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THE VULNERABLE BRAIN AND VERY PRETERM INFANTS

—

FINDINGS FROM THE PIPARI-STUDY

by

Jonna Maunu

TURUN YLIOPISTO
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The Department of Pediatric Neurology, University of Turku, Turku, Finland

Supervised by Docent Liisa Lehtonen, M.D.
Department of Neonatology,
Turku University Hospital
University of Turku
Turku, Finland

Professor Leena Haataja, M.D.
Department of Pediatric Neurology
Turku University Hospital
University of Turku
Turku, Finland

Docent Helena Lapinleimu, M.D.
Department of Neonatology
Turku University Hospital
University of Turku
Turku, Finland

Reviewed by Docent Leena Vainionpää, M.D.
Department of Medicine
Department of Pediatric Neurology
University of Oulu
Oulu, Finland

Timo Saarela, M.D., PhD
Department of Medicine
Department of Neonatology
University of Oulu
Oulu, Finland

Opponent Professor Helena Pihko, M.D.
Institute of Clinical Medicine
Hospital for Children and Adolescents
Department of Pediatric Neurology
University of Helsinki

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To the staff working with preterm infants

Jos siitä voi uneksia,
sen voi myös
toteuttaa.

-Walt Disney-

Jonna Maunu:
The vulnerable brain and very preterm infants – Findings from the PIPARI-Study
Department of Medicine, University of Turku, Turku, Finland
Annales Universitatis Turkuensis

ABSTRACT

Preterm birth is a risk for normal brain development. Brain maturation that normally happens in the uterus is in very preterm infants a developmental challenge during their stay in a neonatal intensive care unit (NICU). Typical brain injuries of preterm infants include ischemic injuries, brain haemorrhages, ventricular dilatation (VD), and reduced brain volumes. Brain injury is a serious complication of prematurity leading to possible long term consequences for the neurodevelopment of the very low birth weight (VLBW) infant, such as cerebral palsy (CP), hearing impairments, vision problems, and delay in cognitive development. There is a need for further studies to ascertain the potential risk factors and their causal relationships to brain vulnerability, growth and development in the increasing number of surviving VLBW infants.

This thesis consists of four studies evaluating the definitions, causes and consequences of brain lesions in VLBW (<1500g) or very low gestational age (VLGA) (gestational age <32 gestational weeks) infants. We showed that the redistribution of fetal blood flow is a risk factor for smaller brain volumes at term. In addition, we showed that brain lesions related to prematurity are not associated with increased spontaneous crying behaviour or circadian rhythm development in infancy. However, the preterm infants began to fuss more often and were held more than term infants at five months of age. Furthermore, we showed that VD is associated with brain lesions and smaller brain volumes. Therefore, brain magnetic resonance imaging can be recommended for infants with VD. VD together with other brain pathology is a risk factor for the onset of developmental impairments in VLBW/VLGA infants at two years of age.

Key words: Preterm, brain imaging, antenatal Doppler, crying behaviour, neuro-developmental outcome

Jonna Maunu:
Pikkukeskosen vaurioherkät aivot – havaintoja PIPARI-tutkimuksesta
Turun Yliopisto, Lastenneurologian klinikka, Turku
Annales Universitatis Turkuensis

TIIVISTELMÄ

Ennenaikainen syntymä on riski aivojen normaalille kehitykselle. Pikkukeskosilla aivojen tärkeitä kehitysvaiheita tapahtuu tehohoidon aikana kohdun ulkopuolella. Pikkukeskosen tyypillisiin aivokomplikaatioihin kuuluvat aivoverenvuodot, hapenpuutteen aiheuttamat vauriot, aivojen sivukammioiden laajentuminen ja aivoaineksen tilavuuksien pieneneminen. Aivovaurio on vakava keskosuuden komplikaatio, koska se voi johtaa pitkäaikaisiin kehityksen ongelmiin, kuten esimerkiksi CP-vammaisuuteen, kuulon tai näön ongelmiin tai älyllisen kehityksen viiveeseen. Koska yhä useampi pikkukeskonen jää henkiin ennenaikaisuudesta huolimatta, on tärkeä tutkia mahdollisia riskitekijöitä pikkukeskosen aivojen vaurioitumiselle.

Tämän väitöskirjan neljä osatyötä käsittelivät aivojen kuvantamistutkimuksissa todetun aivovaurion luokittelua, mahdollisia syitä aivovauriolle ja aivojen vaurioitumisen seurauksia pikkukeskosen (<1500g / <32 raskausviikkoa syntyessä) kehitykselle. Tutkimuksemme osoitti, että sikiön verenkierron uudelleenjakautuminen hapenpuutteen seurauksena aiheuttaa riskin aivojen kasvulle. Osoitimme myös, ettei aivovaurio lisää spontaanin kokonaisitkun määrää tai vaikuta vuorokausirytmin kehittymiseen pikkukeskosilla imeväisiässä. Keskoset aloittivat täysiaikaisia lapsia useammin kitinäjakson viiden kuukauden korjatussa iässä ja heitä pidettiin sen seurauksena enemmän sylissä kuin täysiaikaisia verrokkeja. Lisäksi osoitimme, että keskosilla, joilla aivojen sivukammiot ovat laajentuneet, on usein muitakin poikkeavia aivolöydöksiä ja pienemmät aivojen kokonais- ja osatilavuudet lasketussa ajassa. Mikäli aivojen sivukammiot ovat poikkeavasti laajentuneet ultraäänitutkimuksessa lasketussa ajassa, voidaan tulostemme perusteella suositella aivojen magneettitutkimusta mahdollisten lisälöydösten varmentamiseksi. Aivojen sivukammioiden laajentuminen yhdessä muun aivovaurion kanssa liittyi suurentuneeseen riskiin kehityksen ongelmille kahden vuoden korjatussa iässä pikkukeskosilla.

Avainsanat: keskonen, aivokuvantaminen, raskaudenaikaiset virtausmittaukset, itkukäyttäytyminen, neurologinen kehitys

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ABBREVIATIONS

AI	aortic isthmus
AREDF	absent or reversed end diastolic flow
BAEP	brainstem auditory evoked potentials
BPD	bronchopulmonary dysplasia
BSID-II	Bayley Scales of Infant Development, second edition
BW	birth weight
CLD	chronic lung disease
CP	cerebral palsy
DAo	descending aorta
DV	ductus venosus
ELBW	extremely low birth weight
ELGA	extremely low gestational age
FSE	fast spin echo
GA	gestational age
GMFCS	gross motor function classification system
GMs	general movements
HINE	Hammersmith Infant Neurological Examination
IVH	intraventricular hemorrhage
MCA	middle cerebral artery
MDI	mental developmental index
MRI	magnetic resonance imaging
NDI	neurodevelopmental impairment
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
PI	pulsatility index
PDI	psychomotor developmental index
cPVL	cystic periventricular leukomalacia
PVL	periventricular leukomalacia
SD	standard deviation
SGA	small for gestational age
T	tesla

T1	longitudinal relaxation time
T2	transverse relaxation time
TE	time of echo
TR	time of repetition
UA	umbilical artery
US	ultrasound
V/B	ventricular / brain
VD	ventricular dilatation
VLBW	very low birth weight
VLGA	very low gestational age
WHO	World Health Organization
WM	white matter

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by the Roman numerals (I-IV).

I Maunu J, Ekholm E, Parkkola R, Palo P, Rikalainen H, Lapinleimu H, Haataja L, Lehtonen L and the PIPARI Study Group. Antenatal Doppler Measurements and Early Brain Injury in Very Low Birth Weight Infants. The Journal of Pediatrics. 2007; 150:51-6

II Maunu J, Kirjavainen J, Korja R, Parkkola R, Rikalainen H, Lapinleimu H, Haataja L, Lehtonen L and the PIPARI Study Group. Relation of Prematurity and Brain Injury to Crying Behavior in Infancy. Pediatrics. 2006; 118 (1):e57-65

III Maunu J, Parkkola R, Rikalainen H, Lehtonen L, Haataja L, Lapinleimu H and the PIPARI Study Group. Brain and Ventricles in Very Low Birth Weight Infants at Term: A Comparison Among Head Circumference, Ultrasound and Magnetic Resonance Imaging. Pediatrics. 2009 Feb; 123(2):617-26

IV Maunu J, Lehtonen L, Lapinleimu H, Matomäki J, Munck P, Rikalainen H, Parkkola R, Haataja L and the PIPARI Study Group. Ventricular Dilatation in Relation to Outcome at Two Years of Age in Very Low Birth Weight Infants - A Prospective Finnish Cohort Study. Developmental Medicine and Child Neurology. 2010; in press

INTRODUCTION

Preterm birth is a risk for normal brain development. In very low birth weight (VLBW) infants, the part of brain maturation that normally happens in the uterus is a developmental challenge during their stay in a neonatal intensive care unit (NICU).

The preterm brain is vulnerable to brain injury through different mechanisms. The germinal matrix is a highly vascularized area, located subependymally beside the lateral ventricles, and is prone to injury due to the immature cerebral autoregulation of preterm infants (Inder and Volpe 2000). The small vessels in the germinal matrix are brittle, and the fragmentation of these vessels can lead to hemorrhaging in the brain (Whitelaw 2001). In addition, the vessels that penetrate the cerebral wall are still highly undeveloped during weeks 24 to 28. They form vascular end and border zones in cerebral white matter (WM) regions, which in turn are vulnerable to ischemic injuries (Inder and Volpe 2000, Inder et al. 2003). Brain complications may lead to disturbances in cell migration in preterm infants (Kostovic and Judas 2006), as well as to ventricular dilatation (VD) (Paneth et al. 1990, Whitaker et al. 1996, Leviton and Gilles 1996, Kuban et al. 1999, Ment et al. 1999, Valkama et al. 2000, Panigrahy et al. 2001), and to both reduced brain volumes at term (Inder et al. 1999, Peterson et al. 2003, Vasileiadis et al. 2004, Inder et al. 2005, Lodygensky et al. 2005, Boardman et al. 2006, Srinivasan et al. 2007, Thompson et al. 2007), and at older ages (Stewart et al. 1999, Isaacs et al. 2000, Peterson et al. 2000, Melhem et al. 2000, Allin et al. 2001, Nosarti et al. 2002, Argyropoulou et al. 2003, Reiss et al. 2004, Fearon et al. 2004, Nosarti et al. 2004 and 2008, Ment et al. 2009) when compared to full term controls.

There are several reasons for preterm births. One common reason is pre-eclampsia, which is often associated with the placental insufficiency that can be detected by an abnormal umbilical artery (UA) flow in fetuses. Abnormal blood flow of the UA, in turn, has been associated with increased mortality (Kasdorp and van Vugt 1994, Valcamonico et al. 1994, Voschbeck et al. 2001, Schreuder et al. 2002, Soregaroli et al. 2002, Vergani et al. 2003, Hartung et al. 2005, Kamoji et al. 2008, Byun et al. 2009) and with brain injury in preterm small for gestational age (SGA) infants (Tolsa et al. 2004). The optimal timing of delivery for VLBW infants is often difficult to determine. There is only one study evaluating the consequences of delaying delivery in the case of placental insufficiency in preterm infants. The authors showed that delaying delivery for four days in infants with absent or reversed end diastolic flow (AREDF) of the UA, increased intrauterine mortality and did not improve the two-year cognitive development of the surviving preterm infants (GRIT Study Group 2004).

Perinatal medications may have positive or negative effects in developing the brain of the fetus or the newborn. For example, the benefit of antenatal steroids in maturing the lungs in VLBW infants has been shown in several studies. In addition, antenatal steroids have been shown to have favourable and protective effects on the brain and have been associated with advantageous developmental outcome in VLBW infants (Crane et al. 2003, Roberts and Dalziel 2006). On the other hand, the use of dexamethasone with severe bronchopulmonary dysplasia (BPD) during the neonatal period has been shown to adversely affect brain growth (Yeh et al. 1998, Merz et al. 1999, Murphy et al. 2001, Thompson et al. 2008) and reduce cortical gray matter volume in preterm infants (Murphy et al. 2001).

Both everyday morbidities of VLBW infants and their related treatment practices in neonatal care might be potentially dangerous for the brain, or at least may affect brain development (Gressens et al. 2002). During NICU care, infections of the VLBW infant (Heep et al. 2003, Stoll et al. 2003*)*, nectoritizing enterocolitis (NEC) (Hintz et al. 2005, Stoll and Hansen 2003), and chronic lung disease (CLD) (Vasileiadis et al. 2004, Boardman et al. 2007), have been associated with brain injury and poorer later outcome in VLBW infants. In very preterm or in VLBW infants, incidences of septicaemia, NEC, and CLD were 23 %, 7 % and 34 %, respectively in the Vermont-Oxford Network Database 2008.

Preterm infants with (Brown et al. 2009) or without (Wolf et al. 2002) brain injuries have been shown to be more irritable in test situations when compared to full term controls. Persistent crying problems in infants lasting beyond the first 3 months of life, have been shown to correlate to later sleeping and feeding problems (Wolke et al. 1998 and 2009), to cognitive problems (Rao et al. 2004, Wolke et al. 2009) to hyperactivity, to other behavioural problems, and to academic difficulties at school age (Wolke et al. 2002, Rao et al. 2004). It is not known if brain injury affects the spontaneous crying behaviour of VLBW infants.

Brain injury is a serious complication of prematurity, leading to possible long term consequences for the neurodevelopment of the VLBW infant, such as cerebral palsy (CP), hearing impairments, vision problems, and delay in cognitive development (Emsley et al. 1998*,* Hack and Fanaroff 1999*,* Johnson et al. 2009). The reduction of brain complications in preterm infants is an important goal in the treatment of the increasing number of surviving VLBW infants. There is a need for further studies to ascertain the potential protective and risk factors, and their causal relationships to brain vulnerability, growth and development in VLBW infants.

REVIEW OF THE LITERATURE

1. PRETERM INFANT

Low gestational age, very low gestational age (VLGA), and extremely low gestational age (ELGA) births are defined as a gestational age (GA) of less than 37 weeks, less than 32 weeks and less than 28 weeks, respectively, according to the World Health Organization's (WHO) International Classification of Diseases. Furthermore, low birth weight (LBW) is defined as a birth weight (BW) of less than 2500 g, very low birth weight (VLBW) is defined as a BW of less than 1500 g, and extremely low birth weight (ELBW) is defined as a BW of less than 1000 g. There were 59616 live births in Finland in 2008. 5.5 % (n=3297) of these infants were born preterm, 0.8 % (n=486) very preterm and 0.3 % (n=180) extremely preterm and 4.3 % (n=2575), 0.9 % (n=531) and 0.4 % (n=243) were LBW, VLBW and ELBW infants, respectively (The National Birthregister 2008 /live births). All preterm live births from 2001-2008 at Turku University Hospital are shown in Table 1.

Table 1. Live births at Turku University Hospital, Finland according to birthweight (BW) and gestational age (GA) from 2001-2006.

	2001	2002	2003	2004	2005	2006
All live born infants in TYKS, n	3481	3497	3618	3859	3798	3823
GA <37 weeks, n (%)	311(8.9)	309 (8.8)	251 (6.9)	313 (8.1)	286 (7.5)	259 (6.8)
GA <32 weeks, n (%)	60 (1.7)	57 (1.6)	47 (1.2)	51 (1.3)	37 (1.0)	46 (1.2)
GA <28 weeks, n (%)	20 (0.6)	28 (0.8)	21 (0.6)	18 (0.5)	15 (0.4)	11 (0.3)
BW <2500 g, n (%)	206 (5.9)	210 (6.0)	191 (5.2)	208 (5.3)	215 (5.7)	185 (4.8)
BW<1500 g, n (%)	47 (1.3)	52 (1.4)	38 (1.0)	48 (1.2)	41 (1.0)	33 (0.9)
BW<1000 g, n (%)	19 (0.5)	33 (0.9)	20 (0.6)	24 (0.6)	19 (0.5)	14 (0.4)
All live born infants in Finland, n	55786	55333	56449	57569	57636	58859

Even though survival rates have increased, a very preterm birth is still strongly associated with increased perinatal mortality. Perinatal mortality including stillbirths and deaths during the first seven days of life was 4.9 per 1000 births in Finland (4.8 in South-West Finland) in 2008. Among infants with a birth weight of 1000-1499g and 500-999g perinatal mortality was 118 and 361 per 1000 births, respectively, in 2008 according to the Medical Birth Register at the National Institute for Health and Welfare (THL). Of all birth cohort deaths, 60% have been due to very preterm birth (Slattery and Morrison 2002). Of all live born VLBW or VLGA (infants born before 32 gestational weeks) Finnish infants born between 2000-2003, a total of 11% died during their first year of life (Rautava et al. 2007).

2. ANTENATAL DOPPLER MEASUREMENT OF THE BLOOD FLOW OF PREMATURELY BORN INFANTS

Doppler US measurements are routinely used while monitoring fetus well-being in risk pregnancies (Sonesson and Fouron 1997, Baschat et al. 2000). The pulsatility index (PI) is used to measure the variability of blood velocity in vessels. PI increases when the resistance of the blood flow increases. This results in a decrease in blood flow in that vessel. Vice versa, PI decreases when the resistance of the blood flow decreases and blood flow increases in the respective vessel. The PI is often calculated, as described by Gosling and King (1975), from three waveform measurements of the vessels [PI= (systolic velocity – diastolic velocity)/mean velocity]. The PI ratios between the fetal peripheral vessels (i.e. UA, descending aorta (DAo)) and the central cerebral vessel (i.e. middle cerebral artery (MCA)) are often calculated. The directions of the net blood flow of the aortic isthmus (AI) and the end diastolic flow of the ductus venosus (DV) are defined as antegradic (i.e. normal), retrogradic or absent.

2.1. Blood flow of the different vessels

2.1.1. Umbilical artery (UA)

Fetal blood with low amounts of oxygen, flows in the UA towards the placenta to be reoxygenized. The diastolic component of the blood flow in the UA can be seen from gestational week 12. An abnormally decreased, absent or reversed diastolic component of the flow of the UA, due to increased flow resistance is an early sign of fetal asphyxia (Brezinka 2001, Gudmundsson and Dubiel 2001, Mäkikallio et al. 2002, Baschat 2004). The flow of the UA is sensitive to placental resistance so that the pulsatility index (PI) of the UA increases with increasing resistance in the case of placental insufficiency, which in turn means that the blood flow in the UA decreases.

2.1.2. Middle cerebral artery (MCA)

Increased blood flow to the fetal brain is associated with maternal hypoxia or reduction in the umbilical arterial blood flow. Increased fetal cerebral diastolic blood velocity is related to reduced cerebral vascular resistance and this is a characteristic sign of fetal blood flow redistribution and called the brain sparing effect. The brain sparing effect is defined as the circulatory adaptation of the fetus, resulting in preferential perfusion of the central system. This mechanism is supposed to prevent the fetal brain from developing hypoxia (Scherjon et al. 1993).

In prolonged hypoxia, the fetus redistributes the blood to the brain, myocardium and adrenals through peripheral blood flow. The blood perfusion concentrates on vital organs giving essential supply to the brain, heart and adrenal glands (Mari and Deter 1992,

Baschat 2004). In hypoxia, the diastolic blood flow of the MCA increases (Brezinka 2001, Gudmundsson and Dubiel 2001, Baschat 2004). Increased blood flow of the MCA is a reliable sign of hypoxia and called centralization. However, if the asphyxia worsens and compensatory mechanisms fail, the PI of the MCA may seem misleadingly "normal" again (Pourcyrous 1999, Gudmundsson and Dubiel 2001, Baschat 2004). Similarly, when the hypoxia worsens, the UA/MCA PI ratio increases and this is called brain sparing. The brain sparing effect has been associated in some studies with an increased incidence of IVH (Ertan et al. 2006).

2.1.3. Aortic isthmus (AI) and descending aorta (DAo)

The aortic isthmus (AI) is an arterial shunt in the fetus, connecting the left subclavian artery and ductus arteriosus. It is a link between the left and right ventricles of the heart. In diastole, when the heart valves are closed, the direction of the blood flow in the AI reflects the direction of the blood flow of the cerebrum and placenta. The normal direction of the end-diastolic flow of the AI is antegradic (Bonnin et al. 1993, Räsänen et al. 1996). In hypoxia, the blood flow circulation of the fetal heart increases (Baschat 2004). In fetal blood redistribution flow, the end-diastolic blood flow of the AI reverses in a retrogradic direction (Mäkikallio et al. 2003, Baschat 2004). This change in AI blood flow is a sensitive indicator for increased resistance in placental flow. In hypoxia, low oxygen content blood from the DAo flows towards the brain. The direction of the net blood flow of the AI becomes abnormal earlier in fetal hypoxemia, than the direction of the end-diastolic flow of the DV (Del Rio et al. 2008, Rizzo et al. 2008). A recent study showed that abnormal blood flow of the AI happens on average one week before the blood flow of the DV becomes abnormal (Figueras et al. 2009).

