening instrument for the study of developmental delay in ex-premature infants,[18] and outcome is not influenced by the socio-economic status of the parents.[19] Parents were unaware of the treatment allocation at time of follow-up.

Statistical analysis

The size and power of the study was limited by the number of participants of the APOSTEL II trial. To determine how representative our sample was, we tested for differences in demographic and clinical data between participants and non-participants of the follow-up trial. Furthermore we tested for differences in baseline characteristics

between the nifedipine group and the placebo group. Baseline characteristics were compared using ANOVA for continuous data, χ^2 tests for dichotomous data and Mann-Whitney *U* tests for non-normal distributed data. For outcome at infant level, we used a Generalized Estimated Equation model to test for differences in outcome between infants exposed to nifedipine maintenance tocolysis compared to placebo, accounting for interdependence of scores in siblings. Odds ratio's (OR) and 95% confidence intervals (CI) are reported. We tested for possible subgroup effects for women with and without PPROM, and women with a cervical length <10mm and ≥10 mm at study entry. Subgroup effects were studied using an interaction term between the subgroup and treatment, corrected for gestational age at delivery. When the interaction was found to be statistically significant ($p<0.05$), we performed stratified analysis to investigate the effect of treatment in the different subgroups.
In a cohort of preterm infants, there is a risk for bias because deceased and very disabled infants are not able to participate in follow-up. We therefore performed post hoc sensitivity analyses using a composite of death (n=9), severe disabilities (n=1) or ASQ score <1SD. Because not all parents filled out the questionnaire within the preferred time frame (24 months corrected age ± 1 month), we compared the incidence of poor outcome between infants that were assessed too early, on time, or too late, and we performed sensitivity analysis excluding these cases if there were any differential effects.

Results

Study population

Baseline characteristics of the mothers of placebo group (n=66), nifedipine group (n=78) and the non-participants (n=262) are displayed in Table 1. The women who participated in the follow-up study had a higher percentage of Caucasians compared to the non-participants ($p<0.01$), and a lower percentage of lower maternal education ($p<0.01$). These characteristics did not differ between the nifedipine group and the placebo group. Other baseline characteristics were comparable between the participants and nonparticipants, and between the nifedipine group and the placebo group. Median corrected age (range; interquartile range IQR) at time of completion of the questionnaire was 22.9 (range 21.3-29.1; IQR 22.1-24.0) for the nifedipine group and 22.7 (range 21.1-30.2; IQR 22.2-23.7) for the placebo group (p=0.52).

Table 1. Baseline Demographics and Clinical Characteristics

At entry to APOSTEL II			
Number of women	**Placebo (n =66)**	**Nifedipine (n = 78)**	**No follow-up (n =262)**
Age, y	30.9 (4.6)	30.9 (4.6)	29.8 (5.3)
Body mass index[a]	22.4 (3.2)	24.1 (5.7)	23.3 (4.3)
White European	59 (92.2)	74 (98.7)	188 (74.6)[b]
Lower maternal education	5 (12.2)	3 (7.1)	40 (26.1)[b]
Nulliparous	32 (48.5)	32 (41.0)	111 (42.4)
Prior preterm birth	16 (24.2)	17 (21.8)	60 (22.9)
Gestational age, week	29.1 (1.8)	29.1 (1.7)	29.3 (1.7)
Multiple gestation			
Twins	12 (18.1)	12 (15.4)	58 (22.1)
Triplets	1 (1.5)	0 (0)	5 (1.9)
pPROM	21 (31.8)	17 (21.8)	63 (24.1)
Cervix <10 mm[d]	8 (21.6)	7 (13.0)	22 (12.6)
Short-term outcomes of APOSTEL II			
Number of women	**Placebo (n =66)**	**Nifedipine (n = 78)**	**No follow-up (n =262)**
Adverse perinatal outcome[e]	10 (15.2)	10 (12.8)	32 (12.2)
Perinatal death	1 (1.5)	0 (0.0)	8 (3.1)
Number of infants	**Placebo (n =79)**	**Nifedipine (n = 90)**	**No follow-up (n =333)**
Birth weight, g	2152.7 (100.3)	2304.2 (98.0)	2251.6 (50.4)
NICU admittance	36 (45.6)	37 (41.1)	129 (38.7)
Ventilation support	7 (8.9)	16 (17.8)	69 (13.7)

Data are in mean (SD) or N (%).

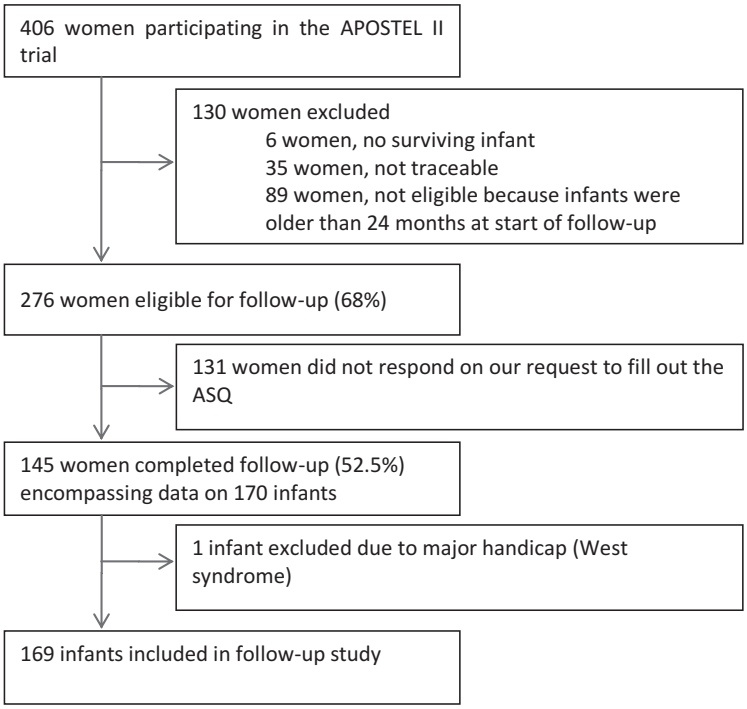
[a] Body mass index is calculated as weight in kilograms divided by height in meters squared; [b] P<0.01 compared to women who participated in follow-up; [c] Based on n= 236 due to missing data on maternal education; [d] Based on n=266 women who had cervical length measurement; [e] Adverse perinatal outcome is a composite of perinatal death, chronic lung disease, neonatal sepsis, IVH > grade 2, PVL > grade 1, and necrotizing enterocolitis, measured per pregnancy.

All other characteristics did not differ between placebo and nifedipine group, or between the follow-up and no follow-up group.

Main outcome

Developmental outcome as measured by ASQ at 2 years of age is shown in Table 2. Overall, infants of women who received nifedipine maintenance tocolysis had a higher incidence of poor outcome on the fine motor scale (22.2% vs. 7.6%, OR 3.43, 95% CI 1.29 – 9.14, p=0.01), whereas these infants did better on the problem solving scale (21.1 % vs. 29.1%, OR 0.27, 95% CI 0.08-0.95, p=0.04).

Figure 1. Flow chart



* 9 infants died in the APOSTEL II trial. However, 3 of them had a surviving twin sibling.

Table 2. Follow-up at age 2

	Nifedipine (n=90)	Placebo (n=79)	OR	95-% CI	*P* value
Communication scale	14 (15.6)	20 (25.3)	0.56	0.25 – 1.26	0.16
Gross motor scale	31 (34.4)	30 (38.0)	0.41	0.15 – 1.14	0.09
Fine motor scale	20 (22.2)	6 (7.6)	3.43	1.29 – 9.14	0.01
Problem solving scale	19 (21.1)	23 (29.1)	0.27	0.08 – 0.95	0.04
Personal social scale	25 (27.8)	26 (32.9)	0.88	0.43 – 1.78	0.72
Developmental delay	53 (58.9)	51 (64.6)	0.50	0.22 – 1.13	0.10

Delayed development is defined as performance <1SD below the mean score. Developmental delay is defined as performance <1SD below the mean score at 1 or more subscales. Data presented as n (%).
OR *odds ratio,* CI *confidence interval.*

Subgroup analysis

The subgroup analysis for possible interaction between the intervention and PPROM at inclusion, corrected for differences in gestational age at delivery (mean gestational age 32.4 weeks for PPROM vs. 34.5 without PPROM, p=0.002) showed that there was a significant interaction effect for gross motor performance (p=0.02) and developmental delay (p=0.02, Table 3).

Table 3. Developmental outcome, interaction with pPROM.

			Subgroup effect		
	Nifedipine (n=90)	**Placebo (n=79)**	**OR**	**95%-C.I.**	***P* value for interaction**
Communication scale					
PPROM	4 / 18 (22.2)	8/28 (28.6)			0.60
No PPROM	10 / 72 (13.9)	12 / 51 (23.5)			
Gross motor scale					
PPROM	8 / 18 (44.4)	8 /28 (28.6)	2.34	0.64 – 8.59	0.02
No PPROM	23 / 27 (31.9)	22 / 51 (43.1)	0.23	0.07 – 0.79	
Fine motor scale					
PPROM	7 / 18 (38.9)	3 / 28 (10.7)			0.70
No PPROM	13 / 72 (18.1)	3 / 51 (5.9)			
Problem solving scale					
PPROM	4 / 18 (22.2)	7 / 28 (25.0)			0.22
No PPROM	15 / 72 (20.8)	16 / 51 (31.4)			
Personal social scale					
PPROM	10 / 18 (55.6)	11 / 28 (39.3)			0.10
No PPROM	15 / 72 (20.8)	15 / 51 (29.4)			
Developmental delay					
PPROM	14 / 18 (77.8)	16 / 28 (57.1)	2.67	0.66 – 10.82	0.02
No PPROM	39 / 72 (54.2)	35 / 51 (68.6)	0.31	0.13 – 0.74	

Data presented as n performing -1SD/total (%), corrected for gestational age.

Infants with PPROM born after nifedipine maintenance tocolysis had a non-significant higher incidence of poor gross motor outcomes than those on placebo (44.4% vs. 28.6%, OR 2.34, 95%-CI 0.64-8.59). Poor gross motor outcomes were less common in infants exposed to nifedipine maintenance compared with placebo in the absence of PPROM (31.9% vs. 43.1%, OR 0.23, 95%-CI 0.07-0.79).

Infants with PPROM born after nifedipine maintenance tocolysis more often had developmental delay than those on placebo (77.8% vs. 57.1%, OR 2.67, 95%-CI 0.66-10.82), although this difference was not significant. Developmental delay was less common in infants without PPROM and nifedipine maintenance tocolysis compared to those on placebo (54.2% vs. 68.6%, OR 0.31, 95%-CI 0.13-0.74). There was no significant interaction effect between treatment group and time interval to delivery (data not shown, p=0.99), i.e. there was no difference in the effect of nifedipine maintenance therapy on time interval to delivery between women with and without PPROM.

Interaction tests of the intervention and cervical length <10 mm at randomization were based on limited numbers (n=63 for nifedipine and n=46 for placebo) because cervical length measurement was not performed in all women. We observed a significant interaction effect on the problem solving scale (p=0.03, Table 4). In both the group of women with a cervix <10mm and ≥10mm at study entry, infants of the nifedipine group had a lower incidence of poor problem solving compared to infants

of the placebo group, but this difference was not significant (22.2% vs. 50%, OR 0.05, 95%-CI 0.00-0.58 for cervix <10 mm at study entry, and 18.5% vs. 27.8%, OR 0.66, 95%-CI 0.22-1.96 for cervix ≥10 mm at study entry).

Table 4. Developmental outcome, interaction with cervical length <10 mm at inclusion.

			Subgroup effect		
	Nifedipine (n=63)	**Placebo (n=46)**	**OR**	**95%-C.I.**	***P* value for interaction**
Communication scale					
Cervix <10 mm	2 / 9 (22.2)	2 / 10 (20.0)			0.36
Cervix ≥10 mm	9 / 54 (16.7)	10 / 36 (27.8)			
Gross motor scale					
Cervix <10 mm	5 / 9 (55.6)	2 /10 (20.0)			0.43
Cervix ≥10 mm	17 /54 (31.5)	15 / 36 (27.8)			
Fine motor scale					
Cervix <10 mm	0 / 9 (0.0)	0 / 10 (0.0)			n.a.
Cervix ≥10 mm	11 / 54 (20.4)	2 /36 (5.6)			
Problem solving scale					
Cervix <10 mm	2 / 9 (22.2)	5 / 10 (50.0)	0.05	0.00 – 0.58	0.03
Cervix ≥10 mm	10 / 54 (18.5)	10 /36 (27.8)	0.66	0.22 – 1.96	
Personal social scale					
Cervix <10 mm	1 / 9 (11.1)	1 / 10 (10.0)			0.98
Cervix ≥10 mm	16 / 54 (29.6)	12 /36 (33.3)			
Developmental delay					
Cervix <10 mm	7 / 9 (77.8)	6 / 10 (60.0)			0.86
Cervix ≥10 mm	27 / 54 (50.0)	22 / 36 (61.1)			

Data presented as n performing -1SD/total (%), corrected for gestational age. Analysis based on n=46 for placebo and n=63 for nifedipine because cervical length measurement was not performed in all women. N.a. not applicable.

Sensitivity analysis

Sensitivity analysis using a composite of poor outcome and perinatal death revealed comparable results, except that the lower incidence of poor outcome on the problem solving scale in the nifedipine group was no longer significant (OR 0.33, 95%-CI 0.11 – 1.02, p=0.06).

Not all parents filled out the questionnaires within the preferred timeframe, therefore we performed additional analysis to test to what extent this might have influenced our results. For fine motor problems, there was a significantly lower incidence of delay in the infants who were tested too late compared to infants that were tested within the correct time frame (0.0% versus 18%). After exclusion of these infants (n=11), the effect of the intervention on fine motor problems remained comparable (OR 3.23, 95%-CI 1.21 – 9.09, p= 0.02). For all other outcome measures there was no significant difference in the incidence of poor outcome between infants that were assessed too early, on time, or too late.

Discussion

Main findings

To our best knowledge, this is the first study to examine the long-term outcome of infants exposed to nifedipine maintenance tocolysis compared to placebo. We observed that these infants at 2 years of age have a higher incidence of fine motor problems, and a lower incidence of poor problem solving. In a specific subgroup of women without PPROM, there was a lower incidence of gross motor problems and developmental delay in infants of the treatment group compared to the placebo group.

Strengths and limitations

Several methodological issues deserve discussion. First, this study uses questionnaire data to measure developmental outcome. Albeit questionnaire data have their inherent limitations, the ASQ is regarded as a validated screening tool for developmental problems.[19] There is some evidence to suggest that the ASQ underestimates the incidence of motor delay in premature infants,[20] yet it is independent of socio-economic status or maternal education.[19] The ASQ has excellent psychometric properties to be used as a screening instrument for abnormal development. [18,19] However, the ASQ is not a diagnostic test and abnormal scores require further examination. Besides, the ASQ does not substitute for clinical examination and sensory screening.[18]

Second, selection bias cannot be excluded. Overall, 169 children from 145 of the 406 women in the APOSTEL II trial could be included in this follow-up study. This may have caused unknown effects. Women participating in the follow-up study more often were Caucasian and less often had a low educational level compared to women who did not participate in the follow-up study. Yet, there was no difference between these baseline characteristics between the nifedipine and placebo groups, and a previous study has shown that the ASQ scores are not significantly associated with socio-economic status or maternal education.[19] Furthermore, other baseline characteristics were comparable between the participants and non-participants of this follow-up study. Therefore, we feel that selection bias may not have affected the results too much. As in most studies involving premature infants, also in this cohort there were infants that were deceased (n=9) or too disabled to participate in the follow-up study (n=1). As this could lead to bias we re-ran all analyses including deceased and severely disabled infants in the poor outcome group, resulting in grossly comparable results.

Third, some of the parents participating in this follow-up study did not fill out the questionnaire within the correct time frame, however follow-up time was equal in both treatment groups and therefore it is unlikely that this influenced the results too much. Besides, sensitivity analyses revealed comparable results after exclusion of infants who were assessed too late.

The subgroup analyses should be interpreted with caution because the study was not powered to conduct long-term follow-up within subgroups. Especially the number of women with a cervical length ≤10 mm (n= 19) is rather limited. Nonetheless, the

results of our subgroup analyses underscore the fact that prematurity is heterogeneous in origin and interventions may well elicit a differential effect in subgroups, which should be taken into account when evaluating neonatal outcome.

Interpretation

To our best knowledge, no previous trials studied the effect of nifedipine maintenance tocolysis on neurodevelopmental outcome compared to placebo. A previous randomized controlled trial[21] on maintenance tocolysis with nifedipine versus ritodrine found no differences in long-term behaviour-emotional outcome and motor functioning between the groups.[22] Despite the lack of effect on short term outcome as found in the APOSTEL II trial[7], maintenance tocolysis may have an effect on long-term development. We indeed found a higher incidence of fine motor problems but better problem solving abilities in infants in the nifedipine group. A possible explanation for the differential effect on motor skills and problem solving skills might be that structures that play an important role in motor functioning are more vulnerable to injury. For example, the cerebellum undergoes the most rapid growth in the third trimester[23] and might therefore be more vulnerable to adverse effects of medication. Besides, disturbance of the myelinisation process is one of the hallmarks of hypoxic-ischemic brain injury,[24] and contributes to poorer corticospinal tract functioning and motor development. Some studies have shown that nifedipine is associated with a decline in uterine artery and middle cerebral artery flow[11], it could therefore be speculated that nifedipine might indeed contribute to poorer motor functioning.

Nifedipine maintenance therapy has a differential effect on infants with and without PPROM. We observed that infants of women with PPROM receiving maintenance tocolysis had a non-significant higher incidence of poor gross motor development and developmental delay compared to the placebo group, whereas infants of women with nifedipine maintenance without PPROM had a lower incidence of poor gross motor development and developmental delay. This did not result from a difference in prolongation of pregnancy or in time interval to delivery. One may speculate that nifedipine maintenance therapy in women with PPROM may actually have an adverse effect through an association with subclinical infection. A possible explanation of the specific effects on motor development might be that the white matter myelinisation process, that is essential for corticospinal tract functioning and motor development might be the most vulnerable to adverse effect of medication or perinatal inflammation.[25,26] There is on-going debate whether any tocolysis should be administered in PPROM. A Cochrane review indicates that tocolysis in women with PPROM before 34 weeks' gestation, is associated with a higher risk of chorioamnionitis, which is in turn associated with poor neonatal and neurodevelopmental outcome.[27] Although we observed less gross motor problems and developmental delay in infants after nifedipine maintenance in the absence of PPROM, given the limited number of women in this study, the loss to follow-up and the low power resulting thereof, and the

lack of short term neonatal benefit, we feel that the results of the study should not be interpreted as a permit to prescribe maintenance tocolysis in women with threatened preterm labour without PPROM.

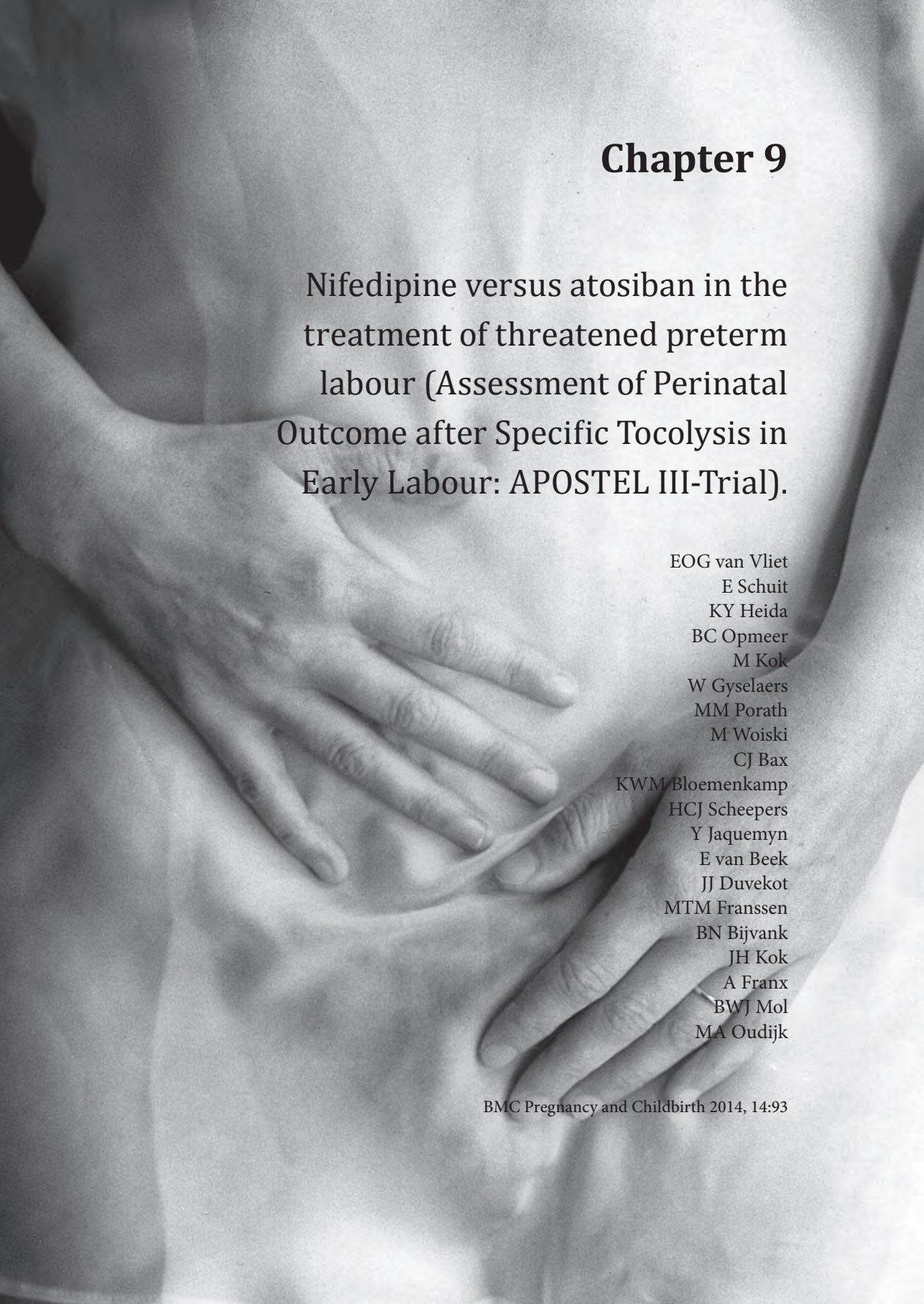
Conclusion

Our 2 year follow-up study of the APOSTEL II trial showed that maintenance tocolysis with nifedipine is associated with a higher incidence of fine motor problems and a lower incidence of poor problem solving. These findings stress the importance of long-term follow-up of intervention studies designed to optimize outcome in preterm infants. As also stated by the authors of the follow-up study of the ORACLE II trial on antibiotics in spontaneous preterm labour[28], the current study emphasizes that we should be cautious about interfering with systems that are poorly understand without clear evidence of the benefit of our intervention in short and long-term. Therefore, long-term follow-up studies of clinical interventions remain of utmost importance.
Since the APOSTEL II trial found no reduction in adverse perinatal outcome, and this follow-up study revealed no clear benefit of nifedipine maintenance tocolysis at age 2, we maintain our conclusion that its use does not appear beneficial.

