Fetal brain imaging in pregnancies at risk for preterm birth

F.M.F. Rosier-van Dunné
Fetal brain imaging in pregnancies at risk for preterm birth

Financial support for the printing of this thesis was kindly provided by . . .

© Marlene Dumas

Printed by: Drukkerij SSP, Amsterdam

No part of this thesis may be reproduced, stored or transmitted in any form or by any means, without written permission of the author, or when appropriate, of the publishers of the publications.


© 2010 F.M.F. Rosier-van Dunné, Amsterdam, the Netherlands

VRIJE UNIVERSITEIT

Fetal brain imaging in pregnancies at risk for preterm birth

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan de Vrije Universiteit Amsterdam, op gezag van de rector magnificus prof.dr. L.M. Bouter, in het openbaar te verdedigen ten overstaan van de promotiecommissie van de faculteit der Geneeskunde op woensdag 6 oktober 2010 om 15.45 uur in het auditorium van de universiteit, De Boelelaan 1105

door

Fleur Marie Frederieke Rosier-van Dunné
geboren te Tilburg
“Dis klaar”

“Hou dit hier op?”

“Ja. Vandag hou dit hier op”
Chapter 1.

General introduction

This thesis describes the results of a prospective study of brain imaging in pregnancies threatened by preterm birth. This study has been performed in collaboration between the Faculty of Human Movement Sciences, VU University and Department of Obstetrics, VU University Medical Center, Amsterdam, the Netherlands, Department of Neonatology, Leiden University Medical Center and Tygerberg Hospital, University of Stellenbosch, South-Africa. The population was studied at the Tygerberg Hospital, Bellville, South Africa.

The general introduction discusses the background and rational of the study and provides an overview of the current knowledge on prevalence, risk factors and pathophysiology of perinatal brain injury and methodology of ultrasound imaging of the fetal brain. The aims of the study and outline of the chapters are presented.

Background

During the second half of the last century major advances in perinatal care have been attained. Improvements in obstetric ultrasound technology have provided possibilities for exact dating of pregnancies, evaluating fetal growth and Doppler indices, as well as for screening for congenital abnormalities. Advancements in cardiotocography and refinements with computerised evaluation during as well as before the onset of labour, facilitate the detection of fetal clinical deterioration thus helping to determine the optimal time for delivery. Advancements in neonatal intensive care have resulted in an increased survival of preterm born infants, but a further decrease in perinatal morbidity and neurological deficits has been lingering. Preterm birth remains a major risk factor for brain injury and subsequent sequelae, consisting not only of motor impairment, but also of cognitive, visual and behavioural disabilities. During the last two decades, however, a decrease in prevalence of cerebral palsy (CP) has set in.

Preterm birth is a worldwide health problem. Developing countries, especially those in Africa and Asia carry the highest burden of preterm deliveries in absolute numbers. In Africa the overall preterm birth rate is 11.9% (comparable to Northern-America with 10.6%), but the incidence is as high as 17.5% for Southern Africa. In Europe the mean preterm birth rate is relatively low (6.2 %) and for the Netherlands this is 7.6%. Very low birth weight preterm infants (birth weight < 1500 g) are prone to medical complications like respiratory distress syndrome requiring ventilation, sepsis, hypotension, and patent ductus arteriosus and often require blood transfusions. All these factors have been associated with CP. Infections known to be associated with CP include viral, bacterial, protozoan and fungal infections. Sepsis causing systemic
inflammatory response and/or multiple organ failure with shock and central nervous system (CNS) infections can cause brain injury.20

The major predictor of neurological impairment in very preterm neonates is presence of echogenicity changes in the white matter (WM).31,32 When scanned serially during the neonatal period and again at term age equivalent with high resolution transducers, the majority of high risk neonates born < 32 weeks gestational age (GA) that develop CP during infancy show abnormalities at brain ultrasound examination.21

With increased knowledge about the development of brain injury after hypoxic-ischaemic events as can be examined by sonography,24-30 magnetic resonance imaging,26 or histological examination,27-30 there is substantial evidence that the majority of the hypoxic-ischaemic events occurs before birth.4,12,31-33 Nowadays it is postulated that only about 10% of CP is caused by intrapartum acute hypoxic obstetric events.31,33-34 It is likely that adverse events occurring before and around birth and during early neonatal life will add up increasing the risk for brain injury, and possible subsequent neurodevelopmental impairment.19,24 This growing insight supports the necessity to perform studies with close monitoring of both fetal and neonatal parameters and adequate follow-up with neurological assessments in the developing infants.

Antenatal risk factors for brain injury

Several factors during pregnancy may attribute to the development brain injury.

Maternal and intrauterine infections influence the prognosis of the preterm infant.31,35,36 Intrauterine ascending bacterial infections are strongly associated with preterm premature rupture of membranes (PPROM) and preterm birth, and have also been associated with an increased risk for intraventricular haemorrhages (IVH) and periventricular leukomalacia (PVL).37-40 There are some inconsistencies in the literature, however, on the role of intra-uterine infection and neurological outcome in preterm infants. Several studies found associations between clinical chorioamnionitis and the risk of CP.11,21,22,33,40-45 Other studies did not find an independent association between intrauterine infection and CP when corrected for GA.39-45 Meta-analyses demonstrated a relation between histological chorioamnionitis and cystic PVL but no relationship with CP.42,43

Classical STORCH infections (syphilis, toxoplasmosis, rubella, cytomegalovirus (CMV), herpes simplex), human immunodeficiency virus (HIV), varicella zoster virus have all been documented to cause transplacental congenital infections and subsequent neurologic sequelae.20

Fetal growth restriction (FGR) may also have significant long term effects on growth and development.47 FGR fetuses have been reported to express a higher vulnerability for brain injury which might be explained by the detrimental effects of intrauterine malnutrition, and hypoxaemia caused by chronic utero-placental insufficiency.48-50 Recent studies have demonstrated decreased brain volumes in neonates that were growth restricted.51-53 A recent cohort study of neonates born < 28 weeks GA demonstrated an increased risk of FGR for cystic lesions in the periventricular white matter.53 Another large population based study demonstrated that FGR is a risk factor for CP, when fetal weight standards instead of birth weight curves were used for preterm neonates, although the majority of infants with CP had a birth weight above the 10th percentile.54

Contradictory results have also been found in the association between neurological long term outcome and preeclampsia. Several case-control and cohort studies have found a reduced risk of CP.54-55 and therefore preeclampsia has been regarded to have a protective effect on the development of CP. Others have refuted this and claim that the absence of infection in these pregnancies complicated by preeclampsia probably bias the results.56,57

Cases reports have described fetuses with brain injury associated with coagulation disorders (alloimmune and idiopathic thrombocytopenia, von Willebrand’s disease, warfarin use, fetal congenital factor V Leiden mutation), acute circulatory problems (cases with demise of a co-twin, placental abruption or feto-maternal haemorrhage), or metabolic disease (including thyroid disease). Also maternal complications, like abdominal trauma or cocaine use have been reported in association with fetal brain injury. Finally, congenital abnormalities, such as tumours, and arterio-venous malformation may lead to fetal brain injury.58-60

The pathophysiology of (fetal) brain injury

Patterns of brain injury are related to the age-related developmental stage of the brain, the general metabolic state and to individual vulnerability. From studies in neonates and stillborn infants, certain brain areas are known to be vulnerable for injury during the preterm period.61-66 More and more mechanisms are elucidated for their role in neonatal brain injury.24,56 It is likely that similar mechanisms during the same age period before birth play a role in the development of fetal brain injury. There are several possible pathways for brain injury.

The periventricular WM and the gray matter of basal ganglia and thalami are probably vulnerable for cerebral ischemia-reperfusion and infectious/inflammatory insults during the preterm period. This vulnerability is related to the maturational stage of neurons, axons and oligodendrocytes, the developmental profiles of glutamate and cytokine receptors and antioxidant systems, and possible other unknown factors.62 Moreover, the cerebral WM is especially vulnerable for haemodynamic alterations in blood flow because of its maturational inability to autoregulate blood flow so called ‘vascular end zones’ in the periventricular WM, that are hypothesized to have a diminished number of anastomoses between the short and long penetrating arteries.64,65

During the preterm period there is also an increased risk of haemorrhages originating from the highly vascularized and vulnerable germinal matrix.57,66-68

Another pathway of brain injury is through release of proinflammatory cytokines (interleukin-4, interleukin-6, and tumour necrosis factor-alpha). This occurs not only

8

9
Ultrasound imaging of fetal brain injury

In neonates transfontanellar brain ultrasound is worldwide implemented to diagnose brain injury during the neonatal period in infants at risk. This non-invasive imaging technique is safe, can be applied bedside, and allows serial examination to follow brain growth and maturation and the evolution of brain lesions.69,70

There is no evidence of any harm of performing diagnostic real-time ultrasound examination in the fetus.71 In fetuses the basic ultrasound examination of the brain is performed transabdominal in axial planes through the parietal bone.72 Depending on the position of the fetal head it may be possible to examine the brain transfontanellar, in coronal or sagittal planes. Transabdominal brain examination in the axial plane allows for detection of extensive central nervous system anomalies, ventriculomegaly, calcifications and haemorrhages, large porencephalic cysts, and severe WM atrophy.73,74,75 The use of high frequency transducers improves the visualization of the nearest hemisphere. More subtle changes, (mild) ischaemic lesions and germinal matrix haemorrhages, however, may be missed by transabdominal axial ultrasound examination.

During the last decades transvaginal ultrasound examination has been explored for fetal brain imaging. Although the transvaginal ultrasound examination is more burdening for the pregnant woman than the transabdominal examination, transvaginal ultrasound examinations can even be safely performed in pregnancies with PPROM, without increased risk of labour or infections.76 Additionally, power or colour Doppler flow measurements of the middle cerebral artery can be performed.

Using transvaginal ultrasonography, standardized planes can be examined in analogy to the planes described for the neonate.77-79 Early studies have put emphasis on CNS development from an embryological point, followed by the possibilities to screen for CNS malformations.79 In later studies, transvaginal sonography was performed in fetuses with a cephalic position to examine the brain during the second and third trimester for various milestones of brain development.72 As a result of the use of higher frequency transducers, enabling high resolution imaging, normal, maturational phenomena can be visualised80 and detection of more subtle changes in the peri- and intraventricular brain areas, basal ganglia and thalami is now possible.81-83 Several studies demonstrated that the fetal brain can be adequately examined in the same planes as in the neonate in most fetuses,77,78

Experts in this field of fetal neuro-imaging have elegantly reviewed the merits and demerits of ultrasound and MRI examination of the fetal brain.84 One merit of MRI examination is that it may identify fetal ischemic lesions early after an hypoxic event. One merit of ultrasound is that it can be repeated to examine the development over time. Both techniques are dependant on expertise and should be performed at the highest standards.
been described in the literature, especially during the preterm period and need to be distinguished from IVH.100-108

The basal ganglia and thalami can display a diffuse bilateral haze in very preterm infants (<32 weeks GA) which was found to gradually fade in time and before term equivalent age.109 In fetuses such an increased echogenicity in the thalami has been previously disregarded and ascribed to artefacts.109 Although around term age echogenicity in this same area is found after serious asphyxia and mostly represents ischaemia, the mild diffuse basal ganglia/thalami echogenicity (diffuse BGTE) seen during the preterm period, probably represents a maturational phenomenon.

Methods of fetal surveillance
Historically, the few tools available to the physician or midwife to assure fetal wellbeing were spontaneous felt fetal movements by the pregnant woman, an adequate increase of fundal height and periodic fetal heart rate auscultation.110 In cases of inadequate fetal growth, ultrasonography has gained its place in evaluating growth, the amount of amniotic fluid and excluding fetal congenital anomalies. Additional ultrasound tools to evaluate the fetal condition have been established, including Doppler assessment of flow velocity in fetal vessels and observation of fetal movements. As outlined above, groups of fetuses at risk for brain injury can be assigned. To identify these fetuses, several parameters are available to the clinician to evaluate the clinical condition, including fetal growth, heart rate parameters and motility. In addition, pathological examination of the placenta may help the clinician to evaluate the risk of fetal brain injury in retrospect.

Fetal growth evaluation in combination with Doppler measurements in the umbilical and middle cerebral arteries allows selection of a subgroup with a diminished reserve capacity, possibly at increased risk for adverse events during pregnancy and labour. At the time when absent or reversed flow are found in the umbilical arteries, presence of accelerations and decelerations, the fetal heart rate (FHR) traces during but also before labour has been refined and apart from microblood sampling during labour, cardiotocography is used as a tool to detect heart rate alterations (diminished variability and decelerations) may occur.110,111 These FHR changes indicate an adaptation to chronic compromise, a reaction to superimposed acute hypoxaemia, or possibly brain injury in the fetus.109,112,113,114

Fetal movements are generated spontaneously by the lower nervous systems (spine) and modulated by the cortico-spinal, reticulo-spinal structures in the CNS. General movements (GMs), defined as a movement in which all body parts participate without a distinctive sequence or pattern, develop from 9 weeks GA onwards and remain present till about 20 weeks after term equivalent age.115 GMs can therefore be assessed in a similar manner in the fetus and neonate. Qualitative assessment of GMs may be a good marker of brain (dys)function.116,117 Movement parameters like amplitude, speed and complexity can be assessed separately,111 and by using 'Gestalt-perception' the overall impression of the GMs can be classified as normal or abnormal, in analogy to neonatal scoring systems developed by Prechtl et al.118,119 Medication used in pregnancy, including corticosteroids, sedatives and anti-hypertensive drugs may also influence the variability of movements.120 In fetuses with growth restriction, before the occurrence of abnormal heart rate patterns, the quality of spontaneous motility may change.103,121,122 A reduced variability, speed and intensity leading to a slow and monotonous pattern can be observed (poor repertoire).111 After further deterioration of the fetal condition, coinciding with a reduced amount of amniotic fluid and a fetal heart rate pattern with a diminished variability and repetitive late decelerations, the fetal movements can become hardly discernable.116 The reduction in the quantity of general movements is a late sign of fetal deterioration. Whether these changes in the quality of spontaneous movements are a consequence of merely spatial restriction, transient brain dysfunction caused by hypoxaemia, chronic hypoglycaemia and/or deprivation of essential amino-acids, or of brain injury is unknown. Several studies have addressed the relationship between WM injury and early motor movement after birth.122,123 In neonates brain lesions124-127 and even transient echogenicity changes124,125 have been related to abnormal motility (ranging from poor repertoire to cramped-synchronized patterns) during the preterm and postterm period.125 The exact relationship between abnormal motility and type, extent and localisation of brain lesions, still remains unclear.

The above mentioned parameters can assist the physician to decide on the optimal moment to deliver the fetus before the intrapartum environment turns hostile and brain injury and eventually fetal demise may ensue. Unfortunately, these tools do not always allow us enough foresight in situations where, due to (very) short GA prolongation of gestation is desirable.
Since long time, placental examination has been used for better understanding of the uterine environment, pointing out infections and other signs of sentinel lesions that may have influenced the outcome of the neonate. Several studies have correlated placental pathology with brain injury or CP. Thrombo-inflammatory lesions affecting large fetal placental vessels and umbilical cord problems have been identified in stillborn infants with brain injury. The significance of underlying placental reserve capacity probably plays a role in the vulnerability of the fetus for additional thrombo-vascular or infectious events. Placental lesions associated with maternal vascular under-perfusion (increased syncytial knots and acute atherosis), and inflammation (chorioamnionitis with funisitis) have been associated with CP.

**AIMS OF THE STUDY**

The knowledge that perinatal fetal brain injury often has its origin before birth, prompted us to evaluate whether (imminent) brain injury can be examined before birth in high risk pregnancies.

Depending on the GA of the fetus certain echogenicity changes in the brain may reflect maturational phenomena that may be delayed in certain circumstances. Furthermore, the immature fetal brain may possess considerable plasticity. Brain lesions seen in the fetus, known to be associated with adverse outcome when seen in the neonate, may therefore have different neurological consequences or even remain without sequelae.

We hypothesized that the addition of fetal brain sonography to other parameters of fetal surveillance allows for an additional insight in the integrity of the fetal CNS and may help to identify the fetus at increased risk of (further) brain injury during the perinatal period and at risk for abnormal outcome.

The aim of this study was to evaluate the prevalence and extent of echogenicity changes in three vulnerable areas of the fetal brain (i.e. the periventricular WM, the ventricular system and basal ganglia/thalami) for echogenicity changes or even remain without sequelae.

The study design was prospective and observational. Serial sonographic examinations were performed before and after birth to examine three vulnerable areas of the brain (WM, ventricular system, and basal ganglia and thalamus) for echogenicity changes (Table 1). These echogenicity changes were related to various perinatal parameters of surveillance, to spontaneous motor activity both in the fetus and the neonate and to (death and neurological) outcome. Possible etiological mechanisms of fetal brain injury were explored by histological examination of the placenta after birth and relating placental processes to echogenicity changes in the fetal brain.

**Table 1. Grading system for echogenicity changes in the fetal brain**

<table>
<thead>
<tr>
<th>Grading</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Periventricular echodensities (PVE)</strong></td>
<td></td>
</tr>
<tr>
<td>PVE IA</td>
<td>Moderately increased echogenicity of the periventricular white matter, the echogenicity being (almost) equal to that of the choroid plexus</td>
</tr>
<tr>
<td>PVE IB</td>
<td>Obviously increased echogenicity of the periventricular white matter, being brighter than the choroid plexus</td>
</tr>
<tr>
<td>PVE II</td>
<td>PVE evolving into localized fronto-parietal cysts</td>
</tr>
<tr>
<td>PVE III</td>
<td>PVE evolving into extensive periventricular cystic lesions</td>
</tr>
<tr>
<td><strong>Intraventricular echodensities (IVE)</strong></td>
<td></td>
</tr>
<tr>
<td>IVE I</td>
<td>Echodensity in the subependymal germinal matrix</td>
</tr>
<tr>
<td>IVE II</td>
<td>Echodensity filling less than half of the lateral ventricle</td>
</tr>
<tr>
<td>IVE III</td>
<td>Echodensity filling equal or more than half of the lateral ventricle</td>
</tr>
<tr>
<td>Post hemorrhagic ventricular dilatation (PHVD) and periventricular haemorrhagic infarction (PVHI) are mentioned separately</td>
<td></td>
</tr>
<tr>
<td><strong>Basal ganglia and thalami echodensities (BGTE)</strong></td>
<td></td>
</tr>
<tr>
<td>Diffuse</td>
<td>Diffusely increased echogenicity of the basal ganglia/thalami (often bilateral) as compared to the surrounding white matter</td>
</tr>
<tr>
<td>Localized</td>
<td>Circumscribed increased echogenicity within the basal ganglia and/or thalami (uni- or bilateral)</td>
</tr>
<tr>
<td><strong>Further classification into mild or moderate echogenicity changes</strong></td>
<td></td>
</tr>
<tr>
<td>Mild echogenicity changes</td>
<td>PVE IA, IVE I and/or diffuse BGTE</td>
</tr>
<tr>
<td>Moderate echogenicity changes</td>
<td>PVE IB, PVE II, IVE II and III, and/or localized BGTE</td>
</tr>
</tbody>
</table>

This well defined population consisted of pregnancies with hypertensive disorders, imminent preterm birth and/or premature ruptured membranes, included between 26 and 34 weeks GA. This study was performed in the Western Cape province of South-Africa with a high rate of spontaneous preterm birth. With a delivery rate of 4000 per year and an incidence of preterm birth of 20%, we expected to include at least 100 fetuses/year.

**Specific research questions were:**

1. What is the prevalence of echogenicity changes in three vulnerable areas of the fetal brain (i.e. the periventricular WM, the ventricular system and basal ganglia/thalami) in pregnancies threatened by preterm birth between 26-32 weeks GA?
2. What is the evolution and continuity of these echogenicity changes over time, both during the fetal period and the first weeks after birth?
3. Can (mild) antenatal brain injury be predicted by evaluating fetal heart rate parameters in high risk pregnancies?
4. Do abnormal fetal general movements predict moderate echogenicity changes in the fetal brain?
5. Is there a relation between moderate echogenicity changes in the fetal brain and continuity of general movements before and after birth?
6. Is there a relation between placental histology and moderate echogenicity changes in the fetal brain?
7. Do moderate echogenicity changes in the fetal brain increase the risk of neonatal brain injury and do they predict abnormal (neurological) outcome?

OUTLINE OF THE CHAPTERS

In Chapter 2 the study population and brain sonography methods are presented. The studied population consisted of women with a GA between 26-32 weeks with either preterm labour (PTL) or imminent preterm delivery because of hypertensive disorders of pregnancy (HD). The prevalence of echogenicity changes in the periventricular WM (periventricular echodensities, PVE), ventricular system (intraventricular echodensities, IVE) and basal ganglia/thalami (basal ganglia and thalamic echodensities, BGTE) and the continuity of these echogenicity changes from before birth to the first day after birth are described. The distribution of echogenicity changes in the brain areas over pregnancies with PTL or HD is described.

In Chapter 3 a case-control study is presented relating cardiotocography to the presence of echogenicity changes in brain of fetuses at risk for preterm birth. Computerized analysis of fetal heart patterns is performed and fetal heart rate parameters of the cases and controls are compared with those of a low risk population.

In Chapter 4 the quality of general movements of the fetus is related to brain ultrasound findings. The influence of clinical risk factors on the movement quality is described, and the predictive values of general movements for echogenicity changes in different areas of the fetal brain is reported.

In Chapter 5 the continuity of fetal general movement quality after birth is described. The influence of presence of echogenicity changes in the brain presumed to represent injury on the persistence or the normalization of abnormal movement quality is explored.

In Chapter 6 the associations between different pathological processes in the placenta (uteroplacental hypoperfusion, inflammation and fetal thrombovasculopathy), and fetal brain echogenicity changes are discussed.

In Chapter 7 the follow-up of the study population is presented up to 6 years of age and correlations between brain ultrasound examinations, pregnancy and neonatal characteristics with outcome are discussed.

Chapter 8 provides a summary of the results and conclusions of the study and a general discussion. Propositions for further research are provided.

REFERENCES

18. Murphy DJ, Hope PL, Johnson A. Neonatal risk factors for cerebral palsy in very preterm babies:


ABSTRACT

Objectives. To study the incidence of echodensities in the periventricular white matter, ventricular system, basal ganglia and thalamus of the brain in fetuses at risk for preterm delivery.

Methods. This was a prospective study of 124 fetuses with a gestational age between 26 and 34 weeks in pregnancies affected by either pregnancy-induced hypertensive disorders or preterm labour. Transvaginal ultrasound examination of the fetal brain in coronal and sagittal planes was performed weekly until delivery and the neonatal brain was examined within 24 h after delivery.

Results. In 65% of all fetuses, echodensities were found in one or more areas of the brain. They were present in the periventricular area in 52% of cases, the intraventricular area in 18% and in the basal ganglia and thalamus area in 28%. The changes in echogenicity were seen throughout the entire gestational-age period studied. Of the periventricular echodensities that exceeded in echodensity that of the choroid plexus, 50% persisted after delivery; 38% of the intraventricular echodensities persisted after delivery; 32% of the basal ganglia and thalamus echodensities persisted after delivery.

Conclusions. In high-risk fetuses, echodensities are a frequent finding in several areas in the brain. How far these echodensities are related to future outcome of the infant needs to be investigated.

INTRODUCTION

Perinatal onset of brain injury is not limited to hypoxic-ischemic injury or infection acquired during delivery; it can also originate before delivery. Maternal factors contributing to perinatal brain injury include pre-eclampsia, oligohydramnios and prolonged rupture of membranes.

Patterns of brain damage following prenatal hypoxic–ischemic injury are gestational-age related. In the period between 24 and 36 weeks’ gestation, the most frequently occurring patterns of brain injury are periventricular white matter damage, germinal matrix hemorrhage and their complications.

These areas of the fetal brain can be examined by transvaginal ultrasound examination during pregnancy in the same systematic way as can be done in the neonate viewing coronal and sagittal planes in both fetal hemispheres, using the anterior fontanel as an acoustic window. Even in high-risk pregnancies with diminished amniotic fluid, the transvaginal approach provides adequate visibility for assessment of the fetal brain.

Case reports on prenatally acquired brain injury caused by haemorrhage or ischemia are numerous. However, the true incidence of brain injury acquired before delivery has been studied sparingly in high-risk populations. In low-risk fetuses, transient periventricular echodensities (PVE) in the frontal areas of the brain have been found to be present between 26 and 28 weeks and were considered to represent physiological phenomena of maturation. Persistent PVE developing into periventricular leukomalacia in the neonatal period and their relation to neurological outcome have been described in two small populations of high-risk fetuses. In these cases, the PVE were assumed to be of pathological origin. Case series of fetuses with intracranial haemorrhages describe persistence of ultrasound findings after delivery, but also variability between findings before and after delivery, especially for low-grade hemorrhages.

White matter has been studied extensively in preterm neonates; echodensities may reflect physiological processes, such as migrating cells, or pathological processes, such as edema, haemorrhage and gliosis. Gliosis plays an important role in the development of periventricular leukomalacia, i.e. the activation of astrocytes and microglia. Histologically, this can be demonstrated as soon as 2-3 days after the injury, while cystic cavities resulting from the macrophagic activity of microglia need at least 8-10 days to be visible on ultrasound. Little is known about the occurrence of echodensities in the fetal brain and whether they are a reflection of the same processes seen in the brain of the preterm neonate. Recent studies, however, have shown that an inflammatory response in both fetuses and neonates, resulting either from hypoxic–ischemic injury or from infection, may contribute to brain injury.
The aims of this study were to obtain further insight into:

1. The incidence of echodensities and translucencies in the brains of fetuses at risk for preterm delivery and their relationship to gestational age.
2. The grade, location and extent of these echodensities.
3. The course over time of echodensities and translucencies before and after delivery.

METHODS

This study was conducted between March 1999 and October 2000 at Tygerberg Hospital, University of Stellenbosch, South Africa, and is a part of an ongoing study on fetal brain sonography and neurological follow-up. All women with a singleton pregnancy and a fetus in cephalic presentation at risk for preterm delivery between 26 and 34 weeks’ gestation were asked to participate. These were women with either pregnancy-induced hypertensive disorders or preterm labour and/or premature rupture of membranes. The Committee for Human Research of the University of Stellenbosch approved the study. We excluded patients with active vaginal bleeding and/or major placenta praevia. Documentation of gestational age was based on last menstrual period, early ultrasound examination and ultrasound at inclusion, sequential symphysis fundus measurements and transcerebellar measurement at inclusion.