2.1.4. Ductus venosus (DV)

The DV originates from the portal sinus, ascending steeply in the direction of the diaphragm. The ostium of the DV is aligned to the outlet of the umbilical vein. The umbilical vein brings oxygenated blood from the placenta through the DV into the fetal heart. The DV shunts and reduces the umbilical flow to the liver. From the DV, blood goes through the foramen ovale into the ascending aorta and into the AI. Retrogradic total end diastolic flow of the DV is visible on US 3-5 days earlier than changes in cardiotocography or in umbilical venal flow. Abnormal blood flow in the DV is a late sign reflecting acute impaired myocardial relaxation and acidemia.

2.2. Abnormal fetal blood flow and outcome

Placental insufficiency is a common finding in SGA fetuses (Baschat 2004) and is associated with chronic hypoxia, preterm birth and perinatal mortality and morbidity (Kasdorp and vanVugt 1994, Mongelli and Gardosi 2000, Buyn et al. 2009).

The AREDF of the UA has been associated with the perinatal mortality and morbidity of the preterm infant (Kasdorp and van Vugt 1994, Valcamonico et al. 1994, Voschbeck et al. 2001, Schreuder et al. 2002, Soregaroli et al. 2002, Vergani et al. 2003, Hartung et al. 2005, Kamoji et al. 2008, Byun et al. 2009). One multicenter study showed that delaying delivery for 4 days in infants with AREDF of the UA, increased intrauterine mortality and also did not improve the 2-year cognitive development of the surviving preterm infants (the GRIT Study Group 2004). Abnormal UA flow has been associated with poorer neurodevelopmental outcome including CP, global delay and poor neurosensory development (Valcamonico et al. 1994, Voschbeck et al. 2001, Wienerroither et al. 2001, Schreuder et al. 2002, Baschat et al. 2009).

An increased UA/MCA PI ratio has been associated with behavioral problems at 18 months of age, measured by the Child Behavior Checklist (Roza et al. 2008). Scherjon et al. (2000) showed that preterm infants who had an antenatally increased UA/MCA PI ratio had lower cognitive levels at 5 years of age than preterm infants without an abnormal UA/MCA PI ratio. On the other hand, a recent study showed no correlation between the brain sparing effect and poorer outcome (Baschat et al. 2009).

Retrogradic net blood flow of the AI has been strongly associated with increased risk for neonatal death (Del Rio et al. 2008) and with poorer neurodevelopmental outcome and/or a nonoptimal cognitive outcome at 2 and 5 years of age (Fouron et al. 2005).

Abnormal end-diastolic umbilical venous pulsation, is a late and ominous sign of severe hypoxia in fetuses (Gudmundsson et al. 1996). Many previous studies have shown, that abnormal venous blood flow identifies most specifically the SGA preterm infants that are at increased risk for neonatal death or an otherwise adverse outcome (Ozcan T et al. 1998, Baschat et al. 2000, Hecher et al. 2001, Bilardo et al. 2004, Baschat et al. 2009, Hernandez-Andrade et al. 2009).

3. BRAIN MATURATION

The cerebrum develops from the germinal matrix (Barkovich 1995, Volpe 1995**a**), which serves as a source of cerebral neuroblasts and provides glioblasts that later will become oligodendroglia and astrocytes. All neurons and glia are derived from this germinal matrix. During the third trimester, the size of the germinal matrix diminishes in a caudocranial direction. This process is completed by the end of the 34th gestational week. Between the 24th and 34th gestational weeks the germinal matrix is particularly prominent in the caudothalamic groove at the level of the head of the caudate nucleus. The germinal matrix involutes by term age (Greisen 1992, Rorke 1992). As the cortex becomes more complex and its gyral pattern develops, blood supply moves away from the germinal matrix to the cortex. The incidence of hemorrhages decreases with increasing maturity

and thus a germinal matrix hemorrhage is very unusual after 34 weeks of gestation (Greisen 1992).

Human cortical maturation, connections and complexity develop between 24 and 32 gestational weeks (Ajayi-Obe et al. 2000). Although cell migration is largely completed by the 25th gestational week, glial migration continues thereafter. This results in an increase of the surface area of the cortex as cortical folding develops (Ajayi-Obe et al. 2000). It has been shown that the cerebral cortex in ELBW infants is less complex and has less cortical surface area when compared to full term controls at term age (Ajayi-Obe et al. 2000).

The vessels that penetrate the cerebral wall are highly undeveloped between weeks 24 and 28. They form vascular end and border zones in cerebral WM regions, which in turn are vulnerable to ischemic injuries (Inder and Volpe 2000). The border zone of poor vascularization is located between the areas of cortical and basal circulation in the periventricular area i.e. in WM regions and between the different cerebral arteries (Pape et al. 1979, Rorke 1992). A well-developed vascular bed is essential for supplying the central part of the cerebrum, and the arteries of the midbrain, brainstem, and cerebellum. Throughout the third trimester, there is a gradual increase in density of cortical and WM vessels (Wigglesworth 1989).

The development of myelin membrane around axons and neurons is called myelination. Brain myelination begins during the fifth fetal month (Brody et al. 1987). Myelination occurs most rapidly during the first two postnatal years and continues more slowly into adulthood. Myelination happens in a caudocranial direction from the brain stem from 29 gestational weeks (McArdle et al. 1987). At the age of 30-34 gestational weeks myelin is found in the brain stem, cerebellum and cortex bordering the hippocampus and central sulcus. During weeks 34 - 36 of gestation, myelin also appears in the lateral part of the posterior limb of the internal capsule and in the corona radiata (Sie et al. 1997).

4. BRAIN IMAGING IN PRETERM INFANTS

4.1. Brain US in preterm infants

US provides an excellent view of the central parts of the brain, ventricles and periventricular areas through the anterior fontanel. It is non-invasive and can be used bedside in NICUs. US is an established method for screening typical brain complications related to prematurity, such as IVH and VD. The accuracy of US in the diagnosis of IVH, especially within the ventricles has been established in previous studies (Carson et al. 1990, Vohr and Ment 1996, Maalouf et al 2001, Horsch et al. 2009). In addition, US accurately predicted the presence of hemorrhagic parenchymal infarction on MRI

(Maalouf et al 2001). The limitations of US include difficulty in imaging the peripheral regions and posterior fossa structures of the brain (Helmke et al. 1987, Carson et al. 1990, Adcock et al. 1998, Kuban et al. 1999, Steggerda et al. 2009) and sensitivity in recognizing all the hypoxic-ischemic WM changes of the brain tissue is poor (Helmke et al. 1987, Carson et al. 1990, Adcock et al. 1998, Kuban et al. 1999). Maalouf et al. (2001) showed that normal WM signal intensities on US, do not predict normal WM signal intensities on MRI, and that US predicted well severe WM changes but that sensitivity was much poorer for mild and moderate WM changes. Similarly, a recent study (Horsch et al. 2009) showed that all severe WM findings were detected alike on US and on MRI, but that ELBW infants with a normal US at term might have milder WM abnormalities seen only on MRI. The presence of echogenic and echolucent areas bilaterally in WM on US has recently been shown to coincide mostly with the normal maturation processes of the preterm brain, although the echogenic areas can also be associated with delayed or abnormal maturation (Leijser et al. 2009).

US is routinely used in NICUs. It has been recommended that preterm infants (< 30 gestational weeks at birth) should be scanned by US at least once between 7 and 14 days, and once between 36 and 40 weeks' postmentrual age (i.e. at term) by the Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. The early US is used to detect most of the IVH's, and the term US for detecting late IVH, PVL, and VD (Ment et al. 2002).

2.2. Brain MRI in preterm infants

A MRI examination provides good anatomical resolution for the whole brain. Parenchymal i.e. white and gray matter abnormalities (Inder et al. 2003), as well as extracerebral abnormalities (McArdle et al. 1987), maturation of the myelinisation (Barcovich 1995) and posterior fossa structures (Steggerda et al. 2009) can all be detected by MRI. Early and late effects of perinatal hypoxic-ischemic insults on brain tissue can also be assessed by MRI. It also allows for more detailed definition of the developmental disorders of brain structure (Hüppi and Barnes 1997).

MRI is a non-invasive method, and with open MRI equipment, infants can be examined without anesthesia or sedation. MRI requires quite a long time (20 min) of immobilization, but in VLBW infants, swaddling after feeding often induces natural sleep. Unstable VLBW infants can be monitored by oxygen saturation, body temperature, or blood pressure during the examination.

Some previous studies have not recommended the routine use of MRI with VLBW infants (Ment et al. 2002, Hart et al. 2008). Both studies argue that there are insufficient data from follow-up studies to show that additional findings seen only on MRI (but not on US) would provide more information about the neurodevelopmental prognosis

than US findings alone. Hart et al. (2008) suggested brain MRI to be used at term age with preterm infants, only if, there is known or suspected brain injury on US or for example if the neurological status of the infant is abnormal. However, some other studies have recommended the routine use of MRI at term for all VLBW infants, as abnormal findings on MRI have been strongly associated with poorer two-year outcome, even when early serial neonatal US examinations have been controlled for as a confounding factor (Mirmiran et al. 2004, Woodward et al. 2006).

5. BRAIN IMAGING FINDINGS IN PRETERM INFANTS

5.1. Structural brain imaging findings on US

IVH in VLBW infants originates usually from the subependymal germinal matrix which ruptures through the ependyma into the ventricles (Whitelaw 2001). Classification of IVH according to Papile et al. (1978) includes four grades based on computer tomography imaging: IVH grade 1 is restricted to the subependymal matrix and occurs without intraventricular extension, IVH grade 2 extends into the ventricle without distending it, IVH grade 3 extends into the ventricle distending it and IVH grade 4 is described as a combination of hemorrhages in the germinal matrix, lateral ventricle and adjacent cerebral parenchyma. The bleed in this case has extended to the brain tissue and distended the ventricles. This grading has been criticized, as this classification is based on anatomical findings rather than on pathogenesis, as IVH grade 4 can quantitatively range from both a minimal to a massive bleed. According to Volpe (1995) IVH grade 4 extending into the parenchyme represents venous infarction with secondary hemorrhage and therefore it is also called periventricular hemorrhagic infarction. Alternatively, bleeding may occur into the parenchyma that is injured by ischemia (Volpe 1998). Whitelaw (2001) divided IVH into three grades: grade 1 confined to the subependymal matrix, grade 2 confined to the ventricles without distending them, and grade 3 extending more than half of the ventricle length and in addition distending the ventricle.

PVL is an ischemic brain injury in the posterior periventricular WM, adjacent to the lateral ventricle and adjacent to the foramina of Monro (Barkovich 1995). PVL can be classified into three categories: Grade 1 PVL is described as increased echogenicity in the periventricular areas and is visible seven days or more after injury. Grade 2 is described as a lesion, where increased echogenicity evolves into small, fronto-parietal cysts and grade 3 is decribed as periventricular echogenicity areas which develop into extensive periventricular cystic lesions, involving the occipital and fronto-parietal WM (de Vries et al. 1993). PVL can form local cysts due to periventricular necrosis in the area of the end zones of the long penetrating arteries adjacent to the lateral ventricles. The cysts are formed 1-3 weeks after the injury (Inder and Volpe 2000, Rees and Inder 2005).

PVL has been associated with impaired cortical development (Inder and Volpe 2000) and deep gray matter injury (Rees and Inder 2005).

Incidences of IVH and cPVL (cystic PVL) decreased to 26 % and 3 % in VLBW/VLGA infants in 2007, respectively (VLBW or <30 GA) (Vermont Oxford Network Database 2007) when compared to earlier studies. It has been shown that infants treated with antenatal steroids, had significantly less grade 4 IVH when compared to infants without the antenatal steroid treatment (Baud et al. 1999, Lee et al. 2006). There are controversial results regarding the association between the quality of antenatal glucocorticoids and cPVL (Baud et al. 1999, Lee et al. 2006). According to Baud et al. (1999) the incidence of cPVL was 4.4%, 11.0% and 8.4% in infants whose mothers had received antenatal betamethasone, dexamethasone or no antenatal glucocorticoids, respectively. In contrast, Lee et al. (2006) showed no difference in cPVL status with respect to antenatal steroid exposure, for example, to dexamethasone, betamethasone or to no steroid exposure.

Horbar et al. 2002 showed that the incidences of any grade of IVH were in 1991 and 1999 27.4% and 24.6%, of which severe IVH (grades 3 and 4) was 10.1% and 8.2%, respectively. The 2005 Vermont Oxford cohort recorded any grade of IVH as 27%, severe IVH as 6% and cPVL as 3% (Chedid et al.2009). A germinal matrix hemorrhage and IVH are much commoner than an intraparenchymal hemorrhage. Diffuse non-cystic PVL has been suggested to be commoner than cPVL in VLBW infants (Volpe 2003, Rees and Inder 2005).

Incidences of VD vary according to definitions but it is one of the commonest findings in preterm populations. In a previous study, the incidence of VD at term recorded using clinical judgement in VLBW infants varied from 30% to 40% with or without hemorrhagic and non-hemorrhagic WM injury, respectively (Paneth et al.1990). The incidence of moderate to severe VD determined by the width of the lateral ventricle from a sagittal scan on US has been shown to be 4% in VLBW infants (Ment et al.2000). The definition of VD varies according to the measurement used. Grasby et al. (2002) showed in their study that ventricular height and diagonal width correlated better with clinical judgements of VD than the ventricular brain (V/B) ratio, especially in mild VD. They recommended measuring the ventricular diagonal width in the coronal plane and the ventricular height at the level of the thalamus (corresponding to the level of foramen of Monro) when defining mild VD. The different definitions of VD are described in detail in the methods section on pages 45-47.

VD has been shown to be associated with WM injury in VLBW infants (Paneth et al. 1990, Whitaker et al. 1996, Kuban et al. 1999, Valkama et al. 2000, Panigrahy et al. 2001), as well as with IVH (Kuban et al. 1999, Ment et al. 1999) and BPD (Ment et al. 1999). In addition, VD in preterm infants has been shown to be a good predictive sign of cortical injury (Leviton and Gilles 1996).

5.2. Structural brain imaging findings on MRI

The most common type of brain injury in VLBW infants is a noncystic diffuse WM injury (Inder and Volpe 2000, Inder et al.2003, Volpe 2003, Rees and Inder 2005, Ruthenford et al. 2010). The possible detection of a diffuse noncystic WM injury using US is limited (Maalouf et al. 2001, Inder et al. 2003), and MRI has been shown to be more sensitive in detecting WM lesions (Inder et al. 2003).

Other typical brain findings among VLBW infants include, for example, hypoplasia of the corpus callosum (Stewart et al. 1999, Peterson et al. 2000, Nosarti et al. 2004). The developing corpus callosum is vulnerable to ischemia and hemorrhage. This may be due to the vulnerability of immature oligodendrocytes in the corpus callosum (Back et al. 2001). In addition, cerebellar lesions (Vollmer et al. 1999, Volpe 2009), a widened extracerebral space (Maalouf et al. 1999, Leijser et al. 2007) and developmental disorders of the cerebral cortex (Ajayi-Obe et al. 2000, Kesler et al. 2004) are other typical brain abnormalities in preterm infants. The incidence of cerebellar hemorrhage has been shown to be 17% in infants with BW below 750g and 2% in those with BW between 750 g and 1500 g (Bednarek et al. 2008). In a recent study, cerebellar injury was diagnosed in 19 % of very preterm infants in the neonatal period (Steggerda et al. 2009). The hypoplasia of the cerebellum has been associated with low GA (Limperopoulos et al. 2005) and with IVH and PVL (Volpe 2009) in preterm infants. A widened extracerebral space is defined as a subarachnoid space over 4 mm in width in the interhemispheric fissure (McArdle et al.1987, Valkama et al. 2000). There are conflicting views regarding its clinical significance, as some studies have argued that there is no significance in the isolated finding of a widened extracerebral space. However, together with other brain injuries it can be seen as a significant risk factor for later development (Lui et al. 1990, Valkama et al. 2000). According to Dyet et al. (2006) the incidence of any grade of VD on MRI images at term in VLBW infants with or without IVH is 50% and 53%, respectively.

Delayed myelination has been associated with preterm birth (Van der Bor 1990, Skranes et al. 1993), but also with hypoxic-ischemic lesions or VD (McArdle et al. 1987, deVries et al. 1989, Leviton and Gilles 1996, Ramenghi et al. 2007, Ruthenford et al. 2010). Ruthenford et al. (2010) showed in diffusion tensor imaging MRI studies, that the posterior limb of the internal capsule and the posterior thalamic radiation were the most commonly affected tracts resulting in delayed myelination.

5.3. Brain volume findings on MRI

In previous studies, preterm birth has been associated with smaller volumes in total brain tissue (Tolsa et al. 2004) and of the cerebellum (Limperopoulos et al. 2005, Allin et al. 2005, Shah et al. 2006, Volpe 2009) at term. In addition, total brain tissue (Nosarti

et al. 2002) and the cerebellum **(**Peterson et al. 2000, Allin et al. 2001, Nosarti et al. 2008) have been shown to be smaller in adolescence. Furthermore, specific regional brain tissue volumes are smaller in VLBW infants than in full term infants at term (Inder et al. 1999, Peterson et al. 2003, Vasileiadis et al. 2004, Inder et al. 2005, Lodygensky et al. 2005, Thompson et al. 2007, Boardman et al. 2006, Srinivasan et al. 2007), at toddler age (Argyropoulou et al. 2003, Peterson et al. 2000, Reiss et al. 2004), at school age (Melhem et al. 2000) and still in adulthood (Stewart et al. 1999, Isaacs et al. 2000, Peterson et al. 2000, Allin et al. 2001, Nosarti et al. 2002, 2004, 2008, Fearon et al. 2004, Ment et al. 2009) when compared to full term controls. In addition, the volumes of the ventricles are larger in VLBW infants when compared to full term controls in adulthood (Fearon et al. 2004). In preterm populations, the brain volumes of SGA infants are smaller at term, than the volumes of appropriate for gestational age preterm infants (Tolsa et al. 2004).

Compared to full term infants, regional smaller brain tissue volumes in preterm infants include cortical volumes (Peterson et al. 2000, Nosarti et al. 2002, Reiss et al. 2004, Thompson et al. 2007, Nosarti et al. 2008, Kesler et al. 2008), WM (Peterson et al. 2000, Fearon et al. 2004, Reiss et al. 2004, Nosarti et al. 2008, Kesler et al. 2008), and deep gray matter (Isaacs et al. 2000, Peterson et al. 2000, Nosarti et al. 2002, 2008, Kesler et al. 2008). Preterm infants have been shown to have both gray matter reduction and less WM gain over time, compared to term infants (Ment et al. 2009). On the other hand, there are studies which show that prematurity by itself without brain injury as a confounding factor, does not affect brain volumes (Mewes et al. 2006, Boardman et al. 2007).

A recent study showed that smaller brain diameters on MRI (bifrontal, biparietal measures of the cerebrum and transverse measures of the cerebellum) at term in VLBW infants, correlated with smaller total brain and cortical gray matter volumes and VD in preterm infants when compared to full term infants. These measures are suggested to be reliable and an easily applicable method to assess brain growth and atrophy in the high risk group of preterm infants (Ngyuen et al. 2009).

All grades of IVH and WM injuries diminish brain tissue volumes at term (Inder et al. 1999, Vasileiadis et al. 2004, Inder et al. 2005, Limperopoulos et al. 2005b, Boardman et al. 2006, Thompson et al. 2007, Srinivasan et al. 2007) and also at toddler age (Melhem et al. 2000). More specifically, severe WM injury is associated with reduced volumes of cortical gray matter (Inder et al. 1999, 2005), hippocampus (Thompson et al. 2008), thalami and lentiform nuclei (Boardman et al. 2006, Srinivasan et al. 2007), myelinated WM (Inder et al. 1999), cerebellum (Limperopoulos et al. 2005a) and volumes of dorsal prefrontal, sensorimotor, midtemporal, parieto-occipital, and inferior occipital regions

(Thompson et al. 2007). Unilateral cerebellar injury has been associated with smaller specific cortical gray and cerebral white matter volumes (Limperoloulos et al. 2010).

