

From the DEPARTMENT OF WOMEN'S AND CHILDREN'S HEALTH
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BRAIN IMAGING AND OUTCOME IN EXTREMELY PRETERM INFANTS

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*Dedicated to Erik
and our wonderful children Julia & August*

*The brain is wider than the sky
Emily Dickinson
1830-1886*

ABSTRACT

In parallel to the dawn of modern neonatal intensive care, the survival after extremely preterm birth has greatly improved. These very immature children are born during a vulnerable phase of brain maturation, and are at high risk of brain injury and subsequent neurodevelopmental impairments. The overall aim of the works compiled in this thesis is to study brain development and damage of extremely preterm (EPT) infants using different neuroimaging techniques, and to investigate the relations to toddler age outcomes.

All infants born before gestational week 27 + 0 days in Stockholm during a 3-year period were invited to participate. Infants underwent Magnetic Resonance Imaging (MRI, n=109) including Diffusion Tensor Imaging (DTI, n=54) at term equivalent age. Paper I describes the brain damage panorama in the cohort, and the rates of major injuries were low; only 14% had moderate or severe white matter (WM) abnormalities. Subtle WM changes, so called DEHSI (diffuse excessive high signal intensities), were found in 56% of infants and were verified as changes on DTI, indicating possible alterations in WM microstructure.

To study functional connectivity in the WM we used non-stimulated functional MRI in EPT infants at rest. In Paper II, we present evidence of five unique resting state networks at term age in healthy preterm infants.

In Paper III we demonstrate that, provided that imaging was performed on the same day, cranial ultrasound (cUS) detected all infants with moderate or severe WM abnormalities on MRI. However, one third of infants with mild WM abnormalities and four infants with small cerebellar haemorrhages on MRI were overlooked with cUS.

Follow-up assessments were performed at age 30 months corrected to study the consequences of extreme prematurity. In Paper IV, infants underwent a neurological examination and were evaluated using the Bayley Scales of Infant and Toddler Development (BSID-III) to assess cognitive, language and motor function. Overall, the preterm group performed within the normal range for test standards, but significantly lower than a full term control group. The rates of severe impairments were low; 2% had a severe cognitive delay, 5% had a severe language delay and 7% had cerebral palsy.

Moreover in Paper IV, we demonstrate a high negative predictive value of a normal MRI at term age. Cystic changes, delayed myelination and severe WM reduction were factors most strongly related to adverse outcomes. DEHSI showed no relation to later cognitive, language and motor performances.

Finally in Paper V, we found poorer cognitive and language function in EPT boys than girls at age 30 months. These differences could neither be explained by an altered WM microstructure assessed with MR-DTI and Tract-Based Spatial Statistics, nor by any individual perinatal factor.

In summary, the rates of brain injuries and later impairments were low in this very high-risk population. The present results suggest that survival without major disability is likely even at extremely low gestational ages. Long-term follow-up after extremely preterm birth is essential.

LIST OF PUBLICATIONS

This thesis is based on the following original articles. They will be referred to by their Roman numerals (I-V).

- I. **Skiöld B**, Horsch S, Hallberg B, Engström M, Nagy Z, Mosskin M, Blennow M & Ådén U (2010). White matter changes in extremely preterm infants, a population-based diffusion tensor imaging study. *Acta Paediatrica*, 99(6), 842-849.
- II. Fransson P, **Skiöld B**, Horsch S, Nordell A, Blennow M, Lagercrantz H & Ådén U (2007). Resting-state networks in the infant brain. *Proc Natl Acad Sci U S A*, 104(39), 15531-15536.
- III. Horsch S, **Skiöld B**, Hallberg B, Nordell B, Nordell A, Mosskin M, Lagercrantz H, Ådén U & Blennow M (2010). Cranial ultrasound and MRI at term age in extremely preterm infants. *Arch Dis Child Fetal Neonatal Ed*, 95(5), F310-314.
- IV. **Skiöld B**, Vollmer B, Böhm B, Hallberg B, Horsch S, Mosskin M, Lagercrantz H, Ådén U & Blennow M (2011). Neonatal magnetic resonance imaging and outcome at age 30 months in extremely preterm infants. *Manuscript submitted to Journal of Pediatrics*
- V. **Skiöld B**, Alexandrou G, Blennow M, Vollmer B & Ådén U (2011). Gender differences in outcome at age 30 months in extremely preterm children and associations with brain structure. *Manuscript*