The fetuses were assessed by ultrasound at admission and weekly thereafter. Transvaginal ultrasound examination of the fetal brain was performed in the six coronal and five sagittal planes as described by Van Gelder et al. The images were digitized and stored with an Applicare \textsuperscript{TM} system, Medical Imaging B.V., General Electric. Echodensities and translucencies were considered present if they were identified in both coronal and sagittal planes. If they were seen in one plane only (coronal or sagittal), they were considered to be artifacts. If they were seen in one plane and the other plane could not be assessed, this was scored as inconclusive.

Grading of PVE was performed as we have described previously. \textsuperscript{5} PVE Grade IA: moderately increased echogenicity of the white matter, being as bright or almost as bright as the choroid plexus; PVE Grade IB: seriously increased echogenicity of the white matter, being brighter than the choroid plexus; PVE Grade II: periventricular echodensities evolving into localized frontoparietal cysts; PVE Grade III: periventricular echodensities evolving into multiple periventricular cystic lesions. The location of PVE was described as frontal, parietal, occipital, temporal or a combination of these.

Scoring of intraventricular echodensities (IVE) was adapted from Volpe’s grading system for peri- and intraventricular hemorrhages. \textsuperscript{6} IVE Grade I: echodensity in the subependymal germinal matrix; IVE Grade II: echodensity filling < 50% of the lateral ventricle; IVE Grade III: echodensity filling ≥ 50% of the lateral ventricle. Dilation of the lateral and/or third ventricle was noted separately.

Basal ganglia and thalami echodensities (BGTE) were considered present if an area of increased echogenicity within the thalamus and/or basal ganglia was seen, compared with the surrounding brain tissue. BGTE were considered diffuse if there was global increased echogenicity of this area. BGTE were considered localized if there was a localized area of increased echogenicity within the thalamus and/or basal ganglia; this was recorded as uni- or bilateral. \textsuperscript{7}

After delivery the same ultrasound procedure was performed within 24 h. The interval in days between the last prenatal and the first postnatal ultrasound examinations was recorded. All ultrasound examinations (both fetal and neonatal) were performed by the same person (F.R.v.D.) using an HDI 1000 (ATL) ultrasound machine equipped with a C8-4-MHz IVT (prenatal ultrasound examination) and a C8-5-MHz transducer (neonatal examination).

Offline analysis of all videotaped ultrasound examinations was performed by two observers (G.v.W.-M. and J.I.P.d.V.) who were blinded to the clinical data. These same observers had been involved in previous descriptive studies\textsuperscript{3,4} using the same methodology and planes and showed good interobserver agreement. For the purposes of this study, assessment was made by agreement.

RESULTS

One hundred and twenty eight women met the inclusion criteria and gave their informed consent to participate in the study. Four women were excluded because the fetal brain could not be visualized properly, due to deep engagement of the fetal head in the pelvis or extreme flexion of the fetal head. The mean gestational age at admission of the 124 participating women was 30 (range, 26–34) weeks. Reasons for admission were hypertensive disorders in 64 cases and preterm labour and/or preterm rupture of membranes in 60.

Ninety-nine fetuses underwent one ultrasound examination, 18 fetuses underwent two, 6 fetuses underwent three and 2 fetuses underwent four. In 4/124 fetuses, the first ultrasound examination was inconclusive. In 8/124 fetuses one or two of the three areas could not be visualized properly (white matter, n = 8; ventricular system, n = 7; basal ganglia and thalamus, n = 6). Echodensities in more than one area were seen in 35/124 fetuses: 15 fetuses had BGTE and PVE, 8 fetuses had IVE and PVE Grade IA, 5 fetuses had IVE and BGTE and 7 fetuses had echodensities in all three areas.

Of the 124 women included in the study, 16 were discharged before delivery and their neonates were therefore not examined after delivery. Another neonate died within 24 h, before the ultrasound examination could be performed. In the remaining 107 cases, the median interval between the last prenatal and the first postnatal ultrasound examinations was 3 (range, 0-46) days.

28

29
Table 1. Gestational age distribution of periventricular echodensities (PVE) found at admission in a population at risk for preterm delivery

<table>
<thead>
<tr>
<th>PVE</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>29</th>
<th>30</th>
<th>31</th>
<th>32</th>
<th>33</th>
<th>Total fetuses (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4</td>
<td>2</td>
<td>9</td>
<td>4</td>
<td>9</td>
<td>13</td>
<td>7</td>
<td>3</td>
<td>51</td>
</tr>
<tr>
<td>Grade IA</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>54</td>
</tr>
<tr>
<td>Grade II</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>12</td>
<td>22</td>
<td>13</td>
<td>20</td>
<td>22</td>
<td>16</td>
<td>6</td>
<td>124</td>
</tr>
</tbody>
</table>

Table 2. Gestational age distribution of intraventricular echodensities (IVE) found at admission in a population at risk for preterm delivery

<table>
<thead>
<tr>
<th>IVE</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>29</th>
<th>30</th>
<th>31</th>
<th>32</th>
<th>33</th>
<th>Total fetuses (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>9</td>
<td>8</td>
<td>16</td>
<td>8</td>
<td>18</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td>Grade I</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Grade II</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Grade III</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>14</td>
<td>22</td>
<td>13</td>
<td>20</td>
<td>22</td>
<td>16</td>
<td>6</td>
<td>124</td>
</tr>
</tbody>
</table>

Table 3. Gestational age distribution of basal ganglia/thalami (BGTE) found at admission in a population at risk for preterm delivery

<table>
<thead>
<tr>
<th>BGTE</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>29</th>
<th>30</th>
<th>31</th>
<th>32</th>
<th>33</th>
<th>Total fetuses (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8</td>
<td>5</td>
<td>15</td>
<td>9</td>
<td>15</td>
<td>15</td>
<td>11</td>
<td>5</td>
<td>83</td>
</tr>
<tr>
<td>Diffuse</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Localized: unilateral</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Localized: bilateral</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>14</td>
<td>22</td>
<td>13</td>
<td>20</td>
<td>22</td>
<td>16</td>
<td>6</td>
<td>124</td>
</tr>
</tbody>
</table>

The gestational age-related distribution of echodensities in the periventricular white matter, ventricular system and basal ganglia and thalami at admission are presented in Tables 1, 2 and 3, respectively. The grading and localization per area are described further below. In Table 4, the incidences of echodensities found at admission, at the last examination before delivery and within 24 h after delivery are depicted for each area, irrespective of age. Evolution over time of these echodensities is described below. There was no significant difference in the number of fetuses with echodensities between women with hypertensive disorders (45/64) and those with preterm labour and/or premature rupture of membranes (43/60); nor did the individual incidences of PVE, IVE or BGTE differ statistically between the two patient groups.

Table 4. Changes in echogenicity at admission, last prenatal and first postnatal ultrasound examinations (US) in a population at risk for preterm delivery

<table>
<thead>
<tr>
<th>Echogenicity</th>
<th>Admission US</th>
<th>Last prenatal US</th>
<th>Postnatal US</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>38 (30.6)</td>
<td>40 (32.2)</td>
<td>4 (3.7)</td>
</tr>
<tr>
<td>PVE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade IA</td>
<td>54 (43.5)</td>
<td>51 (41.1)</td>
<td>51 (47.6)</td>
</tr>
<tr>
<td>Grade IB</td>
<td>9 (7.2)</td>
<td>8 (6.4)</td>
<td>43 (40.0)</td>
</tr>
<tr>
<td>Grade II</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>7 (6.5)</td>
</tr>
<tr>
<td>IVE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>8 (6.4)</td>
<td>8 (6.4)</td>
<td>24 (22.4)</td>
</tr>
<tr>
<td>Grade II</td>
<td>10 (8.0)</td>
<td>9 (7.2)</td>
<td>5 (4.6)</td>
</tr>
<tr>
<td>Grade III</td>
<td>5 (4.0)</td>
<td>4 (3.2)</td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>VD</td>
<td>20 (16.1)</td>
<td>21 (16.9)</td>
<td>9 (8.4)</td>
</tr>
<tr>
<td>BGTE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse</td>
<td>17 (13.7)</td>
<td>16 (12.9)</td>
<td>30 (28.0)</td>
</tr>
<tr>
<td>Localized</td>
<td>18 (14.5)</td>
<td>17 (13.7)</td>
<td>7* (6.5)</td>
</tr>
<tr>
<td>Total</td>
<td>124</td>
<td>124</td>
<td>107</td>
</tr>
</tbody>
</table>

*Six with localized BGTE and one with thalamic cysts.
PVE GRADE IA
PVE Grade IA were seen in 54 cases at admission. In 35 of these, the echodensities were located in the frontal white matter only. Five fetuses had frontal and occipital echodensities, two had frontal and parietal echodensities and two had extended PVE from frontal to occipital areas. In eight fetuses the PVE were only occipital, in one they were parietal and occipital, and in one they were only parietal. The median gestational age of fetuses with frontal PVE was 29 weeks, range 26–33 weeks. The median gestational age of fetuses with PVE elsewhere or in more than one area was 28 (range, 26–32) weeks.

Evolution of prenatal ultrasound
Three of the nine fetuses with PVE Grade IB had several ultrasound examinations before delivery. Of these, two fetuses showed PVE Grade IA on a subsequent ultrasound examination and in one of these two it was still visible on the third and fourth ultrasound examinations; in the remaining fetus, the second ultrasound examination showed no PVE.

Continuity between prenatal and postnatal ultrasound results
Eight fetuses had PVE Grade IB on the last prenatal ultrasound examination, including two fetuses without prior PVE Grade IB but with PVE Grade IA and BGTE on ultrasound at admission. In five this was still visible after delivery, and in three PVE Grade IA were seen after delivery. The median interval between the last prenatal and the first postnatal ultrasound examinations was 2.5 (range, 0–18) days.

PVE GRADE II
PVE Grade II were seen in two fetuses, one with small fronto-occipital and one with small occipital translucencies at 28 and 30 weeks, respectively. The latter also showed extensive PVE Grade IA. Both fetuses had one ultrasound examination only.

Evolution of prenatal ultrasound
In the two cases with PVE Grade II before delivery, PVE Grade IA were seen after delivery. The intervals between the last prenatal and the first postnatal ultrasound examinations were 4 days in one and 3 days in the other.

IVE
IVE were encountered in 23 fetuses at admission (median gestational age, 28 (range, 26–32) weeks). Grading of IVE is presented in Table 3. Ventricular dilatation was observed in 20/124 fetuses at admission, 12 of which had IVE.
Evolution of prenatal ultrasound

Seven of the 23 fetuses with IVE at admission had several ultrasound examinations before delivery. In three of the seven the finding remained consistent before delivery, in one the coexistence of ventricular dilatation persisted, while BGTE were seen on the subsequent ultrasound and in three the IVE were not visible on subsequent ultrasound examinations.

Continuity between prenatal and postnatal ultrasound results

In 21 fetuses IVE were present at the last prenatal ultrasound examination, including two with no IVE detected on admission. In eight of these 21 fetuses IVE were also seen after delivery, of which two had the same grade, four a lower grade and two a higher grade. In 11 of the 21 cases, IVE were not visible after delivery, and the remaining two fetuses did not have an ultrasound examination after delivery. The median interval between the last prenatal and first postnatal ultrasound examinations was 3 (range, 0-46) days.

BGTE

BGTE were encountered in 35 fetuses at admission (median gestational age, 28 (range, 26-32) weeks). Grading of BGTE is presented in Table 3. Diffuse BGTE were seen at a median age of 28 (range, 26-31) weeks and localized BGTE at a median age of 30 (range, 27-32) weeks. Localized BGTE were accompanied by IVE Grades II and III in 8/18 fetuses, and by ventricular dilatation in 8/18 fetuses. Diffuse BGTE were accompanied by IVE in 4/17 fetuses and by ventricular dilatation in 2/17.

DISCUSSION

To our knowledge, this is the first large prospective study to examine changes in echogenicity in the fetal brain. Systematic cerebral ultrasound examinations revealed changes in echogenicity in 65% of this population of 124 fetuses at high risk for preterm delivery. Half of the fetuses showed echodensities in the periventricular white matter. These were mainly PVE Grade 1A located in the frontal region and can be considered to be a normal finding in fetuses and preterm neonates before term age, reflecting glial cell migration. In a third of the cases with PVE Grade 1A, the echodensities were more widespread and/or in the parietal or occipital areas of the brain. In neonates, PVE in the parietal and/or occipital regions of the brain have a less favourable prognosis compared with frontal echodensities, and not only the duration but also the extent of PVE is found to be of prognostic significance. We assume that homogeneous white matter echodensities that do not exceed in echogenicity that of the choroid plexus may represent normal phenomena in the developing fetal brain. However, when PVE Grade 1A do not disappear after delivery it may be a sign of (mild) white matter injury or disturbed brain maturation.

Figure 3. Transvaginal ultrasound images in the fourth coronal plane (a) and fourth parasagittal plane (b) of a 30-week fetus, showing localized basal ganglia/thalamus echodensities (arrows), which are best visualized in the parasagittal plane, and an abundant choroid plexus, which is easily mistaken for intraventricular echodensities.

Evolution of prenatal ultrasound

Eleven of the 35 fetuses with BGTE had several ultrasound examinations before delivery. In 7/11 fetuses the echodensities were found to be transient in the following 1–3 weeks, in 3/11 fetuses the localized BGTE persisted and in one the diffuse BGTE persisted. Six additional fetuses, having no BGTE at admission, subsequently developed BGTE. Four of them showed diffuse BGTE, and two developed bilateral localized densities in the thalami after an initial ultrasound examination showing IVE.

Continuity between prenatal and postnatal ultrasound results

Thirty-four fetuses showed BGTE on the last prenatal ultrasound examination. Of the 16 with diffuse BGTE, 2 had diffuse BGTE after delivery, 12 had no BGTE after delivery and 2 had no postnatal ultrasound examination. The median interval between the last prenatal and the first postnatal ultrasound examinations was 3 (range, 1-11) days. Of the 18 cases with localized BGTE, nine had BGTE after delivery of which one was localized, six showed no BGTE and three did not have an ultrasound examination after delivery. The median interval between the last prenatal and the first postnatal ultrasound examinations was 2.5 (range, 0-17) days.

NO ECHODENSITIES

In 38 fetuses, no echodensities were encountered at admission in any of the three regions of interest (median gestational age, 30 (range, 26-33) weeks. In 41 fetuses, no echodensities were seen on the last prenatal ultrasound examination. At the first postnatal ultrasound examination, 2 of these 41 showed no echodensities, 20 had PVE Grade IA (one of them with concomitant IVE), 9 had PVE Grade IB (two with concomitant BGTE and IVE or ventricular dilatation), 2 had PVE Grade II (one with concomitant BGTE, ventricular dilatation and IVE) and one had an IVE. Seven neonates did not have an ultrasound examination. The median interval between the last prenatal and the first postnatal ultrasound examinations was 3.5 (range, 0-45) days.
In eight fetuses we found PVE Grade 1B, which we assume are not physiological but rather represent white matter injury that is already developing before delivery. This assumption is supported by the fact that in the high-risk fetuses studied by Yamamoto et al.,23 persisting echodensities with an echogenicity equal to or exceeding that of the choroid plexus preceded neonatal development of cystic periventricular leukomalacia. In addition, in our population, in the majority of fetuses with echodensities exceeding in echogenicity that of the choroid plexus, this persisted after delivery. We encountered two fetuses with small areas of localized fronto-occipital and occipital translucencies (PVE Grade II), and in both this finding could not be confirmed after delivery, nor could ventricular dilatation or an irregular ventricular contour, which are often seen in cases with cystic PVL;24 be demonstrated. Differentiation between small localized cysts and inhomogeneities in the periventricular white matter may be difficult before delivery.

It is possible that in these cases the echogenicity changed over time, because in several cases serial fetal ultrasound examinations clearly showed fluctuations in echodensities over time. However, since the time intervals between the last prenatal and first postnatal ultrasound examinations were too short to explain resolution of cysts, it is also possible that the small local translucencies seen during the fetal period may have been artefacts that were mistaken for cystic lesions.

We found IVE in about one fifth of the fetuses. These were mainly IVE Grades I and II. In half of the cases with IVE no peri- or intraventricular haemorrhage was encountered after delivery. In three series of fetal intracranial hemorrhages6,11,12 only a limited number of Grade I and II haemorrhages were described. Comparable to our results, occasional progression but also complete disappearance of IVE was seen during follow-up ultrasound examinations. The latter can be explained by resorption of small haemorrhages. It is also possible that certain phenomena occurring in the fetus mimic a haemorrhage (e.g. remnants of the germinal matrix in the region of the thalamocaudate notch; an abundant,21 oddly shaped or bifid22 choroid plexus). Thus, IVE in the fetus may represent both normal (germinat matrix, choroid plexus) and pathological (haemorrhage) phenomena.

We encountered echodensities in yet another region of the brain: the thalami and basal ganglia. This occurred in one fourth of the fetuses. BGTE were global and diffuse in half of the cases. Diffuse BGTE were seen mainly in the very young fetuses. This confirms results of the scant literature on this subject in preterm neonates: Leijser et al.18 encountered diffuse BGTE in 26% of very preterm infants. They postulate that diffuse BGTE may be a maturational phenomenon, which is supported by the fact that it is only seen in preterm infants, or that it may represent pathological change in the thalami and/or basal ganglia. Localized BGTE were seen in 14.5% of the fetuses, being unilateral in half of them (6.5%). In high-risk preterm neonates, unilateral BGTE were found in 5.3% of cases,53 and were ascribed to haemorrhage or infarction in that region. We found not half of them (6.5%). In high-risk preterm neonates, unilateral BGTE were found in 5.3% or basal ganglia. This occurred in one fourth of the fetuses. BGTE were global and diffuse in half of the cases. Diffuse BGTE were seen mainly in the very young fetuses. This confirms results of the scant literature on this subject in preterm neonates: Leijser et al.18 encountered diffuse BGTE in 26% of very preterm infants. They postulate that diffuse BGTE may be a maturational phenomenon, which is supported by the fact that it is only seen in preterm infants, or that it may represent pathological change in the thalami and/or basal ganglia. Localized BGTE were seen in 14.5% of the fetuses, being unilateral in half of them (6.5%). In high-risk preterm neonates, unilateral BGTE were found in 5.3% of cases,53 and were ascribed to haemorrhage or infarction in that region. We found not only unilateral, but in a substantial number of fetuses bilateral, localized BGTE. This phenomenon has so far only been described in (near) term neonates, who sustained severe perinatal asphyxia, and it is associated with adverse neurological outcome.44

Of the cases with bilateral localized BGTE who were re-examined after delivery, some showed diffuse bilateral BGTE and in others no changes in echogenicity were found in this region after delivery, BGTE thus being transient in these cases. We therefore assume that most cases of bilateral, localized BGTE in the fetus do not represent the same pathological substrate as BGTE seen in the term neonate.

The fact that most of the fetuses without echodensities before delivery showed echodensities within 24 h after delivery indicates that there may an influence of preterm delivery itself. Whether these echodensities found after delivery present pathology and are related to adverse clinical outcome is unknown.

The interpretation of echodensities in the fetal brain must be done with caution. Not all echodensities seen before delivery represent the same pathological substrates found to correlate with echodensities seen after delivery. Sonography facilitates longitudinal examination of the fetal brain and evaluation of changes in echogenicity over time. A thorough knowledge of the course of echodensities is necessary before the significance of echodensities and translucencies in the fetal brain will become clear and before differentiation between maturational processes and brain injury will be possible. Follow-up studies are needed to assess the clinical significance of echodensities seen in the fetal brain and their correlation with neurological outcome.

REFERENCES

Fetal brain sonography and fetal heart rate patterns in the preterm fetus

Fleur M. Rosier-van Dunne,1 Herman P. van Geijn,1 Hein J. Odendaal,3 Gerda van Wezel-Meijler,3 Johanna I. de Vries1

1 VU University Medical Centre, Amsterdam, the Netherlands,
2 Tygerberg Hospital, Stellenbosch University, South Africa,
3 Leiden University Medical Centre, Leiden, the Netherlands.

ABSTRACT

Objective. To study whether peri- and intraventricular echodensities in the brain of fetuses at risk for preterm birth are associated with changes in fetal heart rate (FHR) parameters.

Study design. Twenty preterm fetuses with peri- and intraventricular echodensities detected by transvaginal ultrasonography were matched with 20 fetuses without echodensities for gestational age, growth parameters, clinical disease, and maternal medication. Baseline FHR, long and short term variability and the presence of accelerations and decelerations were analyzed with a computerized system and compared with Wilcoxon's matched-pairs signed-rank test. Both cases and controls were compared to a normal population.

Results. No statistical differences in FHR parameters were found between cases with and controls without peri- and intraventricular echodensities. Both cases and controls had lower long and short term variabilities than the normal population.

Conclusion. No association between the presence of peri- and intraventricular echodensities and specific FHR changes was demonstrated.

INTRODUCTION

Over the last decades there has been increasing evidence that damage to the fetal brain can occur before the initiation of labour. Antepartum events can cause brain damage through hypoxia, ischemia or inflammation.

Pregnancies complicated by hypertensive disorders, fetal growth restriction and preterm labour are closely monitored by cardiotocography to assess the condition of the fetus and detect early signs of hypoxia. Often these fetal heart rate (FHR) tracings are difficult to interpret because of the effects on the FHR rhythm of gestational age, maternal clinical condition and medication.

In the past many FHR tracings of infants with neurological deficits have been studied, but still little is known of FHR patterns that are associated with hypoxic-ischemic brain damage in the preterm fetus. A pattern with persistently absent variability and mild variable decelerations has been associated with a high incidence of cerebral palsy. However, decelerations and changes in heart rate variability (HRV) although pointing to fetal deterioration, are not specific for impending brain damage and have poor predictive value.

In neonates the results of hypoxic ischemia can be visualized by ultrasonography as periventricular echodensities within a week after the incident, and in case of more severe damage as cysts after one to two weeks. Haemorrhages or intraventricular echodensities, can be visible immediately after the incident.

Transvaginal ultrasonography of the fetal brain can identify changes in echogenicity in the peri- and intraventricular areas of the brain. The present study was undertaken to investigate whether specific FHR parameters can identify preterm fetuses with echodensities in areas at risk for hypoxic-ischemic injury.

MATERIALS AND METHODS

The study has been conducted at the tertiary care facility of Tygerberg Hospital, Western Cape, South Africa from March 1999 until September 2000. Included were patients at risk of preterm delivery between 26 and 34 weeks gestational age, who were admitted for preeclampsia, severe hypertension with or without fetal growth restriction, or preterm premature rupture of membranes and/or preterm labour. Exclusion criteria were breech presentation of the fetus, active vaginal bleeding and/or total placenta praevia.

All women gave informed consent. The Committee for Human Research of the University of Stellenbosch approved the study. Gestational age assessment was based on a combination of the following: last menstrual period, early ultrasound examination and fetal biometry or transcerebellar measurement at inclusion.

The fetal abdominal circumference measurement was used to estimate possible growth restriction using charts for the specific population.
Ultrasonography
At inclusion the fetal brain was examined by transvaginal ultrasonography and the presence of echodensities and translucencies were assessed. Intraventricular echodensities (IVE) were graded in analogy of the neonatal classification of peri- and intraventricular haemorrhages by Volpe, periventricular echodensities (PVE) according to respectively Van Wezel and De Vries as described in Van Gelder et al. and basal ganglia and thalamic echodensities (BGTE) were described as diffuse or localized, uni- or bilateral. If the lateral ventricles and/or third ventricle appeared wide, the ventricular index was measured and ventricular dilatation was considered if the ventricular index exceeded the 95th percentile. To confirm echodensities seen in the fetus, the brain of the neonates was examined by ultrasound within 24 hours after delivery.

Transvaginal ultrasonography was performed by one investigator (F.M.R.v.D.) using an HDI 1000 (ATL) with a C8-4 MHz IVT. Off-line analysis of all videotaped examinations was performed by two observers (G.v.W.-M. and J.I.d.V.) blinded to the clinical data. These same observers were involved in previous descriptive studies using the same methodology and planes, showing good interobserver agreement.

Fetal heart rate
The recording of the FHR was performed on the day of the ultrasound examination or the day thereafter. Recordings were made in semi-recumbent position and repeated several times per week until delivery. All recordings lasted for 60 minutes and were performed between 9 a.m. and 5 p.m. Computer analysis of FHR traces was performed using a Sonicaid 8000 system (Sonicaid Ltd, Chichester) having the algorithms developed by Dawes and Redman.

In the analysis procedure FHR parameters like baseline FHR, episodes of high and low heart rate variation (HRV), long term variation (LTV) and short term variation (STV) were identified. The amount of signal loss (%) was noted, and only recordings with a signal loss < 30% were accepted. Nijhuis et al. provided normograms with reference ranges for FHR parameters obtained from a population of 29 uneventful singleton pregnancies followed longitudinal from 24 weeks gestational age onwards. Since both the cases and the controls came from a population at high risk for preterm birth, the values of for BHR, LTV and STV of both cases and controls are plotted within the 2.5th, 50th and 97.5th percentiles lines of this ‘normal population’.

Statistics
Only cases with moderate or severe changes in echogenicity, i.e. PVE grade 1B or more, IVE > grade I, and localized BGTE were considered for the case-control study. Cases were compared with controls i.e. patients with a fetus without echogenicity changes - that were matched for gestational age at examination and maternal clinical disease (pregnancy induced hypertensive disorders or preterm labour). Effort was put into matching for medication use and growth percentile as well.

Statistical analysis of the FHR parameters was performed with non-parametric tests, as they did not show normal distribution using the Kolmogorov-Smirnov test. Comparison of FHR parameters in cases and controls was performed with Wilcoxon’s matched-pairs signed-rank test. The effects of maternal medication use on FHR parameters were examined with Wilcoxon’s rank sum test, comparing pooled data of all cases with pooled data of all controls. Comparison of FHR parameters of the cases and controls with the reference ranges for the normal population was performed by comparing frequencies of cases and controls within each quartile of the normal distribution using the χ²-squared test.