References

1. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller AB, et al. (2013) Born Too Soon Preterm Birth Action Group. Born too soon: the global epidemiology of 15 million preterm births. *Reprod Health.* 10 Suppl 1:S2.
2. Treyvaud K, Lee KJ, Doyle LW, Anderson PJ. (2014) Very preterm birth influences parental mental health and family outcomes seven years after birth. *JPediatr.* 164(3):515-521.
3. Bolisetty S, Bajuk B, Abdel-Latif ME, Vincent T, Sutton L, Lui K. (2006) Preterm outcome table (POT): a simple tool to aid counselling parents of very preterm infants. *Aust N Z J Obstet Gynaecol.* 46(3):189-192.
4. Keirse MJ. The history of tocolysis. (2003) *BJOG.* 110 Suppl 20:94-97.
5. Naik Gaunekar N, Raman P, Bain E, Crowther CA. (2013) Maintenance therapy with calcium channel blockers for preventing preterm birth after threatened preterm labour. *Cochrane Database Syst Rev.* Oct 31;10:CD004071.
6. Parry E, Roos C, Stone P, Hayward L, Mol BW, McCowan L. (2014) The NIFTY study: a multicentre randomized double-blind placebo-controlled trial of nifedipine maintenance tocolysis in fetal fibronectin-positive women in threatened preterm labour. *Aust N Z J Obstet Gynaecol.* 54(3):231-236.
7. Roos C, Spaanderman ME, Schuit E, Bloemenkamp KW, Bolte AC, Cornette J, et al. (2013) Effect of maintenance tocolysis with nifedipine in threatened preterm labour on perinatal outcomes. A randomized controlled trial. *JAMA.* 309(1):41-47.
8. Lyell DJ, Pullen KM, Mannan J, Chitkara U, Druzin ML, Caughey AB, et al. (2008) Maintenance nifedipine tocolysis compared with placebo: a randomized controlled trial. *Obstet Gynecol.* 112(6):1221-1226.
9. Silberschmidt AL, Ku WN, Velten HN, Juon AM, Zimmermann R, von Mandacha U. (2008) Nifedipine concentration in maternal and umbilical cord blood after nifedipine gastrointestinal therapeutic system for tocolysis. *BJOG.* 115(4):480-485.
10. Ferguson JE, Schutz T, Pershe R, Stevenson DK, Blaschke T. (1989) Nifedipine pharmacokinetics during preterm labour tocolysis. *Am J Obstet Gynecol.* 161:1485-1490.
11. Guclu S, Gol M, Saygili U, Demir N, Sezer O, Baschat AA. (2006) Nifedipine therapy for preterm labour: effects on placental, fetal cerebral and atrioventricular Doppler parameters in the first 48 hours. *Ultrasound Obstet Gynecol.* 27: 403–408.
12. de S Lima MM, Souza ASR, Diniz C, Porto AMF, Amorim MMR, Moron AF. (2009) Doppler velocimetry of the uterine, umbilical and fetal middle cerebral arteries in pregnant women undergoing tocolysis with oral nifedipine. *Ultrasound Obstet Gynecol.* 34: 311–315.
13. de Heus R, Mulder EJH, Derks JB, Visser GHA. (2009) The effects of the tocolytics atosiban and nifedipine on fetal movements, heart rate and blood flow. *J Matern Fetal Neonatal Med.* 22(6):485-490.
14. Sribnick EA, Del Re AM, Ray SK, Woodward JJ, Banik NL. (2009) Estrogen attenuates glutamate-induced cell death by inhibiting Ca2+ influx through L-type voltage-gated Ca2+ channels. *Brain Res.* 1276:159-170.
15. Berger R, Garnier Y, Jensen A. (2002) Perinatal brain damage: underlying mechanisms and neuroprotective strategies. *J Soc Gynecol Investig.* 9(6):319-328.
16. Roos C, Scheepers LH, Bloemenkamp KW Bolte A, Cornette J, Derks JB, et al. (2009) Assessment of perinatal outcome after sustained tocolysis in early labour (APOSTEL-II trial). *BMC Pregnancy Childbirth.* 9:42.
17. Squires J, Twombly E, Bricker D, Potter L. (2009) *The ASQ-3 user's guide* (3rd ed.). Baltimore, MD: Brookes.
18. Skellern CY, Rogers Y, O'Callaghan MJ. (2001) A parent-completed developmental questionnaire: follow-up of ex-premature infants. *J Paediatr Child* Health. 37(2):125-129.
19. Flamant C, Branger B, Nguyen The Tich S, de la Rochebrochard E, Savagner C, Berlie I, et al. (2011) Parent-completed developmental screening in premature children: a valid tool for follow-up programs. *PLoS One.* 6(5):e20004.
20. Simard MN, Luu TM, Gosselin J. (2012) Concurrent validity of ages and stages questionnaires in preterm infants. *Pediatrics.* 130(1):e108-114.
21. Papatsonis DN, Van Geijn HP, Adèr HJ, Lange FM, Bleker OP, Dekker GA. (1997) Nifedipine and ritodrine in the management of preterm labour: a randomized multicenter trial. *Obstet Gynecol.* 90(2):230-234.
22. Houtzager BA, Hogendoorn SM, Papatsonis DN, Samsom JF, van Geijn HP, Bleker OP, et al. (2006) Long-term follow-up of children exposed in utero to nifedipine or ritodrine for the management of preterm labour. *BJOG.* 113(3):324-331.

23. Brossard-Racine M, du Plessis AJ, Limperopoulos C. (2015) Developmental Cerebellar Cognitive Affective Syndrome in Ex-preterm Survivors Following Cerebellar Injury. Cerebellum 14:151–164.
24. Back SA. (2014)Cerebral white and gray matter injury in newborns: New insights into pathophysiology and management Clin Perinatol. 41(1): 1–24.
25. Volpe JJ. (2009) Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol*. 8(1):110-124.
26. van Vliet EO, de Kieviet JF, Oosterlaan J, van Elburg RM. (2013) Perinatal infections and neurodevelopmental outcome in very preterm and very low-birth-weight infants: a meta-analysis. *JAMA Pediatr.* 167(7):662-668.
27. Soraisham AS, Trevenen C, Wood S, Singhal N, Sauve R. (2013) Histological chorioamnionitis and neurodevelopmental outcome in preterm infants. *J Perinatol.* 33(1):70-75.
28. Kenyon S, Pike K, Jones DR, Brocklehurst P, Marlow N, Salt A, et al. (2008) Childhood outcomes after prescription of antibiotics to pregnant women with spontaneous preterm labour: 7-year follow-up of the ORACLE II trial. *Lancet.* 372(9646):1319-1327.



Chapter 9

Nifedipine versus atosiban in the treatment of threatened preterm labour (Assessment of Perinatal Outcome after Specific Tocolysis in Early Labour: APOSTEL III-Trial).

EOG van Vliet
E Schuit
KY Heida
BC Opmeer
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MM Porath
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CJ Bax
KWM Bloemenkamp
HCJ Scheepers
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BMC Pregnancy and Childbirth 2014, 14:93

Abstract

Background Preterm birth is the most common cause of neonatal morbidity and mortality. Postponing delivery for 48 hours with tocolytics to allow for maternal steroid administration and antenatal transportation to a centre with neonatal intensive care unit facilities is the standard treatment for women with threatening preterm delivery in most centres. However, there is controversy as to which tocolytic agent is the drug of first choice. Previous trials have focused on tocolytic efficacy and side effects, and are probably underpowered to detect clinically meaningfull differences in neonatal outcome. Thus, the current evidence is inconclusive to support a balanced recommendation for clinical practice.This multicenter randomised clinical trial aims to compare nifedipine and atosiban in terms of neonatal outcome, duration of pregnancy and maternal side effects.

Methods/Design The Apostel III trial is a nationwide multicenter randomised controlled study. Women with threatened preterm labour (gestational age 25 – 34 weeks) defined as at least 3 contractions per 30 minutes, and 1) a cervical length of ≤ 10 mm or 2) a cervical length of 11-30 mm and a positive Fibronectin test or 3) ruptured membranes will be randomly allocated to treatment with nifedipine or atosiban. Primary outcome is a composite measure of severe neonatal morbidity and mortality. Secondary outcomes will be time to delivery, gestational age at delivery, days on ventilation support, neonatal intensive care (NICU) admittance, length admission in neonatal intensive care, total days in hospital until 3 months corrected age, convulsions, apnoea, asphyxia, proven meningitis, pneumothorax, maternal side effects and costs. Furthermore, an economic evaluation of the treatment will be performed. Analysis will be by intention to treat principle. The power calculation is based on an expected 10% difference in the prevalence of adverse neonatal outcome. This implies that 500 women have to be randomised (two sided test, β 0.2 at alpha 0.05).

Discussion This trial will provide evidence on the optimal drug of choice in acute tocolysis in threatening preterm labour.

Background

Preterm birth is the most common cause of neonatal morbidity and mortality worldwide [1]. In the USA, the rate of infants born before 37 weeks gestation is 12-13%; while in Europe and other developed countries these rates vary between 5-11% [2,3]. Preterm birth accounts for approximately 75% of all neonatal deaths and 50% of childhood neurological morbidities[4,5] and puts a financial burden on the public health care system[6]. Neurodevelopmental impairments are frequently present in preterm infants, and are associated with gestational age[7]. Neonatal outcome is enhanced by antenatal corticosteroid administration and in-utero transfer to a tertiary care centre[8,9]. To optimize outcome in threatening preterm delivery, postponing delivery for 48 hours with tocolytic agents is common practice in most perinatal centres[10], to allow maximal effect of maternal steroid administration and transportation of the mother to a centre with neonatal intensive care unit (NICU) facilities.

Several types of tocolytic drugs are commonly used as treatment in preterm labour. These include the β adrenoceptor agonist ritodrine hydrochloride, the oxytocin receptor antagonist atosiban and the calcium channel blocking agent nifedipine. Several meta-analyses indicate that tocolytic drugs are superior to placebo or other tocolytics at delaying delivery by 48 hours and 7 days[11,12].

However, controversy exists as to which tocolytic is the drug of first choice. The ideal drug of choice should be efficient in postponing preterm labour, have a favorable safety profile in both mother and fetus, and should reduce neonatal morbidity and mortality at a reasonable cost.

Studies on β adrenoceptor agonists have shown mixed results for postponing delivery compared to placebo[13]. As β adrenoceptor agonists have substantial side effects, use has been largely abandoned from clinical practice. A Cochrane review on calcium channel blockers for inhibiting preterm labour showed that nifedipine significantly reduced delivery within seven days of receiving treatment as compared with any other tocolytic agent (relative risk (RR) 0.76; 95% confidence interval (CI) 0.60 to 0.97)[14]. In addition, as compared to other tocolytics, calcium channel blockers also reduced the frequency of neonatal respiratory distress syndrome (RR 0.63; 95% CI 0.46 to 0.88), necrotising enterocolitis (RR 0.21; 95% CI 0.05 to 0.96), intraventricular haemorrhage (RR 0.59 95% CI 0.36 to 0.98) and neonatal jaundice (RR 0.73; 95% CI 0.57 to 0.93), and the requirement for women to have treatment ceased for adverse drug reaction (RR 0.14; 95% CI 0.05 to 0.36). The Cochrane review on oxytocin receptor antagonists for inhibiting preterm labour failed to demonstrate the superiority of atosiban over β adrenoceptor agonists (RR 0.98; 95% CI 0.68 to 1.41) or placebo (RR 2.5; 95% CI 0.51 to 12.35) in terms of tocolytic efficacy or infant outcomes[15]. On the other hand, atosiban is thought to be completely safe for the mother, whereas nifedipine may cause severe hypotension and fetal death[16]. Such side effects could however not be demonstrated in a previous nationwide study in The Netherlands[17]. Two small studies did not show a difference in effectiveness between nifedipine and

atosiban,[18,19] however, more side effects were observed with the use of nifedipine, consisting of hypotension, tachycardia headache and vertigo. Neonatal outcome was not reported in one study, the other study did not show a difference.
Recently, a larger study was published, (n=145) and found fewer failures within 48 hours for atosiban compared with nifedipine[20]. However, nifedipine was associated with a longer postponement of delivery. Neonatal morbidity was comparable between the two groups, although the number of neonatal admissions to the NICU and length of postnatal hospital admission was significantly higher in the atosiban group as compared with the nifedipine group. The main outcome measure of these 3 trials were tocolytic efficacy and tolerability, but the trails may be underpowered to detect clinically meaningfull effects in neonatal outcome. Therefore, the evidence remains inconclusive to support a balanced recommendation for clinical practice as the ultimate goal of tocolysis is not only to postpone delivery, but to improve neonatal outcome. This multicenter randomised clinical trial aims to compare nifedipine and atosiban in terms of neonatal outcome, duration of pregnancy, maternal side effects and costs. The study is conducted within the Dutch Obstetric Consortium, a collaborative effort of obstetric clinics in The Netherlands to perform clinical trials.

Methods/design

Aims

The objective of this study is to compare the effectiveness of the tocolytic agents nifedipine and atosiban in the improvement of neonatal outcome in women with threatened preterm labour with a gestational age between 25 – 34 weeks. Outcome is measured in terms of neonatal mortality and morbidity (chronic lung disease, severe intraventricular haemorrhage, periventricular leucomalacia, culture proven sepsis, necrotizing enterocolitis), gestational age at delivery, maternal side effects and costs.

Participants/eligibility criteria

We included women with a high risk of preterm birth. Women, aged ≥ 18 years, with threatened preterm labour and a gestational age between 25 and 34 weeks are eligible for participation in the Apostel III trial. The diagnosis of threatened preterm labour is defined by uterine contractions, at least 3 contractions per 30 minutes, and one of the following: 1) a cervical length of ≤ 10 mm or 2) a cervical length of 11-30 mm and a positive Fibronectin test or 3) ruptured amniotic membranes. Patients with singleton or twin pregnancies are eligible, independent of the position of the fetus.
Exclusion criteria are presence of a contra-indication for tocolysis (severe vaginal bleeding, signs of fetal distress or intrauterine infection, hypertension or use of anti-hypertensive medication, myocardial infarction (<1 month), unstable angina pectoris), cerclage, > 5 cm cervical dilatation, neonates suspected of chromosomal or structural anomalies and tocolytic treatment for >6 hours prior to arrival in a participating centre.

Procedures, recruitment, randomization and collection of baseline data
The study will be a nationwide multicentre randomised controlled trial conducted within the Dutch Obstetric Consortium. The Dutch Obstetric Consortium is a research collaboration of obstetric clinics in the Netherlands. All 10 Dutch perinatal centres with NICU facilities will participate in the trial. In addition, 10 large teaching hospitals in the Netherlands and 2 perinatal centres in Belgium will participate in this trial.

Eligible women will be identified by the staff and/or local research coordinator of the participating hospitals. After counselling and reading the patient information form, patients will be asked for written informed consent. We will provide patient information in Dutch and English. After informed consent, baseline demographics, obstetric and medical history of patient will be entered in a web-based database, which will also facilitate randomisation. Randomisation will be performed by a web based computerized program using permuted-block randomisation. Randomisation allocation will be in a 1:1 ratio for Nifedipine or Atosiban, block size will be 4.

As this is a comparison of oral medication and intravenous medication, and as both group are treated with active medication, the study will not be blinded.

Interventions
Patients are allocated to nifedipine or atosiban for 48 hours. In the nifedipine group, the initial dose will be 2 x 10 mg nifedipine capsules orally in the first hour, followed by 20 mg nifedipine retard per 6 hours for the next 47 hours. In the first hour after starting nifedipine, blood pressure and heart rate will be measured every 15 minutes. If blood pressure remains within the normal limits, treatment will be continued and blood pressure and heart rate will be measured 4 times every 24 hours.
In the atosiban group, a bolus injection of 6.75 mg i.v. in 1 minute, followed by 18 mg/hour for 3 hours, followed by a maintenance dosage of 6 mg/hour for 45 hours.
Antenatal corticosteroids will be administered according to the clinical guideline. Prophylactic treatment with antibiotics is at the decision of the attending physician. When the attending physician considers escape medication, this can be discussed with a perinatologist who will be available for study questions 24 hours per day.

Outcome measures

Primary outcome measures
The primary outcome measure will be a composite of adverse neonatal outcome, including bronchopulmonary dysplasia (BPD), periventricular leucomalacia (PVL) > grade 1, intraventricular haemorrhage > grade 2, necrotising enterocolitis (NEC) > stage 1[21], culture proven sepsis and in-hospital death.

The diagnosis of BPD will be made according to the international consensus guideline as described by Jobe and Bancalari [22] at time of discharge home or at 36 weeks of corrected gestational age. PVL > grade 1 and intraventricular haemorrhage > grade 2 will be diagnosed by repeated neonatal cranial ultrasound by the neonatologist according to the guidelines on neuro imaging described by de Vries [23] and Ment et al. [24]. NEC will be diagnosed according to Bell [21], > stage 1. Culture proven sepsis is diagnosed on the combination of clinical signs and positive blood cultures.

Secondary outcome measures

Secondary outcomes will be time to delivery, gestational age at delivery, days on ventilation support, length of admission in neonatal intensive care, convulsions, apnoea, asphyxia, proven meningitis, pneumothorax, total days in hospital until 3 months corrected age. Furthermore we will examine differences in maternal mortality and maternal side effects leading to discontinuation of study medication.

Follow-up of women and infants

All details of delivery, maternal and neonatal assessments during pregnancy and postpartum are recorded in a web-based Case Report Form (CRF). Details of neonatal admission are also recorded. Long-term follow up of children is dependent on future funding.

Statistical issues

Sample size

The sample size is calculated based on a 10%- reduction of the composite poor neonatal outcome from 25% in the atosiban arm to 15% in the nifedipine arm. With a beta of 0.2 and alpha of 0.05 we have to randomize 500 patients (250 in each arm).

Data analysis

Data will be analyzed according to the intention to treat principle. The main outcome variable, 'adverse neonatal outcome', will be assessed by calculating rates in the two groups, relative risks and 95% confidence intervals as well as numbers needed to treat. To evaluate the potential of each of the strategies, we will also perform a per protocol analysis, taking into account only those women that were treated according to protocol. Time to delivery will be evaluated by Cox proportional hazards regression and Kaplan-Meier estimates, with account for differing durations of gestation at entry, and will be tested with the Log rank test. The other secondary outcome measures will be approached similarly to the primary outcome measure.

Furthermore, we plan to separately report on the treatment effect in the following subgroups: 1) PPROM versus intact membranes 2) GA < 30 weeks versus > 30 weeks, 3) fibronectin positive women only, 4) women with a cervix length < 10 mm, 5) multiple pregnancies, and 6) women with a history of preterm birth.

Interim analysis

An interim analysis is planned after the follow up data of the first 150 women that have been included is obtained. The interim analysis will be performed by an independent person and results will be reported to a data safety and monitoring committee (DSMC) for safety and relevance. As an indication, the trial will be stopped if there is a significant difference in the primary outcome, i.e. a poor neonatal outcome, at $p < .005$ (2-sided). However, the DSMC is free to make its own judgment.

Data safety monitoring committee

Serious Adverse Events (SAEs) and Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported to a Data Safety Monitoring Committee (DSMC). The DSMC can decide to perform an extra interim analysis and, if indicated, terminate the trial prematurely.

Economic evaluation

We plan an economic evaluation of the costs and health effects of nifedipine and atosiban. The economic evaluation will be set up as a cost-effectiveness analysis (CEA) in which we will calculate the cost per prevented case of poor neonatal outcome. To evaluate cost-effectiveness within a long term horizon, downstream costs associated with poor neonatal outcome are estimated, and included in the analysis as a cost-to-benefit ratio. In a cost-utility analysis, with QALYs calculated from average life expectancy and utilities for severe neonatal morbidity, the incremental cost-effectiveness will be expressed as costs per QALY gained. In sensitivity analyses, the impact of parameter uncertainty and stochastic uncertainty is assessed, and the results are visualized in cost-effectiveness planes and cost-effectiveness acceptability curves.

In our economic analysis, we distinguish three cost stages (antenatal stage, delivery/childbirth stage and postnatal stage), and three cost categories: 1) direct medical costs i.e. all costs in the health care sector 2) direct non-medical costs i.e. costs outside the health care sector that are affected by health status or health care, and 3) indirect costs of the pregnant woman and her partner, for example costs of sick leave. For each stage and cost category, costs are measured as the volumes of resources used multiplied with appropriate valuations based on national reference prices, cost-per-unit estimates, or reimbursement fees .

Volumes of health care resource use are measured alongside the clinical study as part of the CRF as well as with questionnaires. Questionnaires will be based on the iMTA Medical Consumption Questionnaire (MCQ) and the Productivity Costs Questionnaire (PCQ) to collect data regarding health care consumption (e.g. number of GP contacts or outpatient visits, hospital admissions, and drug use), travel and time costs and productivity loss during follow-up at 6-month intervals. These questionnaires will be adapted to include only resources relevant to this study, and to document absence from paid work by the partners.

For an evaluation from a societal perspective, valuations of direct medical resources are estimated comprising ' true economic' costs, i.e. including shares of fixed costs and hospital overheads. Dutch reference prices are used where available. Otherwise, costs per unit are estimated for at least one teaching and one non-teaching hospital. Calculations based on reimbursement fees is added to our analysis to represent the payers perspective.
Indirect costs are quantified but remain unvalued. Study-specific costs are excluded from analysis.

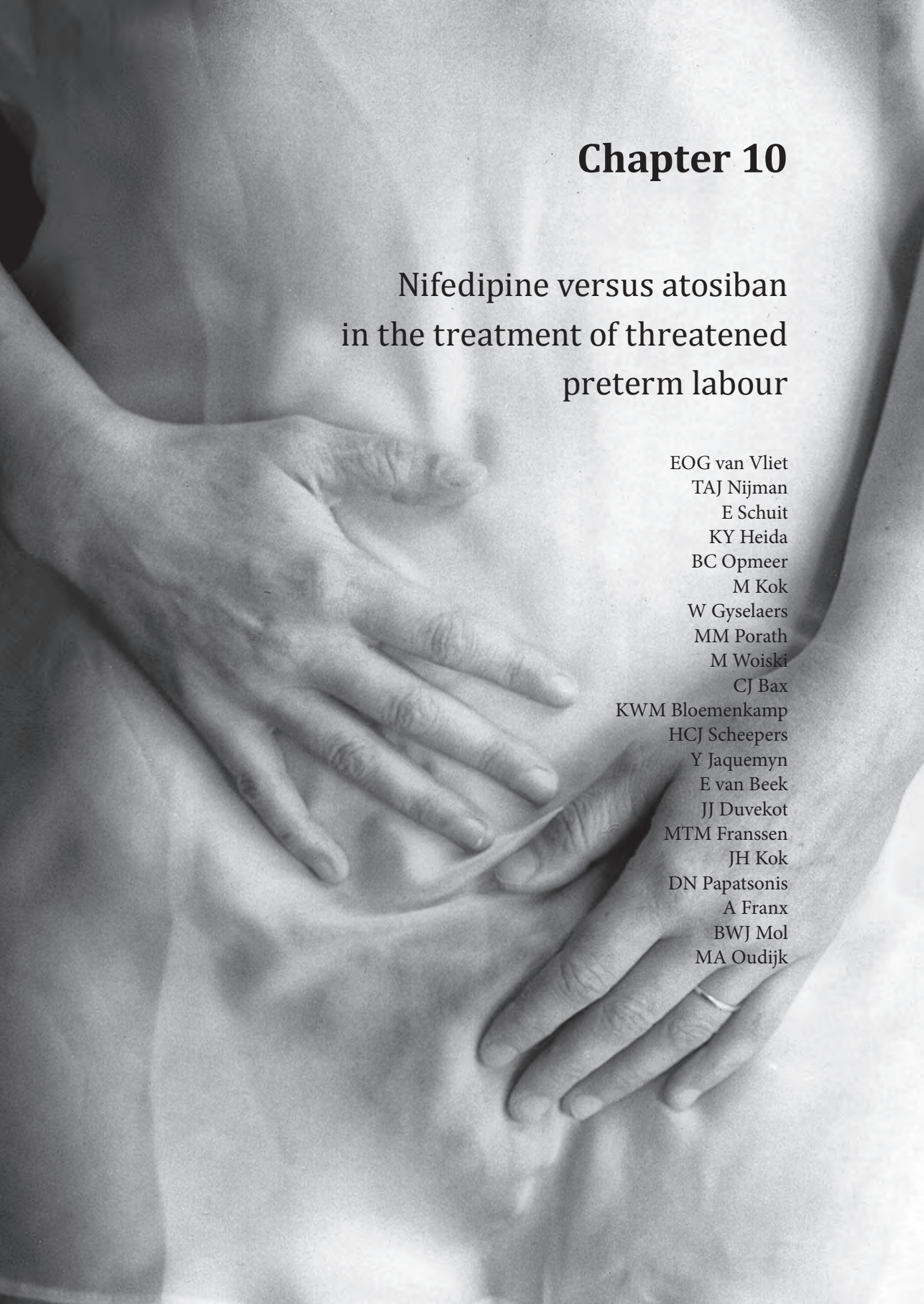
Ethical considerations
This study has been approved by the ethics committee of the Academic Medical Centre Amsterdam (Reference number MEC AMC 09/258) and by the boards of management of all participating hospitals. This trial is registered in the Dutch Trial Register, NTR 2947, http://www.trialregister.nl, date of registration: June 20th 2011.

Discussion

Preterm birth is an important cause of neonatal morbidity and mortality. Outcome of preterm infants can be improved and health care consumption and costs reduced by postponing delivery for 48 hours with tocolytic agents to allow maximal effect of maternal steroid administration and transportation of the mother to a centre with NICU facilities. The optimal type of tocolytic drug should improve neonatal outcome, be effective in delaying delivery, and safe for both mother and fetus. This trial will provide evidence on these subjects on the tocolytic drugs nifedipine and atosiban.