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LIST OF ABBREVIATIONS

ADC	apparent diffusion coefficient
BOLD	Blood-Oxygen-Level Dependent
BSID-III	Bayley Scales of Infant and Toddler Development, Third Edition
BW	birth weight
CC	corpus callosum
CP	cerebral palsy
CI	confidence interval
CPAP	continuous positive airway pressure
DEHSI	diffuse excessive high signal intensities
DMN	default-mode network
DTI	diffusion tensor imaging
EPT	extremely preterm
FA	fractional anisotropy
fMRI	functional magnetic resonance imaging
GA	gestational age
ICA	independent component analysis
IQ	intelligence quotient
IVH	intraventricular haemorrhage
MHz	mega-Hertz
MRI	magnetic resonance imaging
OR	odds ratio
PDA	patent ductus arteriosus
ROP	retinopathy of prematurity
RSN	resting state networks
PHI	parenchymal haemorrhagic infarction
PLIC	posterior limb of the internal capsule
PVL	periventricular leukomalacia
SD	standard deviation
TEA	term equivalent age
TBSS	Tract Based Spatial Statistics
WM	white matter

Advances in perinatal care during the past decades have contributed to an increased survival of extremely preterm infants. These very immature children are born during a vulnerable phase of brain development and maturation, and are at high risk of brain injury and subsequent neurodevelopmental impairments.

Although gratifying, these steady improvements require continuous reflection and scrutiny. The present work contributes to a dynamic field of research by exploring the current brain damage panorama of extremely preterm infants in Stockholm, several aspects of neuroimaging and early childhood outcomes.

1 BACKGROUND

1.1 PRETERM BIRTH

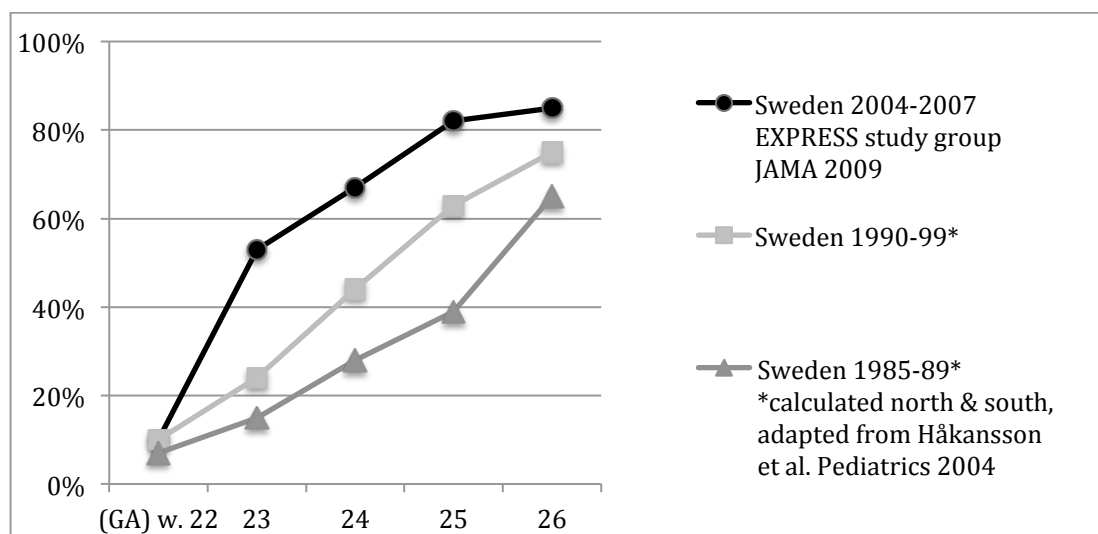
Preterm birth is defined as all births occurring before gestational week 37. Extremely preterm (EPT) births, before gestational week 27, accounts for 2.3 per 1000 live births in Sweden, i.e. approximately 200 infants per year [1].

Most preterm deliveries are spontaneous, starting with preterm labour or preterm rupture of the fetal membranes. A triggering cause such as an infection or a placental haemorrhage, may sometimes be identified; however, the precise causes are often unknown. Medically indicated preterm deliveries (induced labour or caesarean section) are due to fetal or maternal illness, such as infections, intrauterine growth restriction, complications associated to multiple gestations, preeclampsia etc [2].

1.1.1 Survival

Survival after extremely preterm birth has greatly improved during the past decades. Advanced ventilator strategies, the use of antenatal steroids and exogenous surfactant, as well as centralisation of neonatal intensive care, are some factors with immediate effects on short-time survival. There are numerous examples from different populations [3].