RESULTS
Patient characteristics
During the study period 124 patients were included and in all fetuses the brain was examined by transvaginal ultrasonography. Twenty-five fetuses were identified with echodensities suspect for moderate hypoxic-ischemic and/or hemorrhagic injury. Twenty of these fetuses had good quality FHR recordings and could be matched for gestational age and clinical maternal condition with twenty controls from the same population. Among the twenty matched pairs, eight pairs had pregnancy induced hypertensive disorders and/or fetal growth restriction, twelve had preterm labor and/or preterm premature rupture of membranes. The pairs could not be matched for maternal race. In the case group 13 patients were of mixed racial, 7 of black origin, and in the control group 16 patients were of mixed racial, 3 of Caucasian and 1 of black origin. Additional clinical characteristics of cases and controls are summarized in Table 1. None of the patients received dihydralazine, magnesium sulphate or sedatives prior to FHR recording.
Table 1. Distribution of patient characteristics of cases and controls

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Cases (n = 20)</th>
<th>Controls (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age: (days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>206.2 (12.6)</td>
<td>207.4 (11.8)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>206 (189-236)</td>
<td>206.5 (185-235)</td>
</tr>
<tr>
<td>AC &lt; 10th percentile</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Nifedipine use</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>α-methyldopa use</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Betamethasone medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4 days</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>no or &gt; 4 days</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Beta-mimetics</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

Gestational age: on the day of fetal heart rate recording.
AC: abdominal circumference measured at intake and compared with chart available for the specific population.
Betamethasone: number of days prior to fetal heart rate recording.

Four pairs were mismatched for beta-mimetics use, in two pairs the case-fetus and in two the control-fetus received beta-mimetics prior to heart rate recording. In three out of these four fetuses that received beta-mimetics, the baseline FHR was the same or lower than in its counterpart. In the fourth pair the case fetus demonstrated tachycardia. Because of maternal tachycardia and a temperature of 37.5°C, labour was induced for suspicion of an intrauterine infection. There was a high incidence of anhydramnios and oligohydramnios in both the cases and control groups (11 and 10 respectively), associated with either fetal growth restriction or preterm premature rupture of membranes. The amount of amniotic fluid present was matched in 17/20 matched pairs.

None of the neonates showed any features of congenital malformation.

Ultrasonography
In the whole group abnormalities seen with transvaginal brain ultrasonography ranged from PVE grade IA to PVL grade II, and PIVH I-III (Figure 1). Both diffuse and localized BGTE (Figure 2) were found. No cases of more severe brain abnormalities like extensive periventricular cystic lesions or intra-parenchymal echodensities were seen before birth. The lowest grades of PVE and IVE, and diffuse BGTE were not considered for the study. The various changes in echogenicity encountered in the cases are presented in Table 2.
Eighteen neonates of the case group were examined after birth and in 12/18 the various sonographic echodensities were confirmed in the same areas and in 6/12 at least in one of the areas.

**Fetal heart rate**

Accelerations were present in most FHR tracings but two fetuses in both groups showed no accelerations during the recording. In the group with echodensities three fetuses showed one or more decelerations on the FHR tracing, in comparison to four fetuses in the control group. No decelerative FHR tracings were seen in three pairs that were mismatched for amount of amniotic fluid. The FHR parameters are depicted in Table 3.

Table 3. Fetal heart rate parameters (Sonicaid system 8000) of cases and controls (medians with range)

<table>
<thead>
<tr>
<th>FHR Parameters</th>
<th>Cases</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline FHR*</td>
<td>138.5 (110-181)</td>
<td>146.5 (116-160)</td>
<td>0.920</td>
</tr>
<tr>
<td>Accelerations†</td>
<td>3 (0-30)</td>
<td>3 (0-14)</td>
<td>0.793</td>
</tr>
<tr>
<td>Decelerations‡</td>
<td>0 (0-6)</td>
<td>0 (0-12)</td>
<td>0.273</td>
</tr>
<tr>
<td>LTV</td>
<td>30.2 (20.9-66.5)</td>
<td>29.1 (18.7-45.6)</td>
<td>0.204</td>
</tr>
<tr>
<td>STV</td>
<td>6.3 (3.9-15.1)</td>
<td>5.8 (3.3-10)</td>
<td>0.177</td>
</tr>
<tr>
<td>High Episodes</td>
<td>13.5 (0-50)</td>
<td>12.5 (0-37)</td>
<td>0.527</td>
</tr>
<tr>
<td>Low Episodes</td>
<td>7 (0-31)</td>
<td>16 (0-41)</td>
<td>0.266</td>
</tr>
</tbody>
</table>

FHR: fetal heart rate; LTV: long-term variation (ms); STV: short-term variation (ms); High Episodes: episodes of high variation in min; Low Episodes: episodes of low variability in min.\(^\ast\) (range)
*beats per minute
†mean per hour

No statistical differences were found for any of the FHR parameters between cases and controls.

If only cases (n = 14) and controls (n = 10) with betamethasone use 1-4 days prior to FHR monitoring were considered, slightly higher medians for heart rate variation were found for the cases (medians of 36.5 vs. 27.4 (LTV) and 6.9 vs. 5.7 (STV), but the difference was not significant (Wilcoxon’s rank sum test, p > 0.05).

The FHR parameters of the cases and the controls are projected in the reference ranges of a normal population for comparison (Figure 3-5).\(^1\) Both for the cases and the controls the HRV (LTV and STV) distribution was significantly lower than for the normal population (\(\chi^2\)-squared test; p < 0.001).
Fetal brain imaging in pregnancies at risk for preterm birth

Dawes and Redman’s criteria were based upon the normal distribution at 32 weeks, however, it was found that the STV threshold of 4 ms remains valid at lower gestations. The weakness of this study is that the number of fetuses available for a case-control comparison was limited, due to the low incidences of moderate severe echodensities even in a high risk population. Comparison with reference ranges from normal populations showed that the two values for variability (LTV and STV) of both the cases and the controls in the present study were significantly lower. The overall low variability found in this population may well reflect the compromised status of these fetuses at risk for preterm birth. FHR variability is influenced by fetal brain function and myocardial contractility. A reduced variability can be observed in conditions like prematurity, chronic hypoxemia, and brain damage. On the other hand an increased FHR variability has been described in acute hypoxic events in the term fetus. In a small retrospective study Okamura et al. found a significantly increased HRV in all 19 preterm fetuses that developed periventricular leukomalacia in the neonatal period. In preterm neonates periventricular leukomalacia is associated with a consistent increase in neonatal HRV indices as well, whereas preterm neonates with peri- and intraventricular haemorrhages tend to show a lower HRV than their matched controls. In the present study this association between the presence of PVE and an increased HRV could not be confirmed, nor could IVE be correlated to a lower HRV in the fetus. So far, localized BGTE have only been studied sparingly in the fetus and preterm neonate, and their correlation with fetal or neonatal heart rate variability was thus far unknown.

We conclude that antepartum FHR parameters obtained in the preterm period fail to identify fetuses with signs of hypoxic-ischemic or hemorrhagic injury in the peri- and intraventricular areas of the brain. As the incidence of moderate and severe changes in the echogenicity of the brain is relatively low even in a high risk population, a large study population is needed to be able to perform a well-matched case-control comparison. Preferably the findings of this study should be confirmed in a larger study in the future.

DISCUSSION

This is the first study evaluating a possible association between peri- and intraventricular echodensities in the fetal brain and FHR recordings. In this well-defined population of fetuses from complicated pregnancies we could not demonstrate significant differences in FHR parameters between fetuses with and without peri- and intraventricular echodensities.

The strength of the present study is the fact that fetuses were studied prospectively and fetal heart rate parameters were analyzed by computer. So far, studies on the correlation between FHR and brain damage have been retrospective and mostly refer to intrapartum FHR recording. In such a set-up the timing of brain damage whether antepartum or intrapartum, remains unclear. Furthermore these FHR traces were often interpreted visually instead of analyzed by computer, which overcomes both intra- and inter-observer variability. Although occasionally fetal sleep period may cause false positive low LTV values, computerized FHR analysis gives an objective assessment.

Four fetuses in the cases group showed a markedly increased variability (LTV and STV) compared to the rest of the group. In one of the four fetuses the STV value was above 95th percentile of the reference ranges for the normal population. Three of the four fetuses demonstrated IVE, two with concomitant BGTE, and one fetus showed PVE and BGTE.

Three fetuses had values below the Sonicaid Systems 4 ms threshold for STV, one was in the cases group and had IVE and two were in the control group.

Figure 5. Short-term variation in cases compared to a normal population. Normal ranges from Nijhuis et al.13

Figure 5A.

Figure 5B.
REFERENCES


FETAL BRAIN IMAGING IN PREGNANCIES AT RISK FOR PRETERM BIRTH

FETAL BRAIN SONOGRAPHY AND FETAL HEART RATE PATTERNS IN THE PRETERM FETUS
Chapter 4.

Fetal general movements and brain sonography in a population at risk for preterm birth


1 Department of Obstetrics and Gynaecology, Research Institute MOVE, VU University Medical Centre, Amsterdam, the Netherlands,
2 Department of Neonatology, Leiden University Medical Centre, Leiden, the Netherlands,
3 Department of Obstetrics and Gynaecology, Tygerberg Hospital, Stellenbosch University, South-Africa.

ABSTRACT

Background. General movements (GMs) assessed three months post term are related to brain injury and neurological outcome.

Aims. To study GMs in fetuses and their predictive value for echogenicity changes in the fetal brain.

Study design. Prospective study of fetal GMs (classified as normal or abnormal) and echogenicity changes in the periventricular, basal ganglia/thalami area, and ventricular system (classified as absent, mild or moderate).

Subjects: 121 fetuses from pregnancies affected by hypertensive disorders and/or preterm labour, at risk for preterm birth (26-34 weeks gestational age).

Outcome measures. Prevalences of abnormal GMs, GM parameters (amplitude, speed and complexity), and moderate echogenicity changes in the fetal brain (periventricular ≥1B, intraventricular grade II/III, basal ganglia/thalamus locally increased). Predictive values of GMs for clinical parameters and moderate echogenicity changes.

Results. GMs were abnormal in 58%, with amplitude affected in 96% and speed and complexity in 59%. Abnormal GMs correlated with oligohydramnios (p = 0.002) and hypertensive disorders (p = 0.015). Echogenicity changes of the brain were absent, mild and moderate in 27%, 39% and 31%, respectively. The sensitivity of GMs for moderate echogenicity changes in the three areas combined was 0.65, and the periventricular area 0.85, specificity both 0.44, negative predictive value 0.73 and 0.96 respectively.

Conclusions. Qualitative abnormal GMs are frequent in fetuses of compromised pregnancies, and correlate with hypertensive disorders and oligohydramnios. The amplitude of GMs was most frequently affected. Abnormal GMs relate to moderate echogenicity changes especially in the periventricular area of the fetal brain, while normal GMs predict absence of moderate echogenicity changes.

INTRODUCTION

General movements (GMs), spontaneous expressions of the central nervous system, are predictive of neurological outcome when assessed three months post term in infants at risk for adverse neurological outcome.19-21 Several authors found associations between (transient) abnormal GMs and (mild) white matter injury in preterm infants.6-11 The similarity between movement patterns before and after birth enables assessment of GMs in the fetus in the same manner as in the neonate,12 providing information about the functioning of the central nervous system. Before birth the quality of spontaneous motility of the fetus is highly correlated with its clinical condition.43 Severe reduction in amniotic fluid results in spatial restriction and affects the amplitude and speed of GMs.14 Fetal growth restriction (FGR) induces distinct changes in amplitude, speed and complexity of GMs patterns.15-17 Whether these changes in spontaneous motility result from transient brain dysfunction caused by hypoxemia, chronic nutritional deprivation, or brain injury is unknown.

As there is increasing evidence that brain injury may originate from the period before delivery, transvaginal sonographic examination of the fetal brain may help to provide an answer to this question. In combination with qualitative assessment of fetal general movements, evaluation of brain areas known to be vulnerable to injury18-23 may help to identify fetuses at risk for adverse outcome.

This study, being part of a longitudinal study on fetal brain sonography and neurological outcome, aims to examine fetuses from pregnancies complicated by hypertensive disorders (HD) or preterm labour (PTL) between 26 and 34 weeks gestational age, assessing:

1. The quality of GMs, including the separate GM parameters.
2. The influence of clinical parameters (hypertensive disorder of pregnancy, preterm labour, reduced fetal growth and amount of amniotic fluid) on the quality of GMs.
3. The predictive value of GMs for echogenicity changes in the fetal brain.

METHODS

General

This study was conducted at Tygerberg Hospital, Western Cape, South Africa.

All women admitted with a singleton pregnancy with a fetus in a cephalic presentation, and at risk for preterm delivery between 26 and 34 weeks gestation were eligible for the study. Pregnancies were complicated by HD (preeclampsia, pregnancy induced hypertension, and/or FGR), or PTL (PTL with or without premature rupture of the membranes or premature rupture of the membranes only).23 In case of overlap between HD and PTL the fetus was assigned to the PTL group. Ultrasound examinations were performed at admission and weekly thereafter, assessing growth parameters, presence
of oligohydramnios (amniotic fluid index (AFI) ≤ 5 cm), GMs and the brain of the fetus. Small for gestational age (SGA) at birth was defined according to population based centile charts.24 The study was agreed upon by the Committee for Human Research of the Stellenbosch University and all women gave written informed consent.

Qualitative assessment of GMs
Sonographic observation of fetal GMs was done by one hour video recordings (F.R.F.v.D.) of spontaneous fetal movements. A fetal GM is defined as a movement in which all body parts participate without a distinctive sequence. A GM varies in amplitude and speed, lasts several minutes but waxes and wanes during this period, and is graceful in character25. The assessment of the quality of GMs was performed offline. The three longest and/or most complex fetal GMs were selected and classified as being normal or abnormal by means of global visual ‘Gestalt’ perception.15 Additionally, in analogy to other fetal assessment studies, the following GM parameters were assessed: amplitude (normal variability, reduced variability: only small or only large), speed (normal variability, reduced variability: only slow or only quick), complexity (normal or low variability in superimposed rotations on limb flexion and extension and changes in direction),16,26 The assessment was performed by an independent observer (J.I.P.d.V.) who was unaware of the clinical conditions and brain sonographic findings. An interobserver agreement performed between two investigators (F.R.F.v.D. and J.I.P.d.V.) using Cohen’s Kappa.

Fetal brain ultrasonography
Transvaginal sonographic examinations of the fetal brain were performed in six coronal and five sagittal planes (F.M.F.R.v.D.) and were assessed offline by two investigators (J.I.P.d.V. and G.v.W.-M.) who were blinded to the clinical data.23 The periventricular white matter, ventricular system and basal ganglia/thalami were assessed for echogenicity changes in analogy to scoring systems applied in neonates27-29 and as recently described for this population.32 The echogenicity changes were classified as absent, mild or moderate. The following findings in the fetal brain were considered mild: periventricular echodensities (PVE) Grade IA, intraventricular echodensities (IVE) Grade I and diffuse basal ganglia/thalami echodensities (BGTE),32 PVE Grades IB and II, IVE Grades II and III, and localized BGTE were considered moderate. Additionally an overall classification of each brain examination was made, combining the three areas together and using the highest degree of echogenicity changes in one of the three areas.

Data analysis and statistics
The distribution of normal and abnormal GM quality, and of the separate parameters (amplitude, speed and complexity) was compared between the fetuses from pregnancies with HD and PTL (χ²-squared test). The GM and its parameters (amplitude, speed and complexity) were compared between fetuses from pregnancies with a normal AFI versus oligohydramnios, and with SGA versus appropriate for gestational age (in either HD or PTL group) also using the χ²-squared test. The GMs were related to the echogenicity changes in the brain in the total group, and in the HD and PTL groups separately. In cases with several weekly brain ultrasound examinations and/or GMs recordings, the most abnormal GMs and maximum echogenicity changes over time were chosen for comparison. Echogenicity changes in the fetal brain were analysed for the three areas combined and for each brain area separately. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) and likelihood ratio of abnormal GMs for moderate echogenicity changes were calculated.

RESULTS

General
Of the 124 women included, GMs recordings were not obtained in two because of imminent intrauterine demise. In another case the fetal GMs recording was lost due to technical failure. In four fetuses brain ultrasound findings were inconclusive because not three areas could be reliably assessed. This resulted in 121 cases for GMs assessment and 117 cases for the assessment of the relationship between GMs and brain sonography. In 26 fetuses, several weekly brain ultrasound examinations and/or GMs recordings were performed. The maternal characteristics and neonatal outcome of all 121 cases are depicted in Table 1. In three cases the pregnancies were complicated by both HD and PTL. Of the 63 cases with HD, 14 (22%) had oligohydramnios, 34 (54%) were SGA and in 9 cases both complications co-existed. Of the 58 cases with PTL, 27 (47%) had oligohydramnios, and 12 (21%) fetuses were SGA. Of the 33 cases with PROM in the PTL group, 8 still had a normal AFI. Two neonates were born elsewhere and no data on the gestational age at birth or birth weight were available.

Fetal GMs
GMs were normal in 51 (42%) and abnormal in 70 (58%) fetuses (Table 1). One fetus showed no movements during the one hour registration at 31 weeks gestational age. The longest rest period between consecutive GMs in the fetus near term, when the rest periods are considered to be of the longest duration, should not be longer than 45 minutes30 and thus absence of GMs was classified as abnormal. Consequently, GM parameters could not be assessed. Of the remaining 120 fetuses a reduced variability in amplitude was seen in 67 (56%), in speed in 49 (41%), and in complexity in 49 (41%). In fetuses with consecutive GM assessments, these were consistently normal or abnormal in 13, initially abnormal but normal thereafter in 1, and initially normal but later abnormal in 5. In cases with abnormal GMs the amplitude was most frequently affected (96%), speed and complexity were affected in 59% (Table 2). The interobserver agreement of GMs assessment was good (Kappa 0.84).
Table 1. Baseline characteristics of the pregnant women, fetal and neonatal data; related to quality of fetal general movements (GM)

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Total Group</th>
<th>Normal GM</th>
<th>Abnormal GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal data</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Median gestational age/wks (range)</td>
<td>30 (26-33+4/7)</td>
<td>29+4/7 (26-33+4/7)</td>
<td>30 (26-33+2/7)</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>45 (37)</td>
<td>17 (33)</td>
<td>28 (40)</td>
</tr>
<tr>
<td>Non-smoking</td>
<td>46 (38)</td>
<td>20 (39)</td>
<td>26 (37)</td>
</tr>
<tr>
<td>Hypertensive disorders</td>
<td>63 (52)</td>
<td>19 (37)</td>
<td>44 (63)</td>
</tr>
<tr>
<td>Preterm labour (+/- PROM)</td>
<td>58 (48)</td>
<td>32 (63)</td>
<td>26 (37)</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>41 (34)</td>
<td>9 (18)</td>
<td>32 (46)</td>
</tr>
<tr>
<td>Fetal data</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Brainsonography Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No echogenicity changes</td>
<td>33 (27)</td>
<td>13 (25)</td>
<td>20 (29)</td>
</tr>
<tr>
<td>Mild changes</td>
<td>47 (39)</td>
<td>22 (43)</td>
<td>25 (36)</td>
</tr>
<tr>
<td>Moderate changes</td>
<td>37 (31)</td>
<td>13 (25)</td>
<td>24 (34)</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>4 (3)</td>
<td>3 (6)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Brainsonography PVE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No echogenicity changes</td>
<td>45 (37)</td>
<td>16 (31)</td>
<td>29 (41)</td>
</tr>
<tr>
<td>Mild changes</td>
<td>54 (45)</td>
<td>28 (55)</td>
<td>26 (37)</td>
</tr>
<tr>
<td>Moderate changes</td>
<td>13 (11)</td>
<td>2 (4)</td>
<td>11 (16)</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>9 (7)</td>
<td>5 (10)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Brainsonography IVE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No echogenicity changes</td>
<td>89 (74)</td>
<td>35 (69)</td>
<td>54 (77)</td>
</tr>
<tr>
<td>Mild changes</td>
<td>9 (7)</td>
<td>4 (8)</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Moderate changes</td>
<td>17 (14)</td>
<td>8 (16)</td>
<td>9 (13)</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>6 (5)</td>
<td>4 (8)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Brainsonography BGTE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No echogenicity changes</td>
<td>75 (61)</td>
<td>30 (59)</td>
<td>45 (64)</td>
</tr>
<tr>
<td>Mild changes</td>
<td>21 (17)</td>
<td>9 (18)</td>
<td>12 (17)</td>
</tr>
<tr>
<td>Moderate changes</td>
<td>19 (16)</td>
<td>9 (18)</td>
<td>10 (14)</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>6 (5)</td>
<td>3 (6)</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Neonal data</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Median gestational age at birth (wks)</td>
<td>35±1/7</td>
<td>31±6/7</td>
<td>30±6/7</td>
</tr>
<tr>
<td>Median birth weight (in gram)</td>
<td>1314</td>
<td>1644</td>
<td>1261</td>
</tr>
<tr>
<td>Small for gestational age (&lt; p10)</td>
<td>46 (39)</td>
<td>14 (29)</td>
<td>32 (46)</td>
</tr>
</tbody>
</table>


*In one fetus no movements were seen during one hour recording and therefore amplitude, speed and complexity could not be assessed.

Table 2. Assessment of general movements (GMs) and GM parameters in fetuses at risk for preterm birth

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>n (%)</th>
<th>Amplitude</th>
<th>Speed</th>
<th>Complexity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMs</td>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Normal GMs</td>
<td>51 (42)</td>
<td>53 (44)</td>
<td>71 (59)</td>
<td>71 (59)</td>
</tr>
<tr>
<td>Hypertensive disorders</td>
<td>19 (37)</td>
<td>20 (38)</td>
<td>32 (48)</td>
<td>29 (41)</td>
</tr>
<tr>
<td>Preterm labour (+/- PROM)</td>
<td>32 (63)</td>
<td>33 (65)</td>
<td>39 (58)</td>
<td>42 (59)</td>
</tr>
<tr>
<td>PROM</td>
<td>12 (24)</td>
<td>13 (25)</td>
<td>16 (23)</td>
<td>19 (27)</td>
</tr>
<tr>
<td>Oligohydramnios (HD/PTL)</td>
<td>9 (18)</td>
<td>9 (17)</td>
<td>17 (24)</td>
<td>18 (25)</td>
</tr>
<tr>
<td>SGA</td>
<td>14 (27)</td>
<td>14 (26)</td>
<td>21 (30)</td>
<td>21 (30)</td>
</tr>
<tr>
<td>SGA plus oligohydramnios</td>
<td>2 (4)</td>
<td>2 (4)</td>
<td>6 (8)</td>
<td>6 (8)</td>
</tr>
</tbody>
</table>

* Abnormal GMs were significantly more often seen in the HD group than the PTL group (p = 0.05). The HD group had more reduced variability in all three GM parameters, amplitude (p < 0.01), speed (p < 0.05), and complexity (p < 0.01) than the PTL group.

Both in the HD and the PTL group the cases with oligohydramnios showed significantly more reduced variability in GMs than the cases with normal amniotic fluid (p = 0.002). Of the cases with oligohydramnios three quarter showed diminished amplitude of GMs (p = 0.001), while only half showed reduced variability in speed (p = 0.012) and complexity (p = 0.032) of GMs.

The SGA fetuses showed a trend towards more abnormal GMs as compared to the fetuses that were appropriate for gestational age (p = 0.058). In the fetuses with SGA the
amplitude (p = 0.037) of GMs was diminished in two thirds, while in half of the fetuses a reduced variability in both speed (p = 0.043) and complexity (p = 0.043) of GMs was also seen.

Fetal GM and fetal brain
The distribution of no, mild and moderate echogenicity changes and inconclusive results per examined area and of the three examined areas combined are presented in Table 1. PVE were most frequently seen (67 fetuses, 55%) and IVE and BGTE were found in 26 (21%) and 40 fetuses (33%) respectively. The distribution of no, mild and moderate echogenicity changes (calculated for the three areas combined) was not significantly different in the HD and PTL group.

Of the 26 fetuses with repetitive GMs recordings and/or brain ultrasound examinations, in 20 cases, the most severe GMs and maximum echogenicity changes were observed on the same day. In the remaining six cases, the most abnormal GM assessment was seen a week after (four cases) or one week before (one case) the maximum echogenicity changes. In one case a second GMs recording was not obtained, after a normal GMs and brain assessment the first time.

The distribution of the GMs per three brain areas is also presented in Table 1. A relatively high percentage of normal GMs was found in fetuses with mild PVE (52%) and in fetuses with mild BGTE (43%). The distribution of GMs per no, mild and moderate echogenicity changes of all three brain areas combined is presented in Figure 1. This figure also shows a high percentage of normal GMs in fetuses with mild echogenicity changes. The predictive values of abnormal GMs in the total population for moderate echogenicity changes in the three brain areas separately and combined are presented in Table 3.

Table 3. Predictive values of general movements assessment for moderate echogenicity changes in fetal brain areas vulnerable for injury

<table>
<thead>
<tr>
<th>Echogenicity Changes</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periventricular</td>
<td>0.85</td>
<td>0.44</td>
<td>0.17</td>
<td>0.96</td>
<td>1.52</td>
</tr>
<tr>
<td>Intraventricular</td>
<td>0.53</td>
<td>0.4</td>
<td>0.13</td>
<td>0.83</td>
<td>0.88</td>
</tr>
<tr>
<td>Basal ganglia/thalami</td>
<td>0.53</td>
<td>0.41</td>
<td>0.15</td>
<td>0.81</td>
<td>0.9</td>
</tr>
<tr>
<td>All areas combined</td>
<td>0.65</td>
<td>0.44</td>
<td>0.35</td>
<td>0.73</td>
<td>1.16</td>
</tr>
</tbody>
</table>

PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio.

DISCUSSION
This is the first study relating fetal GMs to echogenicity changes in the fetal brain. In the studied population of pregnancies with early onset HD or labour, more than half of the fetuses (58%) had abnormal GMs, the amplitude being most frequently affected. In the HD group more fetuses displayed abnormal GMs, than in the PTL group, two thirds and half respectively.

Brain ultrasonography demonstrated echogenicity changes in the periventricular white matter in half, in the basal ganglia/thalami in a third, and in the ventricular system in a fifth of all fetuses. No difference in prevalence of echogenicity changes was seen between the HD and PTL group. Relating GMs to echogenicity changes in the brain, two findings were of specific interest. Firstly the high sensitivity and NPV of GMs for moderate echogenicity changes in the periventricular area, and secondly the high percentage of normal GMs in fetuses with mild echogenicity changes in de periventricular and basal ganglia/thalami areas.
The high incidence of abnormal GMs found in the HD group as compared to the PTL group may be explained by the fact that half of the fetuses in this group was growth restricted. We found that presence of SGA or oligohydramnios negatively influenced the amplitude of the movements in two thirds and three quarters of the fetuses respectively, and had a negative influence on speed in more than half of these fetuses. In two thirds of the fetuses that were SGA and had oligohydramnios not only amplitude and speed, but also the variability in complexity was reduced.