References

1. Berkowitz GS, Papiernik E: Epidemiology of preterm birth. *Epidemiol Rev* 1993, 15:414-443.
2. Slattery MM, Morrison JJ: Preterm birth. *Lancet* 2002, 360:1489-97.
3. Zeitlin J, Szamotulska K, Drewniak N, Mohangoo A, Chalmers J, Sakkeus L, Irgens L, Gatt M, Gissler M, Blondel B: Preterm birth. *BJOG* 2013, 120:1356–1365.
4. Ananth A, Cintzileos A: Epidemiology of preterm birth and its clinical subtypes. *J Matern Fetal Neonatal Med* 2006, 19:773-82.
5. Goldenberg RL, Culhane JF, Iams JD, Romero R: Epidemiology and causes of preterm birth. *Lancet* 2008, 371:75-84.
6. Gilbert WM, Nesbitt TS, Danielsen B: The costs of prematurity: quantification by gestational age and birth weight. *Obstet Gynecol* 2003, 102:488-92.
7. Moore T, Hennessy, EM, Myles J, Johnson JS, Draper, ES, Costeloe KL,Marlow N. J: Neurological and developmental outcome in extremely preterm children born in England in 1995 and 2006: the EPICure studies *BMJ* 2012, *345:e7961*
8. Roberts D, Dalziel S: Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane database syst rev* 2006, 19:(3):CD004454.
9. Rautava L, Eskelinen J, Häkkinen U, Lehtonen L: 5-Year Morbidity Among Very Preterm Infants in Relation to Level of Hospital Care. *JAMA Pediatr* 2013, 167:(1);40-6.
10. Hehiri MP, O'Connor HD, Kent EM, Robson MS, Keane DP, Geary MP, Malone FD. Early and late preterm delivery rates- a comparison of differing tocolytic policies in a single urban population. *J Matern Fetal Neonatal Me.* 2012, 25(11):2234-6.
11. Haas DM, Imperiale TF, Kirkpatrick PR, Klein RW, Zollinger TW, Golichowski AM: Tocolytic therapy: a meta-analysis and decision analysis. *Obstet Gynecol* 2009, 113:(3):585-94.
12. Haas DM, Caldwell DM, Kirkpatrick P, McIntosh JJ, Welton NJ: Tocolytic therapy for preterm delivery: systematic review and network meta-analysis. *BMJ* 2012, 345:e6226 .
13. Berkman ND, Thorp JM Jr, Lohr KN, Carey TS, Hartmann KE, Gavin NI, Hasselblad V, Idicula AE: Tocolytic treatment for the management of preterm labor: A review of the evidence. *Am J Obstet Gynecol* 2003, 188(6):1648-59.
14. King JF, Flenady VJ, Papatsonis DN, Dekker GA, Carbonne B: Calcium channel blockers for inhibiting preterm labour. *Cochrane Database Syst Rev.*2003, (1):CD002255.
15. Papatsonis D, Flenady V, Cole S, Liley H: Oxytocin receptor antagonists for inhibiting preterm labour. *Cochrane Database Syst Rev.*2005, 20:(3):CD004452.
16. van Veen AJ, Pelinck MJ, van Pampus MG, Erwich JJ: Severe hypotension and fetal death due to tocolysis with nifedipine. *BJOG* 2005, *An International Journal of Obstetrics & Gynaecology*, 509-510.
17. de Heus R, Mol BW, Erwich JJ, van Geijn HP, Gyselaers WJ, Hanssens M, Härmark L, van Holsbeke CD, Duvekot JJ, Schobben FF, Wolf H, Visser GH: Adverse drug reactions to tocolytic treatment for preterm labour: prospective cohort study. *BMJ* 2009, 338:b744.
18. Kashanian M, Akbarian AR, Soltanzadeh M. Atosiban and nifedipin for the treatment of preterm labor. *Int J Gynaecol Obstet.* 2005, 91:10-14.
19. Al-Omari WR, Al-Shammaa HB, Al-Tikriti EM, Ahmed KW. Atosiban and nifedipine in acute tocolysis: a comparative study. *Eur J Obstet Gynecol Reprod Biol.* 2006, 128:129-34.
20. Salim R, Garmi G, Nachum Z, Zafran N, Baram S, Shalev E: Nifedipine compared with atosiban for treating preterm labor. *Obstet Gynecol* 2012, 120:1323-1331.
21. Bell MJ: Neonatal Necrotizing Enterocolitis. *Ann Surg* 1978, 187:1-7.
22. Jobe AH, Bancalari E: Bronchopulmonary Dysplasia. *Am J Respir Crit Care Med* 2001,163: 1723-1729.
23. de Vries LS, Eken P, Dubowitz LM: The spectrum of leukomalacia using cranial ultrasound. Behav Brain Res *1992*, 49:1-6.
24. Ment LR, Bada HS, Barnes P, Grant PE, Hirtz D, Papile LA, Pinto-Martin J, Rivkin M, Slovis TL Practice parameter: neuroimaging of the neonate: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology* 2002, 58(12):1726-38.



Chapter 10

Nifedipine versus atosiban in the treatment of threatened preterm labour

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Abstract

Background In women with threatened preterm labor, delay of delivery for 48 hours allows antenatal corticosteroids to improve neonatal outcome. For this reason, tocolytics are often administered for 48 hours, but uncertainty exists over which tocolytic drug is most effective. The aim of the current study was to compare the effectiveness and safety of nifedipine and atosiban in women with threatened preterm birth.

Methods We randomly assigned 510 women (gestational age $25^{0/7}$ - $34^{0/7}$ weeks) with threatened preterm labor in a 1:1 ratio to tocolytic treatment for 48 hours using nifedipine or atosiban. The primary endpoint was a composite score of perinatal mortality, bronchopulmonary dysplasia, sepsis, intraventricular hemorrhage, periventricular leucomalacia and necrotizing enterocolitis. Secondary outcome measures included prolongation of pregnancy and maternal side effects.

Results The incidence of the primary outcome was 14.2% for nifedipine vs. 15.3% for atosiban (relative risk 0.92; 95%-CI 0.61-1.38). A non-significant higher perinatal death rate was found in the nifedipine group (5.4% versus 2.4%, RR 2.20; 95%-CI 0.91-5.34). No differences in secondary outcome measures were found.

Conclusion and Relevance In patients with threatened preterm labor, tocolysis with nifedipine and atosiban results in comparable adverse perinatal outcome rates.

Trial registration Clinical trial registration: http://www.trialregister.nl, NTR 2947, date of registration: June 20th 2011.

Introduction

Preterm birth is the most common cause of neonatal morbidity worldwide.[1] It affects 5-13% of all deliveries in developed countries[2-5] and is associated with 50% of neonatal morbidity and 50-75% of neonatal mortality.[1,4,5] Additionally, preterm birth is associated with long term physical and developmental impairment and thereby has a substantial impact on the infant, parents, families, and health care costs.[1,2]

To improve outcome in preterm infants, women in labor before 34 weeks of gestation receive antenatal corticosteroids to enhance fetal lung maturation.[6] To allow optimal effect of maternal steroid administration, most perinatal centers attempt to delay delivery by administrating tocolytic drugs for 48 hours.[7] Previous meta-analyses have shown that tocolytic drugs are effective in delaying delivery for 48 hours and for seven days.[8,9]

Several types of tocolytic drugs are used as treatment in preterm labor, including the β adrenoceptor agonists, cyclooxygenase (COX) inhibitors, magnesium sulphate, calcium channel blockers and oxytocin receptor antagonists. Uncertainty remains over which tocolytic is the drug of choice.

Studies on β adrenoceptor agonists have shown contradictory results for postponing delivery and decreasing neonatal mortality compared to placebo,[9,10] and due to substantial side effects their use has been largely abandoned from clinical practice. For COX-inhibitors, no effect on perinatal mortality and morbidity was found and there are some concerns on safety in the administration at late preterm gestational age.[11] For the use of initial tocolysis, calcium channel blockers (i.e. nifedipine) or oxytocin antagonists (i.e. atosiban) for 48 hours are recommended based on the largest effect and most favorable side effect profile.[12]

There is a lack of knowledge on which of these two tocolytical agents, nifedipine or atosiban, has the most favorable effect on neonatal outcome. Three small trials (n=80, n=63 and n=145) comparing nifedipine with atosiban have shown contradictory results.[13-15] One of these studies found a lower incidence of delivery within seven days after nifedipine tocolysis, but a higher incidence of birth within 48 hours after nifedipine compared to atosiban.[15] The two other trials did not find a difference in effectiveness when comparing nifedipine with atosiban.[13,14] Salim et al.[15] found a longer length of stay at the Neonatal Intensive Care Unit (NICU) among the atosiban group as compared with the nifedipine group. Two trials that reported on neonatal morbidities could not show a significant difference, but were underpowered on the topic. [14,15]

In view of this uncertainty, we performed the APOSTEL-III study (Assessment of Perinatal Outcome by use of Specific Tocolytics in Early Labor), a multicenter randomized clinical trial that compared nifedipine and atosiban in women with threatened preterm birth in terms of neonatal outcome, prolongation of pregnancy and maternal side effects.

Methods / Design

The protocol of this study has been published previously, and was approved by the ethics committee of the Academic Medical Centre Amsterdam (Reference number MEC AMC 09/258) and the boards of management of all participating hospitals.[16] The study is registered at the Dutch Clinical Trial Registry as NTR2947, and is reported according to the CONSORT guidelines.

Study design and participants

We performed a multicenter randomized controlled trial in 19 centers (9 secondary and 10 tertiary care centers) in the Netherlands and Belgium that collaborate in the Dutch Consortium for Healthcare Evaluation and Research in Obstetrics and Gynecology[17] Women aged ≥18 years with threatened preterm labor at a gestational age between $25^{0/7}$ weeks and $34^{0/7}$ weeks were eligible for participation. Threatened preterm labor was defined as at least three uterine contractions per 30 minutes, and one of the following: 1) cervical length of ≤ 10 mm, or 2) a cervical length of 11-30 mm and a positive fetal Fibronectin test, or 3) ruptured amniotic membranes. Women with either a singleton or a multiple pregnancy could be included. Maternal exclusion criteria were a contra-indication for tocolysis (severe vaginal bleeding, signs of intrauterine infection), hypertension or use of anti-hypertensive medication, history of myocardial infarction or angina pectoris, cerclage, cervical dilatation > 5 cm, tocolytic treatment for > 6 hours prior to arrival in a participating center, or a previous episode of tocolytic treatment. Women with a fetus showing signs of fetal distress or a fetus suspected of chromosomal or structural anomalies were not included. Eligible women were identified by the local staff and/or research coordinators. After counseling and reading the patient information form, patients were asked for written informed consent.

Randomization and masking

Randomization was performed per center by a web based computerized program using permuted-block 1:1 randomization, with block sizes of 4 participants, rendered by an independent data manager. As the comparison was between oral medication and intravenous medication, the study was not blinded.

Interventions

Patients were allocated to nifedipine or atosiban treatment for 48 hours. Details on the regime can be found in Appendix 1. Antenatal corticosteroids were administered according to national guidelines on management of preterm birth, advising antenatal corticosteroids to women in preterm labor < 34 weeks of gestation. The provision of prophylactic antibiotics was at the discretion of the attending physician.

Study outcome measures

The primary outcome measure was a composite of adverse perinatal outcome, including perinatal mortality and severe perinatal morbidities (bronchopulmonary dysplasia (BPD), culture proven sepsis, intraventricular hemorrhage > grade 2, periventricular leucomalacia (PVL) > grade 1 and necrotizing enterocolitis (NEC) > stage 1).

BPD was diagnosed according to the international consensus guideline as described by Jobe and Bancalari at time of discharge home or at 36 weeks of corrected gestational age.[18] Culture proven sepsis was diagnosed on the combination of clinical signs and positive blood cultures. Intraventricular hemorrhage > grade 2 and PVL > grade 1 was diagnosed by repeated neonatal cranial ultrasound by the neonatologist according to the guidelines on neuro imaging described by de Vries and Ment et al.[19,20] NEC was diagnosed according to Bell > stage 1.[21]

Secondary outcome measures on maternal level were gestational age at delivery, prolongation of pregnancy, maternal mortality and maternal side effects leading to discontinuation of study medication. Secondary outcomes on child level were the individual components of the composite perinatal outcome, neonatal intensive care (NICU) admission, ventilation support, total days in hospital until 3 months corrected age, apnea, asphyxia, proven meningitis and pneumothorax. Data were recorded in a web-based Case Report Form by research nurses and midwives.

All cases of perinatal death were assessed by a panel of two neonatologists and two perinatologists, all not involved in the trial. The members individually reviewed all cases of perinatal death while remaining blinded to the study medication administered. They assessed whether the perinatal deaths could be causally related to the study medication, using the causality categories of the World Health Organization: certain, probable, possible, unlikely, conditional, and non-assessable.[22] When > 75% consensus was reached the conclusion was considered valid.

Statistical analysis

The trial was designed to detect a reduction in the primary outcome rate from 25% to 15%. Enrolling 500 women would provide a power of 80% at a significance level of .05 (2-sided). Continuous variables are presented as mean with standard deviation (SD) or as median with interquartile range (IQR), as appropriate. Categorical and dichotomous variables are presented as a number and percentage of the total allocation group. Data analysis was performed according to the intention-to-treat principle. The primary outcome was assessed on child level, using a binomial generalized estimating equations model (GEEs) with a log-link function and using an unstructured correlation matrix, resulting in a relative risk (RR) with accompanying 95% confidence interval (CI). We accounted for interdependence between outcomes in multiple pregnancies by considering the mother as a cluster variable.[23] Secondary outcomes on the child level were approached similarly to the primary outcome. Continuous outcomes on the child level were assessed using linear quantile mixed models with mother as the grouping variable, resulting in a median difference with 95%-CIs. Prolongation of pregnancy

was evaluated by Cox proportional hazards regression and Kaplan-Meier estimates, accounting for differing gestational age at entry, and tested with the Log rank test. Outcomes on the maternal level were assessed by a binomial regression model with log-link function.
Pre-specified subgroup analyses were performed based on: 1) PPROM status: PPROM versus intact membranes, 2) gestational age at randomization: < 30 weeks versus ≥ 30 weeks, 3) fibronectin test result: positive versus negative/ no fibronectin test, 4) cervical length at randomization: <10 mm versus ≥ 10 mm, 5) number of fetuses: multiple versus singleton pregnancies, and 6) history of preterm birth: yes versus no. Subgroup effects were investigated for adverse perinatal outcome and prolongation of pregnancy. Subgroup effects were assessed by including an interaction term between the subgrouping variable and treatment allocation as covariate to the regression model. When the interaction term was statistically significant ($p < 0.05$) a stratified subgroup analysis was performed to study the effect of treatment in different strata of the subgroups.
A planned interim analysis was performed based on the outcomes of 145 women; the data safety monitoring committee noted no conditions to stop the trial. All analyses were adjusted for the interim analyses with the O'Brien-Fleming alpha spending function. As a result, a nominal p value of less than 0.049 was deemed to indicate statistical significance.
Analyses were performed using R, version 3.1.1 (R Foundation for Statistical Computing). Specifically, GEE was performed using the gee library and linear quantile mixed models using the lqmm library.

Data sharing
Data is available upon request at the University Medical Center Utrecht.

Results

Study population
Between 2012-2014 we enrolled 510 women in the trial, 254 to the nifedipine group and 256 to the atosiban group (Figure 1). Due to withdrawal of informed consent (n=5), baseline characteristics were available for 249 patients in the nifedipine group. Table 1 indicates comparable baseline characteristics between the groups. Median gestational age at study entry was 30.3 weeks in both groups, 36% versus 37% of women had ruptured membranes, and 20% versus 14% of women had a multiple pregnancy. Due to patients being lost to follow-up (nifedipine n=1; atosiban n=1) outcome data were available for 248 women in the nifedipine group and 255 in the atosiban group, corresponding to 296 and 294 children, respectively.

Figure 1. Flow chart

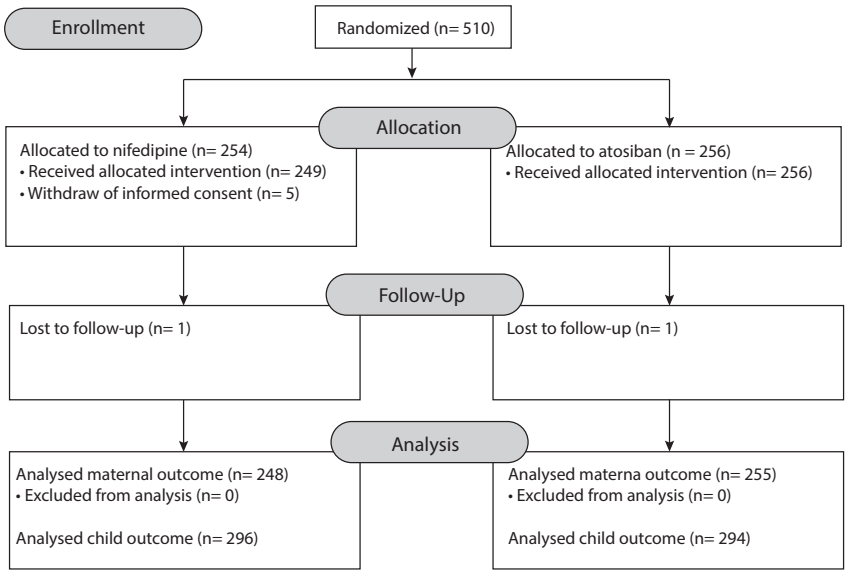


Table 1. Baseline Characteristics

Characteristics	**Nifedipine (n = 249)**	**Atosiban (n = 256)**
Age, mean (SD), years	30.5 (5.2)	30.1 (5.1)
Body mass index, median (IQR)*	23.1 (20.8-25.8)	22.8 (20.6-25.5)
Caucasian, n (%)†	180 (82)	184 (81)
Nulliparous, n (%)	160 (65)	170 (67)
Prior preterm birth, n (%)	33 (13)	30 (12)
Gestational age at study entry, median (IQR), weeks	30.3 (28.4-32.1)	30.3 (28.1-31.7)
Multiple gestation, n (%)		
Twin	49 (20)	37 (14)
Triplet	0 (0)	1 (0)
PPROM at study entry, n (%)‡	84 (36)	88 (37)
Prior tocolytic treatment §	47 (19)	61 (61)
Vaginal examination at study entry, n (%)	114 (47)	121 (48)
Dilatation, median (IQR), cm ¶	1 (1-2)	1 (1-2)
Cervical length, median (IQR), mm ¥	15 (9-22)	14 (8-23)

*Based on n=198 for nifedipine and n=206 for atosiban due to missing data.
† Based on n= 220 for nifedipine and n=227 for atosiban due to missing data.
‡ Based on n= 233 for nifedipine and n=241 for atosiban due to missing data.
§ Based on n=244 for nifedipine and n=255 for atosiban due to missing data.
¶ Based on n=112 for nifedipine and n=120 for atosiban due to missing data.
¥ Based on n=159 for nifedipine and n=153 for atosiban due to missing data.
Abbreviations: SD standard deviation; IQR interquartile range.

Primary outcome

There were 42 children (14.2%) who had adverse perinatal outcome in the nifedipine group and 45 (15.3%) in the atosiban group (RR 0.92; 95%-CI 0.61-1.38; Table 2).

Secondary outcomes

Gestational age at delivery was not statistically significant different between the two groups (Median (IQR) 33.1 weeks (30.5-37.0) for the nifedipine group and 32.4 weeks (30.1-35.8) for the atosiban group, HR 0.86; 95%-CI 0.70-1.05; Table 2). Median (IQR) prolongation of pregnancy was seven days (1.0-41.5) for the nifedipine group and four days (1.0-38.0) for the atosiban group (HR 0.87; 95%-CI 0.71-1.06). The Kaplan-Meier curve (figure 2) indicating gestational age at delivery and prolongation of pregnancy showed no statistically significant differences (log-rank test; p=0.12 and p = 0.17 respectively).

Perinatal death occurred in 16 children (5.1%) in the nifedipine group and in seven children (2.4%) in the atosiban group (RR 2.20; 95%-CI 0.91-5.34). A panel of experts independently assessed the cases of perinatal mortality, and classified all of the cases as unlikely to be caused directly by the study medication (Appendix 2). The rates of bronchopulmonary dysplasia, culture proven sepsis, IVH > grade II, PVL > grade I and NEC > grade 1 analyzed separately were all comparable between the groups.

In the nifedipine group, 154 (58%) infants were admitted to the NICU, compared to 183 (68%) infants in the atosiban group (RR 0.87, 95%-CI 0.75-1.00, Table 2). Forty-two (16%) of the infants in the nifedipine group needed ventilation support, compared to 54 (21%) infants in the atosiban group (RR 0.78, 95%-CI 0.53-1.14). Other neonatal outcomes are displayed in Table 2.

Maternal mortality did not occur in either group. Discontinuation of study medication occurred in 74 women (30%) in the nifedipine group and 74 women (29%) in the atosiban group (RR 1.03, 95%-CI 0.78-1.35), and was mainly due to progression into labor (89% versus 95%, RR 0.97, 95%-CI 0.73-1.30). Side effects leading to discontinuation of study medication were reported in 15 (6%) women of the nifedipine group and 6 (2%) women of the atosiban group (RR 2.57; 95%-CI 1.01-6.55). This difference was mainly driven by more suspected intrauterine infections in the nifedipine group (6 versus 1).

Subgroup analysis

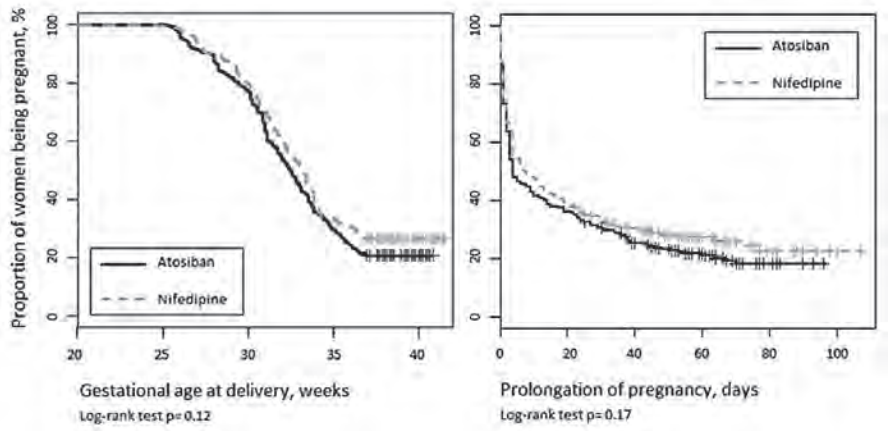
No significant interactions were found between subgroups and allocation on either adverse neonatal outcome or prolongation of pregnancy (table 4); hence no effect sizes were calculated in different strata of the subgroups.

Table 2. Perinatal Outcome

Primary outcome (child level)	**Nifedipine (n=296)**	**Atosiban (n=294)**	**Relative Risk (95% CI)**
Adverse perinatal outcome, n (%)	42 (14.2)	45 (15.3)	0.92 (0.61-1.38)
Perinatal mortality, n (%)	16 (5.4)	7 (2.4)	2.20 (0.91-5.34)
Broncho pulmonary dysplasia, n (%)	11 (3.7)	21 (7.1)	0.55 (0.27-1.15)
Culture proven sepsis, n (%)	24 (8.1)	25 (8.5)	0.93 (0.52-1.63)
IVH > grade II, n (%)	5 (1.7)	2 (0.6)	2.48 (0.48-12.80)
PVL > grade I, n (%)	1 (0.3)	2 (0.7)	0.49 (0.05-5.50)
NEC > grade I, n (%)	7 (2.4)	3 (1.0)	1.73 (0.51-5.85)
Secondary outcomes (child level)	**Nifedipine (n=296)**	**Atosiban (n=294)**	**Relative Risk (95% CI)/ Median difference (95% CI)**
NICU admittance, n (%)*	154 (58)	183 (68)	0.87 (0.75-1.00)
Length of admission at NICU, median (IQR), days	17 (6.3-42.8)	17 (7-40.5)	-1 (-5.60-3.60)
Ventilation support, n (%)†	42 (16)	54 (21)	0.78 (0.53-1.14)
Days on ventilation support, median (IQR)	3 (1.3-9.5)	3 (1.0-7.8)	-0.39 (-2.75 – 1.97)
Total days in hospital until 3 months c.a. ‡	27 (9.0-47.5)	32 (15.5-55.0)	-5.00 (-11.46 – 1.47)
Apneu, n (%)	20 (7)	25 (9)	0.73 (0.41-1.32)
Asphyxia, n (%)	2 (1)	2 (1)	0.99 (0.14-7.06)
Proven meningitis, n (%)	5 (2)	2 (1)	2.45 (0.48-12.53)
Pneumothorax, n (%)	2 (1)	5 (2)	0.40 (0.08-2.04)
Secondary outcomes (maternal level)	**Nifedipine (n=248)**	**Atosiban (n=255)**	**Hazard Ratio (95% CI) / Relative risk (95% CI)**
Gestational age at delivery, median (IQR) - weeks	33.1 (30.5-37.0)	32.4 (30.1-35.8)	0.86 (0.70-1.05)
Prolongation of pregnancy			
Continuous, median (IQR), days	7 (1.0-41.5)	4 (1.0-38.0)	0.87 (0.71-1.06)
≥48 hours, n (%)	167 (69)	168 (66)	1.04 (0.92-1.18)
≥7 days, n (%)	125 (51)	116 (45)	1.13 (0.94-1.36)
Maternal mortality, n (%)	0 (0)	0 (0)	n.a.
Discontinuation of study medication, n (%)	74 (30)	74 (29)	1.03 (0.78-1.35)
Due to progression into labor, n (%)§	66 (89)	70 (95)	0.97 (0.73-1.30)
Due to side effects, n (%)§	15 (20)	6 (8)	2.57 (1.01-6.55)
Unknown, n (%)§	2 (3)	2 (3)	-

Abbreviations: IVH intraventricular hemorrage; PVL periventricular leucomalacia; NEC necrotizing enterocolitis; SD standard deviation; NICU neonatal intensive care unit; c.a. corrected age.
* Based on n=267 for nifedipine and n=271 for atosiban due to missing data.
† Based on n=258 for nifedipine and n=261 for atosiban due to missing data.
‡ Based on n=267 for nifedipine and n=271 for atosiban due to missing data.
§Percentages add up to >100 because study medication could be discontinued for more than one reason.

Figure 2. Kaplan Meier curves for gestational age at delivery and prolongation of pregnancy



Comment

This randomized trial shows that in women with threatened preterm labor 48 hours of tocolysis using nifedipine compared to atosiban resulted in comparable adverse perinatal outcome rates.