In Victoria, Australia, the survival of extremely low birth weight (BW) infants, i.e. BW < 1000 grams, increased threefold over two decades, from 25% in 1979-80 to 73% in 1997 [4]. In a similar way, the survival of infants born <26 weeks in the UK and Ireland improved from 39% in 1995 [5] to 47% 2006 ([6] preliminary data). In Sweden, survival rates have improved correspondingly [1, 7] see below. However, survival rates must be put in relation to neonatal morbidity and outcomes.



Survival rates of extremely preterm infants in Sweden over the last twenty years

1.1.2 Neonatal morbidities

Most organs are very immature in infants born extremely preterm, leading to a diverse range of neonatal problems. EPT infants commonly suffer from respiratory distress, retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), renal failure, nutritional difficulties, invasive infections, persisting fetal circulation/ patent ductus arteriosus (PDA) etc.

During the first days of life, early brain pathologies include germinal matrix haemorrhage, intraventricular haemorrhage (IVH) and parenchymal haemorrhagic infarction (PHI) [8]. Severe IVH occurs in approximately 10% [9] and altogether these conditions affect more than a third of EPT infants [10].

Over the following weeks posthaemorrhagic ventricular dilatation, non-cystic white matter injury and periventricular leukomalacia (PVL) may develop. The focal necrotic white matter lesions evolving into cysts seen in PVL have historically been considered ‘classical’ brain injury of prematurity [11], but is becoming uncommon, affecting approximately 0.5%-6% of preterms [12, 13]. Cerebellar haemorrhage, meningitis, stroke and intracranial malformations are other rare neurological diagnoses in EPT infants.

On the other hand, the incidence of non-cystic white matter (WM) damage is high [14, 15]. Furthermore, regions with diffuse and excessive high signal in the periventricular and subcortical WM on T2 weighted MR images (so called DEHSI, [16]) are present in a majority of EPT infants at term corrected age [17].

1.2 OUTCOME AFTER PRETERM BIRTH

Major disabilities such as cerebral palsy (CP), blindness or deafness, have historically been the main focus of most outcome studies, and are often used as markers of quality of care. Altogether, these conditions affected more than 20% of EPT infants born in the 1990s [3].

As a consequence of the increased survival rates over the past decades, a corresponding increase in the number of severely disabled children was reported [18]. In the large EPICure study 18% of infants born <26 weeks had **cerebral palsy** and 10% were unable to walk without assistance at 30 months [19]. However, this unfortunate trend seems to level off [20] and even cease [21]. In a recent Swedish study of a regional cohort born 1999-2002, the prevalence of CP in children born <28 weeks of gestation, was 55.6 per 1000 live births, compared to 1.43 per 1000 for term born children [22]. For these EPT children, there was a significant decrease in prevalence compared with the previous birth cohort of 1995–1998. Besides CP, preterm infants are at risk of minor neuromotor problems [23] and developmental coordination disorder [24].

In a large Canadian study of infants born with a BW <800 gram, Synnes et al report a changing pattern of **neurosensory impairments** over the past two decades [25]. While 4% of the cohort were deaf or had a severe hearing impairment in the 1980s, there was an increase to 10% by the years 1998-2003. On the contrary, severe visual impairment decreased from 10% to 5% during the same period, attributed to a decrease in sequelae after severe ROP. In total 70% of EPT infants

develop some degree of ROP, and 20% need treatment [26]. Altogether ROP accounts for approximately 5% of childhood blindness while other perinatal origins such as cortical blindness accounts for 17% and has shown no tendency to decline [27].

The panorama of **cognitive and behavioural impairments** in EPT infants includes low intelligence quotient (IQ) and mental retardation, social and behavioural problems, as well as poor academic achievement. These aspects have been investigated in cohorts of different countries;