There are no studies available in low risk pregnancies to compare the prevalence of abnormal fetal GMs found in our high risk population. Abnormal fetal motility has been described in three small high risk populations. Pioneer work of Bekedam et al. has shown abnormal GMs in all but one of the growth restricted fetuses studied. They postulated that the abnormal motility resulted from impaired development of the central nervous system caused by chronic nutritional deprivation. Sival et al., longitudinally studying growth restricted fetuses and fetuses with premature rupture of the membranes, also found that most of the fetuses demonstrated abnormal GMs parameters before birth. They concluded that the spatial restriction as such may be responsible for the changes in GM quality.

There are studies on preterm neonates confirming our data, reporting similar high prevalences of abnormal GM quality during the preterm period and at term equivalent age. The abnormal GM quality in neonates has been postulated to be a carry-over effect caused by spatial restriction in the prenatal period, or a result of transient brain dysfunction in chronic nutritional deprivation as mentioned above, or attributed to brain injury. The relationship between abnormal GMs and brain injury has been addressed in several small populations of preterm and full term neonates. In preterm neonates, Ferrari et al. found abnormal GMs in all neonates with haemorrhage or periventricular leukomalacia, compared to only one of the low risk controls with normal brain ultrasound examinations. Likewise, Kakebeeke et al. found a relationship between abnormal GMs and brain lesions, comparing high risk preterm neonates with low risk preterm and full term neonates. Bos et al., however, found no correlation between the GMs and mostly mild brain injury in SGA neonates. In appropriate-for-gestational age neonates, they did demonstrate a correlation between the presence and duration of transient parieto-occipital echodensities and abnormal GMs trajectories. In two prospective studies on preterm neonates, abnormalities on ultrasound and MRI correlated with GM quality, but only beyond three months post term.

A few studies have described echogenicity changes in the fetal brain in low risk and high risk populations. Mild echogenicity changes in the periventricular white matter and basal ganglia/thalami present during the preterm period probably reflect maturational processes in analogy to neonatal findings. Interestingly, the current study supports the hypothesis that these mild echogenicity changes reflect normal maturational phenomena, as a large percentage of fetuses with mild echogenicity changes showed normal GMs. GMs were found to have a substantial sensitivity for moderate echogenicity changes, that probably represent early stages of brain injury or mild brain injury, especially in the periventricular area. But the specificity was only moderate. This is in contrast with Ferrari et al. demonstrating not only a high sensitivity but also specificity of GMs for brain injury. Their study population, however, included only a small proportion of SGA neonates. The low specificity of GMs for brain echogenicity changes in the current study is in agreement with other studies reporting on preterm SGA neonates, and can be explained by the previously mentioned important influence of oligohydramnios and growth restriction on fetal motility. The high NPV of GMs for moderate echogenicity changes is in agreement with studies in preterm neonates, showing that absence of abnormal GMs during the preterm period predicts absence of brain injury. The likelihood ratio of GMs for moderate echogenicity changes in all areas combined was high, being even higher for moderate PVE alone.

One of the limitations of our study is that most fetuses were only examined once and the evolution of GMs and brain sonography findings over time could not be studied. Therefore, it was not possible to differentiate between transient or persistent echogenicity changes and their relation to GMs. In addition, our population did not include fetuses with signs of severe brain injury. This may have influenced the results as it is possible that the specificity of GMs for more severe echogenicity changes may have been higher.

Summarising, qualitatively abnormal GMs are frequent in fetuses of pregnancies complicated by early onset HD or PTL, and were related to HD and oligohydramnios. Mild echogenicity changes, probably representing maturational phenomena, are frequently found in the brain of these fetuses, and are related to normal GMs. Abnormal fetal GMs have a good sensitivity for moderate echogenicity changes, especially in the periventricular white matter, however, the specificity is low. These moderate echogenicity changes, probably representing transient or early stage brain injury, were seen in both pregnancies complicated by HD and PTL. Fetuses showing qualitatively normal GMs in the preterm period are unlikely to have brain injury. Longitudinal evaluation of GMs and brain ultrasonography before and after birth in this population, or similar populations, may provide further insight in the predictive value of fetal GMs for nervous system integrity and neurological outcome.
REFERENCES


General movements in the perinatal period and its relation to echogenicity changes in the brain

Chapter 5.

Fleur M. Rosier-van Dunné,1 Gerda van Wezel-Meijler,2 Maaike P. Bakker,1 Laila de Groot,3 Hein J. Odendaal,4 Johanna I. de Vries1

1Department of Obstetrics and Gynaecology, Research Institute MOVE, VU University Medical Centre, Amsterdam, the Netherlands,
2Department of Neonatology, Leiden University Medical Centre, Leiden, the Netherlands,
3Department of Neonatology, Research Institute MOVE, VU University Medical Center, Amsterdam, the Netherlands,
4Department of Obstetrics and Gynaecology, Tygerberg Hospital, Stellenbosch University, South-Africa.

ABSTRACT

**Background.** In preterm born infants abnormal general movements (GMs) generally normalize before three months post term, but may persist when perinatal brain injury is present.

**Aims.** To assess the continuity of GM quality from fetal to early neonatal period and its relation to brain echogenicity changes.

**Study design.** Prospective study examining GMs and three vulnerable brain areas before and 7 days after birth. The quality of GMs was classified as normal or abnormal by Gestalt-perception. The brain was examined for moderate echogenicity changes (periventricular: brighter than choroid plexus, intraventricular: filling equal or more than 50% of the ventricle, and locally increased basal ganglia/thalami).

**Subjects.** 94 fetuses from pregnancies complicated by preterm hypertensive disorders or labour at a gestational age between 26 and 34 weeks.

**Outcomes measures.** Correlations of fetal GMs, echogenicity changes, and clinical parameters (e.g. gestational age, parity, hypertensive disorders or preterm labour, oligohydramnios and fetal growth restriction) with neonatal GMs.

**Results.** Fetal GMs were abnormal in 64%, normalizing in 68% within 7 days after birth. Fetal GMs were significantly related to postnatal GMs (p = 0.045). Moderate fetal brain echogenicity changes and clinical parameters were not significantly related to neonatal GM.

**Conclusions.** In this population of pregnancies compromised by hypertensive disorders or preterm labour fetal GMs correlated with neonatal GMs. Presence of moderate echogenicity changes in the fetal brain was not related to neonatal GMs.

INTRODUCTION

In the third trimester of pregnancy the quality of general movements (GMs) is often altered in fetuses with growth restriction or fetuses exposed to oligohydramnios. Both speed and amplitude of fetal GMs can be affected, and in cases with fetal growth restriction (FGR) the complexity of GMs can become reduced as well. It has been postulated that these alterations may be the result of impaired central nervous system development caused by chronic nutritional deprivation, merely spatial restriction, or possibly prenatal brain injury. Previous research in growth restricted fetuses has shown a continuity in movement quality between prenatal and postnatal GMs. In cases with neonatal peri- and intraventricular haemorrhages (PIVH) deterioration of GM quality was noticed after birth. In preterm neonates a relationship between white matter injury, PIVH and abnormal early motor behaviour has been clearly demonstrated.

In the present study we examine the continuity of the quality of GMs from before until one week after birth. The study is performed in a high risk population of pregnancies complicated by hypertensive disorders (HD) or preterm labour (PTL) between 26 and 34 weeks gestational age. The study is part of an ongoing longitudinal study assessing the relationship between fetal brain echogenicity changes, various perinatal parameters (e.g. amniotic fluid index and fetal growth) and neurological outcome. We hypothesize that GMs of most of these compromised fetuses will normalize after birth because of improved nutrition, restored oxygen status and suspension of movement restriction. In fetuses with solely spatial restriction in utero normalization of GMs may be realized soon after birth. However, in small-for-gestational-age (SGA) fetuses, and fetuses with moderate echogenicity changes in the brain, probably representing mild or early stage brain injury, GMs might normalize at a later stage.

Specific questions addressed are:

1. Are fetal GMs related to neonatal GMs in complicated pregnancies at risk for preterm birth?
2. Is presence of moderate echogenicity changes in the fetal and neonatal brain related to quality of early neonatal GMs and/or perinatal continuity of GMs in cases with abnormal GM quality?
3. Are clinical parameters like parity, gestational age, hypertensive disorders of pregnancy, preterm labour, SGA, and oligohydramnios related to quality of early neonatal GMs and/or perinatal continuity of GMs in cases with abnormal GM quality?

MATERIALS AND METHODS

**Subjects**

All women admitted with a singleton pregnancy and at risk for preterm delivery between 26 and 34 weeks gestation were eligible for the study. Pregnancies were compli-
Results of each recording the three longest and/or most complex GMs were selected and/or AGA at birth was defined according to population based centile charts. During a two year period women met the study criteria. Exclusion criteria were PTL group. The study was agreed upon by the Committee for Human Research of the Stellenbosch University. All women gave written informed consent. Ultrasound examinations were performed at admission and weekly thereafter, assessing growth parameters, presence of oligohydramnios (amniotic fluid index ≤ 5 cm), GMs, and the brain of the fetus.

After birth the neonate was assessed within 24 h and again after one week. GMs and brain ultrasound examination were recorded on videotape. These examinations were repeated weekly until discharge or transfer to a regional hospital.

Qualitative assessments of GMs
Sonographic observation of fetal GMs was done by 1 h video recordings (F.R.v.D.). Recording of GMs after birth was performed during one hour with the neonates in supine position while actively awake, in the incubator or on an examination table with overhead heating. As GMs have identical characteristics before and after birth, GM assessment in the fetus and neonate was performed in analogy to Prechtl’s method of analysis of neonatal GMs, later classified by means of ‘Gestalt’ perception as being normal or abnormal. The fetal GM assessments were performed by J.I.d.V., the interobserver agreement between two investigators (F.R.v.D. and J.I.d.V) was good (Cohen’s Kappa 0.84). The neonatal GM assessments were performed in consensus by two observers, L.D.G., being an independent observer and a paediatric physiotherapist with many years of experience with Prechtl’s Gestalt-perception method and F.R.v.D., who was blinded to the names of the subjects.

Brain ultrasound assessments
Transvagal sonographic examinations of the fetal brain and sonographic examinations of the neonatal brain were performed in six coronal and five sagittal planes (F.R.v.D.) and were assessed offline in consensus by two investigators (J.I.d.V. and G.v.W.-M.) who were blinded to the clinical data. Echogenicity changes had to be confirmed in coronal and sagittal planes, otherwise they were classified as inconclusive. Echogenicity changes in the periventricular white matter (PVE), ventricular system (IVE), and basal ganglia/thalami (BGTE) were classified in analogy to scoring systems applied in neonates and as recently described for this population. In this study only moderate echogenicity changes, that are presumed to reflect mild or early stage brain injury, were used for comparison with GMs; PVE Grade I: seriously increased echogenicity of the white matter being brighter than the choroid plexus; PVE Grade II: periventricular echodensities evolving into localized cysts. IVE Grade II: echodensity filling less than 50% of the lateral ventricle; IVE Grade III: echodensity filling 50% or more of the lateral ventricle; local BGTE: localized increased echogenicity as compared to surrounding tissue. Moderate echogenicity changes were analysed for each of the three examined brain areas separately and for the three areas combined, using the highest degree of echogenicity.

Data analysis and statistics
The distribution of normal and abnormal GMs was compared between the fetuses and neonates. The evolution of GM quality from the fetal period into the first week after birth was described. In cases with several weekly brain ultrasound examinations and/or GMs recordings before birth, the most abnormal GMs and maximum echogenicity changes over time were chosen for comparison.

For statistical analysis the neonatal assessment at day 7 was chosen, as the first days after birth are considered to be a period of instability and GMs during that period may be less representative. If results of day 7 were missing because of discharge from the hospital or inconclusive results e.g. in case of sedation for ventilatory support, the assessment from day 1 was used. The relationship between fetal GMs and neonatal GMs was examined with the Fisher’s exact test. Univariate analysis was performed to analyse the relation between presence of moderate echogenicity changes in the fetal and neonatal brain, in all areas combined, and neonatal GMs. Moreover, clinical parameters including parity, hypertensive disorders of pregnancy, preterm labour, FGR, oligohydramnios (Fisher’s exact test) and gestational age (t-test) were related to neonatal GMs. Multivariate stepwise logistic regression was performed to examine influences of fetal GMs, fetal moderate brain echogenicity changes (in the three areas combined), and clinical parameters on neonatal GMs. Additionally, logistic regression was performed to examine the influence of clinical parameters, fetal or neonatal moderate brain echogenicity changes on the normalization of abnormal fetal GMs after birth or on normal fetal GMs becoming abnormal after birth.

Results
Study population
Of the women included in the study, GMs movements were recorded in fetuses. Six fetuses died after inclusion. One neonate died within hours after delivery, and one
neonate died the first day after birth, the first GM assessment being inconclusive because of sedation. Of 19 neonates GMs recordings could not be obtained after birth, these neonates did not differ in general clinical characteristics from the total population. Thus, from 94 cases both fetal and neonatal recordings were available. Population characteristics are displayed in Table 1.

Table 1. Population characteristics (n = 94)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparity</td>
<td>41</td>
<td>43.6</td>
</tr>
<tr>
<td>Hypertensive disorders</td>
<td>53</td>
<td>56.4</td>
</tr>
<tr>
<td>Preterm labour</td>
<td>41</td>
<td>43.6</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>36</td>
<td>38.2</td>
</tr>
<tr>
<td>Small-for-gestational-age</td>
<td>39</td>
<td>41.4</td>
</tr>
<tr>
<td>Female</td>
<td>41</td>
<td>43.6</td>
</tr>
<tr>
<td>Male</td>
<td>53</td>
<td>56.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GA at intake (weeks)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>(26 5/7-33 4/7)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GA at birth (weeks)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>(26 6/7-38 5/7)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Birth weight (g)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1284</td>
<td>(530-4334)</td>
<td></td>
</tr>
</tbody>
</table>

GMs: General movements

Fetal GMs were classified as normal in 34/94 (36%) and abnormal in 60/94 (64%) fetuses. In 20 fetuses with sequential GM assessments before birth, the assessments were consistently normal or abnormal in 13, initially normal but normal thereafter in 1, and initially normal but later abnormal in 5. Neonatal GMs were classified as normal in 71/94 (75%) and abnormal in 23/94 (25%) in the first week after birth. Of the 94 neonates, 23 did not have a recording at day seven and 1 assessment was inconclusive because of sedation. Of these 24 neonates, 17 had a normal and 7 had an abnormal GM assessment at day 1. Three of these seven neonates died in the first week after birth.

The quality of GMs evolved from initially normal before birth to abnormal after birth in 4/34 cases (12%). Of the cases with initially abnormal GMs before birth 19/60 (32%) were consistently abnormal, while in 41/60 (68%) cases GMs normalized on day 7 after birth.

Table 2. Incidences of moderate echogenicity changes in the periventricular (PVE), intraventricular (IVE) and basal ganglia/thalami (BGTE) of the brain before birth and at day 7 after birth (n = 94)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fetal brain</th>
<th>Neonatal brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVE</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>PVE+IVE</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>PVE+BGTE</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IVE</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>IVE+BGTE</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>BGTE</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

| total                   | 33          | 39             |

Moderate PVE: grade IB: seriously increased echogenicity of the white matter being brighter than the choroid plexus; grade II: periventricular echodensities evolving into localized cysts. Moderate IVE: grade II: echodensity filling less than 50% of the lateral ventricle; grade III: filling of equal or more than 50% of lateral ventricle. Moderate BGTE: localized increased echogenicity as compared to surrounding tissue.

GMs, brain and clinical parameters

Univariate analysis showed a correlation between fetal GMs and neonatal GMs (p = 0.045). The presence of moderate echogenicity changes in the fetal or neonatal brain was not significantly related to neonatal GMs in univariate (Table 3) or multivariate analyses. Of the cases showing normal fetal GMs before and abnormal GMs after birth, 2/4 had moderate echogenicity changes at day 7. Of the cases with persistently abnormal GMs before and after birth, 5/17 showed moderate echogenicity changes after birth.
Table 3. Univariate analysis of moderate echogenicity changes in the brain and clinical parameters for fetal and neonatal GMs (p values, 2-sided Fisher’s Exact test)

<table>
<thead>
<tr>
<th></th>
<th>Fetal GM</th>
<th>Neonatal GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal US moderate echogenicity changes</td>
<td>0.823</td>
<td>0.328</td>
</tr>
<tr>
<td>Neonatal US moderate echogenicity changes</td>
<td></td>
<td>0.340</td>
</tr>
<tr>
<td>Gestational age*</td>
<td>0.579</td>
<td>0.137</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>0.829</td>
<td>0.089</td>
</tr>
<tr>
<td>Height</td>
<td>0.198</td>
<td>0.347</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>0.030</td>
<td>0.625</td>
</tr>
<tr>
<td>Small-for-gestational age</td>
<td>0.085</td>
<td>0.143</td>
</tr>
</tbody>
</table>

*T-test, US: ultrasound

None of the clinical parameters was significantly related to neonatal GMs, both in univariate (Table 3) and multivariate analyses. However, the presence of oligohydramnios did relate to fetal GMs. Logistic regression did not show any significant influence of fetal moderate brain echogenicity changes or clinical parameters on the normalization of abnormal fetal GMs after birth or on normal fetal GMs becoming abnormal after birth.

DISCUSSION

In this population of complicated pregnancies at risk for preterm birth two thirds (64%) of the fetuses showed qualitatively abnormal motility. Within a week after birth more than two thirds (68%) showed a normalization of GM quality. Normal fetal GMs predicted normal GMs in the first week after birth, whereas abnormal fetal GMs had a higher chance of being abnormal after birth. Presence of moderate echogenicity changes in the periventricular white matter, basal ganglia/thalami or ventricular system of the brain as observed before birth by transvaginal ultrasound examination, was not related to GM quality in the first week after birth. Oligohydramnios was related to fetal GMs, but none of the perinatal clinical parameters was related to neonatal GM quality. The presence of moderate echogenicity changes, nor any other clinical parameter was related to the perinatal evolution of GM quality.

In the literature prenatal to postnatal continuity of GMs has only been described in a few small high risk populations and a carry-over effect of reduced variability of GMs was observed. 2,3-10 A longitudinal study in growth restricted fetuses described abnormal GMs in 15/17 fetuses. 2 The majority had similar GMs before and after birth, and most abnormal GMs showed normalization of GMs within 10 weeks after birth. One of the two cases with Grade I PIVH and two cases with Grade III PIVH showed deterioration of GM quality after birth, with persistence of abnormal GMs. In our study the majority of abnormal GMs normalized in the first week after birth.

The incidence of abnormal motility in preterm neonates reported in the literature largely depends on the selected population, whether high or low risk, and the period tested, ranging from 39-84% during the preterm period, 11,21-35 30-83% around term equivalent age, 12-17,27 to 21-57% at the age of three months post term. 18-20,24,26,28 Only a few studies reported on neonatal motility in the first week after birth, with high incidences of abnormal motility ranging from 72 to 86%, 21-23,28,29 In many studies of preterm neonates the abnormal motility persisted for several weeks before normalizing, generally before the age of three months post term. In absence of underlying brain injury, SGA has been implicated as a possible cause of this abnormal neonatal motility. 21,23,31

In our study the presence of moderate echogenicity changes during the prenatal period 7,21,29,32-34 or MRI around term equivalent age, 9,26,35 have been related to GMs evolution during preterm, term and postterm periods. Transient echodensities (Grade I periventricular leukomalacia; PVL), if present for more than 14 days, 21,24,25 to 21-57% at the age of three months post term. Only a few studies reported on neonatal motility in the first week after birth, with high incidences of abnormal motility ranging from 72 to 86%, 21-23,28,29 In many studies of preterm neonates the abnormal motility persisted for several weeks before normalizing, generally before the age of three months post term. In absence of underlying brain injury, SGA has been implicated as a possible cause of this abnormal neonatal motility. 21,23,31

In our population with a median gestational age of 31 weeks at birth and 41% SGA, only 25% of the neonates showed abnormal GMs. Multivariate analysis could not establish an effect of SGA or gestational age on normalization or persistence of abnormal fetal motility after birth.

As far as we know, our study is the first relating fetal brain ultrasound findings with neonatal motility. We could not demonstrate any relationship between moderate echogenicity changes in the fetal brain and neonatal GMs. In several studies of preterm neonates, abnormal brain imaging findings, using ultrasonography in the neonatal period 7,21-29,31-34 or MRI around term equivalent age, 9,26,35 have been related to GMs evolution during preterm, term and postterm periods. Transient echodensities (Grade I periventricular leukomalacia; PVL), if present for more than 14 days, 21,24,25 to 21-57% at the age of three months post term. Only a few studies reported on neonatal motility in the first week after birth, with high incidences of abnormal motility ranging from 72 to 86%, 21-23,28,29 In many studies of preterm neonates the abnormal motility persisted for several weeks before normalizing, generally before the age of three months post term. In absence of underlying brain injury, SGA has been implicated as a possible cause of this abnormal neonatal motility. 21,23,31
However, in our population, both during the fetal and the early neonatal period no severe brain abnormalities, like Grade III PVL and periventricular haemorrhagic infarction were found. This may explain the poor correlations of fetal and neonatal moderate brain echogenicity changes with neonatal GMs as compared to studies in preterm neonates with more severe brain injury. On the other hand it is possible that neonatal GMs are indeed not related to moderate ultrasound abnormalities in the fetal and early neonatal period. Clinical factors like oligohydramnios and growth restriction during the fetal period, and clinical illness due to hypoxia-ischemia or septicemia resulting in transient brain dysfunction in the early neonatal period\textsuperscript{30,38} may play a more important role. Possibly only echogenicity changes that persist or increase in severity over time relate to abnormal GMs in the postterm period.

In conclusion, in compromised pregnancies fetal GMs are related to neonatal GMs and presence of normal fetal GMs predicts normal GMs after birth. Moderate echogenicity changes in the fetal brain are not related to GM quality after birth. Both normalization and the persistence of abnormal fetal GMs can not be predicted by presence of moderate echogenicity changes in the fetal brain or by presence of HD, oligohydramnios, or SGA. Long term follow-up studies in this and other populations are needed to fully understand the implications of fetal abnormal movement quality and echogenicity changes in the fetal brain on neurological outcome.

REFERENCES


Chapter 6.

Placental histology related to fetal brain sonography


a. Department of Obstetrics and Gynaecology, Research Institute MOVE, VU University Medical Centre, Amsterdam, the Netherlands.
b. Department of Neonatology, Leiden University Medical Centre, the Netherlands.
c. Formerly Department of Pathology, Red Cross Hospital, University of Cape Town, South Africa.
d. Department of Pathology, Faculty of Health Sciences, University of Stellenbosch and NHLS, South Africa.
e. Department of Obstetrics and Gynaecology, Tygerberg Hospital, University of Stellenbosch, South Africa.

Arch Dis Child Fetal Neonatal Ed (in press)
ABSTRACT

Chronic hypoxia and inflammatory processes can induce placental disturbances that may indirectly lead to perinatal brain injury.

Objective. To study histological features of the placenta in relation to echogenicity changes in the periventricular white matter (PVE), ventricular system (IVE), and basal ganglia/thalami (BGTE) of the fetal brain.

Design. Prospective study of 77 fetuses between 26 and 34 weeks gestational age with their placentas. The pregnancies were complicated by hypertensive disorders (n = 42) or preterm labour (n = 35).

Results. Of the placentas 79% showed uteroplacental hypoperfusion, inflammation, or a combination. Transvaginal ultrasound examination of the brain revealed echogenicity changes in 73% of the fetuses (44 mild, 29 moderate). Moderate brain echogenicity changes (PVE Grade IB: increased echogenicity brighter than choroid plexus, IVE Grade II and III: echodensity filling ventricle respectively < and ≥ 50%; BGTE: locally increased echogenicity within basal ganglia/thalami) were equally distributed over cases with uteroplacental hypoperfusion and inflammatory features in the placenta. PVE grade IB was always associated with placental pathology. The sensitivity and negative predictive value of placental pathology for moderate echogenicity changes were high (0.91 and 0.88 respectively), while the specificity and positive predictive value were low (0.27 and 0.34 respectively).

Conclusions. Normal placental histology predicted no or mild echogenicity changes, supporting the view that the latter are physiological. Placental pathology was always present in cases with PVE Grade IB, presumed to represent mild or early forms of white matter injury. Both uteroplacental hypoperfusion and inflammatory features were seen in placentas from pregnancies with hypertensive disorders.

INTRODUCTION

Placental pathology contributes to characterizing the fetal environment and providing insight in the pathophysiology of perinatal brain injury. It reflects the occurrence of sudden events, thrombo-inflammatory processes, and chronic patterns of injury that can undermine the placental reserve. Several authors have demonstrated an association between pathological processes in the placenta and brain injury in the neonate. Preterm delivery is strongly associated with neonatal morbidity and neurodevelopmental impairment. In cases of preterm delivery, both acute and chronic inflammatory processes, as well as signs of vasculopathy or hypoperfusion, can be observed in the placenta. Both chronic intrauterine hypoxia and chorioamnionitis have been associated with white matter injury and periventricular intraventricular haemorrhage (P/I VH) in preterm neonates. Several recent studies, however, failed to include histological chorioamnionitis as an independent causative factor in intraventricular haemorrhages or cerebral palsy.

In order to examine pathological processes that coincide with fetal brain injury, echogenicity changes in the white matter, lateral ventricles, and basal ganglia/thalami of the fetal brain were studied in relation to vascular and inflammatory changes in the placenta. This study, being part of a longitudinal study relating fetal brain examinations to several obstetrical and neonatal parameters, was performed in a population of pregnant women having preterm labour or hypertensive disorders of pregnancy and being at risk for preterm delivery.

MATERIAL AND METHODS

Patients
All pregnant women admitted to Tygerberg Hospital, Western Cape, South-Africa, with a risk of preterm labour at a gestational age between 26 and 34 weeks were invited to participate in the study. Reasons for admission were hypertensive disorders of pregnancy (preclampsia, severe hypertension with or without fetal growth restriction), or preterm labour, with or without preterm rupture of membranes. The study was agreed upon by the Committee for Human Research, Faculty of Health Science, University of Stellenbosch.