Other factors reducing regional brain volumes, include placental insufficiency in SGA infants (Tolsa et al. 2004), CLD (Vasileiadis et al. 2004, Thompson et al. 2006, Boardman et al. 2007), operated NEC (Hintz et al. 2005) and postnatal dexamethasone treatment for severe CLD (Murphy et al. 2001, Thompson et al. 2008), or indomethacine treatment to close patent ductus arteriosus (Thompson et al. 2008). Brain volumes also differ between genders, in that the volume of the cerebrum is 10% larger in boys when compared to girls until five years of age. This has been suggested to be due to the different cortical neuronal density between genders. After five years of age the volume differences do not differ between genders (Reiss et al. 1996 and 2004).

5.4. Brain findings in relation to adverse outcome

Early brain injury is the most serious complication of prematurity affecting development. It has been found that in preterm infants abnormal findings on brain US during the neonatal period were related to poorer performance on the Dubowitz neurological assessment at term (Molteno et al. 1995). In addition, if the brain US was normal, low gestational weight or fewer completed gestational weeks at birth did not increase the risk for abnormal motor or neurodevelopmental outcome at one year of age (Molteno et al. 1995). Previous studies have shown that there is a 5% risk for CP in preterm infants with normal brain findings according to serial US examinations in neonatal period (Graham et al. 1987, Stewart et al. 1987). The same incidence of CP has been found among infants with isolated IVH (Levene et al. 1990).

The sensitivity of US in predicting CP, ranges from 38 % to 60 % (Valkama et al. 2000)**.** PVL often causes generalized brain injury as it effects the periventricular WM, usually bilaterally. Accordingly, motor disability is usually manifested as spastic diplegia, but when the lesions are wider, all limbs may be affected. IVH of any grade has been associated with poorer cognitive outcome on Bayley tests (Ross et al. 1996). IVH involving brain parenchyma is often unilateral and may result in hemiplegia.

Both transient and stable VD have been shown to be a risk factor for abnormal neurodevelopment (Ment et al. 1999, Leviton and Gilles 1996, Ment et al. 2002, Dyet et al. 2006) and especially for CP (Whitaker et al. 1996, Ment et al. 1999, Valkama et al. 2000, Msall et al. 2006). An increase in cerebrospinal fluid volume has been associated with poorer neurodevelopmental outcome at one year of age (Inder et al. 2005). Typically, children with VD have later problems especially in visuomotor performance (Whitaker et al. 1996, Ment et al. 1999, Vollmer et al. 2006), presenting for example, as specific learning problems in geometric tasks at school age (Ment et al. 1999). A recent study (Brouwer et al. 2008) showed that VLBW infants (GA $\geq$ 25 weeks) with IVH grade III

or IV combined with VD (without progressive VD) had better cognitive outcome at two years of age than reported in previous studies. Progressive VD, i.e. hydrocephalus, has been reported to predict the poorest outcome of all the different forms of VD, with an increased risk for death and abnormal neurodevelopmental outcome in as many as 90 % of the surviving infants, with 56% of them having multiple impairments (Ventriculomegaly Trial Group 1994, Murphy et al. 2002, Brouwer et al. 2008). Moderate and severe VD defined by ventricular midbody diameters of 1.1-1.5, and >1.5 cm, respectively, as measured from an US sagittal scan in the neonatal period, has been shown to correlate moderately with a clinical outcome of CP at 2.5-3 years of age (Allan et al. 1997). In contrast, one study measuring the V/B ratio using MRI failed to predict CP in a cohort of VLBW infants (Valkama et al. 2000). In their study the V/B ratio measured using US was more sensitive than the V/B ratio measured by MRI in predicting CP.

Previous studies have shown that parenchymal abnormalities on brain MRI at term, correlate with CP (Ajayi-Obe et al. 2000, Valkama et al. 2000, Maalouf et al. 2001, Bax et al. 2006, Gkoltsiou et al. 2008, Hoon et al. 2009). Parenchymal abnormalities detected on MRI have been associated with poorer outcome in preterm infants in several previous studies (Hüppi and Barnes 1997, Barkovich et al. 1998, Rutherford et al. 1998, Valkama et al. 2000). Hypoplasia of the corpus callosum on MRI has also been shown to be a risk factor for poorer outcome (Sauerwein and Lassonde 1994, Stewart et al. 1999, Nosarti et al. 2004). In addition, cerebellar injury has been associated with a broad spectrum of neurodevelopmental disabilities in term born infants (Limperopoulos et al. 2009).

In previous studies smaller total and regional brain volumes at term correlated with poorer memory functions (Isaacs et al. 2000, Woodward et al. 2005, Beauchamp et al. 2008) at two years of age. In addition, reductions in WM volumes in the sensorimotor and midtemporal regions at term have been associated with poorer cognitive outcome at 18 and 20 months of age (Peterson et al. 2003) and reduced cerebellar volume at term has been correlated with poorer cognitive and motor development at two years of age (Shah et al. 2006). Smaller total and regional brain volumes at term have also been correlated with poorer performance at school age (Peterson et al. 2000, 2003) and also in adulthood (Nosarti et al. 2008) in VLBW infants. More specifically, decreased cerebral gray matter (Reiss et al. 2004, Inder et al. 2005, Nosarti et al. 2008), cortical (Peterson et al. 2000) and cerebellar (Allin et al. 2001) volumes have been associated with poorer cognitive performance at school age. Furthermore, smaller volumes of nucleus caudatus (Abernethy et al. 2002 and 2004, Nosarti et al. 2005) and thalami (Gimenez et al. 2006) have been associated with developmental and behavioural problems in preterm infants at school age. In addition, there has been an association between smaller cerebellar volumes and impairments in executive and visuospatial functions and language skills in prematurely born infants in adolescence (Allin et al. 2001).

6. NEUROLOGICAL AND BEHAVIOURAL EXAMINATION OF THE PRETERM INFANT

Neurological assessment of the VLBW infant is a challenging task. During the first weeks of life the lability of vital functions and morbidity can lead to both false negative and positive findings. The infants behavioural state during the examination can also affect the results (Prechtl 1982, 1997, Brazelton 1984). Thus, the examination should always be carried out when the infant is alert and awake, not crying. The evaluation of status and behavior is most reliable when it can be done serially (Brandt et al. 2000).

In preterm infants the maturation of the subcortical system is reflected in the progressive appearance of a passive flexor tone in a caudocephalic direction. Lower limbs develop a flexed posture from approximately 28 weeks of gestational age, followed by flexion of the upper limbs towards 34 weeks of gestational age. Postural reflexes against gravity develop also in the same direction. Extension of the legs when the infant is held in a vertical position happens first, and is followed by extension of the trunk. After 34 weeks, the maturation of the subcortical system is almost completed. Between 38 and 40 weeks, balance is achieved between the neck extensors and flexors. Fixation on the target can be detected from 30 weeks of gestation (Dubowitz et al. 1980). According to Mercuri et al. (2003) preterm infants without major brain pathology were more hyperexcitable and tended to have less flexor tone in the limbs, as well as less extensor tone in the neck at term age, when compared to full-term infants.

6.1. Developmental neurological assessments in preterm infants

Dargassies et al. introduced in the 1950's-1960's the concept of the maturation process of tone and primitive reflexes among preterm infants. She showed that the trend in development of the preterm infant was similar outside and inside the uterus. Similarly, Amiel-Tison in the 1960's introduced a neurological examination based on tone and reflexes. It was standardized for preterm infants but couldn't be used in the first few days of life. These assessments were based on tone and reflexes, and thus reflected lower cerebral functions.

Brazelton (1973) developed a Neonatal Behavioural Assessment Scale that can be used with preterm infants, although it has been standardized only for full-term infants. It is mainly used to evaluate the behaviour of an infant but has also been administered in neurological examinations. It is, however, time consuming and thus not so practical for everyday use, consisting of 26 individual criteria with scores on a 9-point-scale.

Prechtl (1977) introduced a version of an examination protocol that could be used with term infants to score on infant's behaviour. The neurological tests in this instence are to be carried out while the infant is awake and content, and not hungry or crying

(Prechtl 1982, Brazelton 1984, Prechtl 1997). These examinations focusing on scoring behavioural functions reflect mostly higher cerebral functions. They are time consuming and need considerable experience to administer. Thus, they have been mostly used as research tools rather than in clinical practice.

General movement (GM) evaluation is based on video observations of the spontaneous movements of the infant. A qualitative observation assessment of a newborns GMs (Prechtl 1990), has been shown in many studies to be a better tool than neurological examination in predicting neurological outcome in preterm infants with brain injury (Ferrari et al. 1990, Geerdink and Hopkins 1993, Hadders-Algra et al. 1997, Cioni et al. 1997). If an infant has transient abnormal neurological symptoms or signs which do not lead to permanent neurological abnormalities, the GMs normalize earlier, than the results of neurological examinations (Cioni et al. 1997). Although the method is inexpensive and highly reliable, it is time consuming, and needs a formally trained person to analyze the tapes. It is not possible to use it in every day practice. Therefore, there are only a few neurological tests valid and feasible for preterm populations (Dubowitz and Dubowitz 1981, Dubowitz et al. 1999).

6.1.1. Dubowitz Assessment

The Dubowitz Neurological Assessment (Dubowitz V and Dubowitz L 1981, Dubowitz et al. 1998) was developed because of the need for an objective, easy and quick neurological test that could be used with preterm infants, right after birth. It can be used in the NICU, and takes only 15 minutes both administer and complete the proforma. The assessment can be used sequentially to follow the neurological signs and maturation of the infant. This method is suitable for both clinical use and research.

Tone is defined as the resistance of the muscle to stretch. It is evaluated by observing the resting posture, and by assessing the resistance of the limbs to passive movement or to changes in posture (recoils). Tone in the hamstrings can be assessed by measurement of the popliteal angle. In the normal preterm infant, arm flexor tone is less than that of the legs. In addition, extensor tone in the neck muscles can be better demonstrated from term age.

The examination consists of 34 selected neurological and neurobehavioural items. The examination proforma contains detailed instructions and diagrams. There are items describing lower and higher cerebral functions. In addition, the behavioural state of the infant is scored according to Brazelton's criteria (1973). The examinations should be administered while the infant is awake and alert, and not hungry or crying.

In addition to the qualitative assessment this test can also be used quantitatively. For newborn infants at term, an optimality score of $\leq$ 30.5-34 has been considered as normal

(Dubowitz et al. 1998, 1999). However, the optimality score cannot be used with VLBW preterm infants (Mercuri et al. 2003). The frequency distribution of findings from the Dubowitz assessment of preterm infants at term has been recently assessed in an European multicenter study (Ricci et al. 2008). The range of normal findings in preterm infants was wide and most of the differences compared to term norms were found in muscle tone. Preterm infants showed more brisk reflexes, startles and tremors than full-term controls (Mercuri et al. 2003). The systematic use of the same neurological test provides an opportunity to identify and follow abnormal neurologic signs some of which may also be transient (Molteno et al. 1995). For example, it has been shown that up to 60 % of VLBW infants have transient abnormal neurologic signs in neonatal examinations. However, the presence of permanent abnormal signs is more important when considering an assessments' positive predictive value with respect to abnormal development (Dubowitz LMS 1988, Brandt et al.2000). Four or more abnormal signs on the Dubowitz Assessment for low risk preterm infants, has been shown, for example, to indicate almost certainly that the infant will have developmental disabilities at one year of corrected age (Molteno et al. 1995). A recent study showed that the severity of outcome of preterm infants with major brain pathology at term, associated with the number of items outside the 90th centile on the Dubowitz Assessment (Ricci et al. 2008).

It has been shown that if the Dubowitz Assessment score is normal at term, the infant probably will develop without any major neurological impairment (Dubowitz et al. 1999). Dubowitz et al. (1984) reported that a cluster of abnormal neurological signs were associated more often with CP or dystonia than with marked hypotonia without any other abnormality. The Dubowitz Assessment has been shown to be as predictive as US in measuring later neurodevelopmental impairment, and there is a strong correlation between the test, the degree of perinatal risk, and brain findings on US (Molteno et al. 1995). In addition, the Dubowitz test has been found to exhibit good sensitivity (negative predictive value 92 %), but poor specifity (positive predictive value 34 %) in identifying the infants with major brain abnormality on MRI (Woodward et al. 2004).

A copy of the Dubowitz Assessment proforma is found in Appendix 1.

6.1.2. Hammersmith Infant Neurological Examination (HINE)

The Hammersmith Infant Neurological Examination (HINE) has been validated for full term infants between 12 and 18 months of age (Haataja et al. 1999). The assessment consists of 37 items that are further divided into three sections. The first section consists of assessments of cranial nerve function, posture, movements, tone and reflexes. The second section consists of descriptions of motor development, and the third section consists of items describing the behavioural state of the infant during examination. Each item can be scored and a global score is the sum of all the item scores. The maximum score from the test is 78. Scores ≥ 73 at 12 months of age, and ≥74 at 18 months of age are

regarded as optimal with full term infants. Scores between 40 -67 at one year of age were associated with restricted mobility and total points <40 at one year of age with severely limited self-mobility at four years of age in full term infants with hypoxic-ischemic encephalopathy (Haataja et al. 2001). The HINE has been shown to be applicable and predictive for VLBW infants at a high risk for developmental impairments between 9 months and two years of corrected age (Frisone et al. 2002, Romeo et al. 2009). The sensitivity of an optimal score above 64 in predicting walking at two years of age was 98%, and the specificity was 85% (Frisone et al. 2002). A recent study (Romeo et al. 2009) also showed that the HINE when administered at 9 to 12 months of corrected age, or even at three months of corrected age, had high predictive value with respect to locomotor function at two years of age.

A copy of the HINE proforma is found in Appendix 2.

6.1.3. Bayley Scales of Infant Development, 2nd edition

The Mental Development Index (MDI) is used to evaluate the level of cognitive performance. In studies of preterm infants the results are often corrected for prematurity (of term age) until two years of age.

BSID-II is a widely used method (Bayley 1993) to assess the cognitive and psychomotor development of preterm infants at two years of age (Wood et al. 2000 and 2005, Woodward et al. 2006, Wilson-Costello et al. 2007, Johnson et al. 2008, Kobaly et al. 2009, Rose et al. 2009). However, the 2nd edition has not been standardized for Finnish populations. In Finnish studies, the original normative data has been used to describe the cognitive performance of VLBW/VLGA infants (Munck et al. 2010). Recently, a third version of the BSID has been published (Bayley 2006), and normative data is now available, has also been validated for use with Finnish infants (Salo et al. 2008). This version was not available during the PIPARI-Study.

6.2. Behavioural examination of the preterm infant

6.2.1. Crying and fussing behaviour of the preterm infant

Healthy preterm infants seem to have similar crying patterns to full term infants when their ages are corrected for term age (Barr et al. 1996). Healthy full term infants increase their total amount of crying during the first two months of life, and they cry most in the evening hours. (Wessel et al. 1954, Brazelton 1962, Hunziker and Barr 1986, Barr et al. 1989, Barr 1990, St James-Roberts and Halil 1991, St James-Roberts et al.1994, Baildam et al. 1995). Full term infants have a crying peak during the second month of their life (mostly at six weeks of age), and thereafter the total amount of crying decreases until five months of age and changes only a little from then on (Hunziker and Barr 1986,

Barr 1990, St James-Roberts and Halil 1991, St James-Roberts et al. 1994). Barr et al showed (1996), that healthy preterm infants had clustered evening crying only at six weeks of corrected age. Studies describing crying behaviour of non-selected VLBW infants are lacking at the present time.

Wolf et al. 2002 showed that preterm infants, compared with term infants, were more irritable in neurobehavioral assessment situations at different age points between term age and 6 months. Oberlander et al. (2002) examined the crying behaviour of preterm infants with or without brain injury after a heel stick procedure, and found no differences with respect to brain status. In these preterm studies, infants with the most severe brain findings on US have been excluded. A recent study has shown, that irritable behaviour in VLBW infants at term in test situations is related to concurrent cerebral WM abnormalities on MRI (Brown et al. 2009).

6.2.2. Baby Day Diary

Parental diaries or questionnaires have been the most commonly used methods to determine the behaviour of an infant in home situations (Barr 1990). Overall amounts of daily crying and fussing measured by parental diaries have been validated against video recordings in home situations (Barr et al. 1982, 1988**,** 1989, St James-Roberts et al. 1993, St James-Roberts and Plewis 1996). As a day-to-day irregularity of crying is common, the diaries cover at least three days in order to measure mean amounts and frequencies per day.

The Baby Day Diary has been used in many previous studies. Baildam et al. showed (1995) that there was no relationship between infants' crying and the socioeconomical status of the family, gender, marital status, or age of the mother. Longer breast feeding has been associated with a lower amount of total crying (Hunziker and Barr 1986, Baildam et al. 1995). In addition, the ignorance of the mother to an infant's crying has been associated with longer daily total crying (Baildam et al. 1995). Whereas crying in early infancy is more related to physiological states, it later becomes more related to communication (Barr 1990). A longer duration of holding has been associated with a lower amount of total crying in the healthy infant. In addition, the crying peak during the second month of the infant's life was not observed if the infant was held more. However, the frequencies of crying and fussing were not affected by carrying the baby more. (Hunziker and Barr 1986)

A copy of the Baby Day Diary is found in Appendix 3.

7. NEUROLOGICAL AND BEHAVIOURAL OUTCOME IN VLBW INFANTS

The group of VLGA and VLBW infants (GA below 32 weeks of gestation or BW less than 1500 g) is the highest risk group for poorer neurodevelopmental outcome, especially the ELGA/ELBW infants (GA below 28 weeks of gestation or BW less than 1000 g) (Hack and Fanaroff 1999, Mercier et al. 2009, Stephens and Vohr 2009, Johnson et al. 2009). Cerebral palsy, cognitive impairment (Doyle and Anderson 2005, Vohr et al. 2005, Wilson-Costello et al. 2005, Johnson et al. 2009) and vision (O'Connor and Fielder 2007, Johnson et al. 2009) and hearing impairments (Vohr et al. 2000, Korres et al. 2005, Johnson et al. 2009) are the most important disabling conditions among VLBW infants.

Universally during this decade, the neurological morbidity rate has decreased. The incidence of CP in ELGA infants in Finland has decreased from 19 % to 9 % (Korvenranta et al. 2009), and in the United States, the incidence of CP among ELBW infants has decreased from 13 % to 5 % (Wilson-Costello et al. 2007). In VLBW/VLGA infants in Finland, the incidences of CP, hearing loss, visual disorder or other ophthalmic problems during this decade have been 6.1 %, 2.5 %, 3.8 % and 13.4 %, respectively (Korvenranta et al. 2009).

Prematurity increases the risk for sensorineural hearing impairment (Vohr et al. 2000, Korres et al. 2005). In addition, early brain injury in preterm infants has been associated with hearing deficits in preterm infants (Marlow et al. 2000, Patra et al. 2006). Furthermore, those preterm infants that suffered from hearing loss have been shown to have a reduced brain stem volume at term, when compared to normal hearing preterm infants (Valkama et al. 2001).

Prematurely born infants have more ophthalmic problems, including refractive error, strabismus, and loss of visual function when compared to full term infants (O'Connor and Fielder 2007). Brain lesions such as cPVL and injuries in the thalami have also been associated with visual problems in preterm infants (Ramenghi et al. 2010). A recent study suggested that visual function at term age in preterm infants is associated with the maturity of the WM in the optic radiations (Bassi et al. 2008).

THE AIMS OF THE STUDY

This thesis consists of four studies evaluating the definitions, causes and consequences of brain lesions in VLBW infants.

The first aim was to assess the effects of abnormal fetal blood flow waveform patterns on brain lesions and volumes at term age. It was hypothesised that abnormal fetal blood flow would disturb the normal brain development of the VLBW infants and might lead to brain lesions and diminished brain volumes.

The second aim was to determine if brain lesions of very preterm infants affect crying behaviour in äearly infancy. It was hypothesised that early brain injury would be associated with more spontaneous irritable behaviour of the infant.

The third aim was to evaluate the associations between ventricular indexes, brain lesions and volumes using exact measurements of the sizes of the ventricles attained using US and MRI. It was hypothesised that VD is a sign of brain injury, and thus related to brain lesions and decreased brain volumes.

The fourth aim was to assess the relationships between the VD indexes and neurodevelopmental outcome at two years of corrected age. It was hypothesised that the occurrence of VD alone or together with additional brain pathology at term, is related to worse neurodevelopmental outcome at two years of age in VLBW /VLGA infants.

The fifth aim was to describe the neurological findings from the structural neurological examinations administered at term and at one and two years of corrected ages for VLBW infants. We hypothesised that the findings of VLBW infants with CP would differ from VLBW infants without CP, severe hearing or vision impairments and normal motor development.