Our study has several strengths. First, our primary outcome measure reflects that the main goal of tocolysis is to improve neonatal outcome, and not prolongation of pregnancy per se. Previous trials were not powered to examine neonatal outcomes.[13-15] Second, to our best knowledge this is the largest RCT directly comparing the effectiveness and safety of the widely used tocolytic drugs nifedipine and atosiban in a multicenter setting. Third, we aimed to include women at high risk of preterm delivery. Indeed, more than half of the women in our study delivered within seven days after inclusion, and >75% delivered preterm, as opposed to previous trials in which the large majority of women did not deliver shortly after randomization.[14,15]

Our study has also some limitations. Due to the nature of the interventions (oral medication versus intravenous medication), our study was not blinded. This might have caused bias, although this is unlikely to have an impact on outcome since our outcome measures were objectively. Second, perinatal death was part of our composite outcome measure. Although composite outcomes can be necessary to make statistical comparisons, it denies in its design differences in clinical or perceived relevance of death over serious possibly disabling morbidities such as BPD or IVH. Also, it might combine outcomes that occur after different mechanisms, on one hand improvement of respiratory perinatal outcome due to prolongation of pregnancy, but more fetal death due to circulatory instability. Our study was not powered to answer this relevant question.

Table 3. **Subgroup analysis**

	Nifedipine	Atosiban	
Adverse neonatal outcome (child level)	**n/N (%)**	**n/N (%)**	***p*-value for interaction**
Ruptured membranes at study entry			
Yes	12 / 98 (12.2)	14 / 106 (13.2)	0.98
No	27 / 178 (15.2)	28 / 173 (16.2)	
Gestational age at randomisation < 30 weeks			
< 30 weeks	33 / 132 (25.0)	36/ 136 (26.5)	0.71
≥ 30 weeks	7 / 158 (4.4)	9 / 158 (5.7)	
Fibronectine test			
Positive	8/ 69 (11.6)	7/ 78 (9.0)	NA
Negative	0/ 13 (0)	0/ 8 (0)	
Cervical length			
< 10mm	14 / 62 (22.6)	9 / 57 (15.8)	0.21
≥ 10 mm	9 / 132 (6.8)	12 / 117 (10.3)	
Multiple pregnancy			
Yes	14/98 (14.3)	11/ 77 (14.3)	0.82
No	28/ 199 (14.1)	34/ 218 (15.6)	
Previous preterm birth			
Yes	2/ 34 (5.9)	7/ 33 (21.2)	0.10
No	40 / 263 (15.2)	38 / 260 (14.6)	
Prolongation of pregnancy (maternal level)	**median (IQR)**	**median (IQR)**	***p*-value for interaction**
Ruptured membranes at study entry			
Yes	2 (0.0-5.5)	3 (1.0-6.0)	0.06
No	27 (4.0-57.0)	14.5 (2.0-53.0)	
Gestational age at randomisation < 30 weeks			
< 30 weeks	12.5 (2.0-58.8)	10.0 (2.0-54.0)	0.20
≥ 30 weeks	5.0 (1.0-32.0)	3.0 (1.0-24.5)	
Fibronectine test			
Positive	142 (10.5-66.0)	38 (4.0-59.5)	0.10
Negative	37 (24.0-58.5)	5 (0.0-15.0)	
Cervical length			
< 10mm	6.5 (1.3-42.5)	2.0 (1.0-12.8)	0.83
≥ 10 mm	29.5 (4.0-59.0)	37.5 (6.0-58.5)	
Multiple pregnancy			
Yes	4 (1.0-24.5)	3.5 (1-20.3)	0.19
No	8.5 (1.0-45.0)	4.0 (1.0-44.0)	
Previous preterm birth			
Yes	30.0 (9.5-64.5)	4.5 (2.0-33.3)	0.55
No	6.0 (1.0-36.5)	4.0 (1.0-38.0)	

Abbreviations: IQR interquartile range.

A non-significant, but possibly clinically relevant higher incidence of perinatal death was seen in the nifedipine group, although the expert panel could not find a direct causal association between the drugs and mortality. A not statistically significant longer prolongation of pregnancy and more infections were noted in the nifedipine group. It has been suggested in the past that prolongation of pregnancy, although the primary mechanism of tocolysis, might not be helpful for the infant.[24] Alternatively, it may be hypothesized that the administration of nifedipine in pregnant women has

an adverse effect on the fetus, for example by lowering maternal blood pressure and reducing placental perfusion. Animal studies describe changes in uterine blood flow and occurrence of fetal acidemia, while studies in humans showed no adverse effects on umbilical artery blood flow or fetal movements.[25-32] There is one case report of fetal death after tocolysis using nifedipine, most likely due to maternal hypotension.[33] A prospective cohort study performed in the Netherlands and Belgium concluded that maternal adverse events, mainly hypotension and tachycardia, were more frequent with the use of nifedipine.[34] In our study, no severe maternal side effects were observed and review of the charts of the perinatal deaths did not reveal any cases of severe hypotension. However, the safety of nifedipine in pregnancy has not been studied extensively, and nifedipine is not registered for use in pregnancy.[35] This is of concern especially since nifedipine is recommended as first-line tocolytical agent in international guidelines.[36,37] Since an expert panel could not find a direct causal association between the drugs and mortality, we could not find evidence in our study for a clinical effect of the proposed pathophysiological mechanism. Most importantly, the debate on the effectiveness and safety of tocolysis in general is inconclusive. There is a lack of proof that tocolysis, and thereby prolongation of pregnancy in preterm labor in general, improves perinatal outcome.[12,38] We therefore support the initiation of large placebo controlled trials in the treatment of preterm labor, with adverse neonatal outcome being the primary outcome.

In conclusion, we found no difference in adverse perinatal outcome rates after tocolysis using nifedipine versus atosiban in women with threatened preterm labor. Nevertheless, the higher perinatal mortality rate in the nifedipine group is of concern and warrants more investigation into the use of this tocolytic drug.

Appendix 1. Treatment regime

In the nifedipine group, the initial dose was 2 x 10 mg nifedipine capsules orally in the first hour, followed by 20 mg nifedipine slow release per 6 hours for the next 47 hours. In the first hour after starting nifedipine administration, blood pressure and heart rate was measured every 15 minutes. If blood pressure remained within the normal limits, treatment continued with blood pressure and heart rate measured 4 times every 24 hours. In the atosiban group, patients received a bolus injection of 6.75 mg intravenous in 1 minute, followed by 18 mg/hour for 3 hours, followed by a maintenance dosage of 6 mg/hour for 45 hours.

Appendix 2. Analysis of perinatal mortality

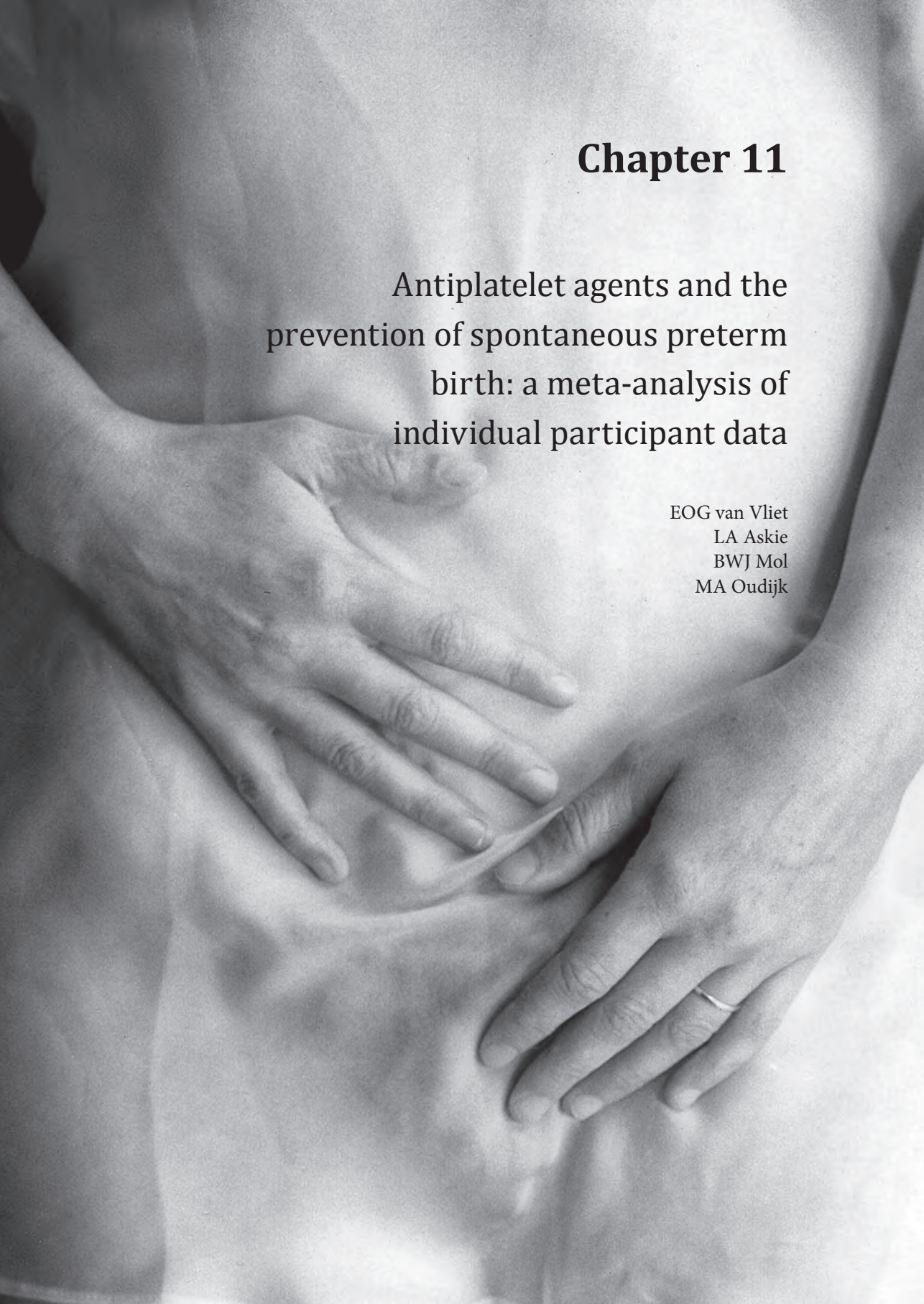
A panel of experts independently assessed the cases of perinatal mortality, and classified all of the cases as unlikely to be caused directly by the study medication. Among the deceased infants, GA at birth was comparable between the groups (median (IQR) of 27.0 (26.3-28.2) weeks in the nifedipine group and 28.3 weeks (25.7-29.9) in the atosiban group). Prolongation of pregnancy was comparable between the 2 groups (median (IQR) 36.0 hours (11.3-87.0) for nifedipine versus 42.0 hours (15.0-320.0) for atosiban). Two infants died in utero or during delivery. For the deceased children, the median time between birth and death was 6 days for the nifedipine group (range 0-40 days) and 5 days for the atosiban group (range 0-45 days). Six (38%) of the deceased infants in the nifedipine group were multiplets, versus 2 (29%) in the atosiban group. Five infants died because of lethal congenital malformations not known at the time of inclusion (three in the nifedipine group and two in the atosiban group). None of the mothers or neonates had clinically relevant hypotension (difference in systolic or diastolic blood pressure > 20mmHg).

	Nifedipine (n = 16)	**Atosiban (n = 7)**
GA at birth, weeks, median (IQR)	27.0 (26.3-28.2)	28.3 (25.7-29.9)
Prolongation of pregnancy, hours	36.0 (11.3-87.0)	42.0 (15.0-320.0)
Interval from birth to death, days, median (IQR)	6 (2-16)	5 (3-15)
Crossover, n (%)	1 (6%)	1 (14%)
Multiplets, n (%)	6 (38%)	2 (29%)
Congenital malformations, n (%)	3 (19%)	2 (29%)
Completed course corticosteroids, n (%)	8 (50%)	3 (43%)

References

1. Blencowe H, Cousens S, Chou D, et al. Born too soon: the global epidemiology of 15 million preterm births. Reprod Health 2013;10 Suppl 1:S2.
2. Treyvaud K, Lee KJ, Doyle LW, Anderson PJ. Very preterm birth influences parental mental health and family outcomes seven years after birth. JPediatr. 2014;164:515-21.
3. Bolisetty S, Bajuk B, Abdel-Latif ME, Vincent T, Sutton L, Lui K. Preterm outcome table (POT): a simple tool to aid counselling parents of very preterm infants. Aust N Z J Obstet Gynaecol. 2006;46:189-92.
4. Ananth A, Cintzileos A. Epidemiology of preterm birth and its clinical subtypes. J Matern Fetal Neonatal Med 2006;19:773-82.
5. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008;371:75-84.
6. Keirse MJ. The history of tocolysis. BJOG. 2003;110 Suppl 20:94-97.
7. Hehiri MP, O'Connor HD, Kent EM, Robson MS, Keane DP, Geary MP, Malone FD. Early and late preterm delivery rates- a comparison of differing tocolytic policies in a single urban population. J Matern Fetal Neonatal Med. 2012;25(11):2234-6.
8. Haas DM, Imperiale TF, Kirkpatrick PR, Klein RW, Zollinger TW, Golichowski AM. Tocolytic therapy: a meta-analysis and decision analysis. Obstet Gynecol 2009;113:585-94.
9. Haas DM, Caldwell DM, Kirkpatrick P, McIntosh JJ, Welton NJ. Tocolytic therapy for preterm delivery: systematic review and network meta-analysis. BMJ 2012;345:e6226.
10. Berkman ND, Thorp JM Jr, Lohr KN, et al. Tocolytic treatment for the management of preterm labor: A review of the evidence. Am J Obstet Gynecol 2003;188:1648-59.
11. King J, Flenady V, Cole S, Thornton S. Cyclo-oxygenase (COX) inhibitors for treating preterm labor. Cochrane Database Syst Rev. 2005;18:CD001992.
12. van Vliet EO, Boormans EM, de Lange TS, Mol BW, Oudijk MA. Preterm labor: current pharmacotherapy options for tocolysis. Expert Opin Pharmacother. 2014;15:787-97.
13. Kashanian M, Akbarian AR, Soltanzadeh M. Atosiban and nifedipin for the treatment of preterm labor. Int J Gynaecol Obstet. 2005;91:10-14.
14. Al-Omari WR, Al-Shammaa HB, Al-Tikriti EM, Ahmed KW. Atosiban and nifedipine in acute tocolysis: a comparative study. Eur J Obstet Gynecol Reprod Biol. 2006;128:129-34.
15. Salim R, Garmi G, Nachum Z, Zafran N, Baram S, Shalev E. Nifedipine compared with atosiban for treating preterm labor: a randomized controlled trial. Obstet Gynecol 2012;120:1323-31.
16. van Vliet EO, Schuit E, Heida KY, et al. Nifedipine versus atosiban in the treatment of threatened preterm labor (Assessment of Perinatal Outcome after Specific Tocolysis in Early Labour: APOSTEL III-Trial). BMC Pregnancy Childbirth. 2014;3:14:93.
17. www.studies-obsgyn.nl
18. Jobe AH, Bancalari E. Bronchopulmonary Dysplasia. Am J Respir Crit Care Med 2001;163:1723-29.
19. de Vries LS, Eken P, Dubowitz LM. The spectrum of leukomalacia using cranial ultrasound. Behav Brain Res 1992;49:1-6.
20. Ment LR, Bada HS, Barnes P, et al. Practice parameter: neuroimaging of the neonate: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology 2002;58:1726-38.
21. Bell MJ. Neonatal Necrotizing Enterocolitis. Ann Surg 1978;187:1-7.
22. Meyboom RH, Hekster YA, Egberts AC, Gribnau FW, Edwards IR. Causal or casual? The role of causality assessment in pharmacovigilance. Drugs Saf 1997;17:374-89.
23. Gates S, Brocklehurst P. How should randomised trials including multiple pregnancies be analysed? BJOG. 2004;111:213-9.
24. Alfirevic Z. Tocolytics: do they actually work? BMJ 2012; 345:e6531.
25. Furuhashi N, Tsujiei M, Kimura H, Yajima A. Effects of nifedipine on normotensive rat placental blood flow, placental weight and fetal weight. Gynecol Obstet Invest 1991;32:1–3.
26. Harake B, Gilbert RD, Ashwal S, Power GG. Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep. Am J Obstet Gynecol 1987;157:1003–8.
27. Holbrook RH, Lirette M, Katz M. Cardiovascular and tocolytic effects of nicardipine HCl in the pregnant rabbit: comparison with ritodrine HCl. Obstet Gynecol 1987;69:83–7.
28. Blea CW, Barnard JM, Magness RR, Phernetton TM, Hendricks SK. Effect of nifedipine on fetal and maternal hemodynamics and blood gases in the pregnant ewe. Am J Obstet Gynecol 1997;176:922–30.
29. Mari G, Kirshon B, Moise KJ, Lee W, Cotton DB. Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor. Am J Obstet Gynecol 1989;161:1514–8.

30. Pirhonen JP, Erkkola RU, Ekblad UU, Nyman L. Single dose of nifedipine in normotensive pregnancy: nifedipine concentrations, hemodynamic responses, and uterine and fetal flow velocity waveforms. Obstet Gynecol 1990;76:807–11.
31. Guclu S, Saygili U, Dogan E, Demir N, Baschat AA. The short-term effect of nifedipine tocolysis on placental, fetal cerebral and atrioventricular Doppler waveforms. Ultrasound Obstet Gynecol 2004;24:761–65.
32. de Heus R, Mulder EJ, Derks JB, Visser GH. The effects of the tocolytics atosiban and nifedipine on fetal movements, heart rate and blood flow. J Matern Fetal Neonatal Med. 2009;22:485-90.
33. van Veen AJ, Pelinck MJ, van Pampus MG, Erwich JJ. Severe hypotension and fetal death due to tocolysis with nifedipine. BJOG 2005;112:509–10.
34. de Heus R, Mol BW, Erwich JJ, et al. Adverse drug reactions to tocolytic treatment for preterm labor: prospective cohort study. BMJ 2009;338:b744.
35. Khan K, Zamora J, Lamont RF et al. Safety concerns for the use of calcium channel blockers in pregnancy for the treatment of spontaneous preterm labour and hypertension: a systematic review and meta-regression analysis. J Matern Fetal Neonatal Med. 2010;23(9):1030-8.
36. Management of preterm labor. American College of Obstetricians and Gynecologist; Committee on Practice Bulletins- Obstet Gynecol. 2012;119:1308-217.
37. Tocolysis for women in preterm labor. London (UK): Royal College of Obstetricians and Gynaecologists (RCOG); 2011. (Accessed May 12, 2015, at https://www.rcog.org.uk/globalassets/documents/guidelines/gtg1b26072011.pdf).
38. Simhan HN, Caritis SN. Prevention of Preterm Delivery. N Engl J Med 2007; 357:477-87.



Chapter 11

Antiplatelet agents and the prevention of spontaneous preterm birth: a meta-analysis of individual participant data

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Abstract

Introduction Spontaneous preterm birth is an important cause of neonatal mortality and morbidity. An increasing body of evidence suggests that uteroplacental ischaemia plays an important role in the etiology of spontaneous preterm birth. We hypothesized that antiplatelet agents may reduce the risk of spontaneous preterm birth.
Methods We analysed data collected in an individual participant data meta-analysis of 17 trials (28.797 women) that had evaluated the effect of antiplatelet agents to reduce pre-eclampsia. Primary endpoints were spontaneous preterm birth <37 weeks, <34 weeks and <28 weeks of gestation.
Results Women assigned to antiplatelet treatment compared to placebo or no treatment had a lower risk of spontaneous preterm birth < 37 weeks (RR 0.93, 95% CI 0.86-0.996) and <34 weeks of gestation (relative risk 0.86, 95% CI 0.76 – 0.99). The relative risk of having a spontaneous preterm birth <37 weeks was 0.83 (95% CI 0.73-0.95) for woman who have had a previous pregnancy, and 0.98 (95% CI 0.89-1.09) for women in their first pregnancy.
The treatment effect was stable in all other pre-specified subgroups.
Conclusion Antiplatelet agents reduce spontaneous preterm birth in pregnant women at risk of pre-eclampsia.

Introduction

Preterm labour is one of the most important obstetric problems worldwide and occurs in approximately 5-10% of all deliveries. It accounts for 70% of perinatal mortality and 40% of severe neurological morbidities.[1] Preterm birth can be classified as spontaneous or iatrogenic preterm birth. Spontaneous preterm birth starts with spontaneous labour or preterm premature rupture of the membranes (PPROM). In iatrogenic preterm birth, pregnancy is prematurely interrupted for maternal or fetal indications, usually pre-eclampsia or intrauterine growth restriction.[2] In industrialized countries, spontaneous preterm birth accounts for two thirds of all preterm births and is considered a heterogeneous syndrome in which different pathological processes prematurely activate the mechanisms of labour.[3,4] Myometrial contractions are activated by an interplay between mechanical and endocrine mechanisms and immune system responses.[4] Intrauterine infections are thought to play a major role in spontaneous preterm labour, as inflammatory cytokines stimulate prostaglandin release thus contributing to preterm myometrial contractions. Furthermore, cytokines and toxins initiate neutrophil activation, leading to the release of metalloproteases that weaken the membranes and cervix.[5] Signs of infection and inflammation are however not always present in spontaneous preterm birth.

An increasing body of evidence suggests that uteroplacental ischaemia also plays a role in the etiology of spontaneous preterm labour, analogous to its role of pre-eclampsia. Placental vascular pathology is found in at least one third of the placentas of women with spontaneous preterm labour or PPROM [6,7] In placental bed biopsies of women with spontaneous preterm labour similarities are found with biopsies of the placenta of women with pre-eclampsia. Failure of physiological transformation of the spiral arteries, necessary for normal placental blood flow, are found in one third of women with preterm labour and PPROM.[7-10] Women with spontaneous preterm labour are shown to have an abnormal angiogenic/anti-angiogenic plasma profile, comparable to the profiles found in women with pre-eclampsia.[11] In addition, women with increased resistance at midtrimester Doppler measurement of uterine artery flow, indicative of disordered placentation, are at increased risk of both spontaneous and iatrogenic preterm birth.[12] Furthermore, women with a history of pre-eclampsia have an increased risk of spontaneous preterm birth and vice versa.

The Perinatal Antiplatelet Review of International Studies Individual Participant Data meta-analysis (PARIS IPD) combined the data of 32,217 women that were randomized to aspirin or placebo, showed that in women at risk of pre-eclampsia, the use of antiplatelet agents during pregnancy is associated with a moderate reduction in the risk of pre-eclampsia (relative risk (RR 0.90), 95% CI 0.84-0.97).[13] Furthermore, this study has shown a reduction in preterm birth < 34 weeks in women treated with antiplatelet agents (RR 0.90, 95%-CI 0.83-0.98). In view of the overlapping underlying mechanisms of pre-eclampsia and spontaneous preterm birth, it would be relevant to know whether this reduction comprises a reduction in iatrogenic preterm birth (e.g.

through lowering the incidence of pre-eclampsia), or also a reduction of spontaneous preterm birth. In view of the scarcity of effective preventive measures for spontaneous preterm birth, this would be very relevant.
The aim of this re-analysis of PARIS-IPD was therefore to evaluate the efficacy of low dose-aspirin for the prevention of spontaneous preterm birth in women at risk of pre-eclampsia, and to explore the effect in pre-specified subgroups.

Methods

Data collection

The PARIS collaboration group conducted an Individual Participant Data (IPD) meta-analysis which included 31 randomized trials on antiplatelet agents for the prevention of pre-eclampsia. The PARIS IPD meta-analysis included studies that randomized women to low-dose aspirin/dipyridamole or placebo/no treatment as a primary preventive strategy for pre-eclampsia.
The studies included women at risk of pre-eclampsia, gestational hypertension or intra-uterine growth restriction, including nulliparous women. The risk classification was based on previous pregnancy history, pre-existing medical condition (eg. renal disease, diabetes, immune disorder, chronic hypertension) or obstetric risk factors early in the current pregnancy (eg. being primigravida, having a multiple pregnancy). Details on search strategy and study selection have been published previously.[13] The current analyses are based on data of 17 trials that supplied data on type of delivery (i.e. spontaneous versus induction / non-labour caesarean section). The PARIS steering group gave permission for the use of the data for the purpose of these analyses.

Outcome measures

We studied three main outcome measures: 1) spontaneous preterm birth of a live born infant between 20 - 37 weeks; 2) spontaneous preterm birth of a live born infant between 20 - 34 weeks; and 3) spontaneous preterm birth of a live born infant between 20 - 28 weeks. Preterm birth was defined as spontaneous when it follows preterm premature rupture of the membranes (PPROM) or spontaneous labour with intact membranes (i.e. no induced labour and no non-labour caesarean section). As the interest of the effectiveness of anti-platelet agents is mainly focused on preterm birth, we assed time to between 20 weeks of gestation and 1) spontaneous preterm delivery, 2) iatrogenic preterm delivery, and 3) any preterm delivery by performing a Kaplan-Meier analysis for those women with a spontaneous preterm birth who started treatment before 20 weeks.

Statistical analysis

We calculated relative risks and 95% confidence intervals for the main outcome measures. Outcomes were analyzed for each trial separately using Chi-square statistics,

and combined in an IPD meta-analysis to calculate an overall effect using a binary logistic regression model. Clustering of data within trials was taken into account by including the trial as a covariate in the model. We calculated numbers needed to treat for statistically significant outcomes. Relative risk ratio's (RR) were calculated from odds ratios based on the prevalence of the outcome in the non-exposed group.
To explore the effect by trial-level characteristics we pre-specified subgroups based on gestational age at trial entry (<16 weeks versus ≥ 16 weeks and <20 weeks versus ≥ 20 weeks), and intended aspirin dose (≤75 mg/day versus >75 mg/day, based on aspirin only trials, n=15 trials with 26,893 women). To explore the effects by participant-level characteristics, we pre-specified subgroups based on (1) risk factors based on medical history, including parity, pre-existing renal disease, diabetes, hypertensive disorders and previous small for gestational age infant, and (2) risk factors in the current pregnancy, including maternal age, pregnancy type (singleton versus multiple gestations) and fetal gender. Furthermore, we tested whether the treatment effect was different within women who developed pre-eclampsia in the current pregnancy. Subgroup effects were analyzed using an interaction term between subgroup and treatment group, and calculating the effect of anti-platelet treatment in different strata of the subgroups. Sensitivity analyses were performed for only studies including a placebo arm, and for studies that used aspirin as an antiplatelet agent. Analyzes were performed in IBM SPSS Statistics 22. *P* values <0.05 were considered to indicate statistically significance.