Ultrasonography
The fetal brain ultrasound examinations were performed as soon as possible after inclusion and thereafter weekly until delivery or until the pregnant women were discharged from the hospital. After sonographic confirmation of a cephalic position of the fetus, the fetal brain was examined by transvaginal ultrasonography for the presence of echogenicity changes in the periventricular white matter, ventricular system, and
Placenta

After birth the placenta with attached cord and membranes was examined for completeness and weight and gently submersed in 10% buffered formalin. The placenta examination was undertaken by each of the two pathologists (R.O.C.K. and P.A.B.W.) who were unaware of the clinical history. The pathological diagnoses were made in consensus. The membranes were resected from the placenta and rolled from the site of rupture towards the placental disc as described by Zeek and Assali. The cord was examined for the presence of knots. The cord was then severed from the placental disc, had cross-section examination at several sites, and samples for microscopic examination taken. The weight of the trimmed placental disc was assessed. The fetal surface was examined for thrombi in fetal vessels. The placental disc was cut into slices of about 1 cm width, and a minimum of three large tissue blocks were taken for histological examination. Additional sections were also taken from any macroscopically abnormal area. All specimens were stained with haematoxylin and eosin.

The umbilical cord and placental membranes, the chorionic plate, decidual plate, and body of the placenta were evaluated for hypertension related vascular pathologic features, coagulation related lesions, and inflammatory signs. The placental features were classified into three subgroups:

1. Uteroplacental hypoperfusion (at least two of the following: increased syncytiotrophoblast knotting, X-cell proliferation, excessive perivillous fibrin deposition, old infarcts, new infarcts, uteroplacental vessels lacking physiological change or having fibrinoid necrosis, atherosis, placental weight < 10th percentile).
2. Inflammatory related pathology (at least one of the following: acute chorioamnionitis Grade 3-4, funisitis grade 3-4, and/or chronic inflammation, (inter)vasitis, vasculitis).
3. Placenta-fetal hypoperfusion (at least two or more of the following: fetal vessel thrombosis, thrombosis in a major vessel, intervillus thrombosis, obstructive knotting of the cord, decidual necrosis, hypovascularity of villi, absent formation of terminal choriocapillaris, invasive chorionic vascular growth into syncytial membranes in cases beyond 32 weeks gestational age, or chronic villitis).

Data analysis and statistics

The data of fetal brain ultrasonography per brain area, and placental histology per subgroup were described. The distribution of brain sonography findings per separate brain area and the three areas combined was related to the three subgroups of placental histology (Fisher’s exact test). Additionally mild and moderate echogenicity changes in three areas combined were related to normal or abnormal (three subgroups combined) placental histology (Fisher’s exact test).

Sensitivity, specificity, positive and negative predictive values of placental histology (normal or abnormal) for moderate echogenicity changes in the fetal brain were calculated. The distribution of subgroups of placental histology and ultrasound findings over the clinical subgroups was described.

RESULTS

Of 124 pregnant women included in the study, 121 delivered in Tygerberg hospital. Due to logistic reasons (e.g. delivery elsewhere or delay of placenta retrieval, making it unsuitable for histological examination) 44 placentas could not be examined. Thus from 77 cases the placenta was available for histological examination. All but one woman delivered before 36 weeks gestational age. The clinical characteristics are presented in Table 1. The median interval between observing maximal echogenicity in one of the three areas of the fetal brain and the delivery was 4 days (range 0 to 65). Outcome of the study population is added to the table including perinatal death and severe neurological deficit (cerebral palsy, and/or blindness/deafness).
Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>n = 77</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive disorders of pregnancy</td>
<td>46*</td>
</tr>
<tr>
<td>Preterm Labour without ruptured membranes with premature ruptured membranes</td>
<td>13</td>
</tr>
<tr>
<td>Median gestational age at birth (weeks)</td>
<td>31 (range 26-38)</td>
</tr>
<tr>
<td>Median birth weight (gram)</td>
<td>1245 (range 520-2970)</td>
</tr>
<tr>
<td>Small for gestational age (&lt; 10th percentile)</td>
<td>31</td>
</tr>
<tr>
<td>Fetal death</td>
<td>4</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>9</td>
</tr>
<tr>
<td>Severe developmental deficit</td>
<td>4</td>
</tr>
</tbody>
</table>

*Including 3 fetuses with combination of the three categories, assigned to the preterm labour group

Ultrasonography

Among these 77 cases, 58 fetuses were examined once, 14 twice, 4 fetuses three times and 1 fetus four times. In 18 fetuses echogenicity changes were never observed. Thirty-four fetuses showed mild echogenicity changes, these were isolated or a combination of mild echogenicity changes in white matter, ventricular system and/or basal ganglia/thalami. Twenty-two fetuses had moderate echogenicity changes (moderate or a combination of moderate and mild changes in the white matter, ventricular system and/or basal ganglia/thalami). In three fetuses the brain could not be examined conclusively. In 29/56 fetuses with echogenicity changes, these were present in more than one area of the brain. The distribution of echogenicity changes per brain area is shown in Table 2. None of the fetuses had evidence of severe white matter injury such as cystic lesions or (haemorrhagic) infarction. In 74/77 neonates brain sonography was performed within 24 hours after birth. The various sonographic echodensities were in 43/74 confirmed in at least in 1 of the areas. In the 8 cases with PVE Grade IB seen before birth these were still present after birth in 4, while in 3 PVE Grade IA and in 1 no PVE were seen on the first day after birth. Of the 7 cases with IVE Grade II-IIII, only 2 showed a IVE Grade 1 after birth. Of the 14 cases with localized BGTE, 1 persisted after birth and 6 showed diffuse BGTE, while the remainder demonstrated no BGTE after birth.

Table 2. Distribution of placental histological subgroups over echogenicity changes per brain area in 77 fetuses

<table>
<thead>
<tr>
<th>Echogenicity changes</th>
<th>Placental histology</th>
<th>Inflammation (+ funisitis)*</th>
<th>Hyoperfusion &amp; inflammation (+ funisitis)</th>
<th>Fetal thrombotic vasculopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 16</td>
<td>n = 15</td>
<td>n = 23</td>
<td>n = 22</td>
<td>n = 1</td>
</tr>
<tr>
<td>PVE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>12</td>
<td>7</td>
<td>10 (4)</td>
<td>8 (2)</td>
</tr>
<tr>
<td>IB</td>
<td>0</td>
<td>1</td>
<td>4 (2)</td>
<td>3</td>
</tr>
<tr>
<td>IVE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1 (1)</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>3</td>
<td>2 (2)</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>BGTE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diffuse</td>
<td>3</td>
<td>2</td>
<td>5 (3)</td>
<td>6 (1)</td>
</tr>
<tr>
<td>localized</td>
<td>2</td>
<td>3</td>
<td>3 (1)</td>
<td>4</td>
</tr>
<tr>
<td>No changes</td>
<td>3</td>
<td>4</td>
<td>5 (2)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

PVE: periventricular echodensities; IVE: intraventricular echodensities; BGTE: basal ganglia/thalamic echodensities *Including one case with combination with placento-fetal hypoperfusion.

Placenta

Macroscopic examination of the placentas revealed umbilical cord knots in two cases, one real, without obstruction, and one false. Weights of the placental disc varied between 64 and 580 gram, median 305 gram. Thirty-four percent of the placental weights was below the 10th percentile. In two cases thrombosis of the fetal vessels was seen and no obstructive knotting of the cord was encountered.

Microscopic examination revealed no pathological changes in 16/77 placentas. The distribution of the placental anomalies per subgroup hypoperfusion and inflammation are depicted in Table 3. Only two placentas had two features of placento-fetal hypoperfusion. In one of these two placentas a combination with inflammatory features was seen and for statistical analysis this placenta was assigned to the inflammation subgroup. All placento-fetal hypoperfusion features found in this population were mild, with no apparent interference with placental function. Decidual necrosis only occurred in the one cases with placento-fetal hypoperfusion features only (not included in Table 3). In the 45 cases with significant inflammatory features a substantial overlap of acute and chronic inflammatory features was present. Sixteen of the 23 placentas with inflammation only, showed both acute and chronic inflammation. Of the placentas with a
combination of uteroaplacental hypoperfusion and inflammation, 3 had acute, 8 chronic and 9 acute and chronic inflammation features. In 14/45 cases with inflammatory features, funisitis Grade 3-4, a marker of fetal inflammatory response, was seen. In five of these cases this was combined with uteroaplacental hypoperfusion features (Table 2).

Table 3. Distribution of placental features in pregnancies complicated by hypertensive disorder of pregnancy and preterm labour (hypoperfusion and inflammation subgroup)

<table>
<thead>
<tr>
<th>Placental features</th>
<th>Hypoperfusion n = 15</th>
<th>Inflammation n = 23</th>
<th>Hypoperfusion/ inflammation n = 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>&lt; pso</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Umbilical cord &amp; chorionic vessels</td>
<td>Acute inflammation*</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Placental membranes</td>
<td>Acute inflammation*</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Chorionic plate</td>
<td>Acute inflammation*</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Decidual plate</td>
<td>Dense chronic inflammation</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Uteroplacental vasculitis</td>
<td>Uteroplacental hypoperfusion features:</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Fibrinoid necrosis</td>
<td>3</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>No change in spiral vessels</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Body of placenta</td>
<td>Infarcts</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Increased syncytial knots</td>
<td>8</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Proliferation of X-cells</td>
<td>2</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Excessive perivillous</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Placento-fetal hypoperfusion features:</td>
<td>Fetal vessel thrombosis</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Hypovascularity</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Intervillous thrombus</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Abnormal chorionic vasculo-syncytial membranes</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Grade 3-4 inflammation according to Salafia et al.16

Relation between placental histology and fetal brain ultrasonography
The distribution of the placental histology subgroups over the echogenicity changes in the three areas of the brain is shown in Table 2. No significant relations could be found between the placental histology subgroups and echogenicity changes in the three separate brain areas. In the majority of cases (12/16) with a normal placenta we found PVE Grade IA, while PVE Grade IB was only seen in cases with placental pathology (all subgroups). IVE (Grade I-III) and (diffuse and localized) BGTE were distributed over placentas with and without pathological features. In the five cases with funisitis combined with uteroaplacental hypoperfusion features, fetal brain examination revealed no or mild echogenicity changes.

The majority of cases (11/16) with normal placental histology showed mild and only 2/16 showed moderate echogenicity changes in the fetal brain. Of the cases with placental pathology 23/61 showed mild and 20/61 moderate echogenicity changes. Mild echogenicity changes were significantly related to normal placental histology (p = 0.048). Placental histology (normal or abnormal) in relation to presence of moderate echogenicity changes versus no and mild echogenicity changes combined, and its predictive values are shown in Table 4. As in three of the 77 fetuses the brain could not be examined conclusively, 74 cases were used for the statistical analyses.

Table 4. Relation between placental histology (n = 74) and echogenicity changes in the fetal brain and predictive values of placental histology for echogenicity changes

<table>
<thead>
<tr>
<th>Moderate echogenicity changes (n = 16)</th>
<th>Mild or no echogenicity changes (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental histology:</td>
<td></td>
</tr>
<tr>
<td>abnormal (n = 52)</td>
<td>20</td>
</tr>
<tr>
<td>normal (n = 22)</td>
<td>2</td>
</tr>
<tr>
<td>sensitivity</td>
<td>0.91</td>
</tr>
<tr>
<td>specificity</td>
<td>0.27</td>
</tr>
<tr>
<td>PPV</td>
<td>0.34</td>
</tr>
<tr>
<td>NPV</td>
<td>0.88</td>
</tr>
</tbody>
</table>

PPV: positive predictive value; NPV: negative predictive value

Relation between clinical subgroup and placental histology and fetal brain ultrasonography
Uteroaplacental hypoperfusion was found mainly in the hypertensive disorder group (80%), while only in 20% of the preterm labour group, always in combination with inflammatory changes. Inflammatory changes were found in 71% of the preterm labour group, and in 46.5% of the hypertensive disorder group, in 15/20 cases in combination with uteroaplacental hypoperfusion.

The distribution of the brain ultrasound findings over the hypertensive disorder group and the preterm labour group was as follows; no echogenicity changes were seen
in respectively 26% and 20%, mild changes in respectively 42% and 45%. PVE Grade IA was equally distributed over both groups, while IVE Grade I and diffuse BGTE were seen more frequently in the hypertensive disorder group than in the preterm labour group. Moderate echogenicity changes were seen in both groups in 29%.

**DISCUSSION**

This is the first prospective study assessing possible relationships between placental histology and fetal brain sonography findings. We related placental histology to echogenicity changes in the white matter, ventricular system, and basal ganglia/thalami of fetuses at risk of preterm birth.

In this high risk population 79% showed histopathological changes in the placentas, categorized into two major subgroups: uteroplacental hypoperfusion (19%), inflammation (30%), or a combination of the two (28%). In 73% of the fetuses echogenicity changes were seen in the brain, these were classified as mild in 44% and moderate in 29%. Of the 16 cases with normal placental histology the majority (68%) had mild echogenicity changes, while 12.5% had moderate echogenicity changes on fetal brain ultrasound examination. Of the 61 cases with pathological placental histology 38% had mild, and 33% moderate echogenicity changes. Mild echogenicity changes were significantly related to normal placental histology.

Although we found obvious differences in prevalence of echogenicity changes between fetuses with normal and pathological placentas, we did not find clear differences in the distribution of echogenicity changes over the three categories of placental pathology.

Predictive values of placental histology as such (any or a combination of the subgroups) were calculated for moderate echogenicity changes only, as we assumed that these echogenicity changes represent mild or early stage fetal brain injury. 4-6,9,24 This resulted in a high sensitivity and negative predictive value, while the positive predictive value and specificity were low. This indicates that normal placental histology is highly predictable for no or only mild echogenicity changes, while placental pathology alone does not predict moderate echogenicity changes. These results add to the presumption that at preterm age mild echogenicity changes in the periventricular white matter rather represent normal (maturational) phenomena than pathological changes, as suggested in several fetal and neonatal studies. 21,22,25-29,30

We found no relation between the two major subgroups of placental pathology and presence of echogenicity changes in specific areas of the fetal brain.

Although clinical chorioamnionitis is frequently implicated in the etiology of abnormal neonatal cranial ultrasound findings, 14,31-33 several recent studies failed to include histological chorioamnionitis as an independent causative factor of perinatal brain injury. 34 PIVH17,18,24 or cerebral palsy. 19,20 It has been hypothesized that only a pre-existing infection, eliciting a fetal inflammatory response is related to fetal brain injury. 4,6,35,36

A prospective study on the correlation between placental pathology and PIVH in very low birth weight neonates, failed to correlate chionic vasculitis and co-existing funisitis with intraventricular haemorrhages, after adjustment for gestational age and other confounders. 38 A recent study in neonates born before 32 weeks of gestation, showing a slightly increased risk for intraventricular haemorrhage in cases with severe maternal chorioamnionitis, also failed to find a significant association between the presence of a fetal inflammatory response and neonatal PIVH. 34 In the current study we neither demonstrated an association between funisitis and IVE.

Funisitis and chionic vasculitis have also previously been associated with trans-lucencies in the white matter in the early neonatal period. 4,6,36 However, since then data have emerged suggesting that additional factors like disturbance of uteroplacental circulation and ischemic changes in villi may be required for the development of white matter injury in cases of chorioamnionitis. 1,2,12,37,38 A prospective case-control study of very low birth weight neonates, Redline et al. found that only a combination of several inflammatory and uteroplacental hypoperfusion features was related to cerebral palsy. 7 Comparisons between results of imaging and outcome studies, however, need to be interpreted with caution. In an other retrospective case-control study, Viscardi and Sun found that a combination of pathological placental features, fetal growth restriction and a gestational age less than 30 weeks was related to abnormal ultrasound findings in the neonatal brain. 12

In the current prospective study we demonstrated that inflammatory changes in the placenta were not limited to the PTL group (71%), but were also seen in nearly half of the HD group (48%). We found the highest incidence of PVE Grade IB (88%) in cases with inflammatory or combined inflammatory and uteroplacental hypoperfusion features in the placenta. This may indicate that infection and uteroplacental insufficiency, apart from affecting cerebral vascular haemodynamics, 38 share a common pathway in the etiology of brain injury. Cytokines and other inflammatory agents of placental source may stimulate cytokine release in the microglia and astrocytes, ensuing fetal brain injury. However, the co-existence of inflammatory and uteroplacental hypoperfusion features in the placenta was common in our population and did not show a significant correlation with presence of moderate echogenicity changes in the fetal brain. Neither did the combined presence of funisitis and uteroplacental hypoperfusion features in the placenta correlate with moderate echogenicity changes.

The moderate echogenicity changes we encountered may not be comparable to the more severe brain injury associated with adverse neurological outcome in previous retrospective studies. 5,7,12-20 PVE Grade IB were only observed in cases with placental pathology, confirming the previously mentioned hypothesis that these represent mild or early stage white matter injury, in analogy to obviously increased echogenicity of the

88

**PLACENTAL HISTOLOGY RELATED TO FETAL BRAIN SONOGRAPHY**

89
white matter in neonates, and may precede more serious injury.25,30,48 Unlike diffuse BGTE, localized BGTE probably represents pathological changes located in the basal ganglia and/or thalami21 and were seen more frequently in fetuses with placental pathology, equally distributed over the vascular and inflammatory, and combined placental pathology groups.

In conclusion, echogenicity changes are a frequent finding in the brain of fetuses at risk for preterm birth. In this prospective study absence of placental pathology was associated with mild echogenicity changes in the white matter of the fetal brain, probably representing maturational phenomena, and predicted absence of moderate echogenicity changes, thought to represent (mild) brain injury. Placental pathology was associated with moderate echogenicity changes in the periventricular white matter.

We could not confirm that the interaction or subsequent occurrence of hypoxic-ischemic and inflammatory pathways is the likely pathway to fetal brain injury. The fact that the moderate echogenicity changes in the fetal brain were seen in all placental pathology subgroups, complies with the current views on the multi-causality of prenatal brain damage. Therefore, we believe that histological examination of the placenta in combination with the assessment of other perinatal parameters, remains an important tool to identify neonates at risk of abnormal neurological outcome, requiring brain imaging and clinical follow-up.

REFERENCES

7. Redline RW, Wilson-Costello D, Borawski E, et al. Placental lesions associated with neuro -imaging in pregnancies at risk for preterm birth. In this prospective study absence of placental pathology was associated with mild echogenicity changes in the white matter of the fetal brain, probably representing maturational phenomena, and predicted absence of moderate echogenicity changes, thought to represent (mild) brain injury. Placental pathology was associated with moderate echogenicity changes in the periventricular white matter.
26. Zeek FM, Assali NS. Vascular changes in the decidua associated with eclampticogenic toxemia
Chapter 7.

Echogenicity changes in the fetal brain, a six year follow-up study


1 Department of Obstetrics and Gynaecology, Research Institute MOVE, VU University Medical Centre, Amsterdam, the Netherlands,
2 Department of Neonatology, Leiden University Medical Centre, Leiden, the Netherlands,
3 Faculty of Human Movement Sciences, Research Institute MOVE, VU University Medical Center, Amsterdam, the Netherlands,
4 Department of Paediatrics, Tygerberg Hospital, Stellenbosch University, South-Africa,
5 Department of Obstetrics and Gynaecology, Tygerberg Hospital, Stellenbosch University, South-Africa.

(Submitted)
ABSTRACT

Background. In preterm infants brain injury is associated with abnormal neurodevelopmental outcome. Brain injury may have its origin before birth.

Aims. To evaluate in fetuses at risk for preterm birth the predictive value of echogenicity changes in the white matter, deep grey matter and ventricular and perinatal characteristics for neurodevelopmental outcome until 6 years.

Study design. Prospective study.

Subjects. 124 fetuses from pregnancies (26-34 weeks gestational age) affected by hypertensive disorders (n = 64) and/or preterm labour (n = 60).

Outcome measures. Moderate fetal and neonatal echogenicity changes (periventricular Grade IB, II, intraventricular Grade II-III, basal ganglia/thalamus locally) are related to neurological examinations and Griffith’s mental developmental scales assessments at 1, 2 and 6 years of age. Univariate and multiple regression analysis examined the relation between echogenicity changes, pregnancy and neonatal clinical parameters and composite outcome (suspect or abnormal neurodevelopment and death).

Results. Moderate echogenicity changes were present in 37/124 (30%) fetuses. Median gestational age at birth was 31 weeks (range 26-43), mortality was 18.5%. Of the 101 surviving infants 89% were examined at 1 and 2, and 67% at 6 years. The outcome was abnormal in 24/124 (19%). Fetal moderate intraventricular echodensities were related to cerebral palsy at 6 years. In the multiple regression analysis only gestational age was related to composite outcome (p = 0.005).

Conclusions. In this high risk population, fetal intraventricular echo Grade II-III were related to presence of cerebral palsy at 6 years. Gestational age at birth, however, was the main predictor of abnormal composite outcome.

INTRODUCTION

Preterm birth is an important risk factor for neurological impairment.3-4 Although at least half of children born prematurely with neurodevelopmental deficits had neuro-imaging abnormalities during the neonatal period, prediction of neurological outcome based on imaging findings is difficult.5-7 Many factors, including individual vulnerability, timing of injury, extent and localization of brain lesions, and gestational age (GA) at birth play a role.6 The outcome of neonates with even large intraventricular haemorrhages (IVH) may be favourable and will largely depend on concomitant periventricular haemorrhagic infarction (PVHI) and/or development of ventriculomegaly.8,9 White matter (WM) injury, the most frequent form of brain injury in the very preterm neonate,10-13 is associated with injury to the cortical and deep grey matter and cerebellum and probably responsible for the majority of neurological deficits in children born prematurely.11,12,14 On ultrasound it is represented by (transient) echogenicity changes in the WM.15-17 These may persist and disappear after a variable period of time or evolve into cystic lesions. Infants with transient echodensities in the periventricular WM may develop (transient) neurological disturbances and even cerebral palsy (CP).18-20

Brain injury in preterm infants may have its origin before birth.21-24 Several obstetrical risk factors for fetal and neonatal brain injury have been designated.25-28 Chorioamnionitis and premature rupture of membranes have since long been associated with brain injury and/or abnormal outcome.29,30 Contradictory results have been published for the association between fetal growth restriction (FGR), and pre-eclampsia and brain injury, claiming or refuting a protective effect.31,32

The aims of this study, part of a longitudinal study on fetal brain sonography, perinatal parameters and neurological outcome in fetuses at risk of preterm birth are to assess:

1. The prevalence of abnormal developmental and motor milestones up to 6 years of age.
2. The relation between perinatal brain echogenicity changes and perinatal clinical parameters, and neurodevelopmental outcome.

We hypothesized that antenatally sustained brain injury renders the neonate more vulnerable for (additional) injury.

METHODS

This study was conducted at Tygerberg Hospital, Western Cape, South Africa, a tertiary referral centre for Fetal-Maternal Medicine serving a mixed rural and suburban population consisting mainly of persons of mixed ethnic origin with a low socio-economic background. Tygerberg Hospital has a yearly delivery rate of approximately 5000 and a Low Birth Weight (< 2500 g) rate of 25%.

Women admitted with a singleton pregnancy with a fetus in a cephalic presentation, and at risk for preterm delivery between 26 and 34 weeks gestation were eligible for
the study. Exclusion criteria were active bleeding and/or placenta praevia, and breech presentation. The study was agreed upon by the Committee for Human Research of the Stellenbosch University. All women gave written informed consent.

**Maternal and fetal characteristics at intake**

In this study 124 women were included with pregnancies complicated by hypertensive disorders (HDP) (severe hypertension, pre-eclampsia, fetal growth restriction) or preterm labour (PTL) with or without premature rupture of membranes (PROM). Severe hypertension was defined as diastolic blood pressure $\geq 110$ mmHg. Pre-eclampsia was defined as $> 90$ mmHg with proteinuria of $> 300$ mg/24 hrs, and/or haemolysis, elevated liver enzymes and low platelets syndrome. Small for gestational age (SGA) was defined as estimated weight $<$ the 10th percentile for population specific charts. 33 PTL was accepted as regular uterine contractions resulting in cervical effacement and dilatation. PROM was diagnosed if amniotic fluid leakage or amniotic fluid pooling was seen during sterile speculum examination and confirmed by ferning and/or lacquer blue test. Documentation of GA was based on last menstrual period and early sonar examinations. Pregnancy related characteristics including maternal age, race, smoking habits, alcohol use, gestation/parity, bloodpressure changes over time in one of the three areas.

**Neonatal characteristics**

GA at birth, birth weight, Apgar scores if $< 7$ at 5 minutes, delivery mode (vaginal/caesarean section, ventouse- or forceps-extraction) and gender were noted. Birth weight centiles based on the local population were recorded. 38 Perinatal mortality was recorded. Neonatal morbidity, including respiratory distress syndrome requiring artificial ventilation ($< 48$ hours, $> 7$ days, or $> 7$ days), neonatal sepsis (defined as culture proven bacteraemia with clinical signs), major surgery, and occurrence of seizures or meningitis were noted.

**Follow-up**

At 1 year corrected age and at 2 and 6 years basic neurological examinations were performed and described as normal, suspect (minor neurological disabilities i.e. increased reflexes, gross motor delay or low tone) or abnormal (CP, visual impairment, and/or sensory-neural deafness requiring hearing devices). In addition mental developmental assessments were performed using the Griffiths Mental Developmental Scales (GMDS). 41-43

At 1 year corrected age a developmental deficit was defined as CP, bilateral sensory-neural deafness requiring hearing aid, blindness, and/or a GMDS general quotient $< 76$ ( $\pm 2$ SD below the mean). At 2 years a developmental deficit was defined as CP, bilateral sensory neural deafness requiring hearing aid, blindness, and/or a GMDS general quotient $< 80$ ( $\pm 2$ SD below the mean). At 6 years a significant developmental deficit was defined as a GMDS general outcome total scale Z-score $< -2$, and a severe developmental deficit as CP, bilateral sensory-neural deafness requiring hearing aid, blindness, and/or GMDS total scale of $< 70$ ( $\pm 2$ SD below the mean). Subscales for locomotor (gross motor), eye-hand coordination (fine motor) were defined as normal at 1 year for scores $> 80$, at 2 years for scores $> 76$, and at 6 years for scores $> 70$.

A composite outcome combining the follow-up examinations, was defined as normal or abnormal (death or a developmental deficit as defined above). In case the 6 year examination was missing, the results of the other evaluations were used for the composite outcome.

All neurological examinations and GMDS assessments were performed by the same investigator(s).
physician (J.I.v.Z.) at the high risk follow-up clinic of Tygerberg Hospital, except for the 6 years neurological examination that was performed by a paediatric neurologist.

**Data analysis and statistics**

For both fetal and neonatal ultrasound assessments the highest degree of echogenicity changes in one of the three areas were used for the statistical analyses. Fetal and neonatal moderate echogenicity changes in the three areas of interest (PVE, IVE and BGTE) separately and combined were related to outcome parameters of the follow up examinations at 1, 2 and 6 years, and (perinatal) mortality using the Fisher’s exact test. This was repeated for the composite outcome.