SUBJECTS AND STUDY DESIGN

1. SUBJECTS

This thesis is part of the regional multidisciplinary follow-up study PIPARI (Development and Functioning of Very Low Birth Weight Infants from Infancy to School age) including all very-low-birth-weight infants (VLBW, birth weight ≤ 1500 grams and gestational age < 37 weeks at birth) who were born at Turku University Hospital between January 2001 and December 2006, whose families were Finnish and/or Swedish speaking and living in the catchment area. The inclusion criteria were modified during the research period of the PIPARI-Study so that full gestational week cohorts up to 31 weeks (VLGA), regardless of BW were also included from the beginning of 2004. Infants with severe congenital anomalies or a diagnosed syndrome affecting developmental outcome were excluded.

There were 63 VLBW infants included in study I. A total of 123 eligible VLBW infants were born in Turku University Hospital between July 2001 and July 2004 and they all participated in the PIPARI -Study. Additional inclusion criteria for study I were 1) participation in an antenatal Doppler US examination in the week before delivery, 2) serial cranial US examinations of surviving infants during their stay in the NICU and at term and 3) that brain MRI at term was carried out. Antenatal Doppler examinations were successfully carried out within a week before delivery for 70 out of the 123 (57 %) infants that participated in the PIPARI-Study. All these 70 infants met the inclusion criteria mentioned above. Seven infants (10 %) died before 40 weeks of GA.

There were 125 VLBW infants and 49 full-term controls included in study II. A total of 151 eligible infants were born between January 2001 and July 2004 from which twenty-two infants died, and four additional infants were excluded according to the main study inclusion criteria. All eligible families decided to participate. One infant was lost during follow-up (0.8 %). The healthy full-term control infants were singleton, firstborn and born to Finnish speaking mothers, they had BW of less than -2.0 SD below the Finnish growth charts mean and no history of an admission to the neonatal care unit. The control infants were recruited two days after birth between October 2001 and April 2002 at Turku University Hospital. The controls were examined at one year of age, and their growth, development and physical examination were normal. Forty-nine of the 80 informed families (61%) agreed to participate in the study. This control group was primarily recruited for another PIPARI sub-study (Korja et al. 2008).

There were 209 VLBW infants included in study III. A total of 272 VLBW infants were born at Turku University Hospital between January 2001 and December 2006. Fifteen

infants (5.5 %) were excluded from the study (based on language criteria or because they lived outside the hospital catchment area). Thirty-nine of the remaining 257 study infants (15.2 %) died prior to term age. A total of 218 infants remained eligible for the study. Nine of the eligible families (4.1 %) refused to participate.

There were 225 VLBW/VLGA infants in study IV. A total of 296 VLBW/VLGA infants were born at Turku University Hospital between January 2001 and December 2006. Forty-two (14.1%) of them died prior to discharge, sixteen infants were excluded according to the inclusion criteria and ten of the eligible families refused to participate. Two infants were withdrawn from the study during follow-up and brain imaging studies were not obtained for one infant.

The PIPARI-Study protocol was approved by the Ethics Review Committee of the Hospital District of South-West Finland in December 2000. All parents gave their informed consent after receiving written and oral information concerning the research project.

2. METHODS

2.1. Data Collection

Neonatal background data was collected using the Vermont-Oxford Network criteria. This will allow for international comparisons to be made between research centers. In addition, the families sociodemographic and interview data from the medical records gathered during pregnancy and intensive care treatment were also collected. During the follow-up visits at one and two years of corrected age, the questionnaires relating to the health status of the infant, its' sleeping and eating habits and family history were recorded (by the researcher Maunu).

2.2. Antenatal Doppler measurements (I)

Fetal Doppler examinations were performed by two experienced perinatologists (authors Ekholm and Palo) according to the study protocol using a 3.5 to 5 MHz convex transducer (Acuson Seqouia, Mountain View, CA, USA). Blood flow velocity waveforms from the UA, MCA and DAo were assessed. The pulsatility index (PI) was calculated as described by Gosling and King (1975). The Doppler measurements used in this thesis were taken during the last week before delivery. The PI's were calculated as means of three waveform measurements of the UA, MCA, and Dao blood flow. The ratios of the PIs between the fetal peripheral vessels (i.e. UA, DAo) and the central cerebral vessel (MCA) were calculated. The directions of the net blood flow of the AI and of the end diastolic flow of the DV were determined as antegradic, retrogradic or absent.

2.3. Brain Imaging (I-IV)

2.3.1. Brain ultrasound

Brain US was performed by the attending NICU neonatologist for all infants once at 3 to 5 days, once at 7 to 10 days, at 1 month of corrected age and thereafter, monthly until discharge from hospital. These serial US examinations were focused on finding IVH (grading according to Papile 1962) and cPVL. cPVL was defined as occurrence of multiple cysts with a typical location in the posterior periventricular WM, adjacent to the lateral aspect of the trigone of the lateral ventricle and in the WM, adjacent to the foramina of Monro. This definition includes grades 2 and 3 PVL according to the classification of de Vries (1992). The instrument used was a 7.5 MHz vector transducer (Aloka SSD 2000, Aloka Co. LTD, Tokyo, Japan) between 1/2001 – 8 /2002 and a 8 MHz vector transducer (General Electric Logic 9) from 9/2002 – 3/2007.

At term age, a cranial US was performed by a pediatric radiologist (author Rikalainen). The radiologist was blind to the clinical data of the infants. At term age, the V/B ratio and the widths of the ventricular horns were measured. In addition, lesions such as IVH, cPVL and caudothalamic cysts were recorded at term.

The V/B ratio was defined as the widths of both ventricular midbodies at the border of the frontal horns and midbodies, in relation to the width of both brain hemispheres from the coronal scan at the level of the foramen of Monro (Figure 1). McArdle et al. (1987) suggested a V/B ratio of < 0.35 as normal. In our study population, the group of infants with a V/B ratio of 0.35 (with accuracy of measurement 0.01 units) did not differ from infants with a V/B ratio of ≤ 0.34 in the number of brain lesions, nor were their brain volumes different. The infants with a V/B ratio of > 0.35 had more pathological brain lesions and smaller brain tissue volumes when compared to infants with a V/B ratio of 0.35. Thus, in this work (III, IV) a V/B ratio of > 0.35 has been defined as abnormal. The same cut-off value has been used in a previous Finnish study of preterm infants (Valkama et al. 2000).

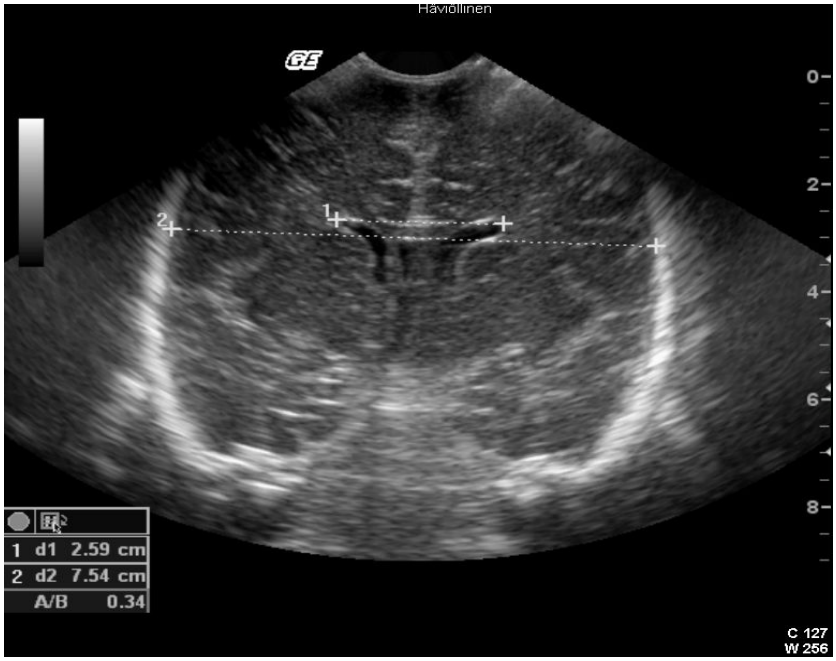

Figure 1. The V/B ratio measured using US. The V/B ratio was defined as the widths of both ventricular midbodies at the border of the frontal horns and midbodies, in relation to the width of both brain hemispheres from the coronal scan at the level of the foramen of Monro.

The oblique widths of the frontal horns of the lateral ventricles were measured from the coronal plane at the level of the foramen of Monro (Figure 2). From the parasagittal view, the widest sagittal measure of both lateral ventricles at the trigonum of the occipital horns was measured (Figure 3). The trigonum is a trigonal area of the lateral ventricles defined by the temporal horn inferiorly, the occipital horn posteriorly, and the body of the ventricle anteriorly. The reference values determined by Virkola (1988) were used to define the VD of VLBW infants at term. The cut-off value used for a dilated frontal horn was 0.3 cm (one SD above the mean for VLBW populations according to Virkola 1988). The same cut-off value has been used in previous studies (Perry et al. 1985, Davies et al. 2000). The cut-off value used for a dilated occipital horn was 1.15 cm (one SD above the mean for VLBW populations according to Virkola 1988).

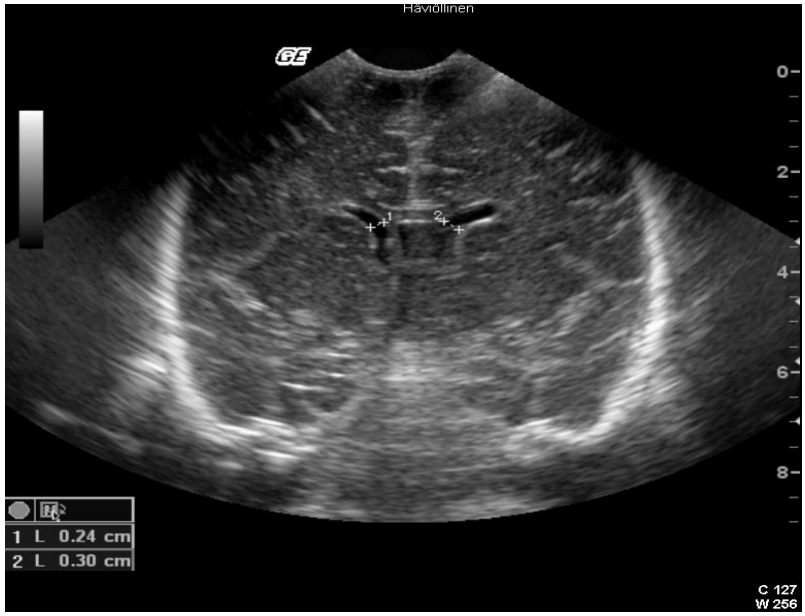

Figure 2. The oblique widths of the frontal horns of the lateral ventricles were measured using US from the coronal plane at the level of the foramen of Monro.

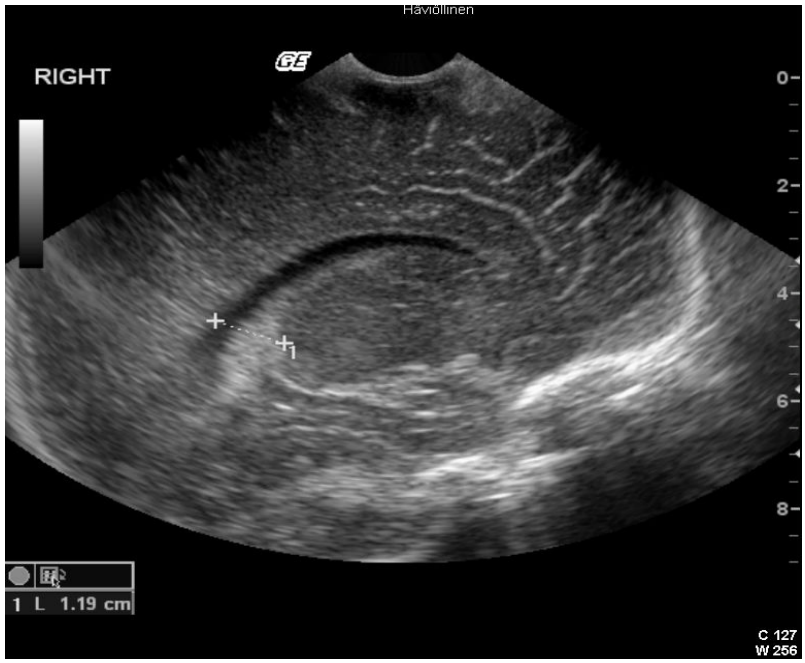

Figure 3. The place of measurement of the widths of the occipital horns of the lateral ventricles as shown by US. From the parasagittal view, the widest sagittal measure of both the lateral ventricles at the trigonum of the occipital horns was measured.

2.3.2. Magnetic Resonance Imaging

The brain MRI was carried out at term on the same day as the US examination. The imaging was performed during postprandial sleep without any pharmacological sedation. The infants were swaddled to calm them and to reduce movement artefacts in the imaging. A pulse oxymeter was routinely used during the MRI examinations. If necessary, a physician attended the examination to monitor the infant. The MRI equipment was an open 0.23 Tesla (T) Outlook GP (Philips Medical Inc., Vantaa, Finland), equipped with a multipurpose flexible coil fitting the head of the infant. The open MR equipment permitted good visual control and easy access to the infant. A total of 125 infants were examined using the 0.23T MRI (I, II, IV) until it was upgraded by the 1.5T (III) Philips Intera (Philips Medical Systems, Best, The Netherlands) for the remainder of the study infants (n=73).

With the 0.23T equipment, axial T2-weighted images, coronal 3D T1-weighted images and coronal T2-weighted images of the entire brain were obtained. With the 1.5T equipment, axial T2-weighted, axial T1-weighted and sagittal T2-weighted images were obtained. All sequences were optimized for imaging of the term infant brain. The total imaging time was approximately 25 minutes.

With the 0.23 T equipment, axial T2-weighted FSE images with a time of repetition (TR) of 9999 ms and time of echo (TE) of 200 ms were obtained. In this sequence, the flip angle was 90º, the slice thickness was 6 mm, the field of view was 220 x 220 mm^2 and the matrix was 256 x 256. T1-weighted FE three dimensional sequences with a TR of 30 ms, a TE of 10 ms, a flip angle of 45º, a slice thickness of 5 mm, a field of view of 220 x 220 mm^2 and a matrix of 256 x 256 were obtained in the coronal plane. Coronal T2-weighted FSE 3D sequences with a TR of 4000 ms, a TE of 350 ms, a flip angle of 90º, a slice thickness of 5 mm, a field of view of 187 x 250 mm^2 and a matrix of 192 x 256 were also obtained. With the 1.5T equipment, axial T2-weighted images with a TR of 5097 ms, a TE of 120 ms, a flip angle of 90º, a slice thickness of 4 mm, a field of view of 200 x 200 mm^2 and a matrix of 256 x 203 were obtained. Axial T1-weighted images with a TR of 496 ms, a TE of 14 ms, a flip angle of 90 º, a slice thickness of 4 mm, a field of view of 200 x 200 mm^2 and a matrix of 256 x 203 were also obtained as sagittal T2- weighted images, with a TR of 4851 ms, a TE of 120 ms, a flip angle of 90º, a slice thickness of 4 mm, a field of view of 200 x 200 mm^2 and a matrix of 256 x 203.

Volume measurement was performed by visually separating the cerebrospinal fluid from the brain tissue image by image. Anatomical differentiation of the brain was based both on anatomical landmarks and signal intensity differences of the brain structures. The volumes of the total brain tissue (total brain volume minus ventricle volumes), the cerebrum, the cerebellum, the frontal lobes, the brain stem (medulla oblongata together with pons), the basal ganglia together with the thalami, and ventricles (lateral ventricles, third and fourth ventricles) were measured. For volume measurements using the 0.23T

equipment, we obtained a T1-weighted FE sequence with a TR of 30 ms, a TE of 10 ms, a flip angle of 45º, a slice thickness of 5 mm, a field of view of 220 x 220 mm^2 and a matrix of 256 x 256 in the coronal plane. With the 1.5 T equipment, we obtained a coronal T1-weighted IR sequence TR of 3500 ms, a TE of 400 ms, a TI of 15 ms, a flip angle of 90º, a slice thickness of 4.8 mm, a field of view of 180 x 180 mm^2 and a matrix of 256 x 256. The postacquisition volume measurement was calculated on a GE workstation (GE AW1.0, GE Medical Systems, Milwaukee, USA). The coronal T1-weighted images were loaded into the Functool 1.0.post-processing software (GE Medical Systems, Milwaukee, USA). The volumes of the ventricles (lateral ventricles, third and fourth ventricles) can also be measured from MRI images. The reference values for brain tissue or ventricular volumes are not available for preterm infants.

The extracerebral space was measured manually from the MRI images. A cut-off value of 4 mm was used as suggested in the study by McArdle et al. (1987). The width of the extracerebral space was measured in front of the frontal lobe, where the extracerebral fluid space is at its widest. We separately analyzed the group of infants with an extracerebral space of 5mm, as the accuracy of measurement was 1 mm. Ventriculitis was defined as high signal intensity and abnormal thickness of the ventricular walls in T1-weighted images. One neuroradiologist (author Parkkola) analysed all the MRI images. The neuroradiologist was blind to both the clinical information and the results of the US examinations of the infants.

The brain MRI imaging findings were scored as shown in Appendix 4.

2.3.3. Pathological brain findings of the study infants

The VLBW study infants were categorized into three groups based on the most pathological brain image detected with either US or MRI during the neonatal ward period or at term: 1) a normal group, 2) an intermediate group based on a finding of unclear clinical significance and 3) a major pathology group based on a finding of clinically significant brain pathology or neonatal death.

The normal group consisted of VLBW infants with normal brain anatomy and a width of extracerebral space $\leq$ 4 mm, a V/B ratio $\leq$ 0.35, and normal lateral ventricular horns. The intermediate brain pathology group consisted of VLBW infants with IVH grades 1 to 2, dilatation of one horn of the lateral ventricles, caudothalamic cysts, or a width of the extracerebral space of 5 mm, or a V/B ratio of 0.35. The infants with IVH grades 3 to 4, WM cysts or VD with 2 to 4 horns dilated, abnormal T1 or T2 signals in the cortex, basal ganglia, thalamus, cerebellum or internal capsule, with hypoplasia of corpus callosum, an increased width of extracerebral space ($\geq$ 6 mm), a V/B ratio >0.35, ventriculitis or other major brain anomaly were included in the major pathology group. The term isolated VD was used if VD was the only deviant brain finding found in the VLBW/

VLGA infant, as defined by either the V/B ratio or by the presence of dilated ventricular horns on the US at term.

2.4. Neurological examination

2.4.1. Baby Day Diary (II)

The Baby Day Diary is a widely used parental diary (Hunziker and Barr 1986, Barr et al 1988). The caregivers (or nurses if the infant was still on the ward at the time of the recording) were asked to fill in the diary for three days (24 hours) on a "normal" day, i.e. when the infant was well, not having a fever, or other acute illnesses, or hadn't had a vaccination that day. The parents were given different symbols to record both infant behavioural states and parental behaviour related to the infant. The shortest unit to be recorded in the diary was five minutes. Two or more events that were distinct from each other for five minutes or less were counted as one episode. The behavioural states of the infant were defined as asleep, awake and content, awake and fussy, awake crying and awake feeding. The parental activities were defined as carrying / holding the infant with body contact, taking care of the infant (ie. changing, bathing, dressing), moving with the infant without body contact (ie. in the car or a pram). The diary was filled in at term, at six weeks of corrected age and at five months of corrected age (Appendix 3).

Crying was defined as "periods of prolonged distressed vocalization". Fussing was defined as "the infant is unsettled and irritable and may be vocalizing negatively, but not continuously crying". The parents of VLBW infants were advised on how to complete the Baby Day Diary by a researcher (author Maunu)

2.4.2. Dubowitz Assessment

The VLBW infants were examined at 36 weeks of gestational age, at term, at one and at two months of corrected age. Infants were either still in hospital or they came on clinical visits. The examinations were carried out by the researcher (author Maunu) and/ or the physiotherapist (Saarinen). The Dubowitz examination consisted of measurement subscales of tone, tone patterns, reflexes, spontaneous movements, abnormal signs, and behaviour. Each item was scored and the infant was examined while awake and alert (Appendix 1).

2.4.3. Hammersmith Infant Neurological Examination (IV)

The children were examined (primarily by author Maunu and physiotherapist Saarinen) at 12 and 24 months of corrected age using the Hammersmith Infant Neurologic Examination (HINE), which has been standardized for full-term infants between 12 and 18 months of age (Haataja et al. 1999). It has also been shown to be a reliable prognostic assessment tool in a preterm cohort (Frisone et al. 2002). The optimal score for the scale is between 74 and 78, for term infants at 18 months of age. The optimal scores for preterm infants at two years

of age have not yet been published, so we used the total scores as a continuous variable in this study. The test consists of sections of assessing cranial nerve function, posture, movements, tone, reflexes and reactions, and behaviour (Appendix 2).