Results

This paper presents the results from 17 randomized trials[14-30] reporting on 28,797 women. Since gestational age at delivery was unknown for 1,287 women (4.5%), data of 27,510 women were included in our analysis. Of these women, 13,825 were randomly assigned to antiplatelet treatment and 13,685 women to placebo or no treatment. Overall, 57% of the women were in their first pregnancy, 96% had a singleton pregnancy and 62% were aged 20-35 years. Aspirin alone was given in 15 trials, in a dose ranging from 60 to 150 mg per day. One trial[21] gave aspirin in combination with dipyridamole and one trial gave dipyridamole alone.[25] Overall, 9.7% (n=2670) of the women had a SPTB before 37 weeks of gestation, 2.8% (n=773) before 34 weeks of gestation, and 0.5% (n=151) of the women had a spontaneous preterm birth before 28 weeks of gestation.
Antiplatelet agents were associated with a significant reduction in the risk of spontaneous preterm birth before 37 weeks (9.3% versus 10.1%, RR 0.93, 95% CI 0.86-0.996) and before 34 weeks (2.6% versus 3.1%, RR 0.86, 95% CI 0.76-0.99) compared with the control group (Table 1). For spontaneous preterm birth before 28 weeks, the relative risk was reduced by 19%, although this effect was not significant (0.49% versus 0.61%, RR 0.81, 95% CI 0.59-1.1). Corresponding numbers needed to treat (NNT) to prevent one case of spontaneous preterm birth was 139 for spontaneous preterm

birth <37 weeks and 242 for spontaneous preterm birth <34 weeks. Figure 1 shows that within women with a spontaneous preterm birth, there was no difference between the two groups in time from 20 weeks of gestation to delivery (Log-rank test: p=0.18). Figure 2 shows the incidence of spontaneous preterm birth, iatrogenic preterm birth, and any preterm birth within women with antiplatelet agents and in women with placebo/no treatment.

For the outcome measure spontaneous preterm birth <37 weeks of gestation, the was a significant interaction with parity status (p-value for interaction=0.04). The relative risk of having a spontaneous preterm birth <37 weeks was 0.83 (95% CI 0.73-0.95) for woman who have had a previous pregnancy, and 0.98 (95% CI 0.89-1.09) for women in their first pregnancy. There was no evidence that women in any of the other subgroups benefited more or less with antiplatelet treatment (Table 2). Sensitivity analyses based on trials using aspirin only (n=15; 28,266 women) and on only trials with a placebo group (n=15; 28,374 women) showed comparable results (data not shown).

Table 1A. Spontaneous preterm birth <37 weeks

Trial	Antiplatelets (n/N)	Control (n/N)	Relative risk (95%-CI)
A	1/44	2/48	0.78 (0.34-1.77)
B	1/52	2/50	0.73 (0.32-1.66)
C	0/32	1/29	0.47 (90.36-0.61)
D	165/1816	167/1814	0.99 (0.89-1.11)
E	3/40	5/44	0.82 (0.46-1.47)
F	3/118	3/75	0.77 (0.34-1.75)
G	152/3397	180/3373	0.91 (0.83-1.01)
H	9/156	5/74	0.89 (0.43-1.86)
I	172/1629	161/1632	1.04 (0.93-1.17)
J	0/43	1/43	0.50 (0.40-0.61)
K	416/3018	429/3024	0.98 (0.92-1.06)
L	13/159	9/142	1.17 (0.70-1.95)
M	16/276	19/278	0.92 (0.67-1.26)
N	15/301	19/301	0.89 (0.65-1.21)
O	97/1470	105/1492	0.97 (0.84-1.11)
P	1/23	2/25	0.77 (0.33-1.79)
Q	228/1251	268/1241	0.90 (0.82-0.99)
	1292/13.825	**1378/13.685**	**0.93 (0.86-0.996)**

Table 1B. Spontaneous preterm birth <34 weeks

Trial	Antiplatelets (n/N)	Control (n/N)	Relative risk (95%-CI)
A	0/44	0/48	
B	1/52	0/50	
C	0/32	0/29	
D	51/1816	53/1814	0.98 (0.81-1.19)
E	0/40	0/44	
F	1/118	1/75	0.77 (0.19-3.13)
G	40/3397	40/3373	1.00 (0.80-1.24)
H	3/156	1/74	1.29 (0.23-7.13)
I	14/1629	20/1632	0.85 (0.64-1.13)
J	0/43	1/43	0.49 (0.40-0.61)
K	124/3018	126/3024	0.99 (0.88-1.13)
L	4/159	2/142	1.42 (0.46-4.44)
M	4/276	6/278	0.83 (0.50-1.39)
N	5/301	3/301	1.34 (0.54-3.29)
O	24/1470	44/1492	0.77 (0.65-0.93)
P	1/23	0/25	
Q	88/1251	116/1241	0.87 (0.76-0.98)
	360/13.825	**413/13.685**	**0.86 (0.76-0.99)**

Table 1C. Spontaneous preterm birth <28 weeks

Trial	Antiplatelets (n/N)	Control (n/N)	Relative risk (95%-CI)
A	0/44	0/48	
B	0/52	0/50	
C	0/32	0/29	
D	9/1816	7/1814	1.14 (0.63-1.99)
E	0/40	0/44	
F	0/118	0/75	
G	4/3397	5/3373	0.90 (0.50-1.61)
H	0/156	0/74	
I	1/1629	5/1632	0.60 (0.42-0.86)
J	0/43	0/43	
K	21/3018	24/3024	0.94 (0.71-1.23)
L	1/159	0/142	
M	1/276	1/278	1.00 (0.25-4.02)
N	0/301	0/301	
O	11/1470	11/1492	1.01 (0.66-1.53)
P	0/23	0/25	
Q	20/1251	30/1241	0.83 (0.66-1.04)
Total	**68/13.825**	**83/13.685**	**0.81 (0.59-1.12)**

Table 2A. Effect in pre-specified subgroups on preterm birth <37 weeks

	Antiplatelets (n/N)	Control (n/N)	Relative risk (95%-CI)	Interaction *p* value
Trial factors				
Gestational age at start of treatment				
<16 weeks	284/3.374	313/3.348	0.90 (0.76-1.06)	0.72
≥16 weeks	1.007/10.445	1.064/10.333	0.93 (0.85-1.02)	
Gestational age at start of treatment				
<20 weeks	668/7.748	710/7.706	0.93 (0.83-1.04)	0.84
≥20 weeks	624/6.071	667/5.975	0.91 (0.81-1.02)	
Intended aspirin dose*				
ASA ≤75 mg	1.076/11.293	1.173/11.289	0.91 (0.83-0.99)	0.40
ASA >75 mg	194/2.174	190/2.137	1.00 (0.81-1.24)	
Obstetric and medical history				
Previous pregnancy				
Yes	469/5819	549/5777	0.83 (0.73-0.95)	0.04
No	823/7968	828/7873	0.98 (0.89-1.09)	
First pregnancy				
With high risk	117/1052	121/1044	0.96 (0.73-1.25)	0.05
Without high risk	706/6916	707/6829	0.98 (0.88-1.10)	
Second or subsequent pregnancy				
With high risk	357/4442	432/4351	0.79 (0.68-0.91)	0.07
Without high risk	112/1377	117/1426	0.99 (0.75-1.29)	
Pre-existing renal disease				
Yes	13/212	11/181	1.03 (0.45-2.38)	0.77
No	859/10331	918/10293	0.92 (0.84-1.02)	
Pre-existing diabetes				
Yes	42/392	44/407	0.99 (0.63-1.55)	0.24
No	1.058/11.407	1.153/11.308	0.90 (0.82-0.98)	
Pre-existing hypertensive disease				
Yes	71/1266	94/1252	0.73 (0.53-0.999)	0.26
No	1030/10584	1105/10512	0.92 (0.84-1.004)	
Previous SGA infant				
Yes	78/1403	89/1273	0.78 (0.57-1.07)	0.32
No	930/10388	957/10387	0.97 (0.88-1.06)	
No previous infant	823/7968	828/7873	0.98 (0.89-1.09)	
Current pregnancy				
Maternal age				
<20 years	414/2.786	404/2.792	1.03 (0.89-1.20)	
20-35 years	500/5.199	574/5.146	0.85 (0.75-0.97)	0.16
>35 years	29/314	32/327	0.94 (0.55-1.59)	
Pregnancy type				
Singleton	1.074/12.985	1.131/12.848	0.93 (0.85-1.02)	0.31
Multiple	218/839	247/835	0.86 (0.69-1.08)	
Fetal gender				
Male	568/6624	590/6542	0.95 (0.84-1.07)	0.70
Female	494/6195	523/6117	0.92 (0.81-1.05)	

Table 2A. Continued

	Antiplatelets (n/N)	Control (n/N)	Relative risk (95%-CI)	Interaction *p* value
Pre-eclampsia				
Women with PE	63/1039	75/1126	0.90 (0.64-1.28)	0.45
Women without PE	1226/12762	1301/12533	0.92 (0.85-0.996)	

* aspirin only trials

Table 2B. Effect in pre-specified subgroups on preterm birth <34 weeks

	Antiplatelets (n/N)	Control (n/N)	Relative risk (95%-CI)	Interaction *p* value
Trial factors				
Gestational age at start of treatment				
<16 weeks	82/3.374	89/3.348	0.91 (0.67-1.23)	0.67
≥16 weeks	278/10.445	324/10.333	0.85 (0.72-0.99)	
Gestational age at start of treatment				
<20 weeks	168/7.748	199/7.706	0.84 (0.68-1.03)	0.74
≥20 weeks	192/6.071	214/5.975	0.88 (0.72-1.07)	
Intended aspirin dose*				
ASA ≤75 mg	332/11.293	382/11.289	0.87 (0.75-1.00)	0.67
ASA >75 mg	21/2.174	27/2.137	0.77 (0.43-1.36)	
Obstetric and medical history				
Previous pregnancy				
Yes	155/5819	189/5777	0.81 (0.65-1.003)	0.55
No	205/7968	224/7873	0.90 (0.74-1.09)	
First pregnancy				
With high risk	44/1052	47/1044	0.93 (0.61-1.41)	0.47
Without high risk	161/6916	177/6829	0.90 (0.72-1.11)	
Second or subsequent pregnancy				
With high risk	117/4442	152/4351	0.74 (0.58-0.95)	0.52
Without high risk	38/1377	37/1426	1.07 (0.67-1.69)	
Pre-existing renal disease				
Yes	4/212	5/181	0.71 (0.19-2.70)	0.22
No	248/10331	269/10293	0.91 (0.77-1.09)	
Pre-existing diabetes				
Yes	10/392	14/407	0.75 (0.32-1.67)	0.93
No	330/44.407	376/11.308	0.87 (0.75-1.01)	
Pre-existing hypertensive disease				
Yes	21/1266	27/1252	0.76 (0.43-1.36)	0.77
No	320/10584	363/10512	0.87 (0.74-1.02)	
Previous SGA infant				
Yes	22/1403	25/1273	0.76 (0.42-1.35)	0.36
No	232/10388	251/10387	0.92 (0.77-1.01)	
No previous infant	205/7968	224/7873	0.90 (0.74-1.09)	

Table 2B. Continued

	Antiplatelets (n/N)	Control (n/N)	Relative risk (95%-CI)	Interaction *p* value
Current pregnancy				
Maternal age				
<20 years	121/2.786	140/2.792	0.86 (0.67-1.10)	
20-35 years	169/5.199	200/2.146	0.84 (0.68-1.03)	0.98
>35 years	7/314	8/327	0.91 (0.33-2.54)	
Pregnancy type				
Singleton	274/12.985	310/12.848	0.87 (0.74-1.03)	0.69
Multiple	86/839	103/835	0.85 (0.62-1.15)	
Fetal gender				
Male	137/6624	154/6542	0.88 (0.69-1.11)	0.78
Female	131/6195	147/6117	0.88 (0.69-1.11)	
Pre-eclampsia				
Women with PE	20/1039	20/1126	1.09 (0.58-2.03)	0.45
Women without PE	340/12762	393/12533	0.85 (0.73-0.98)	

* aspirin only trials

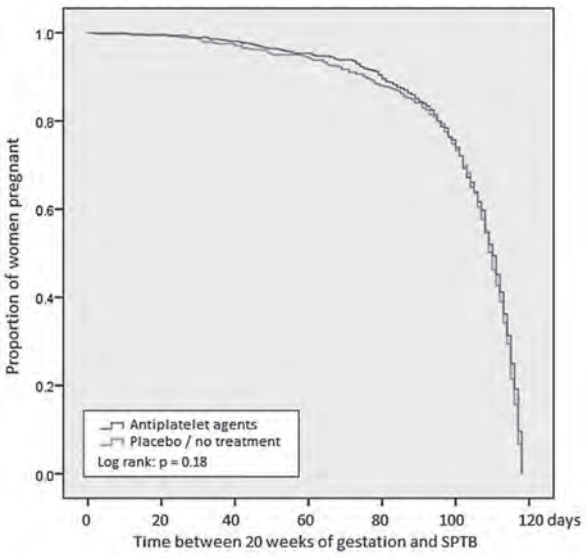
Table 2C. **Effect in pre-specified subgroups on preterm birth <28 weeks**

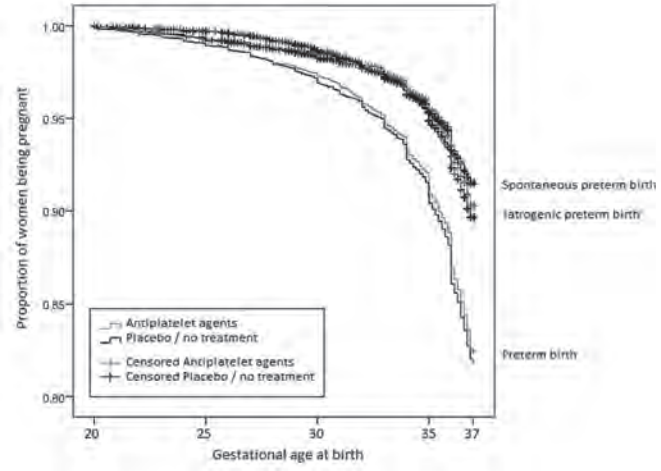
	Antiplatelets (n/N)	Control (n/N)	Relative risk (95%-CI)	Interaction *p* value
Trial factors				
Gestational age at start of treatment				
<16 weeks	20/3.374	24/3.348	0.82 (0.46-1.50)	0.95
≥16 weeks	48/10.445	59/10.333	0.81 (0.55-1.18)	
Gestational age at start of treatment				
<20 weeks	41/7.748	51/7.706	0.80 (0.53-1.21)	0.92
≥20 weeks	27/6.071	32 /5.975	0.83 (0.50-1.38)	
Intended aspirin dose*				
ASA ≤75 mg	65/11.293	77/11.289	0.84 (0.61-1.17)	0.26
ASA >75 mg	2/2.174	6/2.137	0.33 (0.07-1.63)	
Pregnancy and medical history				
Previous pregnancy				
Yes	33/5819	42/5777	0.78 (0.49-1.23)	0.86
No	35/7968	41/7873	0.84 (0.54-1.33)	
First pregnancy				
With high risk	11/1052	10/1044	1.09 (0.46-2.58)	0.85
Without high risk	24/6916	31/6829	0.76 (0.45-1.30)	
Second or subsequent pregnancy				
With high risk	22/4442	35/4351	0.61 (0.35-1.03)	0.92
Without high risk	11/1377	7/1426	1.66 (0.64-4.29)	
Pre-existing renal disease				
Yes	0/212	1/181	-	0.19
No	46/10331	47/10293	0.97 (0.65-1.46)	

Table 2C. Continued

	Antiplatelets (n/N)	Control (n/N)	Relative risk (95%-CI)	Interaction *p* value
Pre-existing diabetes				
Yes	1/392	0/407	-	0.38
No	65/11.407	78/11.308	0.83 (0.59-1.15)	
Pre-existing hypertensive disease				
Yes	5/1266	9/1252	0.56 (0.19-1.68)	0.31
No	61/10584	69/10512	0.88 (0.62-1.24)	
Previous SGA infant				
Yes	4/1403	2/1273	1.71 (0.31-9.45)	0.16
No	39/10388	44/10387	0.89 (0.58-1.36)	
No previous infant	35/7968	41/7873	0.84 (0.54-1.33)	
Current pregnancy				
Maternal age				
<20 years	20/2.786	33/2.792	0.60 (0.34-1.05)	
20-35 years	41/3.199	37/5.146	1.11 (0.71-1.73)	0.23
>35 years	2/314	3/327	1.30 (0.74-2.30)	
Pregnancy type				
Singleton	50/12.985	59/12.848	0.84 (0.57-1.22)	0.75
Multiple	18/839	24/835	0.77 (0.41-1.44)	
Fetal gender**				
Male	23/6624	35/6542	0.65 (0.38-1.10)	0.39
Female	6/6195	21/6117	1.22 (0.69-2.18)	
Pre-eclampsia				
Women with PE	0/1039	2/1126	-	-
Women without PE	68/12762	81/12533	0.83 (0.60-1.14)	

* aspirin only trials
**analysis including singleton pregnancies only, n=25,833


1.0
0.8
0.6
0.4
0.2
0.0
Proportion of women pregnant
Antiplatelet agents
Placebo / no treatment
Log rank: p = 0.18
0
20
40
60
80
100
120 days
Time between 20 weeks of gestation and SPTB


1.00
0.95
0.90
0.85
0.80
Proportion of women being pregnant
Antiplatelet agents
Placebo / no treatment
Censored Antiplatelet agents
Censored Placebo / no treatment
Spontaneous preterm birth
Iatrogenic preterm birth
Preterm birth
20
25
30
35
37
Gestational age at birth

Discussion

This study based on individual participant data of 27,510 women shows that antiplatelet agents in pregnant women at risk of pre-eclampsia reduces the relative risk of spontaneous preterm birth by around 10%. The effect on spontaneous preterm birth <37 weeks is larger in women with a second or subsequent pregnancy, however also primiparous women benefit of the intervention since the effect on spontaneous preterm birth <34 weeks is found not to be different between primiparous and multiparous women. There was no clear evidence that the treatment was more or less effective in any of the other subgroups.-

Previous studies suggested that vascular lesions might play a role in spontaneous preterm birth. The current findings support this hypothesis. Most women in the PARIS IPD were at low to moderate risk of pre-eclampsia.[13] The risk profile of the participants was quite diverse, varying from being primigravida to having pre-eclampsia in history. Our subgroup analysis revealed no difference in treatment effect between women at high or low risk of pre-eclampsia. Therefore, the current findings might be applicable to a broader population of pregnant women. Since there is a poverty of preventive strategies for spontaneous preterm birth, the use of antiplatelet agents might be a promising intervention for women who have a history of spontaneous preterm birth.

One of the main concerns in the administration of medication during pregnancy is safety.

The PARIS IPD[13] revealed no difference in the incidence of antepartum hemorrhage (RR 1.02, 95% CI 0.90–1.15), placental abruption (RR 1.13, 95% CI 0.87–1.48) or infant bleeding (RR 0.93, 95% CI 0.80–1.09) between women who received antiplatelet agents and women who did not. Postpartum haemorrhage was more frequent, but this difference was borderline significant (RR 1.06, 95% CI 1.00–1.13). Long term follow up revealed no effect on infant outcomes at 12 and 18 months[31] and suggest a reduction in neurobehavioral difficulties at 5 years of age among infants who were exposed to aspirin in utero.[32]

This study has some limitations. First, as this study presents secondary analysis the results should be interpreted with caution. Because multiple analyses have been performed, the risk of a type I error, statistically significant results that are based on coincidence, should be considered. However, given the solid evidence for our hypothesis in biomedical research and all outcomes are consistently pointing in the same direction, we feel that these secondary analyses are of clinical relevance. Second, studies published after 2005 were not included in this IPD. There were a few trials published after this date, however all with small sample sizes (n=139, n=152 and n=114). These new studies could be used to test our hypothesis.

In issue the scientific community should ask itself why this finding has not been reported earlier. The trials were conducted between 1987 and 2003. In retrospect, the finding that aspirin reduces spontaneous preterm birth could probably have been found earlier. Then, when meta-analyses including the PARIS IPD were performed,

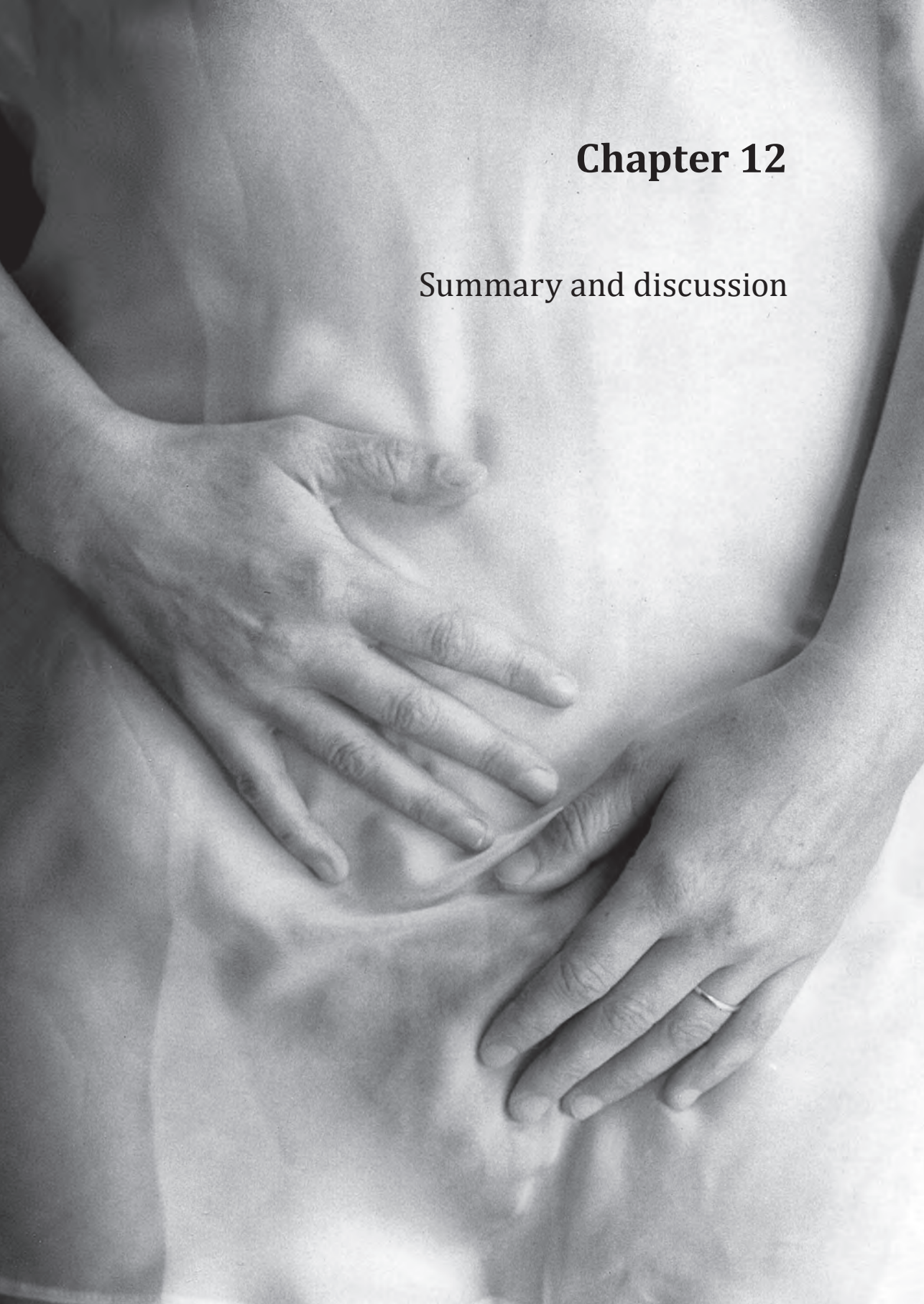
spontaneous preterm birth was not considered as an outcome measure. Standardizing outcomes in obstetrical research could prevent this, and initiatives to do so are ongoing.[33]

Our data show that antiplatelet agents in pregnant women at risk of pre-eclampsia reduce the risk of spontaneous preterm birth by around 10%. This effect was also found in women without an increased risk of pre-eclampsia. As antiplatelet agents in pregnancy are a low-cost and safe intervention, we suggest that antiplatelet agents might also be a promising intervention for women at high risk of a spontaneous preterm birth.