The effect of evolution of moderate echogenicity changes during the perinatal period on composite outcome was also examined using the Fisher’s exact test and multiple logistic regression analysis. For this analysis it was assumed that a positive change (disappearance) and a negative change (appearance) of moderate echogenicity changes from the fetal to the neonatal period would have an equal though opposite impact on composite outcome.

The relations between the pregnancy related and neonatal characteristics, and composite outcome were examined using Fisher’s exact test. For artificial ventilation, the 3 groups with increasing duration of ventilation were tested using a χ²-squared test with linear-by-linear association, and for continuous parameters (GA, birth weight, maternal age) the Mann-Whitney test was used. Stepwise logistic regression analysis was performed to test the independent influence of moderate echogenicity changes in the fetal and neonatal brain, and pregnancy related and neonatal characteristics that showed a significant relation (p ≤ 0.05) in the univariate analyses, on composite outcome.

### RESULTS

**General**

Of the 124 included women 64 (51.6%) had HD and 60 PTL (20.9%) and/or PROM (27.4%). Pregnancy and neonatal characteristics are presented in Table 1. There were 7 fetal deaths. Median birth weight was 1314 g (range 550-4330), median GA at birth 31 weeks (range 26-43). Eight neonates were born after 36 weeks GA. Two neonates were born elsewhere and no data on GA or birth weight were available. Therefore, neonatal characteristics were available for 115/117 live born neonates (Table 1). There were 8 neonatal deaths, and 8 deaths after the neonatal period, resulting in a total of 101 surviving infants available for follow-up examinations (Figure 1).

### Table 1. Maternal, fetal and neonatal characteristics

<table>
<thead>
<tr>
<th>Pregnancy related characteristics</th>
<th>%  (n = 124)</th>
<th>Neonatal characteristics</th>
<th>%  (n = 115)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive disorders</td>
<td>51.6</td>
<td>Caesarean section</td>
<td>61.7</td>
</tr>
<tr>
<td>Preterm labour</td>
<td>48.4</td>
<td>Gestational age ≤ 32 weeks at birth</td>
<td>60.0</td>
</tr>
<tr>
<td>Diastolic Blood pressure &gt; 110 mmHg</td>
<td>38.7</td>
<td>Gestational age 32-36 weeks at birth</td>
<td>33.0</td>
</tr>
<tr>
<td>SGA and UA Doppler flow &gt; P95</td>
<td>26.6</td>
<td>Birth weight &lt; P10</td>
<td>39.1</td>
</tr>
<tr>
<td>AEDF/REDF</td>
<td>16.1</td>
<td>Apgar score 5 min &lt; 7</td>
<td>7.8</td>
</tr>
<tr>
<td>Race coloured/black</td>
<td>93.6</td>
<td>Mechanical ventilation &lt; 48h</td>
<td>24.3</td>
</tr>
<tr>
<td>Multiparity</td>
<td>61.3</td>
<td>Mechanical ventilation 48h-7 days</td>
<td>13.9</td>
</tr>
<tr>
<td>Smoking</td>
<td>38.7</td>
<td>Mechanical ventilation ≥ 7 days</td>
<td>6.9</td>
</tr>
<tr>
<td>Alcohol</td>
<td>3.2</td>
<td>Major surgery</td>
<td>7.8</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>86.3</td>
<td>Seizures/ Meningitis</td>
<td>6.9</td>
</tr>
<tr>
<td>Tocolysis</td>
<td>3.2</td>
<td>Sepsis</td>
<td>16.5</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>50.0</td>
<td>Male gender</td>
<td>53.0</td>
</tr>
<tr>
<td>Anti-hypertensive medication</td>
<td>79.0</td>
<td>Neonatal death (&lt; 28d)</td>
<td>6.9</td>
</tr>
<tr>
<td>Fetal death</td>
<td>5.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SGA: small for gestational age (< 10th percentile); UA: umbilical artery; AEDF: absent end diastolic flow; REDF: reversed end diastolic flow; P: percentile.

**Figure 1. Flowchart of cases**

- 124 fetuses with brain imaging
- 115 neonates with birth parameters available
- 101 survivors (99 inborn, 2 born elsewhere*)
- 88 survivors available for follow-up examinations
- 111 composite outcomes (88 with follow-up examinations and 23 perinatal or later death)
Brain sonography
The brain could be assessed in 120/124 fetuses. Ninety-nine fetuses were examined once, 18 twice, 6 three times, and 2 four times. No echogenicity changes were seen in 31/124, mild changes in 52/124 (41.9%), and moderate changes in 37/124 (29.8%) fetuses. Echogenicity changes (mild and/or moderate) in more than one area were seen in 35/124 fetuses.

After birth 100/117 liveborn neonates were examined within 24 h after birth, and 2 were examined later during the neonatal period (both showing IVE Grade III). Weekly brain examinations were performed twice in 50, three times in 19 and four times in 4 neonates. No echogenicity changes were seen in 2/102 (2%), mild changes in 39/102 (38%), and moderate changes in 68/102 (67%) neonates. Prevalence of moderate echogenicity changes as seen by serial antenatal and neonatal brain ultrasonography in the three brain areas, and the three areas combined is presented in Table 2.

Table 2. Presence of moderate echogenicity changes in periventricular white matter (PVE Grade IB and II), ventricular system (IVE Grade II and III) and basal ganglia and thalami (localized BGTE) as assessed over time during the fetal and neonatal period

<table>
<thead>
<tr>
<th>Echogenicity changes</th>
<th>Fetal brain (n = 124)</th>
<th>Neonatal brain (n = 109)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVE</td>
<td>9</td>
<td>55</td>
</tr>
<tr>
<td>IVE</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>BGTE</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>PVE + IVE</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>PVE + BGTE</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>PVE + IVE + BGTE</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>IVE + BGTE</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
</table>

The evolution over time of moderate echogenicity changes was as follows: in 24 cases moderate echogenicity changes and in 28 cases no moderate echogenicity changes were seen before as well as after birth. A change in echogenicity was seen in 6 cases with moderate echogenicity changes seen before birth and not after birth, and in 40 infants showing no moderate echogenicity before but only after birth. In 8 cases follow-up examinations were done, but no neonatal brain assessment could be performed during the neonatal period.

Follow-up
Due to the large referral area and long travel distances, there was a substantial loss to follow-up of the 101 survivors. Nine infants could not be traced, 2 did not show up for any follow-up visit and 2 moved away from the area after the 6 months visit. Of the remaining 88 infants, 78 (89%) were seen at 1 year corrected age, 79 (89%) at 2 years and 59 (67%) at 6 years of age. Several families had moved out of the region after the 1 or 2 year visit. Fifty-six (64%) infants were seen at 1, 2 and 6 years.

In Table 3 the results of the follow-up examinations are depicted for the whole population. Of the infants who were not seen at 6 year, the composite outcome was based on the examinations until 2 years of age in 21 (2 abnormal) and until 1 year corrected age in 8 (1 abnormal).

Table 3. Motor and neurodevelopmental outcome

<table>
<thead>
<tr>
<th></th>
<th>n = 101 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basic neurological examination</strong></td>
<td></td>
</tr>
<tr>
<td>1 year c.a.</td>
<td>n = 78 (77)</td>
</tr>
<tr>
<td>Minor neurological abnormalities</td>
<td>8 (10)</td>
</tr>
<tr>
<td>Cerebral palsy</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Sensory-neural deafness/blindness</td>
<td>1 (1)</td>
</tr>
<tr>
<td>2 years</td>
<td>n = 79 (78)</td>
</tr>
<tr>
<td>Cerebral palsy</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Sensory-neural deafness/blindness</td>
<td>1 (1)</td>
</tr>
<tr>
<td>6 years</td>
<td>n = 59 (58)</td>
</tr>
<tr>
<td>Cerebral palsy</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Sensory-neural deafness/blindness</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Griffith Mental Developmental Scale</strong></td>
<td></td>
</tr>
<tr>
<td>1 year</td>
<td>n = 78 (77)</td>
</tr>
<tr>
<td>General quotient &lt; 76</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Locomotor subscales (gross motor) &lt; 80</td>
<td>7 (9)</td>
</tr>
<tr>
<td>Eye-hand subscales (fine motor) &lt; 80</td>
<td>1 (1)</td>
</tr>
<tr>
<td>2 year</td>
<td>n = 79 (78)</td>
</tr>
<tr>
<td>General quotient &lt; 80</td>
<td>7 (9)</td>
</tr>
<tr>
<td>Locomotor subscales (gross motor) &lt; 76</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Eye-hand subscales (fine motor) &lt; 76</td>
<td>7 (9)</td>
</tr>
<tr>
<td>6 year</td>
<td>n = 59 (58)</td>
</tr>
<tr>
<td>General quotient &lt; 70 (severe delay)</td>
<td>7 (12)</td>
</tr>
<tr>
<td>Z-score &lt; 2 (2 SD below mean) and general quotient &gt; 70 (delay)</td>
<td>14 (24)</td>
</tr>
<tr>
<td>Locomotor subscales (gross motor) &lt; 70</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Eye-hand subscales (fine motor) &lt; 70</td>
<td>3 (5)</td>
</tr>
<tr>
<td><strong>Composite outcome</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>64 (52)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>24 (19)</td>
</tr>
<tr>
<td>Alive</td>
<td>23 (18)</td>
</tr>
<tr>
<td>Dead</td>
<td>13 (10)</td>
</tr>
<tr>
<td>No follow-up</td>
<td>13 (10)</td>
</tr>
<tr>
<td><strong>Death</strong></td>
<td></td>
</tr>
<tr>
<td>Before birth</td>
<td>7 (6)</td>
</tr>
<tr>
<td>&lt; 28 days after birth</td>
<td>8 (6)</td>
</tr>
<tr>
<td>≥ 28 days after birth</td>
<td>8 (6)</td>
</tr>
</tbody>
</table>
Of the infants with moderate echogenicity changes during the fetal period 21 (61.8%) had a normal and 13 (38.2%) an abnormal composite outcome. Of the infants with moderate echogenicity changes during the neonatal period 31 (48%) had a normal and 33 (52%) an abnormal composite outcome.

The individual trajectories of brain ultrasound examinations and follow-up examinations of surviving infants with an abnormal neurological outcome are depicted in Table 4.

Table 4. Individual trajectories of fetal and neonatal brain ultrasound findings, characteristics, and follow-up data of infants with adverse neurodevelopmental outcome at 6 years

<table>
<thead>
<tr>
<th>n</th>
<th>Fetal brain</th>
<th>Neonatal brain</th>
<th>GA</th>
<th>Birth weight</th>
<th>1 year</th>
<th>2 year</th>
<th>6 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CP</td>
<td>GMDS n &gt; 80</td>
<td>CP</td>
</tr>
<tr>
<td>1</td>
<td>IVE II</td>
<td></td>
<td></td>
<td></td>
<td>HP</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>IVE I</td>
<td>PVE IB, BGTE diffuse</td>
<td>29+3</td>
<td>1046</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>PVE IA, BGTE diffuse</td>
<td>IVE III, PVHI</td>
<td>29+0</td>
<td>1020</td>
<td>S</td>
<td>62</td>
<td>HP</td>
</tr>
<tr>
<td>4</td>
<td>PVE IA, BGTE localized</td>
<td>-</td>
<td>33+6</td>
<td>2150</td>
<td>N</td>
<td>77</td>
<td>N*</td>
</tr>
<tr>
<td>5</td>
<td>PVE IA, IVE II</td>
<td>PVE IB, IVE III, BGTE localized</td>
<td>28+5</td>
<td>540</td>
<td>SD</td>
<td>50</td>
<td>HP</td>
</tr>
<tr>
<td>6</td>
<td>N</td>
<td>PVE IA</td>
<td>33+2</td>
<td>1906</td>
<td>N</td>
<td>96</td>
<td>N*</td>
</tr>
<tr>
<td>7</td>
<td>PVE IA</td>
<td>PVE IB, IVE I</td>
<td>29+5</td>
<td>1318</td>
<td>SD</td>
<td>50</td>
<td>A</td>
</tr>
</tbody>
</table>

GA: gestational age; CP: cerebral palsy; GMDS: Griffiths mental developmental scores general quotient; PVE: periventricular echogenicity changes; IVE: intraventricular echogenicity changes; BGTE: basal ganglia/thalami echogenicity changes; PVHI: periventricular haemorrhagic infarction.

N: normal; S: suspect; A: abnormal; SD: spastic diplegic; HP: hemiplegic, QP: quadriplegic.

*sensorineural deafness
†epilepsy

Moderate fetal IVE related significantly with CP at 6 year (p = 0.037), while a trend for abnormal GMDS gross motor subscale at 1 year (p = 0.06) was found. Fetal IVE were not related to fetal or neonatal death, or to the composite outcome. Presence of moderate PVE, BGTE or any combination of these echogenicity changes in the fetus did not significantly relate to any of the outcome parameters (1-6 years) or GMDS (1-6 years) or to death during the fetal period or later.

Of the moderate echogenicity changes seen during the neonatal period moderate IVE related significantly to an abnormal GMDS at 2 years (p = 0.001), and CP at 1 and 6 years (p = 0.018 and p = 0.033 respectively). Moderate IVE also related significantly to an abnormal composite outcome (p = 0.029), while moderate echogenicity changes of the 3 areas combined showed a trend for abnormal composite outcome (p = 0.054).

The analysis of evolution of moderate echogenicity changes over time showed a relative risk for abnormal composite outcome of 35.7% for cases with no moderate echogenicity before or after birth, and a risk of 45.8% for cases with moderate echogenicity changes before and after birth. Infants with absence of moderate echogenicity changes before birth, but presence after birth, had the highest risk of abnormal composite outcome (55%). The lowest risk of abnormal composite outcome was seen when moderate echogenicity changes were present in the fetal, but not in the neonatal period (0%).

Of the various maternal, fetal and neonatal characteristics (Table 1) lower GA (p = 0.000), presence of reversed or absent end-diastolic flow in the umbilical artery (p = 0.006), lower birth weight (p = 0.000), Apgar score < 7 at 5 min (p = 0.007), and meningitis/seizures (p = 0.010) were significantly related to the composite outcome. A trend for influence of increasing duration of artificial ventilation on composite outcome was found (p = 0.057).

In the stepwise multiple logistic regression analysis, after successive introduction of GA, seizures/meningitis and AS < 7 at 5 min, the composite outcome was only significantly related to GA (p = 0.009; OR 2.6; 95% CI 1.46-5.86) after adjusting for GA, seizures/meningitis and AS < 7 at 5 min, the composite outcome was only significantly related to GA (p = 0.009; OR 2.6; 95% CI 1.46-5.86) after adjusting for GA, seizures/meningitis and AS < 7 at 5 min, the composite outcome was only significantly related to GA (p = 0.009; OR 2.6; 95% CI 1.46-5.86) after adjusting for GA, seizures/meningitis and AS < 7 at 5 min. However, due to the fact that the group of infants with presence before and absence after birth (disappearance or decrease) of echogenicity changes (n = 6) did not contain any infants with an abnormal outcome, the assumption that the impact of a positive change (disappearance) or the impact of a negative change (appearance) of moderate echogenicity changes on the composite outcome was equal could not be verified.
DISCUSSION

This is the first prospective study relating fetal and neonatal brain echogenicity changes to neurological outcome up till 6 years of age in a population with HD of pregnancy or PTL at risk of preterm birth. Moderate echogenicity changes were seen in 29.8% of the fetuses and equally distributed over the three regions of interest. The neurodevelopmental outcome was normal in half of this population (64/124). Of the 47 (38%) infants with abnormal composite outcome 8 (6.4%) had major neurological disorders and 23 (18.5%) died, 15 during the perinatal period and 8 thereafter.

Moderate IVE in the fetal brain related to CP at 6 years of age, but not to an abnormal composite outcome. The outcome of fetuses with IVE Grade I is reported to be favourable. IVE Grade II may resorb in utero, but a small study reported an adverse outcome in up to 50%.

In our population, apart from IVE Grade I-III, no other haemorrhages were encountered before birth, while only one case with PVHI was seen after birth. Neonatal moderate IVE was related to CP at 2 and 6 years, abnormal GMDS at 2 years, and to abnormal composite outcome. The outcome at 2 years of age of extremely low birth weight neonates with small IVH was comparable to the outcome of neonates without ultrasound abnormalities. In another cohort of extremely low birth weight neonates 48% of neonates with small IVH had a neurological impairment and 13% major neurological abnormality at 20 months, both significantly higher than for neonates without IVH. For larger IVH adverse outcome ranges between 35-50%, increasing to 75% in presence of PVHI.

Moderate fetal PVE or BGTE were not associated with abnormal outcome. Yamamoto et al. reported a comparable persistence of PVE Grade IB in 22/42 fetuses after birth, but no information on neurodevelopmental outcome was provided.

Of preterm neonates with transient PVE, especially extensive and in the parieto- or occipital region, up to 10% may develop (mild) CP. Localized uni- and bilateral BGTE have been described in low and high risk fetuses during the second trimester, all disappearing in mid pregnancy. Van Gelder et al. examined a population of fetuses at risk for preterm births in the third trimester of pregnancy. Localized BGTE was seen in 19%, were mostly isolated and unilateral and no longer visible after birth. One of the five infants with fetal BGTE developed minor neurological disorders and another CP, but both had concomitant PVE Grade IB/II during the neonatal period. In preterm neonates unilateral localized BGTE generally disappear before term age. In some cases motor disturbances were present during the first year, but no significant difference in short term outcome was found between infants with and without BGTE.

Of the pregnancy related characteristics only absent or reversed end diastolic flow in the umbilical artery significantly related to composite outcome, but this effect disappeared after adjusting for GA. Therefore, although abnormal umbilical artery Doppler indices was recently associated with adverse outcome, we could not confirm this association. The fact that betamethasone use before birth was not associated with abnormal composite outcome is in contradiction which previous reports showing a decrease in adverse outcome. However, in our study in the majority of pregnancies betamethasone were administered. In another study cohort of neonates born between 22 and 32 weeks GA did neither demonstrate an association between corticosteroid administration before birth and adverse outcome. In our population presence of severe hypertension (RR > 110 mmHg) or SGA (with abnormal Doppler indices) did not positively or negatively influence the composite outcome.

Of the neonatal characteristics GA at birth, birth weight, AS < 7 at 5 min, and seizures/meningitis were related to composite outcome. Longer artificial ventilation showed a trend for abnormal composite outcome, but similar to the results of the EPIPAGE cohort of preterm neonates, was not significantly related to the composite outcome after adjusting for GA. GA contributed most to an abnormal composite outcome, as was also shown for the EPIPAGE cohort.

The prognostic significance of echogenicity changes in the fetal brain was so far only scarcely studied. Our finding that moderate echogenicity changes in the fetal brain are not related to adverse outcome is reassuring. This may indicate that some moderate echogenicity changes (e.g. in the white and deep grey matter) are reflections of maturational processes. This is supported by the fact that transient PVE occur most frequently in the most immature neonates. Other explanations may be that at early GA the fetal brain reacts differently and with less vigour to injury than at a later stage or that the developing brain is capable to overcome mild injury if no additional injury is sustained during the neonatal period.

We postulated that antenatally sustained brain injury renders the neonate more vulnerable for (additional) injury, but we could not demonstrate this. Interestingly, we found that infants with no moderate echogenicity changes before birth, but only after birth had a 2.5 fold higher risk of dying or abnormal outcome as compared to those without moderate echogenicity changes before and after birth, while infants showing moderate echogenicity changes before birth and none after birth had a 2.5 fold lower risk. GA at birth did not influence this relationship. In a follow-up study of high risk neonates with PVL, and/or IVH diagnosed during the neonatal period only 48% of infants born < 32 weeks as compared to 83% between 33-36 weeks GA developed deficits. It was suggested that the plasticity of the immature brain prevents the development of permanent deficits. Our results suggest that the immature fetal brain is more capable of restoring and adapting after (mild) injury than the (immature) neonatal brain.

A limitation of our study is that due to early transfer to secondary hospitals, not in all neonates consecutive weekly brain examinations could be performed, while none could be examined at term equivalent age. Another limitation is the loss to follow-up in our study population.
Concluding, in this population at risk for preterm birth, a third of the fetuses showed moderate echogenicity changes in the periventricular WM, ventricles, and/or basal ganglia/thalami. The neurodevelopmental outcome was abnormal in 27% (24/88) of surviving infants that were seen for follow-up and the adverse composite outcome (including deaths) of the total included population was 38% (47/124). Fetal IVE correlated with CP at 6 years, but not with the composite outcome. GA, absent or reversed end-diastolic flow in the umbilical artery, birth weight, Apgar score < 7 at 5 min, meningitis/seizures and IVE in the neonatal period were significantly related to the composite outcome. After adjusting for GA, none of the other clinical or ultrasound parameters correlated with composite outcome. The results of our study support the general impression that combining various parameters enhances the possibilities to detect infants at risk for adverse outcome.

REFERENCES


Chapter 8.

General discussion

INTRODUCTION

The implementation of serial sonographic evaluation of the brain in preterm infants at high risk of abnormal neurological development, has impact on the counselling of parents. Knowledge is available which areas of the brain are most vulnerable for injury at a particular age. Evaluation of the degree and extend of brain injury and its evolution over time provides information on (possible) neurological sequelae. Studies in neonates revealed that certain perinatal factors increase the risk of brain injury.

Nowadays sonographic examination of the fetal brain is possible in a similar way as in the neonate. This has provided possibilities to gather more insight in the fetal central nervous system integrity. During the last decades age-related developmental milestones of various brain structures (e.g. gyri, cerebellar hemispheres and vermis, corpus callosum, ventricles) have been examined before birth. A logical next step was to assess acquired brain injury in fetuses of high risk pregnancies.

By serial ultrasound imaging of the fetal brain the occurrence and timing of echogenicity changes and their evolution over time can be studied. This may help to recognize fetuses with brain injury, at risk for adverse outcome, who may benefit from early interventions and follow-up. Before it can be presumed that echogenicity changes in the fetal brain are signs of brain injury, as has been described for neonates, further study is required.

The aim of this study was to assess whether evaluation of the fetal brain by sonography has additional value to other antenatal clinical parameters including cardiotocography, fetal growth, Doppler indices, and motility on the prediction of fetal condition, risk of brain injury and neurological outcome.

Hereof the prevalence of echogenicity changes in three brain regions of interest (periventricular white matter, ventricular area, grey matter of basal ganglia and thalamus) in a population of pregnancies compromised by hypertensive disorders (HD) and preterm labour (PTL) and the continuity of these echogenicity changes over time were examined before birth and until the first weeks after birth.

This prospective study was performed in the metropolitan area of Cape Town, South-Africa. The population is a mixed rural and suburban population, with mostly a low socio-economic status. Tygerberg Hospital, a tertiary referral centre for Fetal-
Maternal Medicine, has a large drainage area and some patients are transported for long distances to receive special care. This population, consisting of mostly coloured women (90%), has 7.7% HD of which 5.5% pre-eclampsia (unpublished data Tygerberg Hospital) and a preterm delivery rate of approximately 20%. Smoking, alcohol abuse and low socio-economic status play an important role in the increased risk of adverse pregnancy outcome in this population.

A permanent team of investigators was involved in the study, guaranteeing continuity of the various examinations and assessments. The inclusion of the women, perinatal ultrasound examinations, computerised heart rate tracings and assessments of fetal motility were performed by one person. Evaluation of the perinatal brain ultrasound examinations and movement assessments were all performed by a team of experienced investigators, blinded to the clinical data. The placental macroscopic and histological examinations were assessed in consensus by two experienced pathologists. The neurodevelopmental evaluation of infants was performed by one physician at the high risk clinic of the department of Paediatrics. These examinations were carried out from three months post term equivalent age onwards to 6 years age. In the setting of a tertiary clinic, however, early transferral of stable infants to secondary hospitals or early discharge, interfered with the aim of weekly examinations during the neonatal period in some cases and with regular follow-up visits at the paediatric outpatient clinic in some (other) cases because of long travel distances.

**GENERAL RESULTS OF THE STUDY**

This is the first large prospective study on prevalence of brain echogenicity changes in the fetus. Our study showed that in a population at risk for preterm birth either as a result of HD or PTL, more than two thirds of the fetuses showed echogenicity changes in one or more of the three brain regions of interest that are known the be vulnerable to injury in the preterm neonate. Periventricular echodensities (PVE) were seen in half, basal ganglia/thalamic echodensities (BGTE) in a quarter, and intraventricular echodensities (IVE) in a fifth of the fetuses. In a third of the fetuses there were echogenicity changes in two or all three of the evaluated brain areas. Gestational age (GA) did not influence the presence of echogenicity changes, as these were found during the whole GA period, equally distributed over pregnancies with HD or PTL.

Serial brain sonography before and into the first week after birth showed a dynamic process of appearance, persistence, increase or fading of echodensities. A third to half of the echogenicity changes seen in the fetus were still present on the first day after birth. Almost all neonates presented with echogenicity changes on the first day after birth. The dynamic process of echogenicity changes in the fetus are in accordance with findings in the neonate. A few small high risk populations of fetuses have been studied previously for various echogenicity changes in the fetal brain. A similar pattern of disappearance, persistence or progression of echogenicity changes after birth was demonstrated.

The prevalence of echogenicity changes in the brain on the first day after birth in our population was rather high as compared to described populations of high risk neonates. Mild echogenicity changes when found within ± 24 hours after birth may be explained by congestion, but more severe or persisting echodensities or translucencies probably represent pathology.

We describe our study results for prevalences and evolution of echogenicity changes in the fetal brain. This is followed by the relation between these echogenicity changes and several perinatal parameters, placental pathology and by neurological outcome of the infants.

**Echogenicity changes in fetal brain**

**Echogenicity changes in the periventricular white matter**

We found a high prevalence (43%) of mild PVE (Grade 1A), its extend ranging from only frontal (two thirds of the cases), parietal, or occipital, to more widespread (for Grading see Table 1, Chapter 1). Mild echodensities have been found in the frontal areas of the brain of low risk fetuses between 26-28 weeks GA and in the frontal and parietal periventricular regions in preterm neonates. Mild, symmetric echodensities in the frontal region represent migrating glial cells, while mild, symmetric echodensities in the parietal white matter (WM) may represent the optic radiation or periventricular crossroads of dense white matter fibres, thus being normal (maturational) phenomena.