2.4.4. Bayley Scales of Infant Development, 2nd edition (IV)

The Mental Developmental Index (MDI) was assessed using the Bayley Scales of Infant Development, II edition (BSID-II) (Bayley 1993). The assessment was conducted by a psychologist (primarily author Munck) when the child was at one and two years of corrected ages (- 1 week - +1 month). The original BSID-scales were translated for the purposes of the PIPARI-Study. The original norms were used to calculate the MDI. The psychologist was aware of the preterm birth status of the infant but not of the other perinatal data of the infant.

The MDI was used both as a continuous and as a categorized variable. A significant delay on the MDI was defined as a MDI score below -2.0 SD according to BSID-II norms (below 70).

2.4.5. Diagnoses of CP and Hearing and Vision Assessments (IV)

The diagnosis of CP was made during the follow-up, extending systematically up to two years of corrected age. The infants with CP were categorized by the nature of the neurological abnormality, that is spasticity, dyskinetic movements or ataxia and by the topography of limb involvement including spastic quadriplegia, triplegia, diplegia, and hemiplegia. In addition, the functional level of the infants with CP in gross motor activities was graded according to the Gross Motor Function Classification System (GMFCS) introduced by Palisano et al.1997. The GMFCS has proved to be a valid and reliable tool in classifying gross motor function in infants with CP (Palisano et al.1997 and 2000), and it also remains relatively stable over time (Rosenbaum et al. 2002, Jahnsen et al. 2006, Palisano et al. 2006).

The infants in our study were systematically screened using brainstem auditory evoked potentials (BAEP) from June 2002 and before that if required due to clinical indications. The median age for a BAEP recording was 30 days after term age. Prior to the BAEP recording, a pediatrician removed any possible wax in the ear canal and excluded infants who had a middle ear infection. In case of an infection, the BAEP recording was rescheduled. During the recording, the infant had attached tubal insert phone electrodes (TIPtrode; Nicolet Biomedical Instruments, Madison, WI, USA) that delivered auditory stimuli. The BAEP recording was administered with an eight channel Nicolet Viking IV device (Nicolet Biomedical Instruments, Madison, WI, USA). For both sides, the BAEP was recorded at least twice to ascertain reproducibility. Severe hearing impairment was

defined as hearing loss requiring amplification for at least one ear or hearing impairment with a cut-off of 40 dB.

Ophthalmological status was followed as a part of the clinical follow-up. The patients were referred to an ophthalmologist at two years of age if not needed earlier due to clinical reasons. Severe visual impairment was categorized as a visual acuity below 0.3 or blindness.

2.5. Statistical methods

All data are given as means ((standard deviation (SD) [minimum, maximum]) or percentages. The SPSS for Windows (version 12.0; SPSS, Chicago, IL) statistical package was used in articles I and II and (version 13.0; SPSS, Chicago, IL) in article III and the SAS for Windows version 9.2 (SAS Institute Inc., Cary, NC) was used in article IV. The cut-off point of $p<0.05$ was considered as statistically significant (I-IV).

Data analysis (I)

Possible differences between the characteristics of the study infants and those infants excluded because of unavailable antenatal Doppler measurements were assessed. Statistical analysis was performed using the t-test of independent samples for normally distributed continuous variables and the Mann-Whitney U-test for non-normally distributed continuous variables. Categorical variables were tested using Pearson's Chi-Square test or Fisher's exact test. Similarly, SGA and non-SGA infants within the study group were compared.

The PI of the blood flow of each vessel was classified as normal or abnormal according to Arduini and Rizzo's criteria (1990) for appropriate for gestational age infants. AREDF of the UA, absent or retrogradic net blood flow of the AI and absent or retrogradic end diastolic flow of the DV were also classified as abnormal. In addition to the dichotomized groups, numeric values of the PI were used in further analyses of all other variables except for the UA and the UA/MCA PI ratio. Only the dichotomized groups of normal and abnormal were used in analyses of the UA because the numeric values of the PI of the UA were not available for all patients with AREDF.

The relationships between regional brain volumes and the antenatal Doppler measurements of the MCA, DAo blood flow and the DAo/MCA PI ratio were assessed using correlation analyses. Pearson's correlation coefficient was calculated for normally distributed variables and Spearman's rank-correlation coefficient for other variables. The T-test of independent samples or the Mann-Whitney U-test were considered appropriate to compare the brain volumes between abnormal and normal UA blood flow and the UA/MCA PI ratio.

Analysis of covariance (ANCOVA) was used to further study the effects of an abnormal UA/MCA PI ratio on brain volumes, whilst controlling for the possible confounding

effects of gestational age, gender, SGA, Apgar at 5 minutes, CLD or surgically treated NEC, and sepsis or meningitis during hospital care. Prior to the analysis, the volumes of the ventricles, brain stem, and basal ganglia together with the thalami were log transformed to better meet the assumptions of ANCOVA.

Data analysis (II)

The differences between the VLBW groups were tested using an one-way analysis of variance (ANOVA) or the Kruskal-Wallis Test for continuous variables, followed by multiple comparisons using Tukey's HSD method or the Mann-Whitney U Test with Bonferroni correction. The parental characteristics of full term controls were compared with the VLBW infant's parental characteristics using the t-test for normally distributed continuous variables. Pearson's Chi-Square test or Ficher's exact test were used for the categorical variables.

Data analysis (III)

The measurements of the frontal and occipital horns were dichotomised as normal or abnormal as defined in article III. Preliminary analysis was carried out to compare the infants with either dilated frontal ventricular horns or those with dilated occipital ventricular horns. Since there were no significant differences in brain lesions or brain tissue volumes between the groups, no distinction was made in further analyses regarding the location of the dilatation.

Bivariate analysis of the categorical variables was performed using Pearson's Chi-Square test or Fischer's exact test as appropriate. The brain volumes between the groups were compared using the independent samples t-test for normally distributed variables and by the Mann Whitney U-test for other variables. Simple linear regression analyses were performed, with the number of dilated horns as the independent variable and the different brain tissue volumes as the dependent variables. The brain volumes were used as continuous variables. Spearman's correlation coefficient was used to evaluate the relationships between the V/B ratio on US and ventricular volume on MRI to the other continuous variables.

The V/B ratio classification was compared with the degree of IVH (no IVH and IVH grades 1 to 4) and extracerebral space ($\leq$ 4mm, 5 mm or > 5 mm) using the Pearson's Chi-Square test for trend. The Mann Whitney U-test was used when the volumes of the ventricles and the number of dilated horns were compared between the two groups (the group with brain lesions and the group without them). The Kruskal-Wallis test was used when these variables were compared between several groups (degree of IVH, extracerebral space).

The relationships between head circumference and total and regional brain volumes were assessed using correlation analyses. Pearson's correlation coefficient was calculated

for the normally distributed variables (the volumes of total brain tissue, cerebrum, cerebellum, frontal lobes, and basal ganglia together with the thalami) and Spearman's rank-correlation coefficient for the other variables (the ventricles and brain stem).

Data Analysis (IV)

The measurements of the frontal and occipital horns were dichotomised as normal or abnormal as defined in article IV. A V/B ratio >0.35 was considered as abnormal. Associations between perinatal characteristics and VD were studied using the following methods: Comparisons between two nominal variables were carried out using the Pearson's Chi-Square test or Fisher's exact test, as appropriate. Associations between ordinal and categorical variables were studied using the Pearson's Chi-Square test for trend. Logistic regression was used to test for univariate associations between the dependent variable V/B ratio classification (> 0.35 and ≤ 0.35) and the continuous independent variables. VD (as defined separately by the V/B ratio classification, the number of dilated ventricular horns or ventricular volumes) and brain pathology were used as predictors of the categorical outcome variables (CP, hearing impairment, categorized BSID-II (MDI <85/≥85 and <70/≥70) and NDI) using logistic regression analyses.

Preliminary analysis indicated that the effect of VD on the continuous outcome variables depended on brain pathology, thus the continuous outcome variables were studied separately in infants with and without additional brain pathology. Associations between the V/B ratio classification (≤ 0.35 and > 0.35) and the continuous outcome variables were studied using the Mann-Whitney U-test. Association between VD (the number of dilated ventricular horns or ventricular volumes) and the continuous outcome variables were studied using Spearman's correlation coefficient.

Categorical outcome variables were also studied separately in infants with and without additional brain pathology. Associations between the V/B ratio classification (≤ 0.35 and > 0.35) and the categorical outcome variables were studied using the Pearson's Chi-Square test or Fisher's exact test. Logistic regression was used to test associations between the categorical dependent variables and ventricular volumes. Associations between the number of dilated horns and the categorical outcome variables were studied using the Pearson's Chi-Square test for trend. A receiver operating characteristics (ROC) curve was drawn to evaluate the sensitivity and specificity of the V/B ratio for CP, a MDI below 70 and NDI.

The very preterm infants with CP were compared with very preterm infants without CP, hearing or visual deficit and normal motor milestones in HINE test (Appendix 2) according to the results of both total and subscale scores of cranial nerves, posture, movements, tone and reflexes & reactions on the HINE test at one and two years of corrected age. The Mann Whitney U-Test was used to find possible differences between the groups.

RESULTS

1. PATIENT CHARACTERISTICS

Table 2. Perinatal characteristics of the study infants of studies I-IV. Means (standard deviations) [minimum, maximum] are presented if not otherwise indicated.

	Study I n=70	**Study II** n=123	**Study III** n=209	**Study IV** n=225
Birth weight (g)	1013 (287) [384, 1500]	1058 (275) [400, 1500]	1072 (283) [400,1500]	1133 (333) [400, 2120]
Gestational age at birth (weeks + days)	28+2 (2+5) [24+0, 36+1]	28+4 (2+6) [23+0, 36+1]	28+6 (2+6) [23+0, 36+1]	29+0 (2+5) [23+0, 35+6]
Male n (%)	33 (47.1)	61 (49.6)	114 (54.5)	121(53.8)
SGA n (%)	26 (37.1)	47 (38.2)	86 (41.1)	84 (37.3)
Severe pre-eclampsia n (%)	12 (17.1)	24/119 (20.2)	46/200 (23.0)	49/216 (22.7)
PROM n (%)	18 (25.7)	27 (22.0)	47/201 (23.4)	49/218 (22.5)
Antenatal steroids n (%)	66/69 (95.7)	115/122 (94.3)	190 (90.9)	207 (92.0)
Multiple birth n (%)	21 (30.0)	37 (30.1)	59 (28.2)	67 (29.8)
Distress C-section n(%)	30 (42.9)	58/120 (48.3)	92/206 (44.7)	97/222 (43.7)
Postnatal corticosteroids n (%)	10/68 (14.7)	22/122 (18.0)	33/208 (15.9)	30 (13.3)
RDS	56/68 (82.4)	95 (77.2)	154/208 (74.0)	163/224 (72.8)
CLD	12/66 (18.2)	18/119 (15.1)	34/207 (16.4)	31 (13.8)
Operated NEC	2/68 (2.9)	8 (6.5)	13/209 (6.2)	13 (5.8)
Ventricular shunt	1 (1.4)	4 (3.3)	5/209 (2.4)	6 (2.7)
Normal US	20/63 (31.7)	42 (34.1)	68/205 (33.2)	75/220 (34.1)
Normal MRI	37/63 (58.7)	71/121 (58.7)	118/204 (57.8)	126/221 (57.0)
Normal both US and MRI	14 (20.0)	34 (27.6)	53/207 (25.6)	60 (26.7)
Intermediate brain pathology	31 (44.3)	48 (39.0)	70/207 (33.8)	75 (33.3)
Major brain pathology	18 (25.7)	41 (33.3)	84/207 (40.6)	90 (40.0)

Normal US= Normal serial US examinations once at 3 to 5 days, once at 7 to 10 days, at 1 month of corrected age and thereafter, monthly until discharge from hospital and at term

Normal MRI = Normal MRI at term

2. ABNORMAL ANTENATAL DOPPLER MEASUREMENTS IN RELATION TO BRAIN LESIONS AND BRAIN VOLUMES (I)

Abnormal blood flow patterns in the UA, MCA, DAo, and abnormal UA/MCA and DAo/MCA PI ratios, were found in similar frequencies in the normal, intermediate and major brain pathology groups. None of the infants had retrogradic end diastolic flow of the UA, the net blood flow of the AI was antegradic in all cases (n=52), and only one of 66 fetuses had absent end diastolic flow in the DV. Thus, the most severe changes of abnormal fetal blood flow were absent in our study.

The results did not change when analyzed without the infants who died in the neonatal period. Similarly, when we compared the perinatal characteristics of the study infants

with those excluded because of missing Doppler measurements the results were almost congruent (I). The VLBW infants lacking Doppler measurements recorded during the week before delivery differed from the study infants by having more non-elective C-sections and fewer received antenatal steroids. There were no differences however in the prevalence of preeclampsia, premature rupture of the membranes, multiple pregnancy, sex, gestational age, BW, SGA status at birth, five minutes Apgar, neonatal deaths, brain pathology defined before /or at term, the length of ventilator treatment and hospital stay.

The total brain volume was smaller in infants with an abnormal UA/MCA PI ratio (mean 360 ml, SD 32.5 ml) than in infants with a normal UA/MCA PI ratio (mean 405 ml, SD 51.3 ml), p=0.01. Analysis of the regional brain volumes showed that the infants with an abnormal UA/MCA PI ratio had a smaller cerebral volume (344 ml, SD 28.4 ml) compared to infants without a blood redistribution (368 ml, SD 52.3 ml, p=0.012). Other antenatal Doppler measurements were not associated with total or regional brain volumes.

Other potential confounding factors affecting the brain volumes in addition to an abnormal UA/MCA PI ratio were tested using a multivariate analysis. This showed that an abnormal UA/MCA PI ratio affects independently total brain tissue ($p<0.001$) and cerebrum (p=0.001) volumes. Males had larger volumes of total brain tissue ($p<0.001$), cerebrum ($p<0.001$), cerebellum (p=0.013), and frontal lobes (p=0.042) compared to the females. CLD or surgically treated NEC were associated with lower volumes in total brain tissue (p=0.042), in the cerebrum (p=0.034), cerebellum (p=0.001) and basal ganglia together with the thalami (p=0.029) compared to those without these diagnoses. Interestingly, SGA status did not emerge as a significant factor affecting brain volumes at term.

3. BRAIN IMAGING FINDINGS IN VLBW INFANTS AT TERM AGE (I-IV)

In study I brain US and MRI examinations were successfully administered for all eligible study infants. In study II all the VLBW infants had brain imaging studies performed. Two of the 125 infants had movement artefacts on MR images, but all the 125 US examinations were successfully performed. No imaging studies were performed for the control infants. In study III, the V/B ratio was measured in 206 of the 209 study infants and the widths of the ventricular horns from 205 infants using US.

Percentages of the lesions found on US and on MRI examinations of the VLBW infants in this thesis with respect to the number of gestational weeks or BW are shown in Tables 3a-b.

There were some typical patterns of brain injuries that were more common in VLGA infants such as cPVL, injury of the capsula interna and ventriculitis when compared to older VLBW infants. Irrespective of GA, preterm infants had WM injuries, IVH grades 1 to 4, VD, ventriculitis, anomalies of the cerebellum, cerebral hemorraghes and delayed myelinisation. In addition, severe VD, delayed myelinisation, WM injuries and especially injuries of the capsula interna were also common with VLBW infants with a GA over 32 weeks. If the brain findings were evaluated by BW there was mild IVH and VD, a hypoplasia of the corpus callosum, delayed myelinisation and a wide extracerebral space equally in both groups (<1000g and 1000-1500 g BW). However, infants with a BW<1000g had more major brain pathology, including IVH grade 4, cPVL, severe VD, cerebellar anomaly and WM injuries when compared to infants with a BW of 1000-1500g.

The Tables 3a-b include brain findings from the early US examinations and the MRI examination at term. According to GA at birth, 16%, 36%, 47%, 53% of preterm infants had a normal brain US in the neonatal period, with a GA of ≤27 weeks, 28-29 weeks, 30-31 weeks and ≥ 32 weeks, respectively. Furthermore, 45%, 58%, 67%, and 71% had a normal brain MRI at term with a GA of ≤27 weeks, 28-29 weeks, 30-31 weeks and ≥ 32 weeks, respectively. From the preterm infants with a BW of < 1000g and between 1000-1500g, and for the SGA and non-SGA infants, the percentages of those who had a normal US/MRI was 24%/44%, 40%/68% , 39%/59% and 32%/57%, respectively.

Table 3a. Brain lesions (%) of VLBW/VLGA infants before or at term age according to gestational age at birth (IV).

Brain findings	**Gestational weeks ≤ 27**	**Gestational weeks 28-29**	**Gestational weeks 30-31**	**Gestational weeks ≥32**
On MRI at term,n=227	**n =75**	**n =64**	**n =57**	**n =31**
Normal	45.3	57.8	66.7	71.0
Cortex injury	0	0	0	9.7
Basal ganglia or thalamus injury	1.3	0	0	3.2
Capsula interna injury	18.7	3.1	5.3	12.9
Other WM injury	16.0	14.1	5.3	16.2
Germinal matrix hemorrhage	16.0	4.7	1.8	3.2
Caudothalamic cysts	17.3	10.9	17.5	9.7
Width of the ventricles abnormal	29.3	16.6	3.5	6.5
Ventriculitis	13.3	9.4	5.3	9.7
Frontal horns dilatated of the lateral ventricles	10.7	6.3	1.8	3.2
Occipital horns dilatated of the lateral ventricles	5.3	4.7	0	3.2
All ventricular horns dilatated	13.3	4.7	1.8	0
Corpus callosum hypoplasia	8.0	3.1	0	6.5
Hemorrhage in fossa structures	1.4	1.6	0	3.2
Cerebellum anomaly	5.4	1.6	0	3.2
Delayed myelinisation	22.6	14.5	3.6	9.7
Wide extracerebral space >5 mm	1.3	1.6	5.3	0
V/B ratio >0.35	22.7	7.8	0	0
On US before or at term, n=224	**n =73**	**n =66**	**n =55**	**n =30**
Normal	16.4	36.4	47.3	53.3
No IVH	55.7	85.5	86.9	89.3
IVH grade 1	10.5	14.5	10.9	7.1
IVH grade 2	10.5	0	0	0
IVH grade 3	7.0	0	0	0
IVH grade 4	16.3	0	2.2	3.6
cPVL	6.1	1.4	0	3.0
Caudothalamic cysts	20.5	13.6	21.1	6.7
VD 1 horn dilatated	24.7	18.2	14.5	23.3
VD 2 horns dil	15.1	18.2	12.7	3.3
VD 3 horns dil	11.0	7.6	5.5	0
VD all horns dil	17.8	7.6	1.8	13.3
V/B ratio >0.35	28.8	10.6	5.5	3.2

Table 3b. Brain lesions (%) of VLBW/VLGA infants before or at term age (IV) according to birth weight.