References

1. Hack M, Outcomes of children of extremely low birthweight . Early Hum Dev 1999; 53:193-218.
2. Ananth CV, Getahun D, Peltier MR, Salihu HM, Vintzileos AM. Recurrence of spontaneous versus medically indicated preterm birth. Am J Obestet Gynecol 2006; 195: 643-50.
3. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014;345:760-5.
4. Voltolini C, Torricelli M, Conti N, Vellucci FL, Severi FM, Petraglia F. Understanding spontaneous preterm birth: from underlying mechanisms to predictive and preventive interventions. Reprod Sci. 2013;20:1274-92.
5. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000;342:1500-7.
6. Arias F, et al. Maternal placental vasculopathy and infection: two distinct subgroups among patients with preterm labour and preterm ruptured membranes. Am J Obstet Gynecol. 1993;168:585-91.
7. Kelly R. et al. Placental vascular pathology findings and pathways of preterm delivery. Am J Epidemiol 2009;170:148-158.
8. Kim YM et al. Failure of physiologic transformation of the spiral arteries in patients with preterm labour and intact membranes. Am J Obstet Gynecol. 2003;189:1063-9.
9. Kim YM et al. Failure of physiologic transformation of the spiral arteries in the placental bed in preterm premature rupture of membranes. Am J Obstet Gynecol. 2002;187:1137-42.
10. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol. 2011;204:193-201.
11. Chaiworapongsa T et al. A subset of patients destined to develop spontaneous preterm labour has an abnormal angiogenic profile in maternal plasma. J Matern Fetal Neonatal Med. 2009:1122-39.
12. Misra et al. Placental blood flow and the risk of preterm delivery. Placenta 2009;30:619-624.
13. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA; PARIS Collaborative Group. Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. Lancet. 2007;369(9575):1791-8.
14. Michael CA, Walters BNJ. Low-dose aspirin in the prevention op pre-eclampsia: current evaluation. In : Teoh ES, Shan Ratnam S, Macnaughton M, eds. Maternal physiology and pathology. The current status of gynaecology and obstetric series, volume 4, Carnforth: Parthenon Pub Group Ltd, 1993: 183-89.
15. Morris JM, Jay RA, Ellwood DA, Cook C, Devonald KJ. A randomized controlled trial of aspirin in patients with abnormal uterine artery blood flow. Obstet Gynecol 1996;87:74-8.
16. Gallery ED, Ross MR, Hawkins M, Leslie GI, Gyory AZ. Low-dose aspirin in high risk pregnancy? Hypertens Preg 1997;16:229-38.
17. Rotchell YE, Cruikshank JK, Phillips GM et al. Barbados Low Dose Aspirin Study in Pregnancy (BLASP): a randomised trial for the prevention of pre-eclampsia and its complications. BJOG 1998;105:286-92.
18. Wang ZH, Li WJ. A prospective randomized placebo-controlled trial of low dose aspirin for prevention on intra uterine growth retardation. Chin Med J 1996;109:238-42.
19. Rogers MS, Fung HY, Hung CY. Calcium and low-dose aspirin prophylaxis in women at high risk of pregnancy-induced hypertension. Hypertens Pregnancy 1999;18:165-72.
20. CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) Collabroative Group. CLASP: a randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. Lancet 1994;343:619-29.
21. Uzan S, Beaufils M, Breart G, Bazin B, Capitant C, Paris J. Prevention of fetal growth retardation with low-dose aspirin: findings of the EPREDA trial. Lancet 1991;337:1427-31.
22. ERASME collaborative group. Aspirin (100 mg) used for prevention of pre-eclampsia in nulliparous women: the Essai Regional Aspirine Mere-Enfant study (Part 1). BJGO 2003;110:475-85.
23. Vainio M, KUjansuu E, Iso-Mustajarvi M, Maenpaa J. Low dose acetylsalicylic acid in prevention of pregnancy-induced hypertension and intrauterine growth retardation in women with bilateral uterine artery notches. BJOG 2002;109:161-67.
24. Golding J. A randomised trial of low dose aspirin for primiparae in pregnancy. BJOG 1998;105:293-99.
25. Uzan S, Beaufils M, Bazin B, Danays T. Idiopathic recurrant fetal growth retardation and aspirin-dipyridamole therapy. Am J Obstet Gynecol 1989;160:763-64.
26. Yu CK, Papageorghiou AT, Parra M, Palma D, Nicolaides KH. Randomised controlled trial using low dose aspirin in the prevention of pre-eclampsia in women with abnormal uterine artery Doppler at 23 weeks gestation. Ultrasound Obstet Gynecol 2003;22:233-39.
27. Hauth J. Goldenberg R. Philips J. Copper R. DuBard M, Cutter G. Low-dose aspirin therapy to prevent pre-eclampsia: safety considerations. Am J Obstet Gynecol 1993;168:389.

28. Sibai BM, Caritis S, Thom E et al. Prevention of preeclampsia with low-dose aspirin in healthy, nulliparous pregnancy women. N Engl J Med 1993;329:1213-18.
29. August P, Helseth G, Edersheid TG, et al. Sustained release, low-dose aspirin aeliorates bud does not prevent preeclampsia (PE) in high risk population. Proceedings of 9th International Congress, ISSHP;Sydney, Australia: 1994,72.
30. Caritis S, Sibai BM, Hauth J et al. Low-dose aspirin to preent preeclampsia in women at high risk. J Engl J Med 1998;338:701-05.
31. CLASP collaborative group.Low dose aspirin in pregnancy and early childhood development: follow up of the collaborative low dose aspirin study in pregnancy. Br J Obstet Gynaecol. 1995;102:861-8.
32. Marret S, Marchand L, Kaminski M, Larroque B, Arnaud C, Truffert P, Thirez G, Fresson J, Rozé JC, Ancel PY; EPIPAGE Study Group. Prenatal low-dose aspirin and neurobehavioral outcomes of children born very preterm. Pediatrics. 2010;125:e29-34.
33. Khan KS, Romero R; Chief Editors of Journals participating in CROWN Initiative. The CROWN initiative: journal editors invite researchers to develop core outcomes in women's health. Am J Obstet Gynecol. 2014;211:575-6.



Chapter 12

Summary and discussion

Summary and discussion

The desired outcome of pregnancy is a healthy infant. The partly unravelled pathophysiological processes underlying preterm birth are thought to expose the infant at risk of poor intrauterine and extra uterine development. Both the understanding of the aetiology of preterm birth as the evaluation of current clinical practice in threatened preterm labour will provide new insights to optimize outcome in pregnancy. The aim of the first part of this thesis was to provide insight in the nature of the relations between placenta pathology, brain development and outcome in preterm birth. The second part of this thesis focused on the effects of interventions in pregnancy to prevent preterm birth and to optimize outcome in threatened preterm delivery.

Part I

Infection and inflammation can have a detrimental effect on the developing brain, both by putting the foetus at risk of preterm birth, as by directly interfering with normal brain development. **Chapter 2** provides an overview of the existing literature on perinatal infections and neurodevelopmental outcome in very preterm infants. The study quantitatively aggregates the results of 18 studies that examined neurodevelopment of very preterm infants using the Bayley Scales of Infant Development, the most common method to evaluate neurodevelopment at 2 years of age. Perinatal infections were found to have a negative impact on both mental and motor development, with an average decrease in Mental Developmental Index scores and Psychomotor Developmental Index scores of 0.25 and 0.37 standard deviations respectively. Development was mostly hampered by postnatal infections such as necrotizing enterocolitis and meningitis. These effects of infection add up to the well-known detrimental effects of prematurity. **Chapter 3** studies the association between placenta pathology and neurodevelopmental outcome at age 2 and 7 years in a cohort of very preterm infants who participated in the Glutamine Enriched Enteral Feeding (GEEF) study. At 2 years of age, very preterm infants with placental underperfusion had poorer mental development compared to very preterm infants with histological chorioamnionitis. We found no association of motor development with placental pathology. At 7 years of age, large although non-significant effects were found for better mental and motor development and fewer behavioural problems in infants with histiological chorioamnionitis. This study emphasises that placenta pathology should be taken into account when evaluating neurodevelopmental outcome in preterm infants. To further disentangle the association between prematurity, placenta pathology and neurodevelopment, we performed histological placenta examination of a cohort of very preterm infants who had a cerebral MRI at term equivalent age. Perinatal mortality was significantly higher in infants with inflammatory placental lesions as compared to infants with vascular placental lesions. At 5.5 years of age,

more motor problems were found in infants who had vascular placental lesions as compared to infants with inflammatory lesions or infants with both or none of these lesions (**Chapter 4**). The incidence of moderate/severe white matter injury, IVH > grade II, cerebellar haemorrhage or abnormal ventricular size was not related to type of placenta pathology in this study.
There is a close association between gestational age in two subsequent pregnancies, and although the majority of women with a preterm delivery will deliver at term in a subsequent pregnancy, the most consistently reported risk factor for preterm birth is a previous preterm delivery. **Chapter 5** describes the results of a retrospective cohort study that compared placental pathology between women with a recurrent preterm birth, and women with a subsequent term delivery. Funisitis was found to be inversely related to recurrent preterm birth, i.e. women with funisitis in their index preterm birth tend to have a lower risk on preterm birth in their subsequent pregnancy as compared to women without funisitis. It could be hypothesized that the factors leading to infection and inflammation do not persist in the women and resolve during the inter pregnancy interval.

In summary, the first part of this thesis aimed to provide insight in the nature of the relations between placenta pathology, brain development and outcome in preterm birth. The studies described in the first part of this thesis have shown us that:

1. Perinatal infections, mainly postnatal infections , are associated with poorer mental and motor development in very preterm infants.
2. Very preterm infants with placental underperfusion have poorer mental development compared to very preterm infants with histological chorioamnionitis at 2 years of age.
3. In very preterm infants, the incidence of cerebral MRI abnormalities at term equivalent age was not related to type of placenta pathology. It could be hypothesized that postnatal events such as infections and the need for respiratory support have a larger impact on the development of cerebral damage than placental pathology in their index pregnancy.
4. Women with funisitis in their index preterm birth tend to have a lower risk on preterm birth in their subsequent pregnancy as compared to women without funisitis.

These studies provided more insight in the association of placental pathology and neurodevelopmental outcome, however, the predictive value of placental pathology is currently too small to be of benefit for targeting infants at high risk of poor outcome, or women at high risk of recurrent preterm birth. The findings of these studies stress however that the pathophysiological mechanisms underlying preterm birth are complex and heterogeneous. Although many studies have found associations between placenta pathology, preterm birth and outcome, the discussion whether this relationship is causal is not settled. This is difficult to study since clinical experiments are unethical in human subjects. Animal studies on preterm birth have provided important insights

in the aetiology of preterm birth, and have shown that systemic infections can indeed cause spontaneous preterm birth.[1] As the placenta is a key factor in foetal development it would be interesting to further evaluate the association with neonatal outcome. In clinical trials evaluating management in threatened preterm labour, data on placental pathology could be of additive value to evaluate whether treatment options have differential effects on outcome depending on the type of placental lesions, and whether prolongation of pregnancy has an effect on severity of placental lesions.

Part 2

The second part of this thesis studies the effects of interventions in pregnancy to prevent preterm birth and to optimize outcome in threatened preterm delivery. **Chapter 6** provides an overview of the effectiveness and side effects of commonly used tocolytic agents. According to the current evidence, beta-adrenoreceptor agonists are less effective and have more maternal side effects compared to other tocolytic agents and these antagonists are therefore abandoned from clinical practice. The literature on prostaglandin synthetase inhibitors is scarce, but there is some evidence for a potentially strong beneficial effect. However foetal side effects remain a concern with prostaglandin synthetase inhibitors and should be part of future trials. Although widely used in the United States, magnesium sulphate is not recommended as a tocolytic drug in the Netherlands. However, there may be a role for magnesium sulphate as neuroprotectant in the preterm infant and is recommended as an additional drug to the treatment strategy in preterm labour consisting of tocolytic drugs and corticosteroids. Progesterone has limited benefit in the treatment of acute preterm labour although it might play a role in the prevention of preterm labour or as sensitizer for other tocolytic agents. Based on the current literature, the use of atosiban or nifedipine is preferred based on the largest effectiveness in terms of delay of delivery, perinatal outcomes and most favourable side effect profile.

As neonatal outcome is strongly related to gestational age, maintenance tocolysis after initial treatment for 48 hours seems a reasonable option. Based on Individual Participant Data (IPD) of all randomized controlled trials on nifedipine maintenance tocolysis (**chapter 7**), there is no difference in the incidence of perinatal death, (RR 1.2; 95%CI 0.44-3.4), necrotizing enterocolitis (RR 1.2; 95% CI 0.52-2.6), respiratory distress syndrome (RR 1.00; 95% CI 0.50-2.0) intraventricular hemorrhage ≥ grade 2 (RR 0.66; 95% CI 0.14-3.19) or prolongation of pregnancy (HR 0.86; 95% CI 0.71-1.03) between women who were treated with nifedipine maintenance tocolysis compared to women treated with placebo/no treatment. Within the subgroup of singleton pregnancies nifedipine was associated with a longer prolongation of pregnancy when compared to placebo / no treatment. However also in this subgroup no difference in perinatal mortality was found. Therefore, according to the best available evidence, nifedipine maintenance tocolysis does not improve neonatal outcome. As nifedipine

easily crosses the placenta, maintenance tocolysis might have an effect on long-term infant development. The results of the 2 year follow-up of the infants of the largest trial on nifedipine maintenance tocolysis, the APOSTEL II study, are presented in **chapter 8**. At 2 years of age, intrauterine exposure to nifedipine maintenance tocolysis is associated with a higher incidence of fine motor problems, and a lower incidence of poor problem solving. In a subgroup of women without PPROM, there was a lower incidence of gross motor problems and developmental delay in infants of the treatment group compared to the placebo group. In view of the lack of benefit on neonatal outcome, and no clear benefit of nifedipine maintenance tocolysis at age 2 years, its use does not appear beneficial.

According to the current clinical guidelines for the management of threatened preterm labour, tocolysis for 48 hours using atosiban or nifedipine is recommended. However, there is a lack of studies comparing these two tocolytical agents in terms of perinatal outcome, prolongation of pregnancy and side effects. To fill this gap of knowledge, the APOSTEL III study was conducted. The Apostel III trial is a multicenter randomised controlled study performed in 19 perinatal centres and large teaching hospitals (**Chapter 9**). Five-hundred and ten women with threatened preterm labour were randomised between treatment with nifedipine or atosiban. The incidence of adverse perinatal outcome was 14.2% (42 children) in the nifedipine group, and 15.3% (45 children) in the atosiban group (RR 0.92; 95%-CI 0.61-1.38, **Chapter 10**). Gestational age at delivery was not different between the two groups (median (IQR) 33.1 weeks (30.5-37.0) for the nifedipine group and 32.4 weeks (30.1-35.8) for the atosiban group, HR 0.86; 95%-CI 0.70-1.05). Median (IQR) prolongation of pregnancy was 7 days (1.0-41.5) for the nifedipine group and 4 days (1.0-38.0) for the atosiban group (HR 0.87; 95%-CI 0.71-1.06). Side effects leading to discontinuation of study medication were reported in 15 (6%) women of the nifedipine group and 6 (2%) women of the atosiban group (RR 2.57; 95%-CI 1.01-6.55). This difference was mainly driven by more suspected intrauterine infections in the nifedipine group (6 versus 1). Maternal mortality did not occur in either group. A non-significant but higher incidence of perinatal death was observed in the nifedipine group (5.1% versus 2.4%) and raises questions on the safety of nifedipine use in pregnancy. It is important to note that uncertainty continues on the effectiveness of tocolytic medication in threatened preterm birth. Tocolytic treatment is based on the assumption that postponing delivery for 48 hours enables the administration of antenatal corticosteroids and thereby improves perinatal outcome. Improving perinatal outcome would be the ultimate goal of treating preterm labour, however many studies are underpowered to detect differences on this outcome measure. Tocolytics may have unfavourable side effects that abolish the positive effect of antenatal corticosteroids. Furthermore, it could be questioned whether postponing delivery would have a positive effect on neonatal outcome at all. Although the incidence of neonatal morbidities and mortality decreases as gestational age increases, in threatened preterm birth the foetus may be exposed to a harmful intrauterine environment with unknown effects on neonatal outcome. To optimize

management in preterm labour, there is an urgent need for large placebo controlled trials powered to detect effects –either positive or negative- on neonatal outcome.
There is increasing evidence that the pathophysiological process in preterm birth starts in early pregnancy. Therefore, interventions in early pregnancy might be a promising way to improve perinatal outcome. In the final chapter of this thesis, **Chapter 11**, we present the results of a secondary analysis on an Individual Participant Data meta-analysis of 17 trials (28.797 women) on antiplatelet intervention studies in pregnancy in women at risk of pre-eclampsia. Women assigned to antiplatelet treatment compared to placebo or no treatment had a lower risk of spontaneous preterm birth < 37 weeks (RR 0.93; 95% CI 0.86-0.996) and <34 weeks of gestation (RR 0.86; 95% CI 0.76 – 0.99). The pre-eclampsia risk profile of the participants was quite diverse, varying from being primigravida to having pre-eclampsia in history. Our subgroup analysis revealed no difference in treatment effect between women at high or low risk of pre-eclampsia. Therefore, the findings of this study might be applicable to a broader population of pregnant women. The safety of antiplatelet agents in pregnancy has been studied extensively and revealed no difference in the incidence of antepartum hemorrhage (RR 1.02; 95% CI 0.90–1.15), postpartum haemorrhage (RR 1.06; 95% CI 1.00–1.13), placental abruption (RR 1.13; 95% CI 0.87–1.48) or infant bleeding (RR 0.93; 95% CI 0.80–1.09) between women who received antiplatelet agents and women who did not.[2] Long term follow up of two trials showed no effect on infant outcomes at 12 and 18 months and suggested a reduction in neurobehavioral difficulties at 5 years of age among infants who were exposed to aspirin in utero.[3] Therefore the use of antiplatelet agents might be a promising intervention for women who have a history of spontaneous preterm birth.
In summary, the second part of this thesis focused on the effects of interventions in pregnancy to optimize outcome in threatened preterm delivery and to prevent spontaneous preterm birth. The studies described in this part of the thesis have shown us that:

1. For acute tocolysis, the use of atosiban or nifedipine is preferred based on the largest effectiveness in terms of delay of delivery, perinatal outcomes and most favourable side effect profile.
2. Maintenance tocolysis using nifedipine does not improve neonatal outcome, prolongation of pregnancy nor clear favourable effects on infant outcome at age 2 years.
3. The use of nifedipine and atosiban for acute tocolysis result in similar rates of adverse perinatal outcome.
4. Anti-platelet agents in pregnancy lower the incidence of spontaneous preterm birth in women at risk of pre-eclampsia.
   The most important gap of knowledge remains whether tocolysis optimizes neonatal outcome in threatened preterm birth. Therefore, there is an urgent need for placebo controlled trials large enough to detect effects on perinatal outcome.

Improving pregnancy outcome: future perspectives

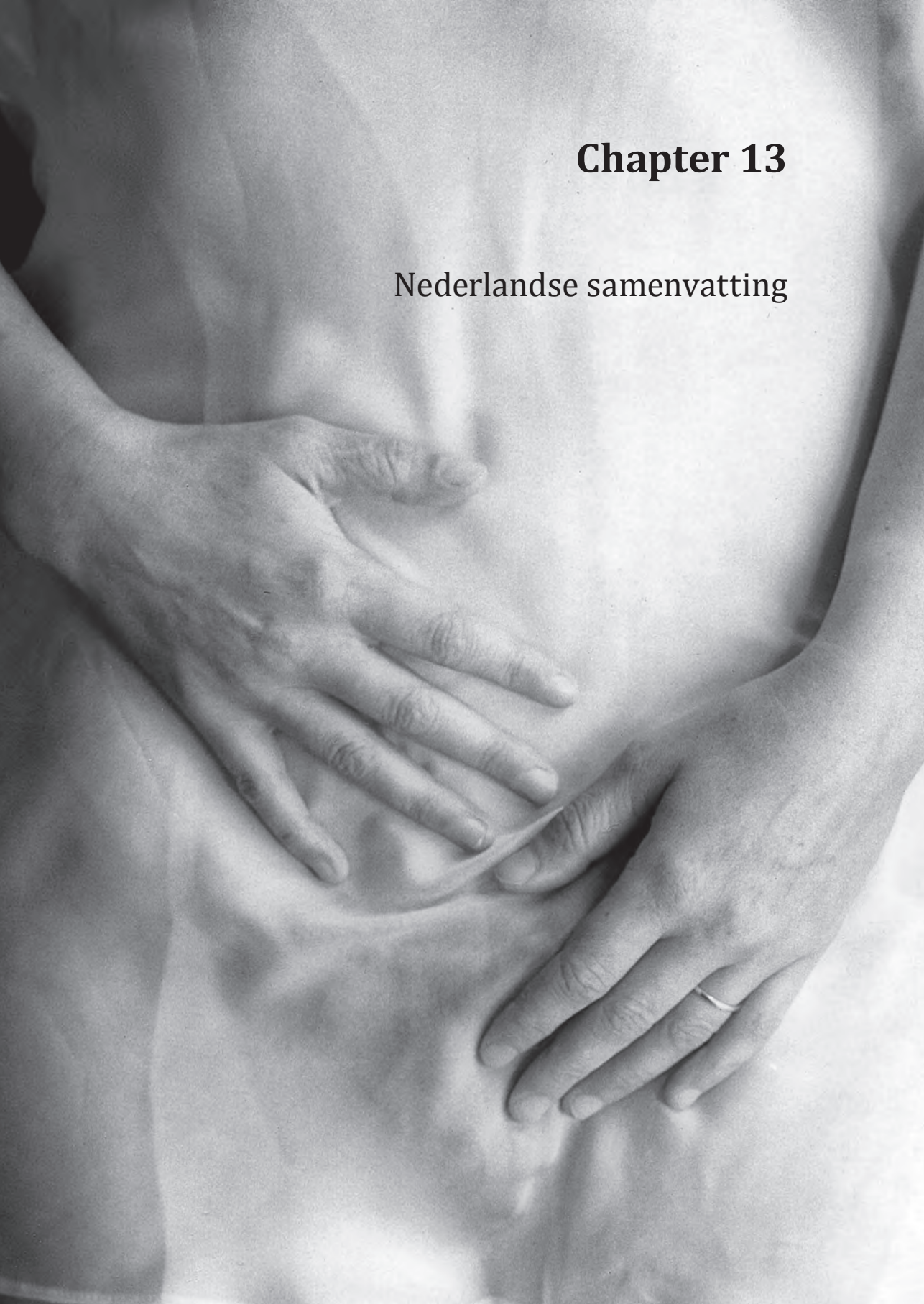
Perinatal outcome in preterm birth has improved over the last decades. Nevertheless it remains one of the great challenges in obstetric care. None of the different treatment options that have been compared in this thesis seems to stand out as the most promising intervention to prevent or postpone preterm delivery. Therefore, new interventions need to be evaluated in RCT's. One of these is a cervical pessary. Previous studies have indicated that a pessary reduces the risk of preterm birth and adverse outcome in women with a short cervix.[4,5] Currently, the APOSTEL VI trial is performed to test whether a cervical pessary would be a useful intervention in women who have been admitted for threatened preterm birth, after the administration of antenatal corticosteroids for 48 hours.[6] Another intervention to prevent or postpone preterm birth is progesterone. The Quadrupple-P study[6] compares vaginal progesterone versus a cervical pessary in women a short cervix at 20 weeks of gestation, and the PC study[6] compares a cervical cerclage with a cervical pessary in women at high risk of preterm birth based on obstetrical history.

The increasing recognition that preterm birth is not just birth before term but a complex interplay of pathophysiological mechanisms has contributed to growing awareness of the need for individualized care. To determine which women are at risk of preterm birth, an individualized risk assessment based on maternal characteristics, foetal characteristics and pregnancy-specific/environmental factors has been proposed[7] and its use should be encouraged in both clinical practice and research. Current and future treatment options should be evaluated in different subgroups of women, for example women with and without PPROM and women with and without cervical shortening. To increase power to detect treatment effects in these subgroups, attempts are made to improve collaboration between research networks worldwide in the Global Obstetrics Network.[8] This is an important step towards establishing a circle of identifying knowledge gaps, conducting clinical trials and observational studies, and implementing knowledge in clinical practice. The combination of both research on the aetiology and epidemiology of preterm birth as well as properly designed clinical trials will help optimizing pregnancy outcome. To further expand the knowledge on the etiology of preterm birth, and to strengthen the evidence for interventions, studies on pathophysiology could be integrated in clinical trials. For example, it would be interesting to study whether women treated with antiplatelet agents indeed have less placental vascular lesions.

Future studies on preterm birth should be designed to detect effects on neonatal outcome. Since interventions in pregnancy can have effect on the developing foetus, long term follow-up is of utmost importance. Based on the experience with the 7 year follow up of the ORACLE II trial,[9] in which a higher risk of cerebral palsy was found in infants who were exposed to antenatal antibiotics, it is important to be cautious about interfering with systems that are poorly understood without clear evidence of the benefit of an intervention at short- and long term.