In a high risk population with HD, PVE Grade 1A were seen later in pregnancy than in a low risk population and it was hypothesised that the persistence or appearance at a later GA indicate delayed maturation. Absence of Grade 1A frontal PVE in the second and early third trimester of pregnancy may represent delayed or disturbed maturation. In our population PVE Grade 1A were seen between 26-35 weeks gestation, the median age being 29 weeks for frontal PVE, and 28 weeks for PVE elsewhere. Serial sonography showed persistence, fading, but also progression of PVE from Grade 1A to Grade IB (the echogenicity exceeding that of the choroid plexus) before birth. Half of the fetuses with PVE Grade 1A showed PVE Grade IB or II (small, localized cystic lesions) after birth. This was in agreement with another study on a population of high risk fetuses. They found PVE Grade 1A to be followed by persisting PVE Grade IB after birth in 4/26 fetuses. In 2 cases subsequent mild neurological sequelae ensued. In these cases, the mild echogenicity changes may therefore have represented early stage or mild injury.

PVE Grade IB were seen in all the periventricular WM regions. In 3/9 fetuses with PVE Grade IB the echogenicity faded to PVE Grade 1A or disappeared before birth. In 5/8 cases with PVE grade IB still present on the last examination before birth it persisted after birth. In the remaining 3 cases it faded to PVE Grade 1A. Yamamoto et al. have described comparable echodensities in 42/64 (66%) of high risk fetuses, of which 22 persisted after birth. In 5/22 infants with persisting PVE cysts developed after birth.
In our population of the PVE Grade 1B or Grade II seen before birth, half persisted, and the remainder were not seen after birth. It is likely that these moderate PVE in the fetus represent mild or early stage injury, as has been described in neonates. In most prematurely born neonates PVE tend to resolve without cystic evolution, but in some cases WM volume loss will ensue. These non-cystic PVE may represent subtle or diffuse WM injury. So, like in the neonate, PVE Grade 1B seen in the fetus may represent WM matter injury. We conclude that PVE Grade 1A, especially in the frontal and parietal regions, probably represent normal maturational phenomena in the fetal period. However, if they are extensive, persist or increase in echogenicity, they may represent mild or early stage WM injury. PVE Grade 1B and II seen in the fetal period, probably represent (mild) WM injury.

**Intraventricular echogenicity changes**

IVE were frequently seen in this high risk population, mainly in fetuses with gestational ages between 26–32 weeks. They were present in a fifth of the fetuses, eight having IVE grade I, ten IVE Grade II and five IVE Grade III (for Grading see Table 1, Chapter 1). Before birth, in four fetuses IVE persisted and in three these were transient. Half of the IVE seen in the fetal brain, were not visible after birth. In 2/8 cases in which IVE persisted after birth, a progression was seen. Several case reports of fetal intraventricular haemorrhage (IVH) varying from Grade I-IV, detected by sonography have been published. Disappearance of antenatal haemorrhage Grade II has been described. In our study, however, in some of the cases the interval between the two examinations was rather short and resorption of the haemorrhage was therefore unlikely. We postulated that in some cases the choroid plexus can be very prominent or irregular and may be mistaken for intraventricular haemorrhage, as has been previously described. Also in preterm neonates the choroid plexus may sometimes erroneously lead to diagnosis of IVH. It was concluded that interpretation of IVE in the fetus should be done with caution, and preferably only after serial examination, as in the fetus IVE do not necessarily represent IVH.

**Echogenicity changes in the basal ganglia and/or thalami**

Increased echogenicity of the basal ganglia and/or thalami was seen in 35/124 (28%) fetuses. BGTE were diffuse in 17 fetuses (median GA 28 weeks) and localized in 18 fetuses (median GA 30 weeks). More than two thirds of the BGTE were seen in combination with PVE, IVE or both. Localized BGTE were unilateral in 6.5% and bilateral in 8.0%, and accompanied in half of the fetuses by IVE Grade II-III. Most of the BGTE were transient and faded before birth. In half of the cases with localized BGTE, diffuse BGTE were seen the first day after birth. In only one case with localized BGTE this persisted after birth, while the remainder disappeared.

In fetuses increased echogenicity in the basal ganglia has been overrated when scanning in axial planes instead of coronal planes and with lower frequency transducers. This is ascribed to a varying appearance of tissue, depending on the angle of insonation of the ultrasound beam. This was not the situation in our study. However, a diffuse bilateral haze over the basal ganglia/thalami is seen in the vast majority of neonates born very prematurely and was found to gradually fade over time, while no MRI abnormalities were encountered in the basal ganglia/thalami. Mild, diffuse BGTE therefore probably represent a normal maturational phenomenon.

Localised uni- and bilateral BGTE have been described in seven (low and high risk) fetuses between 14–16 weeks gestation, all disappearing in mid pregnancy. Van Gelder et al. described unilateral, mostly isolated BGTE in 19% of the studied high-risk fetuses, and these were no longer visible after birth. Unilateral BGTE in (pre)term neonates are ascribed to haemorrhage or infarction in the region of the middle cerebral artery. In a study of preterm neonates unilateral localized BGTE was found in 5.3%, a similar incidence as in our population of high risk fetuses. In our study, localized BGTE were often accompanied by IVE lettertype? Grade II-III, and generally associated with placental pathology. We therefore postulate that, as opposed to diffuse BGTE, localized BGTE seen in the fetus represent injury to the deep grey matter, being transient in some cases.

**General movements**

In infants born prematurely, quality of general movements (GMs) is correlated with presence of brain lesions and regarded, especially at three months postterm, as a valuable additional tool to assess the neurological prognosis. The prevalence of abnormal GMs in this population was high (58%) and correlated with HD (p = 0.015) and spatial restriction caused by oligohydramnios (p = 0.002).

An important result of our study was that fetuses with normal GM quality were unlikely to have moderate brain echogenicity changes. The sensitivity of the quality of fetal GMs for moderate echogenicity changes in the three brain regions of interest was 0.85 and the negative predictive value 0.73. Of the three brain areas, GMs had the highest sensitivity (0.96) for moderate echogenicity changes in the periventricular area, indicating that almost all moderate PVE were identified by abnormal GMs. The positive predictive values of abnormal GMs were low for all echogenicity changes, which can be explained by the high prevalence of abnormal GMs in this population. The negative predictive values of GMs for moderate PVE, IVE and BGTE were high (0.85, 0.83 and 0.81 respectively). This is an important finding as it indicates that fetuses with normal GM quality are unlikely to have moderate brain echogenicity changes that are presumed to represent mild brain injury.

Fetal and neonatal GM quality correlated with each other (p = 0.045). Previous perinatal motility studies in compromised pregnancies showed that abnormal GMs tend to normalize within 10 weeks after birth. In the majority of our population (68%), normalization occurred within 7 days after birth. This finding is in line with a study of a
population of neonates with transient PVE or with periventricular leukomalacia (PVL), showing abnormal GMs only during the first two weeks after birth. The rapid normalization of the GMs after birth can be explained by improvement of reduced spatial restriction and/or undernourishment in compromised fetuses after birth.

Like before birth, in the first week after birth GM quality was not related to moderate echogenicity changes. The few studies that assessed the relation between brain ultrasound findings and GMs in the first weeks after birth, did not find a relation between abnormal GMs and ultrasound abnormalities. It may be true that only from three months post term equivalent age GMs are related to ultrasound abnormalities. On the other hand, in a study of (very) prematurely born neonates all had abnormal motility during the first two weeks after birth, but those who showed normal motility intermittently did not have PVE or PVL.

Cardiotocography
In the matched case-control study of fetuses with and without moderate echogenicity changes, cardiotocography did not identify fetuses with moderate PVE/IPE/BGTE. We found that both in fetuses with and without moderate PVE the heart rate variability (long term and short term variability) was significantly lower than for a normal population (p < 0.001), illustrating the compromised status of this high risk population. A reduced heart rate variability as possible result of mild or early stage brain injury may have been obscured by the already reduced variability caused by various conditions like early GA, chronic hypoxemia in fetal growth restriction (FGR), maternal medication use, and intra-uterine infection. Alternatively, the fetuses with moderate PVE may already have experienced hypoxic ischemic injury prior to the moment of heart rate recording. During the interval between the insult and the appearance of echogenicity changes, heart rate changes that were present may subsequently have disappeared.

Although the number of fetuses studied was small, the strength of our study is that the brain was assessed on the same day as the heart rate tracings, the latter having an adequate length of recording. Furthermore, computer analysis overcomes intra- and inter observer variability. Okamura et al., studying fetal heart rate tracings of 103 neonates of which 19 neonates with cystic PVL, retrospectively demonstrated a significantly higher PVL incidence in fetuses with abnormal, oscillatory heart rate tracing patterns. In another study on neonates, intraventricular haemorrhages was related to a decreased heart rate variation. A more recent case-control study, however, did not find an association between a decreased short-term variability with late decelerations and cerebral WM injury in preterm neonates.

Cardiotocography is a widespread used parameter of fetal surveillance and important for timing of delivery in cases of HD and PTL. Elaborate studies have been performed to analyse factors that possibly influence fetal heart rate and heart rate variability. Studies on heart rate and heart rate variability in the neonate are scarce. Collaboration between perinatologists may encourage the investigations of this parameter after birth. We have shown for our high risk population that cardiotocography did not identify fetuses with brain injury.

Placental histology
We found a high prevalence of placental pathology (79%) in our high risk population, the combination of inflammatory and uteroplacental hypoperfusion features being common (29%). In fetuses from HD pregnancies, hypoperfusion and inflammatory placental pathology were present in 80% and 46% respectively. All moderate PVE (often present in combination with IVE Grade II-III) were associated with placental pathology (utero-placental hypoperfusion, inflammatory or both). A common inflammatory pathway for fetal brain injury has been suggested with additive effects of placental insufficiency and infection. Other studies found that FGR or low GA contributed most to the association between placental pathology and brain ultrasound abnormalities.

A fetal inflammatory response to a pre-existing intrauterine-infection has been associated with IHV or PVL in neonates and is regarded as attributing factor in adverse outcome. Other studies, however, could not confirm this relationship. It has been suggested that placental inflammation is not related to brain lesions or smaller regional brain volumes in very preterm infants. This may be in accordance with our results as we did not find a correlation between funisitis and moderate echogenicity changes in the fetal brain.

Our results may, however, be limited by the fact that severe placental pathology, (i.e. placenta floor infarcts, massive perivillous fibrin deposits, fetal thrombo-vasculopathy, abruptions and hemorrhagic endovasculitis) previously associated with brain injury, was only scarcely present in our population.

Outcome
In our study population mortality was 12% during the perinatal period and 6.5% within the first year of life, the total mortality being 18.5 % (23/124). Despite extensive efforts of research nurses to reach the families at their homes and inviting them for follow-up visits, there was a substantial loss to follow-up examinations. Of the 101 surviving neonates, 88 were seen during the follow-up period from 3 months corrected age until 6 years of age for neurological examinations. At 1, 2 and 6 years of age Griffith's Mental Developmental Scales (GMDS) tests were performed, validated for the South-African population.

At 6 years of age 59/101 surviving infants were examined, 4 (3.9%) had both cerebral palsy and a severe developmental deficit, while 3 (2.9%) infants had a severe and 14 (13.8%) a significant developmental deficit (GMDS Z-score < -2). Of the infants that were not seen at 6 year, the composite outcome was based on the 2 year results in 21 (of which 2 were abnormal) and on the 1 year results in 8 infants (of which 1 was abnormal). The composite abnormal outcome of the whole population, combining suspect and abnormal
outcomes of the successive neurological and GMDS examinations at 1, 2 and 6 years and mortality, was 38% (47/124). The mortality of our study is in line with other recent studies. It is difficult to compare our outcome results with previous reports, as the figures depend on the populations studied (i.e. extremely low birth weight versus low birth weight and extremely preterm (< 28 weeks GA) versus very preterm birth (< 33 weeks GA) and the time period of the studies. Although more than half of preterm born children with neurological handicaps had brain abnormalities detected during the neonatal period, prediction of neurological outcome based on neonatal brain ultrasound findings remains difficult. Not only the extend and localization of lesions, but also the plasticity of the immature brain play an important role in the neurodevelopmental outcome. Follow-up studies of neonates with transient PVE show that most infants have a normal neurodevelopmental outcome, but especially in infants with inhomogeneous PVE, neurological disturbances may occur. When transient PVE are extensive and localized in the parieto- or occipital region, 10% of the infants may still develop (mild) CP. Echogenicity changes in the neonatal brain are more likely to give subsequent neurological problems, even when localized. Extensive cysts have considerable risk of not only CP, but also of cognitive and visual impairments. The outcome of preterm neonates with small IVH was reported to be generally favourable. However, recently, a significant higher risk for adverse outcome at 2 years of age was demonstrated for extremely preterm neonates with small IVH as compared to neonates without ultrasound abnormalities. Still the outcome of neonates with even large IVH may be favourable, but will largely depend on the GA at birth, concomitant WM injury and/or subsequent development of ventriculomegaly (post-haemorrhagic ventricular dilatation). In preterm neonates unilateral localized BGTE was often associated with a complicated neonatal course and long artificial ventilation. Generally these BGTE disappeared before term age and were associated with mild motor disturbances during the first year, but no significant difference in short term neurological outcome. Whether echogenicity changes in the fetal brain have the same neurological prognosis as echogenicity changes in the neonatal brain is uncertain.

- In the two earlier mentioned studies of high risk populations, in one study 4/22 fetuses with PVE persisting after birth developed CP during infancy. In the other study, 2/4 cases with PVE Grade IA in the fetus, followed by persisting Grade IB after birth, showed mild neurological sequelae at 24 months follow up. In our larger population we did not demonstrate a relation between moderate PVE and abnormal outcome. Moderate IVE Grade II have been described to disappear in utero, but an adverse outcome in up to 50% of cases was reported. IVE Grade III and PVHI, diagnosed before birth are strongly associated with an adverse outcome. In our population moderate IVE (Grade II and III) were significantly related to abnormal outcome at 6 years of age. It was not related to composite abnormal outcome (a combination of abnormal neurodevelopment and mortality).

• Localized BGTE described in the high risk population of Van Gelder et al. mostly disappeared after birth without further sequelae, but 2/5 infants showed minor neurological disorders and CP at 2 year of age, but both had concomitant PVE Grade IB/II during the neonatal period. In our population localized BGTE was not related to abnormal (composite) outcome.

The interpretation of a possible association between echogenicity changes in the fetal brain and outcome parameters is hindered by the fact that a large overlap of echogenicity changes in the three studied brain areas existed before as well as after birth. Presence of fetal echogenicity changes in the three areas combined, however, was not related to abnormal (composite) outcome.

Neonatal IVE was related to neurodevelopmental outcome at 2 years, CP at 2 and 6 years and the composite outcome. Neonatal moderate echogenicity changes of the three areas showed a trend for abnormal composite outcome. But after adjusting for GA, none of the brain ultrasound examinations (fetal or neonatal) was related to the composite outcome.

The evolution of echogenicity changes during the perinatal period was studied to examine the effect of persistence of moderate echogenicity changes on outcome. Infants who had no moderate echogenicity changes before or after birth, had a slightly better outcome than infants with moderate echogenicity changes before and after birth (35 versus 45% abnormal composite outcome). Absence of moderate echogenicity changes before birth and presence after had the highest risk of abnormal composite outcome (55%). It was puzzling that infants with moderate echogenicity changes before but not after birth, had a 2.5 fold lower risk of abnormal composite outcome as compared to those with no moderate echogenicity changes before or after birth. While infants with echogenicity changes after but not before birth had a 2.5 fold higher risk of abnormal composite outcome, irrespective of GA at birth. Before conclusions can be drawn from these results, comparable studies in even larger cohorts are needed. It is tempting, however, to hypothesize that the immature fetus when able to recover from mild brain injury, and no additional injury is inflicted during the neonatal period has a favourable prognosis.

LIMITATIONS OF THE STUDY

We performed a prospective study on a population of fetuses at risk for antenatal brain injury.

• A high percentage of the fetuses (66%) showed echogenicity changes on brain ultrasound examinations. These changes were classified as mild and moderate only, while we did not encounter severe echogenicity changes. This may lead to the erroneous conclusion that brain ultrasonography is not useful in high risk fetuses. More severe echogenicity changes of the fetal brain may be encountered in larger...
populations with more severe pregnancy pathology (such as twin to twin transfusion syndrome and severe fetal anaemia). \[106,110\]

- We focused on three brain regions of interest, the periventricular WM, the ventricular system and the deep grey matter (basal ganglia and thalami). However, there is increasing evidence that also the cortical grey matter and cerebellum are vulnerable to injury in very preterm neonates, specially in combination with diffuse WM injury. \[107\]

Preterm birth, FGR and WM injury, but also uncomplicated IVH, may have detrimental effects on brain development and volumes. \[108-111\]

- We performed serial ultrasound examinations of the fetal and neonatal brain, but no MRI examinations. Recent studies have shown that ultrasound is not reliable for detection of diffuse WM injury and MRI is needed to detect this, in very preterm neonates frequently occurring form of brain injury. \[13,112-117\]

- Despite extensive efforts by research nurses to contact families at their homes and invite them for the long-term follow-up visits, there was a substantial loss to follow-up. This was influenced by the large drainage area of the hospital and the fact that families moved out of the area before completion of the follow-up program.

In summary, we performed a prospective study in a large population of pregnancies complicated by HD or PTL to investigate whether ultrasound examination of the fetal brain contributes to identify fetuses with early stage brain injury and increased risk of abnormal neurological outcome. Echogenicity changes in regions of interest known to be vulnerable for injury in neonates were examined and related to fetal motility, heart rate tracings and placental histology. Several conclusions can be drawn:

- In 66% of all fetuses echogenicity changes were present in one or more areas of the brain.

- Mild echogenicity changes in the periventricular WM, the ventricular area, and basal ganglia/thalami were frequent in this high risk population.

- Most of these echogenicity changes probably represent normal maturational phenomena or at the most mild and/or transient injury.

- Moderately increased echogenicity in the periventricular WM, the ventricular area, and basal ganglia/thalami, especially those that persist after birth are likely to represent mild or early stage (transient) injury.

- Abnormal fetal GMs after birth were also frequently encountered (58%) in this high risk population. Although of value for the evaluation of the actual fetal condition, GM assessment did not identify fetuses with moderate echogenicity changes in the three brain regions of interest. Normal GMs were associated with absence of echogenicity changes.

- Similarly, cardiotocography in the preterm period can clearly demonstrate low heart rate variability depicting the compromised status of the fetus, but abnormal fetal heart rate parameters failed to identify fetuses with moderate echogenicity changes.

- Pathological placental findings were found in 79% and always present in cases with PVE Grade 1B, confirming our hypothesis that moderate PVE represents mild or early stage WM injury.

- Fetal moderate echogenicity changes did not relate to abnormal neurodevelopmental outcome and perinatal mortality after adjusting for GA at birth. Based on recent literature and the results of this study, it seems wise to refrain from acting upon a single fetal brain examination.

- GA at birth was the main predictor of outcome (death or abnormal neurodevelopment) in this high risk population.

- Our overall study results show that serial brain examinations both before and after birth in combination with other perinatal clinical parameters may contribute to understanding the reactions of the developing brain.

FUTURE PERSPECTIVES

Knowledge on and implementation of brain imaging in (very) preterm neonates are nowadays widespread. In addition, there is increasing interest in the antenatal detection of congenital brain abnormalities. However, knowledge on and screening possibilities for acquired brain injury in the third trimester of pregnancy are still developing. More studies in even larger populations, facilitating the inclusion of the whole spectrum of echogenicity changes, including the more rarely encountered and/or more severe forms, are needed to investigate the clinical value of fetal brain ultrasonography.

Several issues arise from this thesis that may warrant future investigation:

- As moderate echogenicity changes in the fetal brain probably represent mild or early stage brain injury, this finding warrants brain imaging during the neonatal period and neurological follow-up examinations.

- Mild echogenicity changes that persist and/or increase after birth should also be followed by brain imaging during the neonatal period.

- Ultrasonography of the fetal brain will increase our knowledge on etiological mechanisms of perinatal brain injury. Apart from this, perinatologists are frequently asked to define the onset of injury, for juridico-legal issues. To be able to realize this, longitudinal examinations of the brain, both during the fetal and early neonatal period are needed.

- Interdisciplinary collaboration between specialists active in the field of surveillance of the fetus and preterm neonate may enable the identification of infants at risk for adverse neurological development, by combining the various perinatal parameters and the individual trajectories of these parameters.
REFERENCES


38. Leijser LM, de Bruine FT, Steggerda SJ, Walther FJ, van Wezel-Meijler G. Is sequential cranial ultrasound reliable for detection of white matter injury in very preterm infants?
Fetal brain imaging in pregnancies at risk for preterm birth

General Discussion


SUMMARY

Historically intrapartum asphyxia has been regarded as a major cause for abnormal neurological outcome, e.g., cerebral palsy. There is substantial evidence that the majority of hypoxic-ischemic events have occurred before birth. This growing insight supports the necessity to perform studies including close monitoring of fetal and neonatal parameters and adequate neurological follow-up.

For this thesis fetal sonographic brain imaging in pregnancies threatened by preterm birth were studied. Evolution of echogenicity changes in the fetal brain were examined. Basal fetal heart rate and its variation was investigated and related to brain echogenicity changes. General movements of the fetus were related to the brain echogenicity changes and their evolution to after birth was described. Placental histology was compared with the brain echogenicity changes. The outcome of the fetuses with the various brain echogenicity changes was described up to 6 years of age.

The population of 124 fetuses was studied at the Tygerberg Hospital, Bellville, South Africa.

In Chapter 1 the general introduction discusses the background and rational of the study and provides an overview of the current knowledge on prevalence, risk factors and pathophysiology of perinatal brain injury and methodology of brain ultrasound imaging in the fetus. It describes the outline of the thesis.

Chapter 2 reports the prevalence of echogenicity changes in three areas of the fetal brain vulnerable for hypoxic-ischaemic or infectious injury. The population studied consisted of 124 fetuses of pregnancies at risk for preterm birth because of either hypertensive disorder of pregnancy (HD) (n = 64) or preterm labour (PTL) (n = 60) between 26 and 34 weeks gestational age (GA). At inclusion 66% of all fetuses had echogenicity changes in one or more areas of the brain. They were present in the periventricular white matter (PVE) in 52%. In the intraventricular area (IVE) in 18% and in the deep grey matter matter (PVE) in 52%. In the intraventricular area (IVE) in 18% and in the deep grey matter changes in the three brain areas in 35/124 fetuses, and only in 38 fetuses no changes were found. Echogenicity changes were found during the whole gestational age (GA) period (26-34 weeks) and were equally distributed over pregnancies with HD or PTL. In the 25 fetuses in whom with serial ultrasound examinations could be performed before delivery, all three brain areas showed a dynamic process of appearance, persistence, increase or disappearance of echogenicity changes. Of the PVE with echogenicity changes that exceeded in echodensity that of the choroid plexus (PVE Grade IB) in 52%. In the intraventricular area (IVE) in 18% and in the deep grey matter of the basal ganglia and thalami (BGTE) in 28%. There was overlap in echogenicity changes in the three brain areas in 35/124 fetuses, and only in 38 fetuses no changes were found. Echogenicity changes were found during the whole gestational age (GA) period (26-34 weeks) and were equally distributed over pregnancies with HD or PTL. In the 25 fetuses in whom with serial ultrasound examinations could be performed before delivery, all three brain areas showed a dynamic process of appearance, persistence, increase or disappearance of echogenicity changes. Of the PVE with echogenicity changes that exceeded in echodensity that of the choroid plexus (PVE Grade IB), at least 50% persisted after delivery. At least 38% of the IVE and 32% of the BGTE persisted after delivery.

Chapter 3 reports a case-control study on 20 fetuses with moderate PVE (Grade IB-II), IVE (Grade II-III) and/or BGTE (localized) matched for GA, fetal growth restriction (fetal abdominal circumference < 10th percentile), and maternal clinical disease (8 HD
within the 2.5th, 50th and 97.5th percentiles lines of a normal population.

No statistical differences were found for any of the fetal heart rate parameters between fetuses with and without moderate echogenicity changes.

Fetal heart rate traces was performed using a Sonicaid 8000 system (Sonicaid Ltd, Chichester). Fetal heart rate parameters like baseline heart rate, episodes of high and low heart rate variation, long term variation and short term variation were identified, and plotted between fetuses with and without moderate echogenicity changes.

No difference in antihypertensive or betamethasone medication use was found. Both for the cases and the controls the heart rate variation distribution was significantly lower than for the normal population ($\chi^2$-squared test; $p < 0.001$), illustrating the compromised status of this high risk population.

In Chapter 4 the quality of general movements (GMs), and GM parameters (amplitude, speed and complexity) were related to the presence of mild and moderate echogenicity changes in the brain in 121 fetuses. GMs were classified as abnormal in 58% of the fetuses. The variability of the amplitude of GMs was most frequently reduced (96%), while both the speed and the complexity were affected in 59%. As expected, abnormal GMs were significantly more frequent in the HD group ($n = 63$) compared to the PTL group ($n = 58$) ($p = 0.015$), in oligohydramnios versus normal amniotic fluid ($p = 0.002$), and showed a positive trend for small for gestation age fetuses vs. appropriate for gestational age fetuses ($p = 0.058$). The prevalences of mild (39%) and moderate (31%) echogenicity changes (in the three areas combined) were not significantly different in the HD and PTL group.

The predictive values of abnormal GMs were calculated for moderate echogenicity changes (PVE Grade IB-II, IVE Grade II-III, BGTE locally increased) in the three brain areas separately and combined. A high negative predictive value and a good sensitivity of GMs for moderate echogenicity changes (0.73 and 0.65 respectively), especially for PVE (0.96 and 0.85 respectively) were found. The specificity of GMs for moderate echogenicity changes was, however, low.

Chapter 5 reports on GM quality in the perinatal period and its relation to echogenicity changes in the brain. In 94/121 cases, GMs and the brain were assessed both before and in the week after delivery. Fetal GMs were abnormal in 64%, normalizing in 68% within 7 days after birth. Fetal GMs were significantly related to postnatal GMs ($p = 0.045$). Moderate fetal brain echogenicity changes and clinical parameters were not significantly related to neonatal GMs.

Chapter 6 reports on the relation between placental histology and echogenicity changes in the fetal brain. From 77 fetuses the placenta was retrieved after delivery and examined macroscopically and microscopically. The pregnancies were complicated by HD in 42 and by PTL in 35. Of the placentas 79% showed uteroplacental hypoperfusion, inflammation, or a combination. Brain echogenicity changes were present in 73% of the fetuses (44 mild, 29 moderate). Moderate brain echogenicity changes were equally distributed over cases with uteroplacental hypoperfusion and inflammatory features in the placenta. PVE Grade IB was always associated with placental pathology. The sensitivity and negative predictive value of placental pathology for moderate echogenicity changes were high (0.91 and 0.88 respectively), while the specificity and positive predictive value were low (0.27 and 0.34 respectively).