- France: In the EPIPAGE study (Etude Epidémiologique sur les Petits Ages Gestationnels), cognitive abilities were assessed in infants born 1997. At five years of age 40% of children born <27 weeks had a mental processing composite score (equivalent to IQ) below 85 and 17% below 70 [28].
- Canada: In children with BW<800 grams born 1998-2003 the incidence of cognitive impairment at school-entry age was 21% [25]. In Canadian adolescents with a BW < 750 grams as many as 72% had school difficulties, and problems were apparent even in children without neurosensory impairments and normal IQ [29].
- UK and Ireland: In the EPICure study the mean Mental Development Index in children born <26 weeks in 1995 was low: 84 ± 12 (standardized mean 100) and 10 % were classified as having a severe cognitive disability without severe neuromotor or sensory and communication problems. A speech delay was also reported in nearly one quarter of the cohort [19]. At age 11 years, the EPT children were 4.3 times more likely to have attention-deficit hyperactivity disorder (ADHD) compared with classmates, but they were also at higher risk of emotional disorders and autism [30].
- USA: Eight-year old children born 1992-95 with BW <1000 grams showed significantly more symptoms of attention-deficit hyperactivity disorder (17%) and social phobia (7%) compared to controls. They also had more depressive symptoms (2%), generalized anxiety (3%), autistic symptoms (2%) and Asperger's disorder (1%) although numbers were small [31].
- Sweden: In children born 1988-93 with BW <1500 grams, cognitive performances were within the normal range at 5.5 years, although lower than in the full term controls [32]. In another region of Sweden, 49% of 15-year-old children with BW <1500 grams, had an IQ <85, and 12% <70 [33]. Investigations of school age outcomes in 11-year-old children born <26 weeks of gestation in 1990-1992, showed that nearly a third were found to have poor learning skills and 49% had poor academic performance [34]. Interestingly, there are important psychological outcome aspects; for example, recent data show that adolescents born EPT describe a self-estimated quality of life comparable to their term born peers [35].

In a longer perspective, cardiovascular and metabolic consequences of preterm birth have been of increasing concern. The rapid catch-up growth, often more accentuated for weight than height [36], in combination with findings of higher blood pressure in ex-preterm infants [37] demonstrate the need for other than neurodevelopmentally oriented long-term follow-up studies.

1.2.1 Perinatal factors affecting outcome

Mortality and morbidity are inversely related to gestational age (GA) and infants born at the border of viability are at greatest risk of adverse outcomes [3]. However, GA alone is not a powerful determinant for later outcome. Brain damages (discussed in detail below) are independently more strongly associated with adverse neurodevelopmental outcome than GA at birth [38, 39].

Perinatal mortality and morbidity are higher in both fullterm and preterm boys compared to girls [40]. The fact that preterm boys have poorer cognitive, language and motor performances during early infancy is only partly explained by the higher rates of early acquired brain damages [41]. In follow-up studies, male gender has been identified as a separate risk factor for adverse outcome [19, 41, 42].

Other perinatal and social factors have been shown to affect outcome:

- no tocolysis [13]
- multiple birth [42]
- longer duration of mechanical ventilation [13, 42]
- postnatal corticosteroid use [43]
- postnatal infection [39, 44]
- patent ductus arteriosus [13]
- pneumothorax [42]
- low maternal education and lack of maternal health insurance [42]

1.2.2 Brain Imaging and Outcome

In neonatal clinical practice today EPT infants are routinely examined with cranial ultrasound (cUS) to discover bleedings and major structural abnormalities [45]. During the past decade Magnetic Resonance Imaging (MRI) has become increasingly available, and is now widely used to investigate the preterm brain [46], not only in research settings. Subtle changes are however not always detected with conventional MRI but require more sophisticated imaging techniques, such as Diffusion Tensor Imaging (DTI) [47]. One of the newest forms of neuroimaging is functional MRI (fMRI), providing information on brain connectivity and function [48].

1.2.2.1 Cranial ultrasound

1. Background

The principles of sending and receiving sound wave echos have been used for more than a century. For example, after the sinking of Titanic, Paul Langevin invented the hydrophone to detect icebergs. In the late 1930s, Dr Karl Dussik, an Austrian psychiatrist became the first to use ultrasound pictures in an attempt to diagnose brain tumors. The method was developed and improved in 1970s and is today the most commonly used neuroimaging technique for neonates.

cUS is based on the use of high-frequency sound waves emitted into the brain through the cranial fontanel. The echoes are detected and the reflection signature is processed to be visualised in images. Neonatal cUS standard views include sagittal and coronar projections through the anterior fontanel. In order to visualize the posterior fossa and brain stem, the posterior or mastoid fontanel may be used [49].

2. Applications

cUS reliably detects germinal matrix- and intraventricular haemorrhages, hydrocephalus and cysts. It is an inexpensive neuroimaging technique that may be performed bedside and repeated several times during the hospital stay. However, cUS is user dependent, retrospective interpretations may be difficult and the anatomy of the posterior fossa and convexity of the brain is suboptimally visualized if the examination is performed only through the anterior fontanel. In addition, cUS is less accurate in determining diffuse white matter injury, brain maturation (myelination and gyration), migrational disorders and cortical dysplasias [49].