Brain findings	Birth weight < 1000g	Birth weight 1000-1500g	SGA yes / no
On MRI at term,n=227	**n =90**	**n =116**	**n = 87/140**
Normal	44.4	68.1	58.6/57.1
Cortex injury	2.2	2.7	2.2/2.1
Basal ganglia or thalamus injury	1.1	0.9	1.1/0.7
Capsula interna injury	15.5	6.9	9.1/10.7
Other white matter injury	15.6	12.1	10.2/14.3
Germinal matrix hemorrhage	14.4	3.4	5.7/8.6
Caudothalamic cysts	15.6	11.2	12.6/15.7
Ventriculitis	15.6	5.2	8.0/10.7
Frontal horns dilatated of the lateral ventricles	11.1	3.4	9.2/4.3
Occipital horns dilatated of the lateral ventricles	4.4	3.4	2.3/4.3
All ventricular horns dilatated	8.9	4.3	2.3/8.6
Corpus callosum hypoplasia	8.9	1.7	5.7/3.6
Hemorrhage in fossa structures	1.1	1.7	0/2.9
Cerebellum anomaly	6.7	0	4.6/1.4
Delayed myelinisation	21.3	9.6	9.3/15.7
Wide extracerebral space	1.1	2.6	2.3/2.2
V/B ratio >0.35	20.0	3.4	8.0/10.7
On ultrasound before or at term, n=224	**n =87**	**n =119**	**n =85/139**
Normal	24.1	40.3	38.8/32.4
No IVH	64.0	79.2	82.9/70.0
IVH grade 1	10.3	12.8	11.0/10.2
IVH grade 2	8.2	0.9	0/6.6
IVH grade 3	6.2	0.9	1.2/4.4
IVH grade 4	11.3	4.6	4.9/8.8
cPVL	5.2	1.6	3.2/1.8
Caudothalamic cysts	18.4	12.6	9.4/21.3
VD 1 horn dilatated	20.7	20.2	20.0/20.1
VD 2 horns dil	19.5	10.1	14.1/13.7
VD 3 horns dil	9.2	5.9	8.2/6.5
VD all horns dil	13.8	9.2	9.4/10.8
V/B ratio >0.35	22.7	9.2	7.0/18.7

4. VENTRICULAR DILATATION

4.1. Comparison of the ventricular dilatation indexes by ultrasound and MRI (III, IV)

An abnormal V/B ratio (>0.35) associated with an increasing number of dilated horns of the lateral ventricles ($r=0.42$, $p<0.001$), and with a larger ventricular volume on the MRI ($r=0.42$, $p<0.001$). An increasing number of dilated horns of the lateral ventricles correlated with a larger ventricular volume on the MRI ($r= 0.23$, $p=0.002$)

4.2. Ventricular dilatation in relation to brain lesions in VLBW infants (III)

In infants whose V/B ratio was abnormal, the incidence of brain lesions detected by US and MRI was higher (Table 2). The infants had more often severe IVH (grades 3 to 4) ($p<0.001$), WM injury ($p<0.001$), cortical injury ($p=0.013$), hypoplastic corpus callosum ($p<0.001$) or ventriculitis ($p<0.001$). The incidences of IVH grades 1 to 2, cPVL, caudothalamic cysts on either the US or on the MRI, or the width of extracerebral space were not increased in children with a V/B ratio of > 0.35.

An increasing number of dilated horns of the lateral ventricles associated with severe IVH ($p<0.001$), cPVL ($p=0.02$), WM injury ($p<0.001$), cortical injury ($p=0.004$), hypoplastic corpus callosum ($p<0.001$) and ventriculitis ($p<0.001$), but not with caudothalamic cysts on the US or on the MRI or with the width of the extracerebral space (Table 4).

Larger ventricular volumes measured on MRI were found in VLBW infants with WM injuries ($p=0.02$), with IVH with increasing grades ($p=0.02$), with cortical injury ($p=0.01$), with hypoplasia of the corpus callosum ($p=0.006$) and with a width of the extracerebral space over 5 mm ($p=0.02$) compared to infants without these findings. Ventricular volumes did not correlate with ventriculitis, caudothalamic cysts on US or on MRI, or with cPVL.

Table 4. Brain lesions in relation to VD in VLBW/VLGA infants (IV). One infant may have several findings. The infants were classified according to the worst grade of IVH.

	V/B ≤0.35 on US	V/B >0.35 on US	All horns normal	One horn dilated	Two horns dilated	Three horns dilated	Four horns dilated
	n= 174	n= 31	n= 95	n= 40	n= 24	n= 15	n= 22
Structural brain findings on US n (%)							
No IVH	136 (78.2)	13 (41.9)	79 (82.3)	34 (81.0)	18 (62.1)	9 (60.0)	9 (40.9)
IVH grades 1 to 2	34(19.5)	6 (19.4)	16 (16.7)	6 (14.3)	9 (31.0)	2 (13.3)	7 (31.8)
IVH grades 3 to 4	4 (2.3)	12 (38.7)***	1 (1.0)	2 (4.8)	2 (6.9)	4 (26.7)	6 (27.3) §§§
cPVL	1(0.6)	2 (6.5)	0	0	1 (3.4)	1 (6.7)	1 (4.8) §
Caudothalamic cysts	25 (14.4)	6 (19.4)	17 (17.7)	5 (11.9)	3 (10.3)	4 (26.7)	2 (8.7)
Structural brain findings on MRI n (%)							
Caudothalamic cysts	23 (13.9)	26 (13.2)	11 (11.6)	6 (15.0)	5 (20.8)	2 (13.3)	2 (9.1)
WM injury	17 (10.2)	16 (51.6)***	3 (3.2)	4 (10.0)	7 (29.2)	6 (40.0)	12 (54.5) §§§
Cortical injury	1 (0.6)	3 (9.7)**	0	0	0	0	3 (13.6) §§
Corpus callosum hypoplasia	2(1.2)	7 (22.6)***	0	1 (2.5)	1 (4.2)	2 (13.3)	4 (18.2) §§§
Ventriculitis	8 (4.8)	11(35.5)***	3 (3.2)	1 (2.5)	6 (25.0)	5 (33.3)	3 (13.6) §§§
Extracerebral space							
≤ 4mm	135 (91.8)	26 (83.9)	74 (90.2)	32 (86.5)	22 (100)	12 (85.7)	20 (90.9)
5 mm	10 (6.8)	3 (9.7)	6 (7.3)	5 (13.5)	0	1 (7.1)	1 (4.5)
≥ 6 mm	2 (1.4)	2 (6.5)	2 (2.4)	0	0	1 (7.1)	1 (4.5)

** = p≤ 0.01, compared to V/B≤ 0.35, Pearson's Chi Square Test or Fisher's Exact test

*** = p≤ 0.001, compared to V/B≤ 0.35, Pearson's Chi Square Test or Fisher's Exact test, Chi Square test for trend (IVH)

§ = p≤0.05, increasing number of dilated horns increases the incidence of lesions, Chi Square Test for trend

§§ = p≤0.01, increasing number of dilated horns increases the incidence of lesions, Chi Square Test for trend

§§§ =p≤0.001, increasing number of dilated horns increases the incidence of lesions, Chi Square Test for trend

4.3. Ventricular dilatation in relation to brain volumes in VLBW infants (III)

A V/B ratio above 0.35 measured by US at term was associated with a smaller volume of total brain tissue (p=0.02), cerebrum (p=0.03), and cerebellum (p=0.01) measured using MRI at term. In linear regression analyses, the volumes of total brain tissue (p=0.03), cerebellum (p<0.001), frontal lobes (p=0.003), brain stem (p=0.01), and basal ganglia together with thalami (p=0.004) decreased with an increasing number of dilated horns of the lateral ventricles. The association between an increasing number of dilated horns of the lateral ventricles and the volumes of the cerebrum was not statistically significant (p=0.07) at term.

The larger volume of the ventricles measured by MRI was significantly related to the smaller volumes of the cerebellum (r=0.21, p=.003), frontal lobes (r=0.23, p= 0.001), brain stem (r=0.48, p<0.001), and basal ganglia together with thalami (r=0.22, p=0.002), but not with the smaller volumes of total brain tissue or the cerebrum.

An abnormal V/B ratio (>0.35) correlated with an increasing number of dilated horns of the lateral ventricles (r=0.42, p<0.001), and with a larger ventricular volume as measured by MRI (r=0.42, p<0.001). An increasing number of dilated horns of the lateral ventricles correlated with a larger ventricular volume as measured by MRI (r= 0.23, p=0.002).

4.4. Ventricular dilatation in relation to 2-year outcome in VLBW infants (IV)

An abnormal V/B ratio as an isolated finding did not associate with adverse outcome in VLBW/VLGA infants. However, an abnormal V/B ratio in infants with brain pathology associated with CP, NDI and the MDI score. When the effect of brain pathology was controlled for, the associations between an abnormal V/B ratio and both CP (p=0.003) and NDI (p=0.001) remained significant.

An increasing number of dilated ventricular horns without additional brain pathology did not increase the risk for adverse outcomes. However, in VLBW/VLGA infants with additional brain pathology, an increasing number of dilated ventricular horns associated with CP, a MDI score below 70, NDI, a lower total score on the Infant scale, and the MDI score in general. When the effect of brain pathology was controlled for, associations with an increasing number of dilated ventricular horns and both CP (p=0.002) and NDI (p=0.002) remained significant.

Isolated larger ventricular volumes measured by MRI did not associate with any adverse outcome. Larger ventricular volumes in infants with other brain pathology associated with CP and a MDI score below 70. When the effect of brain pathology was controlled for, the association between larger ventricular volumes and CP (=0.04) remained significant.

5. HEAD CIRCUMFERENCE MEASURE AT TERM (III)

There were 18 (8.7 %) infants with head circumference < -2.0 SD according to the Finnish growth charts (III). A smaller head circumference at term correlated significantly with smaller volumes of total brain tissue (r=0.79, p<0.001), cerebrum (r=0.78, p<0.001), cerebellum (r=0.51, p<0.001), frontal lobes (r=0.47, p<0.001), brain stem (r=0.19, p=0.009), and basal ganglia together with thalami (r=0.42, p<0.001). There was no correlation between head circumference and the volume of the ventricles at term. Thus, larger head circumference did not correlate with VD. Even though head circumference at term did not differ between the infants with post-haemorrhagic hydrocephalus treated with a ventriculoperitoneal shunt and the other study infants, the infants with a shunt had smaller total and regional brain volumes.

6. NEUROLOGICAL AND BEHAVIOURAL ASSESSMENTS OF VLBW INFANTS

6.1. Infant behaviour of the VLBW infant in relation to brain injury (II)

Infant crying behaviour was assessed using the Baby Day Diary (Barr et al 1988). The diaries were completed and returned from 117 of 125 (94%) families of the VLBW infants at term, from 121 (97%) at six weeks of corrected age and from 117 (94%) families at five months of corrected age. All 49 families of the control infants completed and returned the diaries at five months of age.

In VLBW infants at term, the duration of combined fussing and crying was significantly higher in the 'intermediate brain pathology group' compared to the 'normal group' ($p=0.017$). There were no other differences in total daily duration of fussing and crying between the groups. The daily duration of fussing and crying decreased with increasing age in all the study groups (Figure 4).

The number of fussing and crying bouts increased from term to 6 weeks of age and then decreased up to the first five months of age in all the VLBW groups. There were no differences when fussing and crying were explored separately. No consistent differences in circadian rhythm were found between the groups.

The behavior of VLBW infants was compared to the full term controls at five months of age. There were no differences in the combined duration of fussing and crying or, crying or fussing alone at five months of age between the preterm and full-term infants. However, the number of combined fussing and crying bouts and fussing bouts, alone, were higher ($p= 0.009$ and $p=0.006$, respectively) in VLBW infants compared to full term controls. When the bout frequency was compared between all the four study groups, the number of combined fussing and crying bouts did differ between the groups ($p=0.033$). In pair-wise comparisons, the only significant difference was found between the 'brain pathology group' and full-term infants ($p=0.003$).

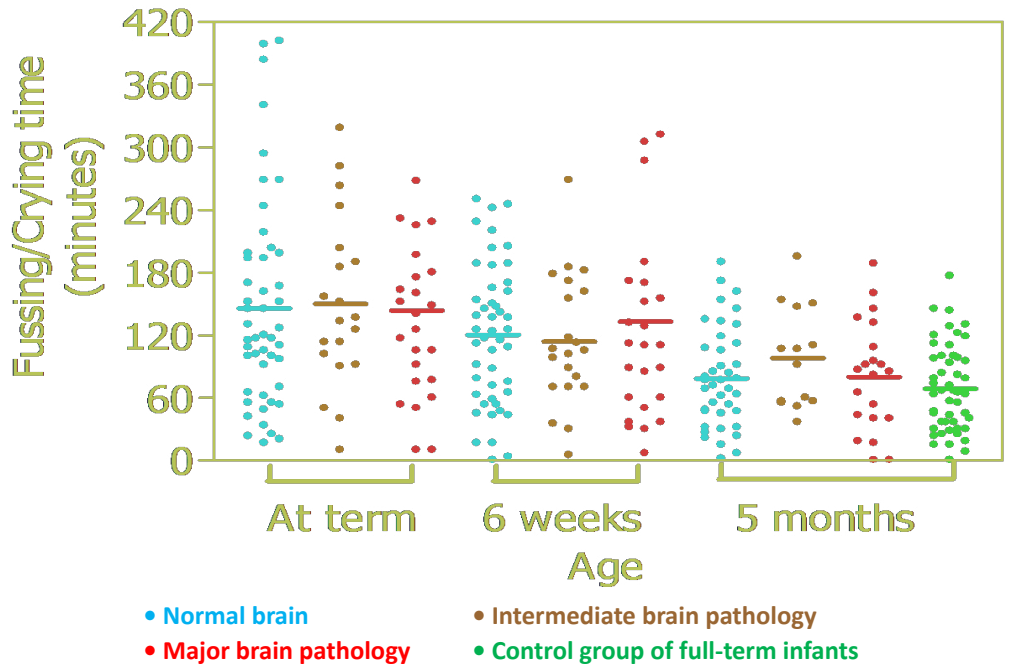

Figure 4. Total amount of combined crying and fussing between the VLBW infants with a normal brain, and intermediate and major brain pathology and the control group of full-term infants

At term, the VLBW infants in the 'brain pathology group' were held significantly more ($p=0.009$) compared to the other VLBW groups. This difference ($p=0.012$) persisted, even if the hospitalized VLBW infants were excluded. Although the infants in the 'brain pathology group' were held most at each measurement point, the difference was statistically significant only at five months of corrected age. No other differences were found in parental behavior (moving with the baby, or taking care of the baby) between the VLBW infants. When all the VLBW infants were compared to the full-term infants, the VLBW infants were held significantly more than the full term infants. In contrast, the VLBW infants were moved (in a stroller or in a car) less ($p<0.001$), compared to the full term controls at five months of corrected age.

The scores for crying and consolability on the Dubowitz Assessment at term and the behaviour scores on the HINE were compared between the VLBW infants with major brain pathology and other infants. In test situations, the VLBW infants with major brain pathology did not differ from other study infants at term, one or two years of corrected age according to crying behavior.

6.2. Neurological examination of the VLBW infants at term age (Dubowitz Assessment) (unpublished data)

The infants were examined by the researcher and physiotherapist (mostly Maunu and Saarinen together for the first 126 infants) using the Dubowitz Assessment at 36 weeks of corrected age, at term, at one and two months of corrected age. The results from the term age examination (n=126) were compared to the previous study data published by Ricci et al 2008. In their study, the reference range for 90th centile frequency distributions values for each item were published for low risk preterm infants.

The scores of each item on the Dubowitz Assessment at term were analyzed for VLBW infants with CP. There were 13 out of 126 (10.6%) infants with CP in this study cohort. The preterm infants who developed CP had a median of 5 items (range 1-9) outside the 90th centile of the reference values for low risk VLBW / VLGA infants at term age. 62 % of the infants with CP had more than 4 items outside the 90th centile. From the applied 34 items, posture (6/13), head lag (6/13), Moro reflex (3/13), palmar grasp (3/13), startles (4/13), visual orientation (4/13) and irritability in response to stimuli (3/13) were the items that were most frequently outside the 90th centile limits. In addition, the tone patterns in infants with CP were outside the 90th centile limits on three items: 7/13 (54%) infants had intermittent strong arm flexion with strong leg extension, 4/13 (31%) infants had a score for leg traction higher than the score for popliteal angle and 4/13 (31%) infants had a score for ventral suspension lower than head lag. Increased extensor tone and poorer head control were thus most often abnormal in infants with CP.

The auditory orientation of VLBW infants was tested on the Dubowitz Assessment using a rattle. Five out of the eight infants with severe hearing impairment (63 %) did not react to auditory stimuli during the test situation at term. Notably, 3/8 (38 %) of them startled frequently during the test situation.

6.3. Results of the neurological and behavioural assessments of VLBW infants at one and two years of age (HINE) (unpublished data)

The total and subscale scores on the HINE were compared between the groups of VLBW infants with CP (n=13) and VLBW infants without CP, hearing impairments or vision impairments at one (n=101) and two years of age (n=103). We used the total scores as a continuous variable rather than the optimal scores used for low risk full term infants (Haataja et al 1999).

At one year of corrected age the groups differed significantly on all subscales (cranial nerves, posture, movements, tone, reflexes and reactions sections and asymmetries). Thirteen out of 15 infants with CP participated in the neurological assessments at one year of corrected age. Nine out of 13 (69%) infants with CP had total scores of 58 or under, while none of the infants in the control group had such low scores. Asymmetries

were found in 6/13 (46%) of CP infants on six or more test items whereas none of the infants in the control group had over 4 asymmetries ($p<0.0001$). Individually, total scores were suboptimal (< 73) on the HINE in all infants with CP at one year of corrected age. 10 out of 13 infants with CP (77%) had four or five out of five subscale scores that were suboptimal (Table 5).

At two years of corrected age, the groups differed significantly in total scores ($p<0.001$). Eleven out of 15 infants with CP were tested. The VLBW infants with CP had total scores between 38 and 62, except one infant with spastic hemiplegia who scored 74. The group of VLBW/VLGA infants without CP or severe hearing or vision impairments had total scores ranging from 66 to 78. All subscales (cranial nerves, posture, movements, tone and reflexes & reactions sections) differed significantly between the groups (cranial nerves 0.002, other $p<0.0001$). The infants in the control group had 0-2 asymmetries on the test, except one infant who had four asymmetries. In the group of infants with CP, the number of asymmetries varied; five infants had 0-2 asymmetries, two infants had 4 and four infants with CP had ≥ 8 asymmetries. Individually, ten out of 11 infants with CP (91%) had suboptimal total scores on the HINE at two years of corrected age (< 74). Nine out of 11 infants with CP (75 %) had four or five out of five suboptimal subscale scores. One infant with CP had two and another three suboptimal subscale scores on the HINE at two years of corrected age. The motor milestones section and behavior section were not analyzed. (Table 5)

Table 5. Comparisons between VLBW infants with CP and other VLBW infants (without CP, severe hearing or vision impairments) according to the total and subscale scores on the Hammersmith Infant Neurological Examination (HINE) at one and two years of corrected ages. Median (range) given if not otherwise indicated.

	Infants with CP at 1 year n=13	**Other VLBW infants at 1year n=101**	**Infants with CP at 2 years n=11**	**Other VLBW infants at 2 years n=103**
Total scores	55.0 (38.5, 71.0) ###	74.5 (58.5, 78.0) ###	54.0 (38.0, 74.0) ###	74.5 (67.5, 78) ###
Cranial nerves	15.0 (8.0,15.0)	15.0 (12.0,15.0)	15.0 (7.0,15.0)#	15.0 (12.0,15.0)#
Posture	11.25 (5.5, 15.5) ###	16.5 (11.5, 18)###	10.125 (7.5, 16) ###	17.0 (13.5, 18) ###
Movements	3.0 (0,6.0) ###	6.0 (2.0,6.0) ###	3.0 (0, 6.0) ###	6.0 (3.0, 6.0) ###
Tone	21.0 (11.0,24.0) ###	23.0 (14.0,24.0) ###	19.25 (9.0, 24.0) ###	24.0 (18.0, 24.0) ###
Reflexes&reactions	9.0 (1.5, 14.0) ###	14.0 (11.0,15.0) ###	9.0 (4.5, 14.0) ###	14.0 (9.0, 15.0) ###
Asymmetries	4 (1, 15) ###	0 (0,4) ###	4 (0,14) ###	0 (0,4) ###
Total scores ≤58/78 (1y), ≤63/78 (2 y), n (%)	9 (69)	0	10 (91)	0
Posture ≤ 12/18, n (%)	9 (75)	0	10 (91)	0
Tone ≤14/24, n (%)	2 (17)	0	5 (45)	0

p= 0.05, Mann Whitney U-test
p<0.0001, Mann Whitney U-Test

DISCUSSION

The prospective, multidisciplinary PIPARI cohort study with VLBW infants has provided new information regarding the definitions, causes and consequences of brain injury in VLBW infants. Brain injury was a common complication of prematurity as has also been shown in previous studies (Inder and Volpe 2000, Ment et al. 2002, Inder et al. 2003, Volpe 2003). Although most of the brain injuries were detected in VLBW infants with GA <28 weeks at birth, there were several brain injuries common in all VLBW infants regardless of GA at birth. According to these results, it is essential to include all VLBW/VLGA infants MRI imaging procedures at term in addition to routine US examinations, to detect all common types of brain injuries that might affect later infant development.

Abnormal antenatal Doppler measurements in relation to brain lesions and brain volumes (I)

Abnormal blood flow patterns of the UA, MCA or UA/MCA were not related to structural brain pathology as seen on the US or MRI at term in VLBW infants. This is in agreement with previous studies (Scherjon et al. 1994, Inder et al. 1999, Sterne et al. 2001, Soregaloli et al. 2002, Habek et al. 2004). Even though we did not find any relationship between mild fetal blood flow abnormalities and brain lesions at term, there is a possibility that abnormal fetal blood flow patterns may result in such subtle neuroanatomical or functional changes, that they may correlate with later cognitive development changes and might be seen by other imaging methods like diffusion tractography not used in this study. In our study, however, the most severe abnormal blood flow patterns were not present.