References

1. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O et al. The preterm parturition syndrome. BJOG. 2006;113 Suppl 3:17-42.
2. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA; PARIS Collaborative Group. Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. Lancet. 2007;369:1791-8.
3. Marret S, Marchand L, Kaminski M, Larroque B, Arnaud C, Truffert P, Thirez G, Fresson J, Rozé JC, Ancel PY; EPIPAGE Study Group. Prenatal low-dose aspirin and neurobehavioral outcomes of children born very preterm. Pediatrics. 2010;125:e29-34.
4. Goya M, Pratcorona L, Merced C, Rodó C, Valle L, Romero A et al.; Pesario Cervical para Evitar Prematuridad (PECEP) Trial Group. Cervical pessary in pregnant women with a short cervix (PECEP): an open-label randomised controlled trial. Lancet. 2012;379:1800-6.
5. Liem S, Schuit E, Hegeman M, Bais J, de Boer K, Bloemenkamp K et al. Cervical pessaries for prevention of preterm birth in women with a multiple pregnancy (ProTWIN): a multicentre, open-label randomised controlled trial. Lancet. 2013;382:1341-9.
6. www.studies-obsgyn.nl
7. Kazemier BM. Improving perinatal outcome: towards individualized care. 2015 PhD Thesis, University of Amsterdam, the Netherlands.
8. Mol BW, Ruifrok AE; Global Obstetrics Network. Global alignment, coordination and collaboration in perinatal research: the Global Obstetrics Network (GONet) Initiative. Am J Perinatol. 2013;30:163-6.
9. Kenyon S, Pike K, Jones DR, Brocklehurst P, Marlow N, Salt A, Taylor DJ. Childhood outcomes after prescription of antibiotics to pregnant women with spontaneous preterm labour: 7-year follow-up of the ORACLE II trial. Lancet. 2008;372:1319-27.



Chapter 13

Nederlandse samenvatting

Nederlandse samenvatting

De gewenste uitkomst van een zwangerschap is de geboorte van een gezond kind. De pathofysioloigische mechanismen die ten grondslag liggen aan vroeggeboorte (geboorte <37 weken zwangerschapsduur) zijn deels nog onbekend. Gedacht wordt dat deze onderliggende mechanismen bijdragen aan een suboptimaal verlopende ontwikkeling van het kind, zowel in de baarmoeder als na de geboorte. Het vergroten van de kennis van de etiologie van vroeggeboorte alsook het evalueren van het huidig medische beleid bij dreigende vroeggeboorte zal nieuwe inzichten opleveren die de uitkomst van zwangerschappen kan verbeteren. Het doel van deze these was tweeledig. Het eerste deel van deze these beschrijft onderzoek naar de relatie tussen placenta pathologie (afwijkingen aan de moederkoek), hersenontwikkeling en lange termijn uitkomst bij vroeggeboorte. Het tweede deel van deze these beschrijft studies naar de effectiviteit en veiligheid van interventies in de zwangerschap die als doel hebben om vroeggeboorte te voorkomen en de uitkomst bij dreigende vroeggeboorte te verbeteren.

Deel I

Infectie en inflammatie hebben een negatief effect op de ontwikkeling van de hersenen van te vroeg geboren kinderen. Cytokinen (stoffen die vrijkomen bij infectie/inflammatie) interfereren met de normale hersenontwikkeling doordat ze de permeabiliteit van de bloed-hersenbarriere vergroten en schade toebrengen aan myeliniserende cellen. Bovendien vergroot een intrauterine infectie (een infectie in de baarmoeder tijdens de zwangerschap) de kans op vroeggeboorte. **Hoofdstuk 2** geeft een overzicht van de bestaande literatuur op het gebied van perinatale infecties (infecties in de zwangerschap of kort na de geboorte) en neuropsychologische ontwikkeling bij extreem vroeg geboren kinderen (<32 weken zwangerschapsduur). Dit hoofdstuk vat de uitkomsten samen van 18 studies die de relatie bestuderen tussen perinatale infecties en neuropsychologische ontwikkeling gemeten met de Bayley Scales of Infant Development. Perinatale infecties bleken een negatief effect te hebben op zowel de mentale als de motorische ontwikkeling van te vroeg geboren kinderen. Kinderen die een infectie doormaakte in de perinatale periode bleken 0.25 standaard deviaties lager te scoren bij testen die de mentale ontwikkeling meten, en 0.37 standaard deviatie lager te scoren op testen die de motorische ontwikkeling meten. De ontwikkeling van deze kinderen was het meest aangedaan wanneer zij een postnatale infectie (een infectie na de geboorte) doormaakte, zoals necrotizerende enterocolitis (een ontsteking van de darmen) of meningitis (hersenvliesontsteking). **Hoofdstuk 3** bestudeert de associatie tussen placenta pathologie en neuropsychologische ontwikkeling op 2- en 7 jarige leeftijd in een cohort van extreem vroeggeboren kinderen (<32 weken zwangerschapsduur) die meededen in de Glutamine Enriched Enteral Feeding (GEEF)

studie. De GEEF studie onderzocht of het toedienen van glutamine aan de voeding van te vroeg geboren kinderen het risico op een infectie deed afnemen. Op de leeftijd van 2 jaar hadden de kinderen waarbij in de placenta tekenen van een slechte doorbloeding (vasculaire placenta laesies) werden gevonden een slechtere mentale ontwikkeling in vergelijking met kinderen bij wie tekenen van ontsteking in de placenta (histologische chorioamnionitis) werden gevonden. Er werd geen associatie gevonden tussen afwijkingen aan de placenta en de motorische ontwikkeling van deze kinderen. Op 7 jarige leeftijd werden grote, maar niet significante effecten gevonden waarbij er een betere mentale en motorische ontwikkeling en minder gedragsproblemen gezien werd bij kinderen bij wie tekenen van ontsteking in de placenta werden gevonden. Deze studie benadrukt dat placenta pathologie van invloed kan zijn op de latere ontwikkeling van te vroeg geboren kinderen en dus betrokken moet worden bij onderzoek naar de neuropsychologische ontwikkeling van te vroeg geboren kinderen. Om de relatie tussen placenta pathologie, vroeggeboorte en neuropsychologische ontwikkeling verder te onderzoeken, zijn de placenta's onderzocht van te vroeg geboren kinderen die een MRI scan van de hersenen hebben ondergaan op de a terme leeftijd (40 weken zwangerschapsduur). De resultaten van deze studie staan beschreven in **hoofdstuk 4**. De perinatale mortaliteit was hoger in de groep met kinderen waarbij in de placenta tekenen van ontsteking werden gevonden ten opzichte van kinderen bij wie in de placenta tekenen van slechte doorbloeding werden gevonden. Op 5.5 jarige leeftijd hadden kinderen met vasculaire placenta laesies vaker problemen met de motorische ontwikkeling ten opzichte van kinderen met inflammatoire placenta laesies of kinderen met beide typen of geen van deze typen laesies. In deze studie was de incidentie van matige tot ernstige witte stof schade, intraventriculaire bloedingen > graad II, cerebellaire bloedingen of abnormale ventrikelgrootte niet gerelateerd aan het type placenta laesie.

Er is een sterke correlatie tussen zwangerschapsduur van twee opeenvolgende zwangerschappen, en hoewel de meerderheid van de vrouwen met een vroeggeboorte de volgende zwangerschap niet te vroeg bevalt, is een belangrijke risicofactor voor een vroeggeboorte toch het doorgemaakt hebben van een eerdere vroeggeboorte. **Hoofdstuk 5** beschrijft de resultaten van een retrospectieve cohort studie die placenta pathologie vergelijkt tussen vrouwen die een herhaalde vroeggeboorte doormaken, en vrouwen die na een vroeggeboorte bij een normale zwangerschapsduur bevallen. Funisitis, een ontsteking van de navelstreng vaten, was omgekeerd gecorreleerd met herhaalde vroeggeboorte. Met andere woorden, vrouwen die bij de eerste vroeggeboorte funisitis hebben, hebben een bij een volgende zwangerschap een kleinere kans om weer een vroeggeboorte door te maken dan vrouwen die dat niet hebben.

Samenvattend had het eerste deel van deze these tot doel om inzicht te verkrijgen in de aard van de relatie tussen placenta pathologie, hersenontwikkeling en neuropsychologische ontwikkeling bij vroeggeboorte. De studies die beschreven worden in het eerste gedeelte van deze these laten zien dat:

1. Perinatale infecties, en met name postnatale infecties, geassocieerd zijn met slechtere mentale en motorische ontwikkeling bij vroeg geboren kinderen.
2. Vroeg geboren kinderen met tekenen van slechte doorbloeding in de placenta hebben een slechtere mentale ontwikkeling op 2 jarige leeftijd vergeleken met vroeg geboren kinderen met tekenen van ontsteking in de placenta.
3. Bij vroegeboren kinderen is de incidentie van cerebrale MRI afwijkingen niet gerelateerd aan het type placenta pathologie. Mogelijk hebben postnatale gebeurtenissen, zoals het doormaken van infecties en de noodzaak tot beademing, een grotere impact op de ontwikkeling van hersenschade.
4. Vrouwen met een vroeggeboorte waarbij funisitis wordt gevonden in de placenta hebben een kleinere kans om bij hun volgende zwangerschap weer te vroeg te bevallen vergeleken met vrouwen bij wie geen funisitis werd gevonden.

Deze studies verschaffen inzicht in de associatie tussen placenta pathologie en neuropsychologische ontwikkeling. Echter, de predictieve waarde van placenta pathologie is momenteel te gering om in individuele counseling uitspraken te doen over welk kind een vergroot risico heeft op een slechte uitkomst, of welke vrouwen een groot risico hebben op herhaalde vroeggeboorte. De bevindingen van deze studies benadrukken dat de pathofysiologische mechanismen die ten grondslag liggen aan vroeggeboorte complex en heterogeen zijn. Ook eerdere studies vonden relaties tussen placenta pathologie, vroeggeboorte en neuropsychologische ontwikkeling, echter het blijft onduidelijk of deze relatie ook een causaal verband betreft. Deze vraag is lastig te onderzoeken, gezien het onethisch zou zijn om klinische experimenten uit te voeren die deze vraagstelling zou kunnen beantwoorden. Dierstudies hebben laten zien dat systemische infecties tijdens de zwangerschap inderdaad kunnen leiden tot spontane vroeggeboorte.[1] Omdat de placenta een belangrijke rol speelt in de ontwikkeling en bescherming van het ongeboren kind is het interessant om de relatie tussen placenta pathologie en uitkomst verder te bestuderen. In klinische studies naar de effectiviteit van interventies bij dreigende vroeggeboorte zou het van toegevoegde waarde kunnen zijn om te kijken of behandeleffecten anders zijn bij vrouwen met verschillende typen placenta pathologie.

Deel II

Het tweede deel van deze these bestudeert het effect van interventies in de zwangerschap die vroeggeboorte zouden kunnen voorkomen en de uitkomst bij dreigende vroeggeboorte verbeteren. **Hoofdstuk 6** verschaft een overzicht van de effectiviteit en veiligheid van veel gebruikte vormen van tocolyse (weëenremmers). Gebaseerd op de huidige literatuur zijn beta-adrenoreceptor agonisten (een bepaald type weeënremmer) minder effectief als tocolyticum en hebben deze middelen meer bijwerkingen in vergelijking met andere vormen van tocolyse. Het gebruik van

beta-adrenoreceoptor agonisten is daarom obsoleet geraakt. Er is weinig literatuur beschikbaar over prostaglandine synthese remmers, de beschikbare literatuur laat echter een potentieel gunstig effect zien. Echter, de veiligheid van prostaglandine synthese remmers voor de foetus is nog onvoldoende onderzocht en moet in toekomstig onderzoek nader geëvalueerd worden. Magnesium sulfaat wordt niet geadviseerd als tocolyticum, hoewel dit middel in de Verenigde Staten nog veelvuldig gebruikt wordt als zodanig. Magnesium sulfaat heeft echter wel een neuroprotectieve werking en kan derhalve dus aangeraden worden als aanvullende therapie bij dreigende vroeggeboorte, naast tocolyse en medicatie ter bevordering van de longrijping. Progesteron heeft een beperkt nut in de behandeling van acute dreigende vroeggeboorte, maar zou een rol kunnen spelen in de preventie van vroeggeboorte. Gebaseerd op de huidige literatuur zou het gebruik van nifedipine of atosiban de voorkeur hebben als tocolytica, gezien deze middelen het meest gunstige bijwerkingenprofiel hebben, en de grootste effectiviteit hebben wat betreft het uitstellen van de geboorte en het verbeteren van de perinatale uitkomst.

Gezien neonatale uitkomst sterk gerelateerd is aan de zwangerschapsduur, zou het mogelijk zijn dat onderhoudstocolyse (tocolyse langer dan 48 uur) een goede behandelingsstrategie kunnen zijn. Gebaseerd op Individual Participant Data (IPD) van alle gerandomiseerde klinische trials naar onderhoudstocolyse middels nifedipine (**hoofdstuk 7**), werd er geen verschil gevonden in de incidentie van perinatale sterfte (relatief risico (RR) 1.2; 95% betrouwbaarheidsinterval (BI) 0.44-2.0), necrotizerende enterocolitis (RR 1.2; 95% BI 0.52-2.6), respiratoir distress syndroom (RR 1.00; 95% BI 0.50-2.0), intraventriculaire bloedingen ≥ graad 2 (RR 0.66; 95% BI 0.14-3.19) of verlenging van de zwangerschapsduur (HR 0.86; 95% BI 0.71-1.03) tussen vrouwen die behandeld werden met nifedipine onderhoudstocolyse vergeleken met vrouwen die behandeld werden met placebo/geen behandeling. Binnen de subgroep van vrouwen die zwanger waren van een eenling, bleek nifedipine geassocieerd met een langere verlenging van de zwangerschap in vergelijking met placebo/geen behandeling. Echter, ook in deze subgroep werd geen effect gevonden op perinatale sterfte. De conclusie is daarom dat, gebaseerd op de best beschikbare evidentie, nifedipine onderhoudstocolyse de neonatale uitkomst niet verbeterd. Doordat nifedipine gemakkelijk de placenta passeert, zou onderhoudstocolyse middels nifedipine een effect kunnen hebben op de lange termijn ontwikkeling van kinderen die daar in de baarmoeder aan zijn blootgesteld. De 2 jaars follow-up van kinderen uit de grootste studie naar nifedipine onderhoudstocolyse, de APOSTEL II studie, wordt beschreven in **hoofdstuk 8**. Op de leeftijd van 2 jaar hebben kinderen die in de baarmoeder blootgesteld zijn aan nifedipine onderhoudstocolyse een hogere incidentie van problemen met de fijne motoriek, en een lagere incidentie van slechte probleemoplossingvaardigheden. In de subgroep van vrouwen zonder te vroeg gebroken vliezen werd er een lagere incidentie gevonden van problemen met de grove motoriek en een lagere incidentie van algemene ontwikkelingsproblemen bij kinderen die blootgesteld waren aan nifedipine onderhoudstocolyse in vergelijking met kinderen uit de placebo groep.

Gezien onderhoudstocolyse met nifedipine de neonatale uitkomst niet verbetert, en er ook geen eenduidig bewijs is dat nifedipine onderhoudstocolyse de uitkomst van kinderen op 2 jarige leeftijd verbetert, wordt het gebruik hiervan niet aangeraden voor de klinische praktijk.
Volgende de huidige richtlijnen ter behandeling van dreigende vroeggeboorte wordt tocolyse middels atosiban of nifedipine gedurende 48 uur aangeraden. Echter, er is een gebrek aan studies die deze twee tocolytica vergelijken in termen van perinatale uitkomst, verlenging van de zwangerschapsduur en bijwerkingen. Hiertoe werd de APOSTEL III studie uitgevoerd. De APOSTEL III studie is een multicenter gerandomiseerde gecontroleerde studie uitgevoerd in 19 perinatale centra en grote ziekenhuizen. Het studieprotocol wordt beschreven in **hoofdstuk 9** en de resultaten van deze studie worden beschreven in **hoofdstuk 10**. Vijfhonderd en tien vrouwen werden gerandomiseerd tussen behandeling met nifedipine of atosiban. De incidentie van ongunstige perinatale uitkomst was 14.2% (42 kinderen) in de nifedipine groep, en 15.3% (45 kinderen) in de atosiban groep (RR 0.92; 95% BI 0.61-1.38). De zwangerschapsduur bij geboorte was niet verschillend tussen de twee groepen (mediaan (interkwartiel range IQR) 33.1 weken (30.5-37.0) in de nifedipine groep en 32.4 weken (30.1-35.8) in de atosiban group, HR 0.86; 95% BI 0.70-1.05). De mediane (IQR) verlenging van de zwangerschapsduur was 7 dagen (1.0-41.5) in de nifedipine groep en 4 dagen (1.0-38.0) in de atosiban group (HR 0.87; 95% BI 0.71-1.06). Bijwerkingen die leiden tot het staken van de studiemedicatie werden gerapporteerd bij 15 (6%) vrouwen in de nifedipine groep en bij 6 (2%) vrouwen in de atosiban groep (RR 2.57; 95% BI 1.01-6.55). Dit verschil werd voornamelijk veroorzaakt door het hogere aantal vermoedelijke intrauterine infecties in de nifedipine groep (6 versus 1). Maternale sterfte kwam niet voor in beide groepen. Een niet significante maar hogere incidentie van perinatale sterfte werd gezien in de nifedipine group (5.1% versus 2.4% in de atosiban groep). Het is van belang te benadrukken dat er onzekerheid bestaat over de effectiviteit van tocolyse bij dreigende vroeggeboorte. Behandeling middels tocolyse stoelt op de assumptie dat het uitstellen van de bevalling met ten minste 48 uur de mogelijkheid biedt om corticosteroïden in te laten werker, en zo de rijping van de longen en de perinatale uitkomst te verbeteren. Het verbeteren van de perinatale uitkomst is het belangrijkste doel van de behandeling bij dreigende vroeggeboorte. Echter, veel studies naar de effectiviteit van tocolyse zijn niet groot genoeg om een verschil in deze uitkomst te detecteren. Tocolyse zou bovendien ongunstige bijwerkingen kunnen hebben, die het positieve effect van antenatale corticosteroïden teniet kunnen doen. Tot slot zou het in twijfel getrokken kunnen worden of het uitstellen van de bevalling een positief effect zou hebben op de neontale uitkomst. Hoewel de incidentie van neonatale morbiditeit en mortaliteit afneemt bij een toenemende zwangerschapsduur, zal bij een dreigende vroeggeboorte de foetus mogelijk worden blootgesteld aan een schadelijke intrauterine omgeving, waarbij de effecten op de neonatale uitkomst niet bekend zijn. Er is daarom een dringende behoefte aan grote placebo gecontroleerde studies die groot genoeg zijn om zowel positieve als negatieve effecten van tocolyse op

de neonatale uitkomst te detecteren, om op deze manier de behandeling van dreigende vroeggeboorte te optimaliseren.
In toenemende mate is er bewijs dat de pathofysiologische processen die ten grondslag liggen aan vroeggeboorte reeds vroeg in de zwangerschap optreden. Interventies vroeg in de zwangerschap zouden daarom veelbelovend kunnen zijn om de perinatale uitkomst te verbeteren. In het laatste hoofdstuk van deze these, **hoofdstuk 11**, worden de resultaten beschreven van een secundaire analyse met Individual Participant Data van 17 studies (28.797 vrouwen) naar het gebruik van trombocyten aggregatie remmers (bloedplaatjes remmers zoals aspirine) in de zwangerschap bij vrouwen met een verhoogd risico op pre-eclampsie (zwangerschapsvergiftiging). Vrouwen die behandeld werden met trombocyten aggregatie remmers hadden in vergelijking met vrouwen met placebo/geen behandeling een lager risico op spontane vroeggeboorte <37 weken (RR 0.93; 95% BI 0.86-0.996) en <34 weken (RR 0.86; 95% BI 0.76-0.99). De mate van verhoging van het risico op pre-eclampsie van de deelnemende vrouwen was erg heterogeen, en varieerde van zwanger zijn van het eerste kind tot het eerder doorgemaakt hebben van pre-eclampsie. Subgroep analyses lieten geen verschil in behandel effect zien tussen vrouwen met een zeer of matig verhoogd risico op pre-eclampsie. De bevindingen uit deze studie zouden daarom ook van toepassing kunnen zijn op een bredere populatie van zwangere vrouwen. De veiligheid van trombocyten aggregatie remmers tijdens de zwangerschap is uitvoerig onderzocht en laat geen verschillen zien in de incidentie van antepartum bloedingen (RR 1.02; 95% BI 0.90-1.15), postpartum bloedingen (RR 1.06; 95% BI 1.00-1.13), abruptio placentae (RR 1.13; 95% BI 0.87-1.48) of bloeding bij het kind (RR 0.93; 95% BI 0.80-1.09) tussen vrouwen die behandeld werden met trombocyten aggregatie remmers vergeleken met vrouwen met placebo/geen behandeling.[2] Lange termijn follow-up van twee van deze studies lieten geen effect op uitkomst van de kinderen zien op de leeftijd van 12 en 18 maanden, en liet bovendien een vermindering van het aantal gedragsproblemen zien op de leeftijd van 5 jaar bij kinderen die blootgesteld waren aan de trombocyten aggregatie remmer aspirine.[3] Het gebruik van trombocyten aggregatie remmers tijdens de zwangerschap zou dus mogelijk een veelbelovende interventie kunnen zijn bij vrouwen met een spontane vroeggeboorte in de voorgeschiedenis.
Samenvattend gaat dit laatste gedeelte van deze these in op de effecten van interventies tijdens de zwangerschap om de uitkomsten bij dreigende vroeggeboorte te verbeteren en om spontane vroeggeboorte te voorkomen. Die studies die beschreven zijn in deze these lieten zien dat:

1. Bij acute tocolyse gedurende 48 uur het gebruik van nifedipine of atosiban de voorkeur heeft, gebaseerd op literatuur over de effectiviteit, perinatale uitkomst en bijwerkingen.
2. Onderhoudstocolyse middels nifedipine leidt niet tot verbetering van de neonatale uitkomst, verlenging van de zwangerschaspduur of duidelijke verbetering van de neuropsychologische ontwikkeling op 2 jarige leeftijd.

3. Het gebruik van nifedipine en atosiban als tocolyse leidt tot vergelijkbare incidenties van slechte perinatale uitkomst.
4. Het gebruik van trombocyten aggregatieremmers in de zwangerschap verlaagt de incidentie van spontane vroeggeboorte bij vrouwen met een verhoogd risico op pre-eclampsie.

Het belangrijkste kennis hiaat blijft of tocolyse de neonatale uitkomst verbeterd. Er is daarom dringend behoefte aan placebo gecontroleerde studies die groot genoeg zijn om effecten op perinatale uitkomsten te bestuderen.

Het verbeteren van zwangerschapsuitkomsten: aanwijzigen voor toekomstig onderzoek

De perinatale uitkomst bij vroeggeboorte is sterk verbeterd in de afgelopen jaren. Desalniettemin blijft vroeggeboorte een van de grootste vraagstukken in de obstetrie. Geen van de verschillende behandelingsmogelijkheden die zijn besproken in deze these lijkt het meest veelbelovend als interventie om vroeggeboorte uit te stellen of te voorkomen. De effectiviteit van nieuwe interventies zal daarom onderzocht moeten worden in gerandomiseerde klinische trials. Een mogelijk zinvolle interventie is het plaatsten van een pessarium. Eerdere studies hebben laten zien dat een pessarium het risico op vroeggeboorte en op een ongunstige neonatale uitkomst doet verkleinen bij vrouwen met een korte cervix (baarmoedermond).[4,5] De APOSTEL VI studie onderzoekt momenteel of een pessarium zinvol is bij vrouwen die opgenomen zijn geweest in verband met een dreigende vroeggeboorte, als interventie na het toedienen van tocolyse en corticosteroïden.[6] Een andere mogelijke interventie om vroeggeboorte te voorkomen of uit te stellen is progesteron. De Quadrupple-P studie[6] vergelijkt vaginale toediening van progesteron met een pessarium bij vrouwen met een korte cervix bij 20 weken zwangerschapsduur, en de PC-studie vergelijkt een cervicale cerclage (een bandje om de baarmoedermond) met een pessarium bij vrouwen die op basis van een eerder doorgemaakte vroeggeboorte een hoog risico hebben op vroeggeboorte.