3. cUS safety

cUS involves no mutagenic ionizing radiation, however, other possible biological effects have been investigated. In mice, ultrasound has been shown to influence neuronal migration during cortical formation [50]. In humans, most studies evaluating the safety aspects of ultrasound on the developing brain involve prenatal examinations. Newnham et al [51] conducted a large prospective randomised controlled trial of repeated prenatal ultrasound examinations in western Australia. Almost 3000 infants attended five follow up appointments until age 8 years. No negative effects could be identified on childhood speech, language, behaviour, or neurological development at any age.

In Sweden and Norway researchers found a weak association to non-right handedness in boys [52, 53]. The same groups have over the years investigated a wide range of other possible short- and long-term effects of repeated prenatal ultrasounds. No negative effects have been identified on growth, vision or hearing [54], neurologic development [55], intellectual performance [56], schizophrenia and other psychoses [57], risk of brain tumours [58] or overall school performance [59].

In a recent metaanalysis, Torloni et al reviewed data from 41 different studies and concluded that exposure to diagnostic ultrasonography during pregnancy appears to be safe [60]. These results from prenatal examinations have been used in neonatal clinical practice, with recommendations to avoid unnecessary examinations as a precaution.

4. Abnormalities on cUS and outcome

Many studies have investigated the predictive value of neonatal cUS for adverse outcome at different ages. In the recent review of El-Dib et al [61], the following conclusions are drawn on this subject:

- Major abnormalities on cUs, such as severe IVH, PHI and PVL, predict neuromotor delay and cerebral palsy (odds ratio; OR ranges between 5 and 10.5, sensitivity up to 0.86, and specificity up to 0.99).

- The predictive value of major abnormalities on cUS for cognitive outcome is less clear, especially when controlling for the effects of significant motor delay on cognitive testing.

The authors also state that prognostic conclusions about normal or mildly abnormal ultrasounds are problematic, depending on how data are interpreted. For example, Laptook et al 2005 report outcome data of nearly 1500 preterms with birth weight <1000g born in the US 1995-99 [42]. Nearly 30% of preterms with a normal cUS developed either CP or had a low Mental Developmental Index. Inversely, in the large EPIPAGE cohort (born <32 weeks 1997 in France), one third of infants with CP at 2-year follow-up had no abnormalities on neonatal cUS [62].

Multiple neuroimaging studies were combined into single estimates in the metanalysis by Nongena et al 2010 [63]. With a normal cUS scan, the calculated pooled probability for a normal neuromotor outcome was 94% (95% CI: 92-96), including the EPIPAGE study mentioned above. The calculated probability of CP was 9% for low grade IVH (grades 1-2) but the confidence interval was wide (95% CI: 4-22) and the pooled probability of a normal cognitive outcome with a normal ultrasound scan was 82% (95% CI: 79-85) [62, 64-68].

Clearly, cUS is a useful neuroimaging tool to identify major abnormalities and foremost valuable in the prediction of motor impairments. However, cUS proves less ideal in evaluation of mild-moderate brain injury and for prediction of cognitive outcome. Instead, as MRI has become increasingly available for examining the newborn brain, its advantages have become evident. However, further studies comparing cUS and MRI, as well as evaluations of the recent advanced applications of MRI are needed.

1.2.2.2 *Magnetic Resonance Imaging*

1. **Background**

In the 1940s, two US researchers, Felix Bloch (Stanford) and Edward Purcell (Harvard) independently described the principles of nuclear magnetic resonance in their experiments investigating chemical compounds. 30 years later, Paul Lauterbur became the first to publish a paper on the applications of nuclear magnetic resonance to produce images. For their discoveries, Bloch and Purcell were awarded the Nobel Prize in 1952, and Lauterbur in 2003. In the early 1980s, the first human MRI scanners became available.

MRI is based on the properties of protons, i.e. water molecule nuclei, and their behaviour in a strong external magnetic field. Image contrast is generated through the excitation of the protons by a radiofrequency pulse, the subsequent relaxation and emission of a signal recorded by the scanner.

2. Diffusion Tensor Imaging

DTI is based on traditional MRI and the so-called ‘Brownian motion’ of water molecules. Robert Brown was a Scottish botanist who in 1827 noted that pollen grains suspended in water were constantly moving in a random fashion.