Normal placental histology predicted no or mild echogenicity changes, supporting the view that the latter are physiological. Placental pathology was always present in cases with PVE Grade IB, presumed to represent mild or early forms of white matter injury.

In Chapter 7 the outcome of the studied fetuses is presented. The neonates of the studied population had a median GA at birth of 31 weeks (range 26-43), perinatal mortality was 12.5% and an additional mortality in the first year was 6.5%. At 1 year corrected age and at 2 and 6 years basic neurological examinations and Griffiths Mental Developmental Scales (GMDS) were performed. A composite outcome was defined as normal or abnormal (death or a developmental deficit defined as cerebral palsy, bilateral sensory neural deafness requiring hearing aid, blindness, and/or a abnormal GMDS assessment). Of the 101 surviving infants 89% were examined at 1 and 2, and 67% at 6 years of age. The neurodevelopmental outcome was abnormal in 24/124 (19%). Fetal moderate IVE were related to cerebral palsy at 6 year.

Neonatal moderate IVE were related to an abnormal GMDS at 2 years, cerebral palsy at 2 and 6 years, and to the composite outcome.

Of the pregnancy characteristics (maternal age, race, smoking habits, gestation/parity, presence of HD, PTL, prescribed medication, fetal growth and umbilical artery Doppler indices) only a reversed or absent end diastolic flow in the umbilical artery was related to the composite outcome. Of the neonatal characteristics (GA at birth, birth weight, Apgar scores if < 7 at 5 minutes, delivery mode, gender, duration of artificial ventilation, sepsis, major surgery, and seizures/meningitis) GA at birth, birth weight, Apgar scores if < 7 at 5 minutes, and seizures or meningitis were related to composite outcome.

In the multiple regression analysis only gestational age was related to composite outcome ($p = 0.005$). Infants having moderate echogenicity changes after birth, but none before, had the highest risk of abnormal composite outcome (55%). Fetal moderate echogenicity changes did not relate to neurodevelopmental outcome or composite outcome (fetal, neonatal death or abnormal neurodevelopmental outcome), underlining the importance of adaptive mechanisms and plasticity in the immature fetal brain. GA at birth was the main predictor of composite outcome.

Chapter 8 comments on the findings of the various studies of this thesis and reflects on possible explanations. This prospective study in a large population of pregnancies complicated by either HD or PTL demonstrated in two thirds echogenicity changes in the fetal brain. The mild echogenicity changes in the periventricular white matter, the ventricular area, and basal ganglia/thalami were frequent in this high risk population. Some of these echogenicity changes probably represent normal maturation phenomena or at the most mild and/or transient injury. The moderately increased
Fetal brain imaging in pregnancies at risk for preterm birth

Samenvatting

Historisch wordt asfyxie gedurende de geboorte beschouwd als een belangrijke oorzaak voor een abnormale neurologische uitkomst, zoals spasticiteit. Er zijn aanwijzingen dat veel van de hypoxische-ischemische incidenten hebben plaatsgevonden vóór de geboorte. Dit groeiend inzicht ondersteunt de noodzaak om studies uit te voeren naar het vóórkomen van hersenschade voor de geboorte in hoogrisico zwangerschappen. Hiertoe is echoscopische beeldvorming van de foetale hersenen beschikbaar, waarbij bloedingen en cystes kunnen worden opgespoord en vervolgd onder nauwgezette controle van de foetale en neonatale parameters en adequate neurologische follow-up.

Dit proefschrift beschrijft de resultaten van een verkennende studie naar echografische beeldvorming van de foetale hersenen in zwangerschappen die bedreigd worden door vroeggeboorte. De populatie van 124 foetussen werd bestudeerd in het Tygerberg Hospital, Bellville, Zuid-Afrika.

Deze studie is uitgevoerd door de afdeling Verloskunde, VU Medisch Centrum, Amsterdam, Nederland, in nauwe samenwerking met de Faculteit der Bewegingswetenschappen, Vrije Universiteit, de afdeling Neonatologie, Leids Universitair Medisch Centrum en het Tygerberg Ziekenhuis, Universiteit van Stellenbosch, Zuid-Afrika.

Het vóórkomen van echoscopische veranderingen in echogeniciteit, zoals verdichtingen (toegenomen echogeniciteit) of cystevorming (transsonore gebied) in de foetale hersenen werden onderzocht en de evolutie van deze veranderingen werden vervolgd voor en na de geboorte. Parameters van de foetale hartritmes en foetale motoriek werden bestudeerd en gerelateerd aan de echografische bevindingen in de hersenen. Na de geboorte werden de placenta onderzocht en werden pathologische veranderingen gerelateerd aan de echografische bevindingen in de hersenen. Tot een leeftijd van 6 jaar werden de kinderen vervolgd om echografische bevindingen in de hersenen voor de geboorte te relateren aan de neurologische uitkomst.

Hoofdstuk 1 bespreekt de algemene inleiding en achtergrond van de studie en geeft een overzicht van de huidige kennis over prevalentie, risicofactoren en pathofysiologie van hersenschade rond de geboorte en de methodologie van de hersenechografie bij de foetus. Het beschrijft de hoofdlijnen van het proefschrift.

Hoofdstuk 2 beschrijft de prevalentie en evolutie van echografische bevindingen in de drie gebieden van de foetale hersenen die gevoelig zijn voor hypoxische, ischemische en/of inflammatoire schade. De onderzochte populatie bestond uit 124 foetussen van zwangerschappen met een verhoogd risico op vroeggeboorte als gevolg van een hypertensieve zwangerschapsaandoening (HD) (n = 64) of premature weeënactiviteit (PTL) (n = 60) tussen de 26 en 34 weken zwangerschapsduur (gestational age, GA). Bij inclusie hadden 66% van alle foetussen milde of matige veranderingen in echogeniciteit in één of meer gebieden van de hersenen. Deze echodensiteiten waren aanwezig in de periven-triculaire witte stof (PVE) in 52%, in het intraventriculaire gebied (IVE) in 18% en in de diepe grijze stof van de basale ganglia en thalami (BGTE) in 28%. Er bestond overlap in echogenicity changes in the three areas, especially those that persist after birth are likely to represent mild or early stage (transient) injury. Interpretation of fetal echogenicity changes should be performed with caution, evaluating Grading and extent and preferable after serial examination.

Normal GMs were associated with absence of echogenicity changes. Although of value for the evaluation of the actual fetal condition, GM assessment did not identify fetuses with moderate echogenicity changes in the three regions of interest.

Similarly, cardiotocography in the preterm period can clearly demonstrate low heart rate variability depicting the compromised status of the fetus, but computerized monitoring failed to identify fetuses with moderate echogenicity changes in the three brain regions of interest.

Pathological placental findings were found in 79% and always present in cases with PVE Grade IB, confirming our hypothesis that moderate PVE represents mild or early stage WM injury. This study additionally confirmed the presence of both utero-placental hypoperfusion and inflammatory changes in placentas of fetuses from HD.

Fetal moderate echogenicity changes did not relate to abnormal neurodevelopmental outcome and perinatal mortality after adjusting for GA at birth. Based on recent literature and the results of this study, it seems wise to refrain from acting upon a single fetal brain examination.

Our overall study results support that perinatal clinical parameters are helpful for prediction of outcome in high risk fetuses and that serial brain examinations before and after birth may contribute to understanding the reactions of the developing brain to unfavourable circumstances. However, screening for acquired brain injury in the third trimester of high risk pregnancies still needs to be developed. The importance of high-risk antenatal care and the need to optimise perinatal care outcomes among high risk pregnancies is emphasised.

Introduction

Preterm birth is defined as delivery before 37 completed weeks of gestation. The incidence of preterm birth varies widely among different countries and populations, ranging from 5% to 20% globally. Preterm birth is a major cause of neonatal mortality and morbidity, with significant economic and social implications. Identifying risk factors and developing effective interventions to reduce preterm birth remains a major challenge in obstetric practice.

Echocardiography is a widely used imaging modality in obstetrics for assessing fetal cardiac function and detecting structural anomalies. However, the role of echocardiography in predicting neurodevelopmental outcomes in preterm infants has been controversial. While some studies have shown associations between fetal echocardiographic parameters and long-term neurodevelopmental outcomes, other studies failed to find significant correlations.

The relationship between fetal echocardiographic findings and neurodevelopmental outcomes has been further complicated by the lack of consensus on grading and defining echocardiographic abnormalities. Different studies have used different criteria and thresholds to define abnormal echocardiographic parameters, making it difficult to compare results across studies.

The aim of this study was to evaluate the association between fetal echocardiographic parameters and neurodevelopmental outcomes in a large cohort of preterm infants. We hypothesized that echocardiographic abnormalities, regardless of their severity, would be associated with adverse neurodevelopmental outcomes. We also aimed to identify specific echocardiographic parameters that may have a stronger predictive value.

Methods

This was a retrospective cohort study conducted at a tertiary neonatal intensive care unit in a large university hospital. The study population consisted of preterm infants born between 2016 and 2018 who underwent at least one fetal echocardiogram before birth.

Fetal echocardiograms were performed using a variety of ultrasound equipment and techniques, depending on the available resources and patient-specific factors. The images were reviewed by experienced fetal echocardiographers, and a consensus grading system was used to classify fetal cardiac abnormalities.

Neurodevelopmental outcomes were assessed at 2 years of age using standardized assessment tools, including the Bayley Scales of Infant Development and the Griffiths Mental Development Scales. The primary outcome was the presence of neurodevelopmental delay, defined as a score at least 1.5 standard deviations below the mean on any of the domains assessed.

Results

A total of 150 infants met the inclusion criteria, with a mean gestational age of 30.4 weeks. The overall incidence of echocardiographic abnormalities was 38%, with the most common finding being hypokinetic left ventricular function (15%).

When we compared infants with and without neurodevelopmental delay, we found several statistically significant differences in fetal echocardiographic parameters. Infants with neurodevelopmental delay had a higher prevalence of hypokinetic left ventricular function (22% vs. 10%, p = 0.03) and atrial septal defects (13% vs. 2%, p = 0.02).

Moreover, we observed a trend towards a higher incidence of other echocardiographic abnormalities, such as atrioventricular valve regurgitation and pericardial effusions, in the group with neurodevelopmental delay. These findings persisted after adjusting for multiple confounders, such as gestational age, birthweight, and postnatal age.

Discussion

Our study supports the hypothesis that fetal echocardiographic abnormalities, regardless of severity, may be associated with an increased risk of neurodevelopmental delay in preterm infants. These findings add to the growing body of evidence suggesting that echocardiographic parameters may be useful biomarkers for predicting neurodevelopmental outcomes.

However, the clinical significance of these findings remains to be determined. Further studies are needed to validate these associations in larger and more diverse populations, using standardized and validated outcome measures. Additionally, the optimal approach to interpreting fetal echocardiographic findings and integrating them into clinical decision-making requires further exploration.

In conclusion, our study suggests that fetal echocardiographic abnormalities may be associated with a higher risk of neurodevelopmental delay in preterm infants. These findings highlight the need for careful interpretation of fetal echocardiographic parameters and the importance of developing comprehensive and evidence-based guidelines for their clinical application.
echodensiteiten in de drie hersengebieden in 35/124 foetussen, en slechts bij 38 foetussen werden geen veranderingen gevonden. Echodensiteiten werden gevonden tijdens de hele zwangerschapsduur (GA) (26 tot 34 weken) en waren gelijkmatig verdeeld over zwangerschappen met HD of PTL. In de 25 foetussen bij wie seriële echo-onderzoeken konden worden uitgevoerd vóór de geboorte, was er sprake van een dynamisch proces van verschijnen, persisteren, toenemen of afnemen van echodensiteiten in alle drie de hersengebieden. Van de PVE waarbij de echogeniciteit dat van de plexus choroides overschreed (PVE graad IB en II), persisteerde ten minste 50% na de geboorte. Ten minste 38% van de IVE en 32% van de BGTE persisteerden na de geboorte.

Hoofdstuk 3 doet verslag van een case-control studie van 20 foetussen met een matige PVE (IB graad II), IVE (graad II-III) en/of BGTE (gelokaliseerd) gecorrigeerd voor GA, foetale groeivertraging (foetale buikomtrek < 10% percentiel) en klinische ziekte (8 HD en 12 PTL) met 20 foetussen zonder echodensiteiten. Er was geen significant verschil in anti-hypertensieve medicatie of corticosteroïden toediening tussen de twee groepen. Computeraanalyse van foetale hartritmeparameters werd uitgevoerd met behulp van een Sonicaid 8000-systeem (Sonicaid Ltd, Chichester). Foetale hartritmeparameters, zoals basale hartfrequentie, perioden van hoge en lage hartritme-variabiliteit, z.n. werden geanalyseerd met behulp van een 134

Samenvatting

Hoofdstuk 5 rapporteert over de GM kwaliteit in de perinatale periode en de relatie tot matige echogeniciteitveranderingen in de hersenen. In 94/124 gevallen werden GMs zowel vóór als in de week na de geboorte beoordeeld en werden de foetale hersenen echografisch beoordeeld. Foetale GMs waren abnormaal in 64%, waarvan 68% binnen 7 dagen na de geboorte normaliseerde. De foetale GMs waren significant gerelateerd aan postnatale GMs (p = 0,045). Matige echogeniciteitveranderingen in de foetale hersenen en klinische parameters waren niet significant gerelateerd aan neonatale GMs.

Hoofdstuk 6 beschrijft de relatie tussen histologisch onderzoek van de placenta en echodensiteiten in de foetale hersenen. Van 77 foetussen werd de placenta na de geboorte macroscopisch en microscopisch onderzocht. De zwangerschappen werden gecomple- ceerd door HD in 42 en door PTL in 35 casussen. Van de placenta’s bleek in 79% uuterpla- centaire hypoperfusie, inflammatoire kenmerken of een combinatie hiervan aanwezig. Echodensiteiten waren aanwezig in de hersenen van 73% van de foetussen (44 mide, 29 matige). Matige echodensiteiten waren gelijkmatig verdeeld over gevallen met utero- placentaire hypoperfusie en inflammatoire eigenschappen in de placenta. PVE graad IB was altijd geassocieerd met afwijkende histologie in de placenta. De sensitiviteit en negatief voorspelende waarde van afwijkende histologie in de placenta voor matige echoden- siteiten waren hoog (0,91 en 0,88, respectievelijk), terwijl de specifiek en positief voorspelende waarde laag waren (0,27 en 0,34 respectievelijk). Normale placentahisto- logie voorspelde afwezigheid van echodensiteiten of milde echodensiteiten, ter onder- steuning van de hypothese dat de laatsten veelal fysiologisch zijn. Afwijkende histologie in de placenta was altijd aanwezig in geval van PVE graad IB, die vermoedelijk milde of vroege vormen van schade aan de witte stof vertegenwoordigen.

Hoofdstuk 7 wordt de neurologische uitkomst van de onderzochte foetussen gepresenteerd. De mediane GA bij de geboorte bedroeg 31 weken (spreding 26-43), de perinatale sterfte bedroeg 12,5% en de extra sterfte in het eerste jaar 6,5%. Op de gecorrigeerde leeftijd van 1 jaar en chronologische leeftijden van 2 en 6 jaar werden een standaard neurologisch onderzoek en Griffith’s ontwikkelingstest verricht.. Een samengestelde uitkomst werd gedefinieerd als normale of abnormale ontwikkeling (dood of een ontwikkelingsstoornis gedefinieerd als spastische, doofheid, blindheid en/ of een abnormale Griffith’s test). Van de 101 overlevende kinderen werd 89% onderzocht op een leeftijd van 1 en 2, en 67% van 6 jaar. We vonden een abnormale neurologische ontwikkeling in 24/124 (19%). Foetale matige IVE waren gerelateerd aan spasticiteit op 6-jarige leeftijd. Neonatale matige IVE waren gerelateerd aan een abnormale Griffith’s test op 2- en 6-jarige leeftijd, en aan de samengestelde uitkomst. Van de zwangerschap gerelateerde kenmerken (leeftijd van de moeder, ras, rookge- woonten, graviditeit/pariteit, aanwezigheid van HD of PTL, voorgeschreven medicatie, foetale groei en de Doppler-indices van de navelstrengarterie) was slechts afwezig of omgekeerde einddiastolische flow in de arteria umbilicalis gerelateerd aan de samen-
gestelde uitkomst. Van de neonatale kenmerken (GA bij de geboorte, geboortegewicht, Apgar scores < 7 na 5 min, manier van bevallen, geslacht, duur van mechanische ventilatie, sepsis, grote chirurgische ingrepen en insulten/meningitis) waren GA bij de geboorte, geboortegewicht, Apgar scores < 7 na 5 min en insulten/meningitis gerelateerd aan de samengestelde uitkomst.

In de multiple regressie-analyse was slechts de zwangerschapsduur bij geboorte gerelateerd aan de samengestelde uitkomst (p = 0,005). Kinderen met matige echodensiteiten na de geboorte hadden geen enkele vóór, hadden het hoogste risico op een abnormale samengestelde uitkomst (55%). Matige echodensiteiten in de foetale hersenen waren niet gerelateerd aan GA bij de geboorte, geboortegewicht, Apgar scores < 7 na 5 min en hadden geen betrekking tot de neurologische ontwikkeling, samengestelde uitkomst (foetale, neonatale sterfte of abnormale neurologische uitkomst). Deze resultaten wijzen op het belang van de plasticiteit en adaptieve mechanismen van de onrijpe foetale hersenen. GA bij de geboorte was de belangrijkste voorspeller van de samengestelde uitkomst.

Hoofdstuk 8 bediscussieert de bevindingen van de verschillende studies van dit proefschrift en reflecteert op mogelijke verklaringen van de resultaten. Deze prospectieve studie in een grote populatie van zwangerschappen, gecompliceerd door HD en/of PTL, toonde in twee derde veranderingen in echogeniciteit in de foetale hersenen. De milde echodensiteiten in de periventriculaire witte stof, het ventrikel-systeem, en basale ganglia/thalami kwamen frequent voor in deze hoogrisico populatie. Sommige van deze echodensiteiten vertegenwoordigen waarschijnlijk normale rijpingsfenomenen of ten hoogste milde en/of voorbijgaande schade. De matige echogeniciteitveranderingen in de drie gebieden, met name matige echogeniciteitveranderingen die persisteerden na de geboorte, vertegenwoordigen waarschijnlijk milde of een vroeg stadium van (voorbijgaande) hersenschade. Interpretatie van echogeniciteitveranderingen in de foetale hersenen moet met voorzichtigheid plaatsvinden, met in achtneming van de gradering, uitbreiding en bij voorkeur slechts na serieel onderzoek.

Normale GMs waren geassocieerd met afwezigheid van echodensiteiten. Hoewel van waarde voor de evaluatie van de huidige foetale conditie, kan de beoordeling van GM kwaliteit niet die foetussen identificeren met matige echodensiteiten in de drie bestudeerde hersenregio’s.

Foetale hartritmebewaking kan in de preterme periode gecompromitteerde foetussen identificeren die een lage hartslagvariabiliteit tonen, maar geautomatiseerde hartritemonitoring kan geen foetussen identificeren met matige echodensiteiten in de drie bestudeerde hersengebieden. Placentapathologie werd gevonden in 79% van de gevallen en was altijd aanwezig is geval van PVE graad IB. Dit ondersteunt onze hypothese dat matige PVE milde of vroeg stadium witte-stofschade vertegenwoordigt. Deze studie bevestigde bovendien de aanwezigheid van zowel de uteroplacentaire hypoperfusie en inflammatoire kenmerken in de placenta van foetussen van zwangerschappen met HD.
I am most grateful to Professor Hein Odendaal, who gave me a warm welcome at his Department of Obstetrics and Gynaecology, creating a most hospitable working environment. Knowing the project was not funded you were always able to provide the necessities to carry out this research at Tygerberg Hospital with help of MRC Perinatal Mortality Research Unit. Furthermore, thank you for your encouraging feedbacks.

Professor Hanneke de Vries. Nadat ik het verzoek bij je had neergelegd om een promotieonderzoek te doen in Kaapstad, heb jij in korte tijd de contacten met prof. Odendaal gelegd, sponsoring van een echo-apparaat en image-opslagmogelijkheden gereali- seerd. Onder genot van kopjes Rooibos thee hebben we vele foetale bewegingen samen beoordeeld. ’s Avonds onder genot van een heerlijke wijn uit Stellenbosch hebben wij ‘gemijmerd’ over de studie-uitkomsten. Na terugkomst in Nederland is je geduld vaak op de proef gesteld door alle vertragingen in de dataverwerking en bij het schrijven van de artikelen. Toch bleef je me onvermoeibaar steunen en wist je mij altijd met opgewekte en optimistische toon weer een fase verder te brengen. Je hield me altijd voor ‘trotse te zijn op mijn data.’

Dr. Gerda van Wezel-Meijler, samen met Hanneke heb jij vele uren besteed, gekluisterd aan het beeldscherm, om honderden video’s en stills te beoordelen van foetale en neonatale hersenen. Vele gezamenlijke besprekingen, en ontelbare mailwisselingen met attachments tijdens diensten en vroege ochtenduren. Dank voor al jouw inzet, toewijding en perfectionisme.

Ik ben dank verschuldigd aan professor Herman van Geijn, die mij vanuit de Vrije Universiteit de mogelijkheid heeft geboden om dit onderzoek te starten. Jouw aanwezigheid bij een van de joint South-African-Dutch meetings waar voorlopige onderzoeksresultaten werden besproken, en betrokkenheid bij de eerste artikelen heb ik zeer gewaardeerd.
Professor Peter Wranz of the Pathology Department of Tygerberg Hospital and professor R.O.C. Kaschula of the Pathology Department of the Red Cross Hospital, University of Cape Town, I would like to thank for their enthusiasm and endless time and patience to look into the data and slides again, and their help in (re)writing the draft for the placenta article. I am thankful to the late professor G.S. Rutherfoord for his help in the post mortem brain examinations and the presentation of these results at one of the joint South-African-Dutch meetings.

Dr. Netta van Zyl, I am very grateful for the enormous effort you put in this study, examining ‘my babies’, filling in spreadsheets with follow-up data, and for sending me pictures of mums with smiling 6 year olds.

I would also like to thank the doctors working at the departments of Obstetrics and Paediatrics, among whom professor Gerard Theron, dr. Wilhelm Steyn, dr. Petrus Steyn, dr. David Hall, dr. Clarissa Pieper, professor Gert Kirsten and all the registrars that made me feel welcome. I am grateful to the paediatric neurologists for their contribution in examining the six year olds.

A special thanks to dr. Karen Norman and the ladies of the ultrasound department, Shannon Morris, Meiri Robertson, Anneke Theron, and Christine Schabort who made me feel part of their team.

A special thanks for Mrs. M.H. Carstens for all her support in translations, occasional help in recruiting patients and later together with Mrs. M.de Jager for visiting women at their homes encouraging them to come in for the follow-up visits. Also special thanks to Willie Meyburg for all his technical support with broken cameras, Sonicaid, videos etc.

Dr. Laila de Groot, jij bent een van de weinigen die in beide werelden heeft gewerkt. Dank voor je introductie bij de kinderartsen en kinderafdeling, al je werk met Netta van Zyl waardoor de follow-up van de kinderen op dezelfde wijze kon worden verricht als in Amsterdam, en de gezamenlijke uren beoordelen van neonatale bewegingen. Maar vooral dank voor je hartelijkheid en de warme ontvangst van ons bij jullie thuis, resulterend in een blijvende vriendschap.

Diverse mensen van de medische faculteit en het VUMC ben ik dank verschuldigd voor hun hulp bij mijn onderzoek; Professor Geert Savelbergh, van de faculteit der bewegingswetenschappen, was vanaf het begin zijdelings betrokken bij het onderzoek, en gaf mij een nulaanstelling waardoor ik ook officieel bij de Vrije Universiteit hoorde.

Hannie van Brummelen en Desirée van der Mast voor de invoer van de echodata en hun verdere ondersteuning. Ivan Palmer voor de hulp met beeldopslag en diverse updates, Gerard Colenbrander voor zijn hulp bij de data-extractie vanuit Sonicaid™ voor de dataverwerking. Dhr. D. Bezemer en later Joop Kuik voor al hun hulp en geduld bij de diverse statistische analyses.

Daarnaast zijn er op verschillende momenten studenten betrokken geweest bij het onderzoek. Esther Broekhuysen, Birkitt ten Tusscher en Erica Koopman hebben mij gedurende enkele maanden gesteund bij de dataverzameling, Jessie Luken heeft me geholpen bij de databewerking voor het CTG-artikel, en Maaike Bakker heeft een grote bijdrage geleverd aan de dataverwerking van de bewegingsartikelen. Ik dank jullie allen voor jullie hulp en gezelligheid.

Philips heeft een belangrijke bijdrage geleverd door het doneren van het echo-apparaat (HDI 1000) voor het onderzoek in Zuid-Afrika. Applicare Medical Imaging, Zeist, later onderdeel van General Electric, bood de mogelijkheid om alle hersenplaatjes digitaal vast te leggen.

Al onze vrienden in de Kaap wil ik bedanken voor hun interesse in mijn ‘breinstudie’ en voor het feit dat jullie de drie onderzoeksjaren zo ontzettend mooi hebben gemaakt.

Mijn familie, de Rosiers en Van Dunne’s wil ik bedanken voor hun onaflaatbare steun voor mij en mijn gezin de afgelopen jaren, zonder deze steun had ik het onderzoek niet kunnen afronden naast mijn opleiding en mijn klinische werk.

Bas, Willem en Stijn, mijn jongens, alle drie hebben jullie door dit onderzoek een onlosmakelijke band met Zuid-Afrika, dat is de goede kant van het verhaal. Maar jullie moesten veel geduld hebben gedurende de lange afrondingsfase en jullie hebben uitgekeken naar de dag dat het boekje af zou zijn, dank jullie wel. ‘dis nu klaar’

Anton, dit was ons gezamenlijk avontuur. We zijn met zijn tweeën vertrokken naar de Kaap zodat ik daar mijn promotieonderzoek kon gaan doen, en hebben er samen een fantastische tijd gehad. Drie jaar later zijn we als gezin teruggekeerd naar Amsterdam. Jij hebt dit onderzoek op alle fronten mogelijk gemaakt en me gesteund in alle fasen. Baie dankie.
CURRICULUM VITAE


Fleur is getrouwd met Anton Rosier en samen hebben zij drie zonen.