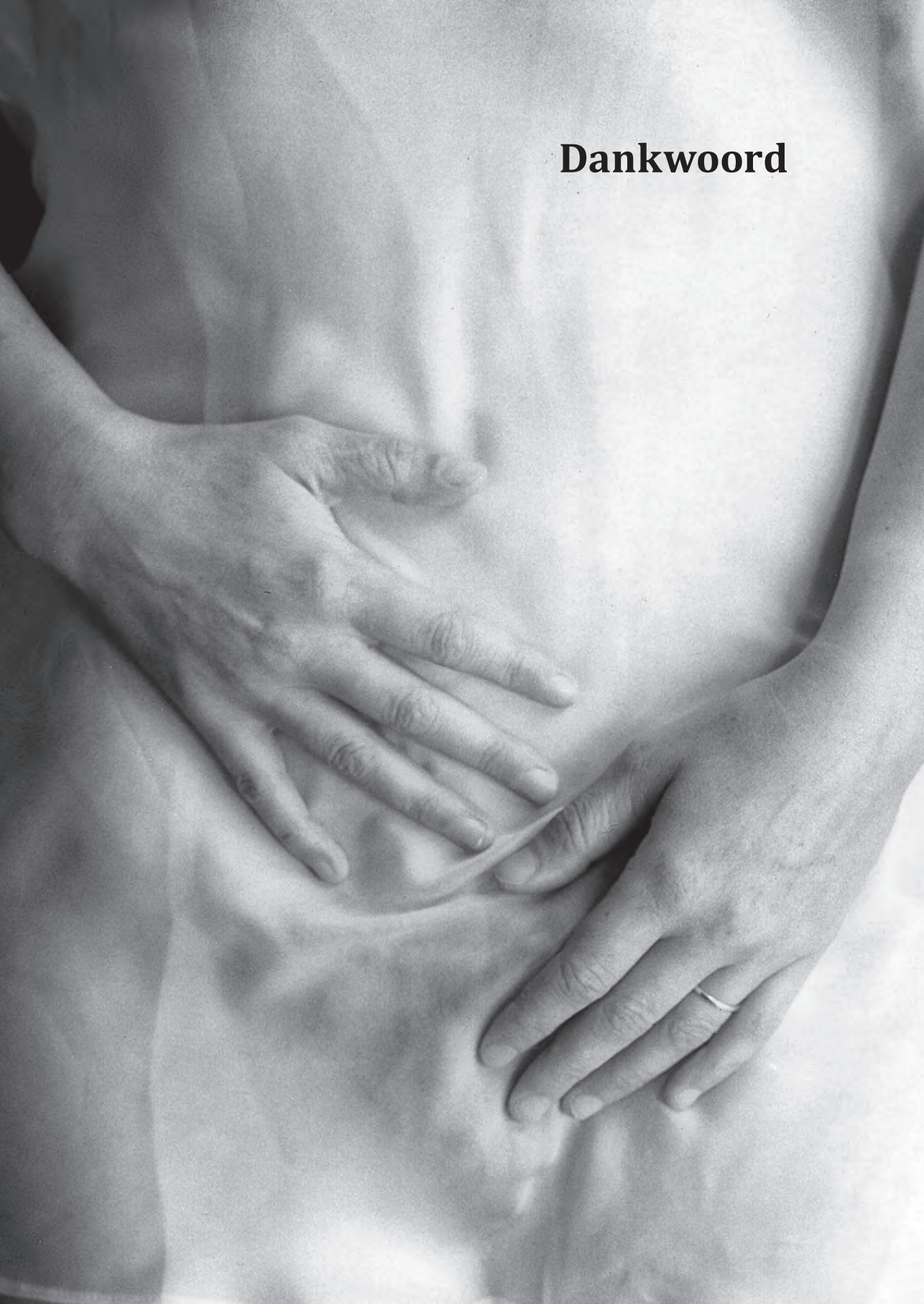
De toegenomen bewustwording dat vroeggeboorte niet simpelweg een geboorte voor de uitgerekende datum is, maar een complexe aandoening waarbij verschillende pathofysiologische mechanismen een rol spelen, heeft ertoe geleid dat er steeds meer aandacht is voor het individualiseren van de behandeling om de uitkomst bij (dreigende) vroeggeboorte te verbeteren. Een individuele risico inschatting om te bepalen welke vrouwen een verhoogd risico hebben op vroeggeboorte moet gebaseerd zijn op maternale, foetale en zwangerschap/omgevingsspecifieke factoren.[7] Het gebruik van zo'n model in onderzoek en klinische praktijk zou aangemoedigd moeten worden. De huidige en toekomstige behandelopties zouden geëvalueerd moeten worden in verschillende subgroepen, bijvoorbeeld in vrouwen met vroegtijdig gebroken vliezen en vrouwen met en zonder cervix verkorting. Hiertoe is het belangrijk om resultaten van verschillende studies te kunnen vergelijken, waarvoor samenwerking tussen verschillende onderzoeksnetwerken wereldwijd onontbeerlijk is. Om dit te

bewerkstellingen is het Global Obstetrics Network opgericht.[8] Dit is een belangrijke stap om ervoor te zorgen dat het identificeren van kennis hiaten, het opzetten van klinische studies, en het implementeren van de resultaten in de dagelijkse praktijk naadloos op elkaar aansluiten. De combinatie van klinisch en epidemiologisch onderzoek naar vroeggeboorte, alsook het uitvoeren van goed ontworpen klinische studies zullen bijdragen aan het optimaliseren van de zwangerschapsuitkomst. Onderzoek naar de pathofysiologie zouden geïntegreerd kunnen worden in de klinische trials. Zo zou het bijvoorbeeld interessant kunnen zijn om te onderzoeken of vrouwen bij wie trombocyten aggregatie remmers worden gegeven inderdaad ook minder vasculaire laesies hebben in de placenta.

Toekomstige studies naar vroeggeboorte zouden gericht moeten zijn op het verbeteren van de neonatale uitkomst. Gezien interventies in de zwangerschap effect kunnen hebben op de ontwikkelende foetus is het van groot belang dat er ook lange termijn follow-up plaats vind. Als lering uit de ORACLE II studie,[9] waar een hogere incidentie van cerebrale parese werd gevonden bij kinderen die antenataal waren blootgesteld aan antibiotica, zou het uitgangspunt voor de klinische praktijk moeten zijn dat voorzichtigheid geboden is bij het interfereren in de zwangerschap zonder overtuigend bewijst van effectiviteit en veiligheid op korte en lange termijn.

Referenties

1. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O et al. The preterm parturition syndrome. BJOG. 2006;113 Suppl 3:17-42.
2. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA; PARIS Collaborative Group. Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. Lancet. 2007;369:1791-8.
3. Marret S, Marchand L, Kaminski M, Larroque B, Arnaud C, Truffert P, Thirez G, Fresson J, Rozé JC, Ancel PY; EPIPAGE Study Group. Prenatal low-dose aspirin and neurobehavioral outcomes of children born very preterm. Pediatrics. 2010;125:e29-34.
4. Goya M, Pratcorona L, Merced C, Rodó C, Valle L, Romero A et al.; Pesario Cervical para Evitar Prematuridad (PECEP) Trial Group. Cervical pessary in pregnant women with a short cervix (PECEP): an open-label randomised controlled trial. Lancet. 2012;379:1800-6.
5. Liem S, Schuit E, Hegeman M, Bais J, de Boer K, Bloemenkamp K et al. Cervical pessaries for prevention of preterm birth in women with a multiple pregnancy (ProTWIN): a multicentre, open-label randomised controlled trial. Lancet. 2013;382:1341-9.
6. www.studies-obsgyn.nl
7. Kazemier BM. Improving perinatal outcome: towards individualized care. 2015 PhD Thesis, University of Amsterdam, the Netherlands.
8. Mol BW, Ruifrok AE; Global Obstetrics Network. Global alignment, coordination and collaboration in perinatal research: the Global Obstetrics Network (GONet) Initiative. Am J Perinatol. 2013;30:163-6.
9. Kenyon S, Pike K, Jones DR, Brocklehurst P, Marlow N, Salt A, Taylor DJ. Childhood outcomes after prescription of antibiotics to pregnant women with spontaneous preterm labour: 7-year follow-up of the ORACLE II trial. Lancet. 2008;372:1319-27.



Dankwoord

Dankwoord

Het belangrijkste van doel van klinisch wetenschappelijk onderzoek is het verbeteren van zorg voor patiënten. De beslissing om deel te nemen aan een klinische trial wordt vaak gemaakt op momenten van grote onzekerheid; bij vroegtijdige weeën of net na een (veel) te vroege bevalling. Door deelname aan wetenschappelijk onderzoek is het mogelijk behandelingen te evalueren en hiermee de uitkomsten voor andere moeders en kinderen te optimaliseren. In de eerste plaats wil ik alle daarom vrouwen en kinderen die mee hebben gedaan aan de GEEF-studie, de APOSTEL II follow-up studie en APOSTEL III studie hiervoor danken.

Voor de wetenschapper is een tweede doel het ontwikkelen van academische kwaliteiten. Velen hebben hierin voor mij een belangrijke rol gespeeld, hen wil ik graag op deze plaats bedanken.

Prof. Dr. Franx, beste Arie, toen ik begin 2013 voor het eerst in het WKZ kwam wist ik direct dat dit de plek was waar ik mijn proefschrift wilde schrijven en ben zeer blij dat ik daar de kans voor heb gekregen. Je vertrouwen, betrokkenheid, en efficiënte besprekingen waardeer ik enorm.
Prof. Dr. van Elburg, beste Ruurd, bedankt dat je ook tijdens mijn promotie in het WKZ bij mijn onderzoek betrokken bent gebleven als promotor.
Prof. Dr. Mol, beste Ben Willem, ik heb veel van je geleerd, over geneeskunde, wetenschap, subsidies aanvragen, samenwerking, en zelfs over voetbal. Je hebt de bal vaak voor mij voor het doel gelegd, heel veel dank daarvoor!
Dr. Oudijk, beste Martijn, ik kan me geen betere samenwerking voorstellen dan met jou in de afgelopen jaren, en hoop dan ook dat er nog vele projecten zullen volgen. Dankjewel voor het altijd bereikbaar zijn, altijd enthousiast en vol vertrouwen. Ik wens iedere promovendus een Martijn toe.
Dr. Nikkels, beste Peter, tijdens mijn studie aan de VU heb ik een presentatie over je placenta studie bijgewoond, en was direct aangestoken door je enthousiasme. Mijn dank ik dan ook groot dat je bereid was om naast je klinische werk honderden placenta's te beoordelen voor mijn onderzoek.
Prof. Dr. Oosterlaan, beste Jaap, dank voor je enthousiasme en zeer heldere commentaren op mijn eerste manuscripten, waar ik ook bij latere manuscripten veel aan heb gehad!
Prof. Dr. M.J. Jongmans, Prof. Dr. J.A.M. van der Post, Prof. Dr. C.J.M. de Groot, Prof. Dr. P.J. van Diest en Prof. Dr. S. Durston, hartelijk dank dat jullie zitting hebben willen nemen in de leescommissie.

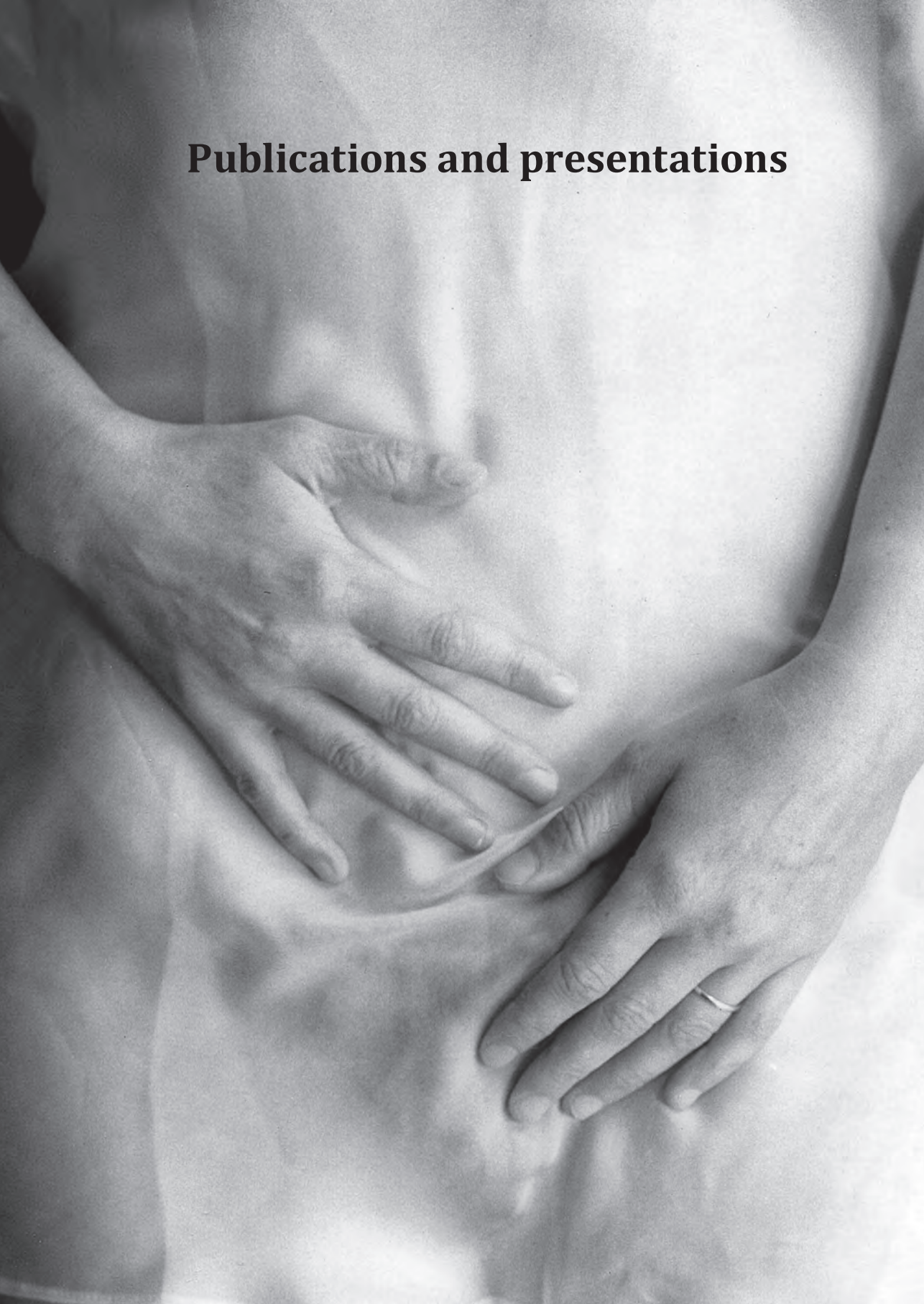
Graag wil ik alle mede auteurs bedanken voor hun bijdrage aan de manuscripten in dit proefschift. Met name Manon Benders, dank voor alle tijd die je hebt vrijgemaakt naast je vele klinische werk. Jorrit de Kieviet, dank voor je bijdrage aan het honourstraject.

Mijn lieve collega onderzoekers in het WKZ en van 'de overkant' dank voor jullie gezelligheid tijdens de lunch, koffie, borrels en safari's. Jan, als jij dat briljante plan niet had gehad voor de Apostel III…?! Lieve Daphne, wat ben ik blij dat ik jou heb leren kennen, als collega en vriendin. Mede dankzij jouw enthousiasme kwam ik in het WKZ, en heb ik er een top tijd gehad. Mijn collega's uit het Gelre ziekenhuis, bedankt voor de fijne samenwerking!
Thank you dr. Lisa Askie for the wonderful time at the Clinical Trial Center, I have learned a lot and you made me feel most welcome. Many thanks to you and all my colleagues in Sydney.

Mijn paranimfen; Lieve Esmeralde, ik ben trots om jou als zus en paranimf te hebben. Veel eigenschappen die promoveren in zo'n korte tijd mogelijk maken heb ik van jou geleerd. Ik bewonder je fantastische zakelijke en organisatorische talent. Vanaf de eerste seconde ben ik de trotste tante van Hugo en Juliette, de mooiste baby's uit het WKZ. Lieve Eva, dank je wel dat je er altijd bent, en dat je mijn paranimf wil zijn! Ik hoop dat we nog veel mooie reizen gaan maken samen.
Lieve Agaath en Hanneke, wat fijn om jullie als grote zussen te hebben. Zo vanzelfsprekend als dat lijkt, zo bijzonder vind ik het. Flora, Lotte en Koen, wat jullie ook doen jullie tante is trots! Carla, dank je wel dat jouw werk de voorkant van mijn proefschrift mag sieren.
Lieve Fleur, Dana, Eva, Wendela, Rozemarije, Koen en Tilaka, dank jullie wel voor jullie vriendschap! Lieve Joanne, in al mijn plannen en ambities heb je me altijd gesteund en was je vol vertrouwen dat het goed zou komen. Je had weer eens gelijk!

Lieve Robbert en Eleonore, bedankt dat jullie mij hebben geleerd altijd nieuwsgierig, kritisch en zelfstandig te zijn, om te dromen en die dromen te volgen.

Publications and presentations



Publications

van Vliet EOG, de Kieviet JF, van der Voorn JP, Been JV, Oosterlaan J, van Elburg, RM. Placental Pathology and Long Term Neurodevelopment of Very Preterm Infants. Am J Obstet Gynecol. 2012;206:489.e1-7.

van Vliet EOG, de Kieviet JF, Oosterlaan J, van Elburg RM. Perinatal Infection and Neurodevelopmental Outcome in the Very Preterm and Very Low-Birth-Weight Infant: a Meta- Analysis. JAMA Pediatr. 2013;167:662-8.

van Vliet EOG, Boormans EM, de Lange TS, Mol BW, Oudijk MA. Preterm labour: current pharmacotherapy options for tocolysis. Expert Opin Pharmacother. 2014;15:787-97.

van Vliet EOG, Schuit E, Heida KY, Opmeer BC, Kok M, Gyselaers W, Porath MM, Woiski M, Bax CJ, Bloemenkamp KW, Scheepers HC, Jaquemyn Y, van Beek E, Duvekot HJ, Franssen MT, Bijvank BN, Kok JH, Franx A, Mol BW, Oudijk MA. Nifedipine versus atosiban in the treatment of threatened preterm labour: APOSTEL III-Trial. BMC Pregnancy Childbirth. 2014;14:93.

Hopmans TE, van Houten C, Kasius A, Kouznetsova OI, Nguyen LA, Rooijmans SV, Voormolen DN, **van Vliet EOG**, Franx A, Koster MP. Increased risk of type II diabetes mellitus and cardiovascular disease after gestational diabetes mellitus: a systematic review. Ned Tijdschr Geneeskd. 2015;159:A8043. [Dutch]

van Vliet EOG, Seinen L, Roos C, Schuit E, Scheepers H, Bloemenkamp K, Duvekot JJ, van Eyck J, Kok JH, Lotgering FK, van Baar A, van Wassenaer-Leemhuis AG, Franssen MT, Porath MM, van der Post J, Franx A, Mol B, Oudijk MA. Maintenance Tocolysis with Nifedipine in threatened preterm labour: a 2 year follow up study. BJOG. 2015 Aug 27. [Epub ahead of print]

van Vliet EOG, Schuit E , Dijkema GH, Heida KY, Roos C, van der Post JAM , Parry EC, Mc Cowan L, Lyell DJ, El-Sayed YY, Barry DB, Clark AL, Mahdy ZA , Uma M, Sayin NC, Varol GF, Mol BWJ, Oudijk MA. Nifedipine maintenance tocolysis and perinatal outcome: an individual participant data meta-analysis. Submitted

van Vliet EOG, Nijman TAJ, Schuit E, Heida KY, Opmeer BC, Kok M, Gyselaers W, Porath MM, Woiski M, Bax CJ, Bloemenkamp KWM, Scheepers HCJ, Jaquemyn Y, van Beek E, Duvekot JJ, Franssen MTM, Papatsonis DN, Kok JH, van der Post JAM, Franx A, Mol BWJ, Oudijk MA. Nifedipine versus atosiban in the treatment of threatened preterm birth (Assessment of Perinatal Outcome after Specific Tocolysis in Early Labor:
APOSTEL III-Trial): a multicenter randomized controlled trial. Submitted

van Vliet EOG, Askie LA, Mol BWJ, Oudijk MA. Antiplatelet agents and the prevention of spontaneous preterm birth: a meta-analysis of individual participant data. Submitted

Veerbeek JHW, Brouwers L, Koster MPH, Koenen SV, **van Vliet EOG**, Nikkels PGJ, van Rijn BB, Franx A. Spiral artery remodeling and maternal cardiovascular risk: the SPAR study. Submitted

Presentations

PSANZ Annual meeting 2015, Melbourne

Nifedipine versus atosiban in the treatment of threatened preterm birth: the APOSTEL III trial. Oral presentation

PSANZ Annual meeting 2015, Melbourne

Antiplatelet agents and the prevention of spontaneous preterm birth: a meta-analysis of individual participant data. Oral presentation

SMFM Annual meeting 2015, San Diego

Nifedipine versus atosiban in the treatment of threatened preterm birth: the APOSTEL III trial. Oral presentation (plenary session)

SGI Annual meeting 2014, Florance

Placenta pathology, cerebral MRI and neurodevelopmental outcome in preterm birth. Poster presentation (late breaking abstract)

NVK Congres 2012, Veldhoven

Placental pathology and long term neurodevelopment of very preterm infants. Oral presentation (in Dutch)

The 4th Congress of the European Academy of Peadiatric Societies, Istanbul

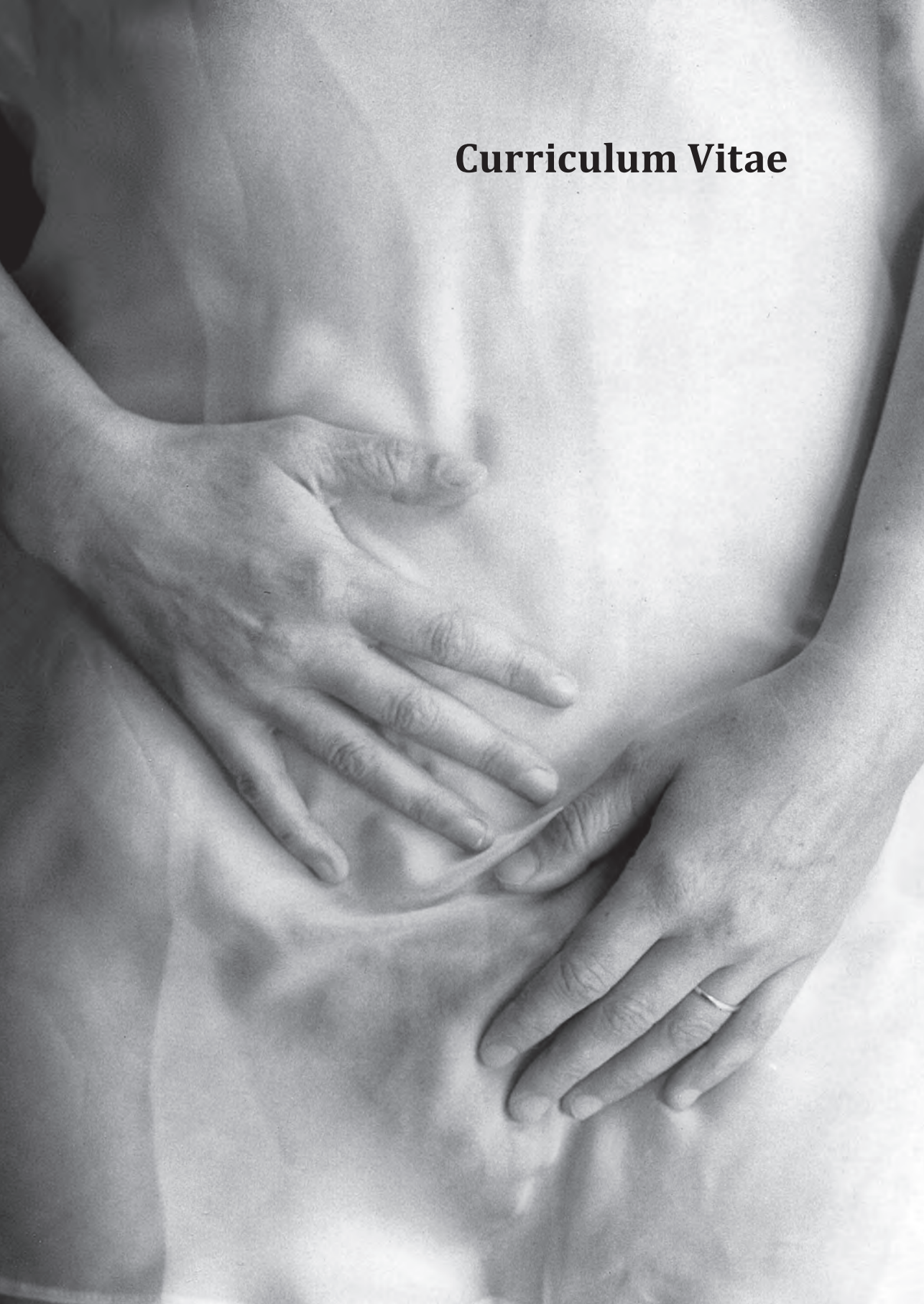
Neurodevelopmental Outcome in the Very Preterm and Very Low-Birth-Weight Infant: a Meta-Analysis. Short oral presentation

Landelijk Neonatale Follow-up Symposium 2011, Amsterdam

Placental pathology, neonatal morbidity and Neurodevelopmental outcome in very preterm infants. Oral presentation (in Dutch)

12e Landelijke Neonatale Neurologie symposium, Utrecht

Placenta pathologie en neuropsychologische ontwikkeling bij premature kinderen. Oral presentation (in Dutch)



Curriculum Vitae

Curriculum Vitae

Elvira Odette Geraldine van Vliet, geboren 25 april 1986, startte in 2007 met haar studie Geneeskunde na het behalen van haar bachelordiploma Biologische psychologie. In het derde jaar van haar studie begon zij in het kader van het *Honoursprogramma* aan een wetenschappelijke stage bij de afdeling Neonatologie en Klinische Neuropsychologie. Onder supervisie van dr. van Elburg en prof. dr. Oosterlaan verrichte zij onderzoek naar de lange termijn ontwikkeling van prematuur geboren kinderen. Tijdens haar studie volgde zij een extra coschap in het Princess Basma Hospital te Irbid, Jordanië. Na het behalen van haar artsendiploma in april 2013 werkte zij als arts-onderzoeker aan de afdeling Obstetrie en Gynaecologie in het UMC Utrecht onder supervisie van prof. dr. Arie Franx en dr. Martijn Oudijk. Het onderzoek naar placenta pathologie bij prematuren en het onderzoek naar de effectiviteit van tocolyse bij vroegtijdige weeën vormen de basis van dit proefschrift. Na het afronden van dit onderzoek ontving zij een beurs van het Ter Meulen Fonds van de Koninklijke Nederlandse Akademie van Wetenschappen (KNAW) waarmee ze aan de *University of Sydney* aan een project werkte over aspirine ter preventie van spontane vroeggeboorte. In december 2014 startte zij als arts-assistent Gynaecologie in het Gelreziekenhuis te Apeldoorn. Naast haar werk als arts en onderzoeker heeft ze een balletschool waar ze met veel plezier les geeft aan kinderen van 2.5 t/m 14 jaar.