DTI uses the fact that the free diffusion of water molecules is restricted within the brain by structural barriers such as macromolecules, cell membranes and white matter fibres [69, 70]. Mean diffusivity, or apparent diffusion coefficient (ADC) is high in the immature brain, and decreases during maturation. The directional preference of water diffusion in the brain tissue occurs preferentially along the direction of axons and is restricted perpendicular to them [71] and is evident even before myelination takes place [72]. Fractional anisotropy (FA) is a measure of directional preference and increases with age and maturation.

ADC and FA may be measured in selected anatomical regions of interest [73]. Tract-Based Spatial Statistics (TBSS) is a recent technique based on DTI, that provides objective whole-brain diffusion data analysis allowing for comparisons across many subjects [74].

3. Functional MRI

In 1890 Roy and Sherrington conducted a series of experiments on dogs, cats and rabbits investigating the blood supply of the brain. They suggested, by speculation, that neural activity was accompanied by a regional increase in cerebral blood flow. One hundred years later, it was discovered that the oxygenation level of haemoglobin could be used as a contrast agent in MR images. Taken together, these are the basic principles of fMRI, describing which areas of the brain are “active” at any given time.

Much of what is known about brain function originates from studies where a specific task has been performed, or when different kinds of stimuli have been administered. However, the brain is very active even in the absence of explicit input or output [75]. Constituting only 2% of the total body weight in adults, the brain accounts for almost 20% of its energy expenditure- at rest. When “active”, the metabolism increases only a few percentages from baseline values [76]. So called ‘Resting state networks’ are such haemodynamic spontaneous fluctuations, in a non-stimulated setting. In addition, functional MRI may be used to study regions that routinely decrease their activity during task performance, and are more active during rest than during tasks, the so-called ‘Default Mode’ of brain function.

In newborns, task studies evidently become difficult to conduct. Stimulated fMRI in infants is also troublesome due to the risk of motion artefacts. Nevertheless, there is great interest, and still quite little knowledge, regarding the basic functioning of the developing brain.

4. Applications

Conventional MRI accurately visualizes the anatomical brain structures, including the posterior fossa and brain stem. MRI allows determination of the stage of development [77] and reliably detects both overt [8] and diffuse [78] brain injuries. The use of new advanced MR techniques provides not only morphological, but also functional information [79].

In term born infants, DTI plays an important clinical role in the early detection of ischemia and neonatal stroke. In preterm infants, DTI has been used to assess development and maturation [80] and in the explorations of mechanisms involved in white matter injury of prematurity [81]. MRI is user-independent and provides excellent high-resolution images of soft tissues. However, MRI is far more expensive and time-consuming than cUS. In addition, the infant must be transported to the scanner, and is required to lie still, and sedation is sometimes needed [82].

5. MR safety

Like cUS, MRI involves no ionizing radiation. The risks of MRI and possible negative effects on the developing brain may instead be related to the presence of the strong magnetic fields and the electromagnetic radiofrequency pulses. Animal studies have shown conflicting results [83, 84] and have not been considered clinically relevant in humans [85]. However clinical fetal MRI scanning is not recommended in the first trimester as a precaution against yet unknown harmful effects.

Tissue heating is another possible harmful effect in clinical MR scanning [86] and consequently there are strict regulations regarding the maximum amount of electromagnetic energy deposited by the MR scanner. In addition, the sound levels in the scanner may be problematic when scanning newborns. For a typical 1.5 Tesla scanner, the noise ranges between 80 and 120 decibel. Hearing protection is always used for both infants and adults. In fetal MRI the maternal surroundings offer a corresponding hearing protection, and no relations to later hearing impairments have been found [87]. Others have investigated effects of fetal MRI on intrauterine growth [88] and later infants' development [89] and found no harmful effects.

At present there are no known health risks of fetal or early postnatal MR imaging when following current safety procedures [90].

6. Abnormalities on MRI and outcome

In most preterm MR studies, imaging is performed to compare **“the preterm phenotype”** with a term born control group in relation to outcome:

- Children and adolescents born preterm, have smaller brain volumes, larger lateral ventricles, smaller corpus callosum and reduced grey and white matter volumes compared to term born infants [91-94].