A previous study (Tolsa et al. 2004) showed that healthy SGA preterm infants with normal brain MRI and neurological status, but abnormal UA blood flow, had smaller total brain and cerebral cortical gray matter volumes, compared to preterm infants with normal growth and without placental insufficiency. In contrast, in our cohort, abnormal UA pattern alone or fetal growth restriction did not affect brain volumes at term age. However, we did not measure separately cortical gray matter volumes.

In this study, we found that an abnormal UA/MCA PI ratio associated with decreased total and cerebral brain volumes of VLBW infants at term. In addition, CLD or surgically treated NEC in VLBW infants, led to decreased volumes of total brain tissue, of the cerebrum, cerebellum, and basal ganglia together with the thalami. The finding of an association between CLD and decreased volumes is in agreement with a previous study which showed the relation between CLD and smaller volumes of deep nuclear gray matter (Peterson et al. 2003). To our knowledge, this study is the first one to show associations between intrauterine blood flow abnormalities and smaller brain

volumes at term, as well as NEC and smaller brain volumes in a non-selected cohort of VLBW infants.

In conclusion, in this study it has been shown that even a mild compromise of placental blood flow is associated with decreased brain volume, especially in the cerebrum of VLBW infants. According to our results, the redistribution of the blood flow is a significant sign of threat to the fetal brain and not sufficient as a compensatory mechanism to maintain normal brain growth and development. Blood flow patterns indicating redistribution, resulted in a more than 10% reduction of the total brain volume, which is likely to be a significant risk for later development. Furthermore, chronic inflammation (as in this study CLD and NEC) seem to be an independent risk factor for brain development. Further neurological follow-up is needed in order to explore the prognostic value of abnormal fetal blood flow patterns, CLD and operated NEC on the long-term development of VLBW infants.

Crying and fussing behaviour and holding of the VLBW infant in relation to brain injury (II)

Our main finding was that severe structural brain injuries in VLBW infants do not affect the amount (either duration or frequency) of fussing and crying or the development of the circadian rhythm of the infant. In addition, prematurity, in itself, does not increase the total duration of fussing or crying when compared to full term controls at five months of corrected age. In addition, those VLBW infants who were still in hospital at term age (n=11), cried significantly more than the infants at home (n=106). Furthermore, the frequency of fussing bouts was higher in preterm infants than in full term controls at five months of corrected age. In pair-wise comparisons, the only significant difference was that the VLBW infants with brain pathology fussed more often than their full-term controls.

Our hypothesis was that in home situations the amount of holding could be one explanatory factor and could be used as an intervention in order to prevent distress behavior. As the duration of holding was not longer in infants with brain injury after term age compared to those without brain injury, it is not likely that holding was masking a persistent difference in crying behavior related to brain injury. When all VLBW infants were compared to the control group at five months of corrected age, the VLBW infants were held significantly more than full term infants. It has not been well shown if increased holding can decrease the amount of crying. One study in healthy full term infants showed that a longer duration of holding prophylactically decreased the total amount of crying but did not affect the frequencies of either crying or fussing (Hunziker and Barr 1986). The finding of more frequent fussing bouts in preterm infants compared to full term infants may be explained by the dysregulation of behavioral states in preterm infants, who react with fussing to internal or external stimuli. However, as the duration of total fussing was not increased, the more fragmented fussing behavior in preterm

infants may simply reflect more frequent parental intervening with holding resulting in interruptions in fussing. Physical contact may help an infant to regulate her behavior. It has also been shown that holding of the infant is related to more optimal mother-infant interaction later in life (Korja et al. 2008).

The strengths of our study included that 1) we used a reliable proforma of The Baby Day Diary which has been shown to be reliable in estimating the spontaneous crying behavior of infants (Barr et al 1982). 2) We had a long enough recording period as at each time point we used a recording from three subsequent days to minimize the effect of day-to-day variability in behavior. 3) The completion and return rate of the diaries was high. 4) The proportion of missing data was small, ranging from 0.2% to 0.9% of the total minutes of a day. 5) The estimation of crying at several time points made it possible to explore developmental trends. 6) The inclusion of a control group of healthy term infants at five months of age.

The development of the circadian rhythm was reflected by less crying at night compared to the day at six weeks and five months of age in all VLBW groups, whereas, expectedly, no clustering of crying was seen at term. These trajectories confirm the development of the circadian rhythm at the same corrected age as in full term infants (Kirjavainen 2004, Harrison 2004). These data are consistent with an earlier report showing the diurnal clustering of crying in the evening hours in healthy preterm infants at six weeks of corrected age (Barr et al. 1996). Sleeping time was concentrated at night as age increased (Data not shown). We did not use the sleeping data, as a previous study by Kirjavainen (2004) showed that infants may be content and awake at night but parents can misrecognize such behaviour as a sleeping period if they don't hear negative vocalizations. Therefore, the sleep diary data is not reliable.

In our study, the preterm infants with brain pathology did not differ in their behavior in test situations (Dubowitz Assessment, HINE), when compared to VLBW infants with intermediate brain pathology or normal brain findings at term, at one or at two years of corrected age. Our finding is in contrast to a previous study which showed preterm infants to be more irritable than full term infants in test situations (Wolf et al. 2002). In contrast, those study infants still in hospital at term, were more irritable than the infants at home but brain pathology was not separately tested for those still staying in hospital.

Ventricular dilatation indexes (III, IV)

The systematic practice of using reproducible indexes of VD has been absent in most previous studies. One of the most frequently used methods identifying VD has been the subjective visual estimation of the sizes of the ventricles. This may be useful in severe VD, but the milder forms of VD cannot be compared without reproducible measures.

It would be important to find consensus regarding VD definitions both on US and on MRI.

No cut-off values for abnormal ventricular volumes in MRI in preterm infants at term are available. In our study, the exact cut-off values of 0.35 for the V/B ratio, of 0.3 cm for the frontal horn and of 1.15 cm for the occipital horn were used on US. An abnormal V/B ratio correlated significantly with an increasing number of dilated horns of the lateral ventricles, and with a larger ventricular volume on the MRI. In addition, an increasing number of dilated horns of the lateral ventricles correlated with larger ventricular volume on the MRI. The ventricular indexes used in this thesis, can all be viewed as reliable methods in defining VD, as the correlations between them were significant.

The reliability of US in determining VD is clinically important, as US is relatively easy to perform and available universally in NICUs. In further analyses, the inter-observer reliabilities in the measurement of the V/B ratio using US and the ventricular volumes using MRI were high (0.97 and 0.99, respectively). The inter-observer reliability analysis in the measurement of all four ventricular horns using US was not assessed.

Using a cut-off value of 0.35 for the V/B ratio, we showed that an abnormal V/B ratio was associated with several brain injuries, smaller volumes of total brain tissue, of the cerebrum and the cerebellum. In infants whose V/B ratio was abnormal, the incidences of severe IVH, WM injury, cortical injury, hypoplastic corpus callosum or ventriculitis were higher when compared to those VLBW infants with a normal V/B ratio. WM injury (Stewart et al. 1999, Melhem et al. 2000), cortical injury (Peterson et al. 2000) or a hypoplastic corpus callosum (Nosarti et al. 2004, Anderson et al. 2006, Narberhaus et al. 2008) related to VD are known to be associated with poor neurodevelopmental outcomes in VLBW infants. Accordingly, we suggest that VD defined by an abnormal V/B ratio on US could be used as an indicator for the need of a brain MRI.

Our study showed that an abnormal V/B ratio, an increasing number of dilated horns of the lateral ventricles on US, and smaller head circumference are practical and reliable indicators of reduced volumes of total brain tissue in VLBW infants at term. Although the widths of the horns of the lateral ventricles on US may be more complicated to measure than the V/B ratio, the widths of the horns better predict regional brain volumes, for example, the possible loss of tissue in the frontal lobe. Our results confirm the hypothesis that mild and severe VD is a sign of brain injury.

Ventricular dilatation in relation to 2-year outcome in VLBW infants (IV)

Our study showed that VD is a common finding in VLBW/VLGA infants as shown also in previous studies (Paneth et al. 1990, Ment et al. 1999, Dyet et al. 2006). VD was a risk for later developmental problems only in those infants with other additional brain

pathology, which is in agreement with other studies on preterm infants (Vollmer et al. 2006, Dyet et al. 2006).

Compared to US, measuring the total volumes of ventricles using MRI did not provide additional information to help to predict later neurodevelopmental impairments in VLBW infants. The V/B ratio and the individual sizes of the ventricular horns are alternative methods, providing maximum information when used together. However, the V/B ratio is technically more practical to measure.

Our study is in agreement with an earlier study that showed a correlation between VD in preterm infants and CP (Whitaker et al. 1996, Allan et al. 1997, Ment et al 1999, Valkama et al. 2000, Msall et al. 2006). We showed that the risk for CP increased if two or more of the ventricular horns were dilated or if the V/B ratio was abnormal. In our study, cognitive outcome was severely impaired only when all four horns of the ventricles were dilated, but the association was not significant when adjusted for brain pathology, suggesting that other brain pathology than VD explains the cognitive delay. However, the assessment of cognition at two years of age has its known limitations. Therefore, we have reported only severe cognitive delays (< -2 SD) which can be reliably diagnosed at two years of age. Milder cognitive impairments will be diagnosed more reliably at older ages. Although none of the study infants had major visual impairments, there might have been present, for example, milder visuomotor difficulties or defects in the visual field that could be diagnosed when the children get older.

In conclusion, VD in the group of VLBW/VLGA infants with other brain pathology increased the risk of poor neurodevelopmental outcome at two years of age. Accordingly, VLBW/VLGA infants with detected VD on US, we suggest, should also be imaged using MRI to determine possible additional brain lesions commonly related to VD but not seen on US. Our hypothesis was, that isolated VD as well as VD related to other brain pathology may be associated with poorer neurodevelopmental outcome. However, according to our study, isolated VD was not associated with poorer neurodevelopment of the VLBW/VLGA infants.

Perinatal characteristics in VLBW/VLGA infants

Even though the MRI equipment and the US vector transducer used in NICU were upgraded during this thesis, the perinatal characteristics of study infants remained almost similar in different substudies (Table 1). The proportions of normal findings on the serial US examinations and on MRI examinations at term age were 31-34% and 57-58%, respectively. As expected, the most immature infants with lower GA and smaller BW had more brain findings than more mature infants as shown in Tables 3a and 3b. US examinations revealed more deviant findings than single MRI at term age since US

was based on serial images catching the most common early brain findings of preterm infants, IVH and isolated VD. On the other hand, MRI completes US examinations by detecting white matter and cerebellar lesions not visible on routine US examinations.

SGA status at birth in relation to the brain findings and volumes at term (I)

Interestingly, the SGA infants at birth had by term age caught up their normal brain growth. Neither the total or regional brain volumes or head circumference of SGA infants differed from non-SGA infants at term. In addition, there were no significant differences in pathological brain findings between the groups. One explanation might be the lack of the most severe blood flow abnormalities on antenatal Doppler measurements in this cohort as a result of the obstetrical practice to deliver in case of antenatal fetal distress. One can hypothesize that the absence of the most severe blood flow abnormalities might also have protected brain development and growth in SGA infants. Another explanation may be, that improved neonatal nutrition of the VLBW infants had optimized the brain growth of SGA infants and, therefore, they were able to reach their normal growth velocity even by term age. There may also be other perinatal and neonatal treatment practices, not discussed here, that protect the brain growth of SGA infants compared to those reported in earlier literature.

Neurological examinations of VLBW infants at term age (Dubowitz Assessment) (unpublished data)

The results from a Dubowitz test are recorded on proforma, which provide a visual record of neurological findings and an objective tool for follow-up examinations of VLBW infants. It aids a clinician to follow the changes over time in the status of an infant in hospital when used repeatedly. The tone pattern section is a valuable tool when estimating the normality of the flexor and extensor tone balance in a newborn.

Ricci et al. (2008) found that the number of items falling outside the 90th centile at term was a good predictor of later neurological impairments. In their study, over 90 % of the low risk preterm infants had less than 4 items outside the 90th centile. In our study population, nine out of 13 (70%) infants with CP had four or more items outside the 90th centile. In these cases, the examination supported clinical judgement in predicting increased risk for abnormal neurological outcome. If the test is repeated systematically several times, transient abnormal signs in infants will be able to be more clearly documented. Our hypothesis was, that the infants with CP would differ in their neurological status from VLBW infants without CP or severe hearing or vision impairments. However, in contrast to our hypothesis, infants with CP cannot be found as early as at term. A longer follow-up is needed for CP diagnosis.

Results of the neurological examinations of VLBW infants at one and two years of age (HINE) (unpublished data)

The HINE was a practical and easy test to perform during the follow up visits of the VLBW infants. On total scores, as well as on all subscale scores, the infants with CP differed significantly from control infants without CP, or severe hearing or vision impairments at both ages. The systematic use of the same neurological test in the same hospital is recommended. Although normative data for the optimal scoring system has only been published for low-risk full term infants at 12 and 18 months of age, the applications of the total and subscale scores were useful with VLBW infants at 12 and 24 months of age, respectively. We hypothesised that the findings of VLBW infants with CP would differ from VLBW infants without CP or severe hearing or vision impairments. It was very interesting to demonstrate that there was little overlapping between the total scores of infants with and without CP. The infants with CP had significantly lower total scores at one and two years of ages on the HINE when compared to VLBW infants without CP/severe hearing or vision impairments as seen in Table 5. The HINE aids the early detection of suspected CP, especially when used sequentially.

Strengths and limitations

We were fortunate that our study infants and their families were fully committed to the research project, as the participating percentages for the PIPARI-Study were high and the drop-out percentage was very small during the follow-up. Information gathered during neonatal care was fully complete including background information. The diaries were also filled out by our nurses if the infants were still in hospital at the time of the Baby Day Diary recordings.

There were a few changes that did occur during the six year-long recruitment period. First, we expanded the inclusion criteria in the middle of the recruitment period to cover full gestational week cohorts up to 32 weeks. Second, MRI equipment was upgraded from 0.23 T to 1.5 T in the middle of the study. After upgrading the MRI equipment more WM injuries were reported in all gestational week cohorts when compared to the images obtained by 0.23T equipment. However, there were no statistically significant differences between the groups imaged by the 0.23T and 1.5T equipment with respect to WM injuries. Accordingly, we cannot exclude some inconsistencies in our findings due to the upgrading, because of the small total number of WM injuries in our study.

Furthermore, this resolution improvement could have affected the results of the volume measurements if we had measured white and gray matter volumes separately. However, the measurements of brain volumes were performed manually by clearly defined anatomical landmarks to avoid inconsistencies due to the change of the MRI equipment.

One of the strengths of the study was that the antenatal Doppler measurements were performed by two experienced obstetricians. The trade-off was that not all patients admitted to the hospital could be evaluated by Doppler US within the required time limits.

The US vector transducer used at term, was a 7.5 MHz transducer from 1/2001 to 8/2002 and a 8.0 MHz tranducer from 7/2002 to 3/2007. It is not likely that the change in the US vector transducer affected the results, because we used the vectors to diagnose well distinguished lesions such as IVH, caudothalamic cysts, and PVL or fluid containing ventricles. We decided not to analyze resolution sensitive parenchymal lesions using US due to the change of the US vector transducer and also because we used MRI imaging as well as US imaging at term.

The Vermont Oxford Network aims to improve the quality and safety of medical care for newborn infants. The NICU at Turku University Hospital is one of over 800 Neonatal Intensive Care Units around the world which regularly report their care indices to the Vermont Oxford Network database, and which in turn receive detailed data on their own results compared to the other units. As Turku University Hospital belongs to the international Vermont Oxford Network, we are able to compare our neonatal outcomes of VLBW/VLGA infants with international incidences. When compared to the Vermont Oxford Network, the standardized incidences of cPVL and IVH were higher than the average during the 3-year period from 2001 to 2003. However, the incidences of cPVL and IVH decreased to below average during the following 3-year period, from 2004 to 2006. The mortality rates of the VLBW/VLGA infants remained below the average throughout the study period 2001 to 2006.

In addition, it is too early at two years of age to predict the final cognitive outcome of VLBW infants. The assessment of cognitive outcome at two years of age can only reveal the most severely delayed infants. A more detailed cognitive and neuropsychological outcome has to be measured later at school age. However, it should be possible to distinguish moderate or severe abnormality in motor development, spontaneous movements and muscle tone by one year of age using serial systematical neurological examinations. It has also been recently reported that CP can be diagnosed even in extremely low birth weight infants with a sufficient reliability in most cases by two years of age (Voss et al. 2007). The PIPARI-Study is ongoing, aiming to assess the long-term functional outcome of the study infants and to elucidate the long-term effects of perinatal factors.

SUMMARY AND CONCLUSION

Brain injury is a serious complication of prematurity. It is important to also study prenatal factors that affect brain development, so that we might better understand the injury mechanisms and optimize the care of preterm infants, and thereby improve their developmental outcome. Thus, multidisciplinary, prospective, and longitudinal follow-up studies bridging from fetal life to long term outcome are valuable. In the future, new brain imaging techniques, for example diffuse weighted imaging MRI, functional MRI, diffusion tensor imaging, and diffusion tractography will provide us with more detailed information concerning brain injuries and their predictive value for VLBW infants.

This study has important clinical implications. Antenatal fetal blood flow redistribution was shown not to be sufficient to maintain normal brain growth. This new finding has been integrated into clinical practice at Turku University Hospital, as the UA/MCA ratio - in addition to the traditional PIs of the UA and MCA – is also determined in fetuses when considering the need for preterm delivery. In addition, the fact that the VLBW infants with brain pathology were not more irritable compared to the other VLBW infants is a comforting message for their parents. Our studies have also shown that using the V/B ratio to evaluate the degree of VD provides an easy and clear marker for identifying other brain findings in VLBW/VLGA infants. VD increases the risk of a poorer neurolodevelopmental profile when combined with other brain pathology. Furthermore, the systematic clinical use of the standardized neurological tests in the neonatal period (Dubowitz Assessment) provided a clinically useful prognostic tool. Similarly, the application of the HINE aided the early detection of suspected CP. All the CP diagnoses in our cohort have been done during the first two years of corrected age. However, the final classification of the specific type and the functional severity of CP have to be reassessed later.

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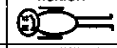
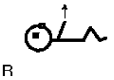
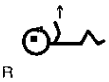
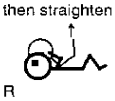
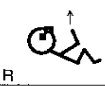
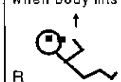
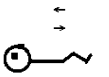
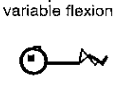
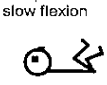
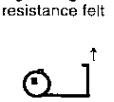
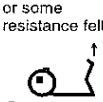
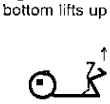
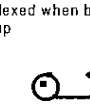
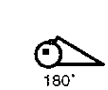
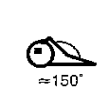
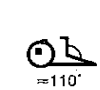
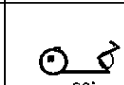
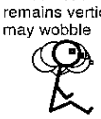
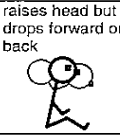
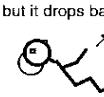
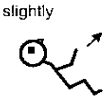
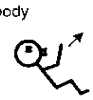
Appendix 1. The proforma of the Dubowitz Assessment used at 36 weeks, 40 weeks, 1 month and 2 months of corrected age in VLBW/VLGA infants in the PIPARI-Study.