- Neurodevelopmental testing have demonstrated that specific regional brain volumes (sensorimotor and midtemporal cortices) are associated positively with full-scale, verbal, and performance IQ scores in 8 year-old preterms [91] as well as the size of the corpus callosum and motor performance [95]. Others have shown alterations in hippocampi, thalami and cerebellar volumes and associations to poorer adolescent neurodevelopmental outcomes [96].
- A number of DTI studies have in a similar way compared preterm infants scanned at TEA with term-born controls, and demonstrated decreased FA and increased ADC within different regions of the WM [97-99]. Whole brain analysis using TBSS have shown lower FA in the centrum semiovale, the corpus callosum, the external capsule and the posterior aspect of the PLIC in preterm infants without focal brain lesions, compared to healthy term born controls [100].
- DTI in preterm infants performed later in childhood has demonstrated disturbances in the WM microstructure that are still detectable at 11 y of age, most pronounced in the posterior CC and the internal capsule [101]. Altered WM integrity has been demonstrated all the way into adulthood using TBSS [102].
- Moreover, DTI has been shown to relate to outcome when the MRI and the developmental assessment is performed at the same age both with regard to motor performance [103, 104] and cognitive impairments [102, 105].

However there are studies investigating **the predictive value of MRI** at term and outcome later in childhood. Similarly to major abnormalities on cUS, a clearly abnormal MRI at term, such as presence of PVL or extensive ventriculomegaly is highly associated to CP and neuromotor delay [39, 106-108]. Cystic PVL and parenchymal lesions are also associated to adverse cognitive outcome [39, 43, 109].

Similarly diffuse WM abnormalities have been related to neurodevelopmental outcomes but associations are less clear [17]. Hyperintense foci in the periventricular WM observed on T1-weighted MR images is another form of possible diffuse WM injury, but reports regarding associations to outcome are conflicting; Cornette et al. 2002 found no association to adverse outcome [110], whereas Nanba 2007 report significant associations to motor sequelae [107].

Others have used combinations of MR imaging appearances in different grading systems. In the presence of moderate-severe MR abnormalities at TEA, Miller et al [39] report a significantly increased risk of abnormal neurodevelopmental outcome at 12-18 months (RR = 5.3; 95% CI: 1.2-24.5; OR= 7.3). Using another scoring system, moderate-severe WM abnormalities were predictive of motor delay (OR 10.3; 95 % CI: 3.5-30.8), cerebral palsy (OR 9.6; 95 % CI: 3.2-28.3), neurosensory impairment (OR 4.2; 95 % CI: 1.6-11.3) and cognitive delay (OR 3.6; 95% CI: 1.5-8.7) at 2 years of age [43]. Overall, the calculated predictive value of moderate-severe WM abnormalities for abnormal motor and cognitive impairment were similar and low (motor: 31%, 95% CI: 17-49 versus cognitive: 34%, 95% CI: 20-52) [63].

As MRI provides excellent morphological information, selected anatomical structures have been studied individually, in relation to outcome (review, see [111]).

- Asymmetrical appearance of the posterior limb of the internal capsule (PLIC) at TEA is strongly related to future hemiplegia [112, 113].
- Cerebellar haemorrhagic injuries, isolated or in combination to supratentorial lesions, are related to both motor and cognitive impairments [114] and have a poor prognosis [115].
- Regional brain volumes (sensorimotor and parieto-occipital regions) at TEA are associated to developmental outcome in early infancy [116]. Furthermore, smaller hippocampal volumes in preterms at TEA are related to poorer performance on working memory tasks at age 2 years [117].

Fewer studies have explored **the predictive value of DTI findings** at TEA and association to neurodevelopmental outcome [118-120]. Interestingly, Boardman et al 2010 used a combination of conventional MRI and DTI to define an “image phenotype” of preterm infants [121]. In 66 of 80 preterm infants at TEA, diffuse white matter injury (ADC >2 SD of the mean of the controls), and tissue volume reduction of the thalamus, the globus pallidus, periventricular white matter, the corona radiata and the central region of the centrum semiovale were demonstrated. This abnormal image phenotype was further associated to a reduced median developmental quotient at age 2 years compared with control infants.

Clearly, there is growing knowledge in the field of neonatal neuroimaging research. The constant improvements of clinical neonatal practices require careful evaluation of both short-term and long-term outcomes. In essence, what is the panorama of early acquired brain injuries and subsequent sequelae in extremely preterm infants in Stockholm today? How do we compare with similar international centra?

Prediction of later disabilities is a delicate task [122]. There is still great need for studies aiming at answering the question “what are the consequences of specific focal or diffuse brain injuries?”. Over the past decade, new promising neuroimaging techniques, such as MRI, are becoming readily available, but larger scale studies are few. What additional information can be gained by using MRI instead of the standard cUS? And in a similar way, may we better understand the underlying mechanisms of preterm brain damage by using DTI? Moreover, being aware of the early gender differences with regard to neonatal morbidities and the male disadvantage in outcomes, may DTI also be used for this purpose?