Hammersmith Neonatal Neurological Examination CODE_____ D.O.E._____

NAME _____ SEX___ RACE_____ D.O.B._____ AGE_____ G.A._____ BW _____

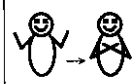
STATE

Posture and tone

POSTURE Infant supine. Look mainly at position of legs but also note arms. *Score predominant posture.*	arms & legs extended or very slightly flexed 	Legs slightly flexed 	legs well flexed but not adducted 	legs well flexed & adducted near abdomen 	abnormal posture: a) opisthotonus b) marked leg extension, strong arm flexion 
ARM RECOIL Take both hands, quickly extend arms parallel to the body, Count to three. Release. Repeat 3 times.	arms do not flex 	arms flex slowly, not always; not completely 	arms flex slowly; more completely 	arms flex quickly and completely 	arms difficult to extend; snap back forcefully
ARM TRACTION Hold wrist and pull arm upwards. Note flexion at elbow and resistance while shoulder lifts off table. *Test each side separately.*	arms remain straight; no resistance felt  R L	arms flex slightly or some resistance felt  R L	arms flex well till shoulder lifts, then straighten  R L	arms flex at approx 100° & maintained as shoulder lifts  R L	flexion of arms <100°; maintained when body lifts up  R L
LEG RECOIL Take *both* ankles in one hand, flex hips + knees. Quickly extend. Release. Repeat 3 times.	No flexion 	incomplete or variable flexion 	complete but slow flexion 	complete fast flexion 	legs difficult to extend; snap back forcefully
LEG TRACTION Grasp ankle and slowly pull leg upwards. Note flexion at knees and resistance as buttocks lift. *Test each side separately.*	legs straight - no resistance felt  R L	legs flex slightly or some resistance felt  R L	legs flex well till bottom lifts up  R L	knee flexes remains flexed when bottom up  R L	flexion stays when back+bottom up  R L
POPLITEAL ANGLE Fix knee on abdomen, extend leg by gentle pressure with index finger behind the ankle. Note angle at knee. *Test each side separately.*	 180° R L	 ≈150° R L	 ≈110° R L	 ≈90° R L	 <90° R L
HEAD CONTROL (1) ***(extensor tone)*** Infant sitting upright. Encircle chest with both hands holding shoulders. Let head drop forward.	no attempt to raise head 	infant tries: effort better felt than seen 	raises head but drops forward or back 	raises head: remains vertical; it may wobble 	
HEAD CONTROL (2) ***(flexor tone)*** Infant sitting upright. Encircle chest with both hands holding shoulders. Let head drop backward.	no attempt to raise head 	infant tries: effort better felt than seen 	raises head but drops forward or back 	raises head: remains vertical; it may wobble 	head upright or extended; cannot be passively flexed
HEAD LAG Pull infant towards sitting posture by traction on both wrists & support head slightly. Also note arm flexion.	head drops & stays back 	tries to lift head but it drops back 	able to lift head slightly 	lifts head in line with body 	head in front of body
VENTRAL SUSPENSION Hold infant in ventral suspension. Observe back, flexion of limbs, and relation of head to trunk. If it looks different, DRAW.	back curved, head & limbs hang straight	back curved, head ↓, limbs slightly flexed	back slightly curved, limbs flexed	back straight, head in line, limbs flexed	back straight, head above body

FLEXOR TONE (1) **(on traction: arm versus leg)** Compare scores of arm traction with leg traction.		score for arm flexion less than leg flexion	score for arm flexion equal to leg flexion	score for arm flexion more than leg flexion but difference 1 column or less	score for arm flexion more than leg flexion but difference more than 1 column
FLEXOR TONE (2) **(arm versus leg)** Posture in supine.			arms and legs flexed	strong arm flexion with strong leg extension *intermittent*	strong arm flexion with strong leg extension *continuous*
LEG EXTENSOR TONE Compare scores of leg traction and popliteal angle.		score for leg traction more than score for popliteal angle	score for leg traction equal to score for popliteal angle	score for leg traction less than score for popliteal angle, by 1 column only	score for leg traction less than score for popliteal angle, by more than 1 column
NECK EXTENSOR TONE (SITTING) Compare scores of head control 1 and 2.		score for head extension less than head flexion	score for head extension equal to head flexion	score for head extension more than head flexion. but difference 1 column or less	score for head extension more than head flexion but difference more than 1 column
INCREASED EXTENSOR TONE (HORIZONTAL) Compare scores of head lag and ventral suspension.		score for ventral suspension less than head lag	score for ventral suspension equal to head lag	score for ventral suspension more than head lag but difference 1 column or less	score for ventral suspension more than head lag but difference more than 1 column

Reflexes

TENDON REFLEX Test biceps, knee, and ankle jerks.	absent	felt, not seen	seen	"exaggerated" (very brisk)	clonus
SUCK / GAG Little finger into mouth with pulp of finger upwards.	no gag / no suck	weak irregular suck only No stripping	weak regular suck Some stripping	strong suck: (a) irregular (b) regular Good stripping	no suck but strong clenching
PALMAR GRASP Put index finger into the hand and gently press palmar surface. Do not touch dorsal surface. *Test each side separately.*	no response R L	short, weak flexion of fingers R L	strong flexion of fingers R L	strong finger flexion, shoulder ↑ R L	very strong grasp; infant can be lifted off couch R L
PLANTAR GRASP Press thumb on the sole below the toes. *Test each side separately.*	no response R L	partial plantar flexion of toes R L	toes curve around the examiner's finger R L		
PLACING Lift infant in an upright position and stroke the dorsum of the foot against a protruding edge of a flat surface. *Test each side separately.*	no response R L	dorsiflexion of ankle only R L	full placing response with flexion of hip and knee & placing sole on surface R L		
MORO REFLEX One hand supports infant's head in midline, the other the back. Raise infant to 45° and when infant is relaxed let head fall through 10°. Note if jerky. Repeat 3 times.	no response, or opening of hands only	full abduction at shoulder and extension of the arms; no adduction 	full abduction, but only delayed or partial adduction 	partial abduction at shoulder, and extension of arms followed by smooth adduction 	• minimal abduction or adduction • no abduction or adduction; only forward extension of arms • marked adduction only  or

SPONTANEOUS MOVEMENT (quantity) Watch infant lying supine.	no movement	sporadic and short isolated movements	frequent isolated movements	frequent generalized movements	continuous exaggerated movements
SPONTANEOUS MOVEMENT (quality) Watch infant lying supine.	only stretches	stretches and random abrupt movements; some smooth movements	fluent movements but monotonous	fluent alternating movements of arms + legs; good variability	• cramped, synchronized; • mouthing • jerky or other abnormal movements
HEAD RAISING PRONE Infant in prone, head in midline.	no response	infant rolls head over, chin not raised	infant raises chin, rolls head over	infant brings head and chin up	infant brings head up and keeps it up

Abnormal signs/patterns

ABNORMAL HAND OR TOE POSTURES		hands open, toes straight most of the time	intermittent fisting or thumb adduction	continuous fisting or thumb adduction; index finger flexion, thumb opposition	continuous big toe extension or flexion of all toes
TREMOR		no tremor, or tremor only when crying or only after Moro reflex	tremor occasionally when awake	frequent tremors when awake	continuous tremors
STARTLE	no startle even to sudden noise	no spontaneous startle but reacts to sudden noise	2-3 spontaneous startles	more than 3 spontaneous startles	continuous startles

Orientation and behaviour

EYE APPEARANCES	does not open eyes		full conjugated eye movements	*transient* • nystagmus • strabismus • roving eye movements • sunset sign	*persistent* • nystagmus • strabismus • roving eye movements abnormal pupils
AUDITORY ORIENTATION Infant awake. Wrap infant. Hold rattle 10 to 15 cm from ear.	no reaction	auditory startle; brightens and stills; no true orientation	shifting of eyes, head might turn towards source	prolonged head turn to stimulus; search with eyes; smooth	turns head (jerkily, abruptly) & eyes towards noise every time
VISUAL ORIENTATION Wrap infant, wake up with rattle if needed or rock gently. Note if baby can see and follow red ball (B) or target (T).	does not follow or focus on stimuli B T	stills, focuses, follows briefly to the side but loses stimuli B T	follows horizontally and vertically; no head turn B T	follows horizontally and vertically; turns head B T	follows in a circle B T
ALERTNESS *Tested as response to visual stimuli* (B or T).	will not respond to stimuli	when awake, looks only briefly	when awake, looks at stimuli but loses them	keeps interest in stimuli	does not tire (hyper-reactive)
IRRITABILITY In response to stimuli.	quiet all the time, not irritable to any stimuli	awakes, cries sometimes when handled	cries often when handled	cries always when handled	cries even when not handled
CONSOLABILITY Ease to quiet infant.	not crying; consoling not needed	cries briefly; consoling not needed	cries; becomes quiet when talked to	cries; needs picking up to be consoled	cries; cannot be consoled
CRY	no cry at all	whimpering cry only	cries to stimuli but normal pitch		High-pitched cry; often continuous

Appendix 2. The proforma of the Hammersmith Infant Neurological Examination (HINE) used at 12 and 24 months of corrected ages in VLBW/VLGA infants in the PIPARI-Study.

HAMMERSMITH INFANT NEUROLOGICAL EXAMINATION

Name: **Date of birth:**

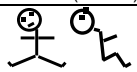
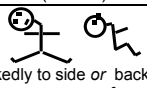
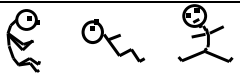
Gestational age: **Date of examination:**

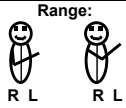
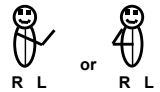
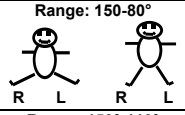
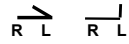
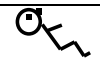
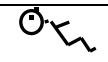
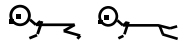
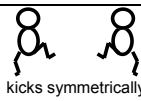
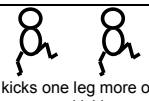
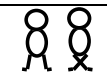
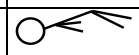
SUMMARY OF EXAMINATION
No of asymmetries in section 1:
Neurological items score:
Behavioural score:

COMMENTS:
Cranial nerves functions
Posture
Movements
Tone
Reflexes and reactions
Behaviour

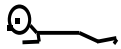
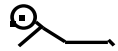
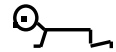
Assessment of cranial nerve function

	column 1 (score 3)	c 2 (sc.2)	col. 3 (score 1)	col. 4 (score 0)	A	comment
Facial appearance (at rest and when crying or stimulated)	smiles or reacts to stimuli by closing eyes and grimacing		closes eyes but not tightly poor facial expression	expressionless, does not react to stimuli		
Eye appearance	normal conjugated eye movements		**Intermittent** deviation of eyes or abnormal movements	**continuous** deviation of eyes or abnormal movements		
Auditory response test the response to rattle or bell	reacts to stimuli on both sides		doubtful reaction to stimuli or asymmetrical	does not react to stimuli		
Visual response test the ability to follow a red ball or moving object	follows the object for a complete arc		follows the object for an omplete arc or asymmetry	does not follow the object		
Sucking/swallowing watch the infant suck on breast or bottle	good suck and swallowing		poor suck and/or swallowing	no sucking reflex no swallowing		

	Column 1 (score 3)	c 2 (sc.2)	column 3 (score 1)	column 4 (score 0)	**A**	
Head in sitting	 straight; in midline		 slightly to side *or* backward *or* forward	 markedly to side *or* backward *or* forward		
Trunk in sitting	 straight		 slightly curved or bent to side	 very rounded rocketing back bent sideways		
Arms at rest	in neutral position central straight *or* slightly bent		**slight** internal rotation *or* external rotation **intermittent** dystonic posture	**marked** internal rotation *or* external rotation or dystonic posture hemiplegic posture		
Hands	hands open		**intermittent** adducted thumb *or* fisting	**persistent** adducted thumb *or* fisting		
Legs in sitting	able to sit with straight back and legs straight or slightly bent (long sitting) 		sit with straight back but knees bent at 15-20 ° 	unable to sit straight unless knees markedly bent (no long sitting) 		
in supine and in standing	legs in neutral position straight *or* slightly bent	**slight** internal rotation or external rotation	internal rotation *or* external rotation at hips	**marked** internal rotation *or* external rotation *or* fixed extension or flexion or contractures at hips and knees		
Feet in supine and in standing	central in neutral position toes straight midway between flexion and extension		**Slight** internal rotation *or* external rotation **intermittent** tendency to stand on tiptoes or toes up or curling under	**marked** internal rotation *or* external rotation at the ankle **persistent** tendency to stand on tiptoes *or* toes up or curling under		
Movements						
Quantity watch infant lying in the supine	Normal		excessive or sluggish	minimal or none		
Quality	free, alternating, smooth		jerky, slight tremor	• cramped & synchronous, • extensor spasms, • athetoid; • ataxic, • very tremulous, • myoclonic spasm • dystonic movement		

Tone	**Column 1 (score 3)**	**c. 2 (sc2)**	**column 3 (score 1)**	**column 4 (score 0)**	**A**	
Scarf sign Take the infant's hand and pull the arm across the chest until there is resistence. Note the position of the elbow.	**Range:**  R L R L		 R L	 R L or R L		
Passive shoulder elevation Lift arm next to the infant's head. Note resistance at shoulder and elbow.	Resistance but overcomed  R L	resistance difficult to overcome	no resistance  R L	resistance,not overcomed  R L		
pronation/supination steady upper arm while pronating and supinating forearm, note resistance	Full pronation and supination, no resistance,		full pronation and supination but resistance to be overcomed	full pronation and supination not possible, marked resistance		
Adductors With the infant's legs extended, open them as far as possible. The angle formed by the legs is noted.	**Range: 150-80°**  R L R L	**150-160**  R L	**>170°**  R L	**<80°**  R L		
Popliteal angle Legs are flexed at the hip simultaneously on to the side of the abdomen, then extended at the knee until there is resistance. Note angle between lower and upper leg.	**Range: 150°-110°**  R L R L	**150-160**  R L	**~90° or > 170°**  R L R L	**<80°**  R L		
Ankle dorsiflexion with knee extended, dorsiflex ankle. Note the angle between foot and leg.	**Range: 30°-85°**  R L R L	**20-30**  R L	**<20°or 90°**  R L R L	**> 90°**  R L		
Pulled to sit Pull infant to sit by wrists						
Ventral suspension hold infant in ventral suspension; note position of back, limbs and head						
Reflexes and reactions						
Tendon Reflexes	easily elicitable biceps knee ankle	mildly brisk bic knee ank	brisk biceps knee ankle	clonus or absent biceps knee ankle		
Arm protection Pull the infant by one arm from the supine position and note the reaction of the opposite side.	 arm & hand extend R L		 arm semi flexed R L	 arm fully flexed R L		
Vertical suspension hold infant under axilla make sure legs do not touch any surface	 kicks symmetrically		 kicks one leg more or poor kicking	 no kicking even if stimulated or scissoring		
Lateral tilting (describe side up)**.** Infant held vertically tilt quickly to horizontal. Note spine, limbs and head	 R L	 R L	 R L	 R L		
Forward parachute Infant held vertically and suddenly tilted forwars . Note reaction of the arms.	 (after 6 months)		 (after 6 months)			

SECTION 2: MOTOR MILESTONES

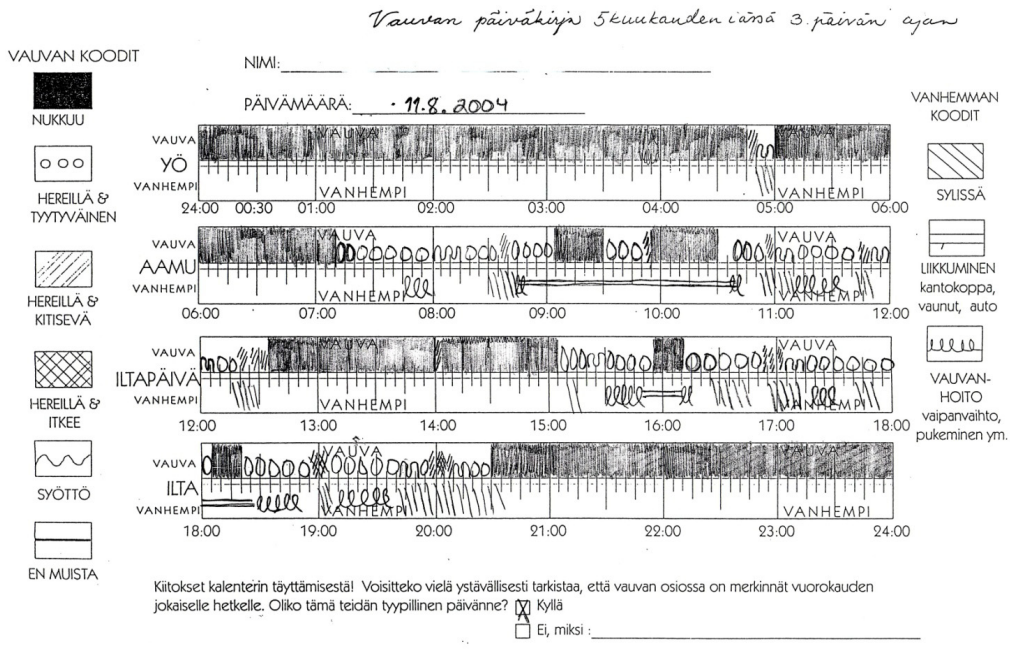
Head control	unable to maintain head upright (normal < 3 m)	Wobbles (normal at 4 m)	all the time maintained upright (normal at 5 m)			
Sitting	cannot sit	with support at hips  (normal at 4m)	props  (normal at 6 m)	stable sit  (normal at 7-8 m)	pivots (rotates)  (normal at 9 m)	Observed: Reported (age):
Voluntary grasp	no grasp	uses whole hand	index finger and thumb but immature grasp	pincer grasp		Observed: Reported (age):
Ability to kick : (in supine)	no kicking	Kicks horizontally legs do not lift	upward (vertically)  (normal at 3 m)	touches leg  (normal at 4-5 m)	touches toes  (normal at 5-6 m)	Observed: Reported (age):
Rolling	no rolling	Rolling to side (normal at 4 m)	prone to supine (normal at	supine to prone (normal at		Observed: Reported (age):
Crawling	does not lift head	On elbow  (normal at 3 m)	on outstretched hand  (normal at 4 m)	crawling flat on abdomen  (normal at 8 m)	crawling on hands and knees  (normal at 10 m)	Observed: Reported (age):
Standing	does not support weight	Supports weight (normal at 4 m)	stands with support (normal at 7 m)	stands unaided (normal at 12 m)		Observed: Reported (age):
Walking		Bouncing (normal at 6 m)	cruising (walks holding on) (normal at 12 m)	walking independently (normal at 15 m)		Observed: Reported (age):

SECTION 3: BEHAVIOUR

	1	2	3	4	5	6	Comment
State of consciousness	Unrousable	drowsy	sleepy but wakes easily	awake but no interest	loses interest	maintains interest	
Emotional state	irritable, not consolable	irritable, mother can console	irritable when approached	neither happy or unhappy	happy, smiling		
Social Orientation	Avoiding, withdrawn	hesitant	accepts approach	friendly			

Score for behaviour:

Appendix 3. An example day of the Baby Day Diary filled at five months of corrected age. Baby Day Diary has been used in the PIPARI-Study at term, at six weeks of corrected age and at five months of corrected age for three days at each time point.



Appendix 4. Scoring of the brain magnetic resonance imaging at term in the PIPARI-Study.

PIPARI-Study: Brain Structures
Brain Magnetic Resonance Imaging at term
Analyzed by Parkkola R, MD, PhD, Turku University Hospital

A. Cortex
    1. Normal
    2. Grade 1 (One area affected)
    3. Grade 2 (Two areas affected, describe which)
    4. Grade 3 (Three areas affected, describe which)

A. Basal Ganglia and Thalami
    1. Normal
    2. Grade 1 (Focal lesions but posterior part of internal capsule not affected)
    3. Grade 2 (Focal lesions in lentiform nuclei or thalami and abnormal signal in poster part of internal capsule)
    4. Grade 3 (Large changes in basal ganglia or thalami and abnormal poster part of internal capsule)

B. Posterior Limb of Internal Capsule (PLIC)
    1. Normal (high signal intensity in at least one third of the area in T1-weighted image and low signal intensity in at least one third of the area in T2-weighted image)
    2. Grade 1 (Asymmetrical poster limbs of internal capsules in T2-weighted images)
    3. Grade 2 (Low signal intensity in T1-weighted images and high signal intensity in T2-weighted images)

C. White matter
    1. Normal
    2. Grade 1 (Small hemorrhages and/or abnormal high T1 and T2 signal intensities in white matter but white and gray matter can be visually separated)
    3. Grade 2 (Large hemorrhages or abnormal high T1 and T2 signal intensities extending to gray matter)

D. Germinal Matrix
    1. Normal
    2. Grade 1 (High-intensity signals due to small, local hemorrhages on one or both sides)
    3. Caudothalamic cysts: side and size (mm)

E. Width of lateral ventricles
    1. Normal
    2. Dilatation of frontal horns
    3. Dilatation of occipital horns
    4. Dilatation of all the horns of the lateral ventricles

F. Ventriculitis
    1. No
    2. Yes (High signal intensity and abnormal thickness of the ventricular walls in T1-weighted images)

G. Corpus Callosum
    1. Normal
    2. Hypoplastic (Both thin corpus callosum and congenitally hypoplastic corpus callosum)

I. Posterior fossa structures
    1. Normal
    2. Hemorrhage: Define the location
    3. Anomaly: Define
J. Myelination pattern corresponds to ____ gestational weeks
K. Ventricular/brain ratio
L. The width of extracerebral space (mm)
M. Other findings