Exploring brain pathologies naturally necessitate a thorough understanding of the normal functioning of the newborn brain. However, many basic scientific aspects of brain development are poorly explored. What can we learn by using functional MRI in these very immature infants? Are fMRI studies at all interpretable in the present setting? If yes, how do data compare with older children and adults? Many questions, some almost bordering philosophy, remain to be answered when dealing with infants born at the limits of viability.

2 AIMS

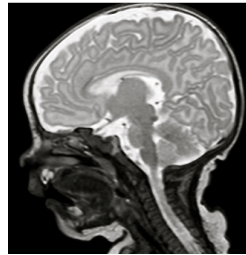
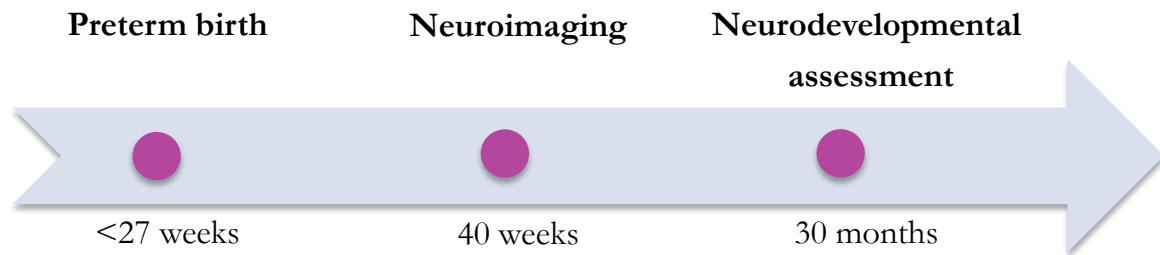
The overall aim of the works compiled in this thesis is to study brain development and brain damage of extremely preterm infants at term equivalent age, and investigate the relations to toddler age outcomes.

The specific aims of the included papers were:

- To study cerebral white matter abnormalities and diffuse and excessive high signal intensities in a cohort of extremely preterm infants born in Stockholm during a 3-year period, using magnetic resonance diffusion tensor imaging, MR-DTI (paper I).
- To identify perinatal risk factors for white matter abnormalities on MRI at term equivalent age (paper I).
- To investigate which neuronal networks are active at rest in extremely preterm infants at term, using functional MRI (paper II).
- To compare the findings of two different imaging modalities, MRI and cranial ultrasound, when performed on the same day at term age in extremely preterm infants (paper III).
- To study the consequences of extreme prematurity at 30 months corrected age, and investigate how cerebral white matter abnormalities on MRI relate to outcome (paper IV).
- To investigate differences in outcomes between extremely preterm boys and girls, and potential early dissimilarities of brain microstructure as assessed by MR-DTI and Tract-Based Spatial Statistics (paper V).

3 PATIENTS AND METHODS

3.1 STUDY DESIGN



3.2 ETHICAL CONSIDERATIONS

The regional ethical committee in Stockholm approved all studies included in this thesis and informed consent was obtained from all parents of the participating infants.

All results of individual MR and cUS examinations were given to the parents by the neonatologist in the ward, as part of the clinical follow up of EPT infants. The neuroimaging involved no discomfort for the infants and no adverse events were reported during the three year study period.

3.3 PATIENTS

3.3.1 Preterm infants

For all studies, infants born in Stockholm with a gestational age of less than 27 weeks + 0 days were included. The main study period was three years, from 1st January 2004 to 31st March 2007. Study III included a subgroup of preterms born between August 2004 and November 2006.

Children with malformations, chromosome aberrations and congenital infections were excluded (n=8); a pair of twins with congenital cytomegalovirus infection, one infant with Downs Syndrome, one with oesophageal atresia, one with myelomeningocele, one with hemophagocytic lymphohistiocytosis, one with intracranial vascular malformations, and one infant with cleft lip and palate. Another four families moved from the region and 9 declined participation in the MRI examination. One infant was too unstable for MRI at TEA.

In total, 108 EPT infants underwent MRI at term-equivalent age and were included in Paper I. However, when analysing follow up data for Paper IV, another child meeting exclusion criteria was identified (having intracranial vascular malformations) resulting in 107 infants for Paper IV. See Flowchart (fig 1) in Paper IV.

3.3.2 Term born control infants

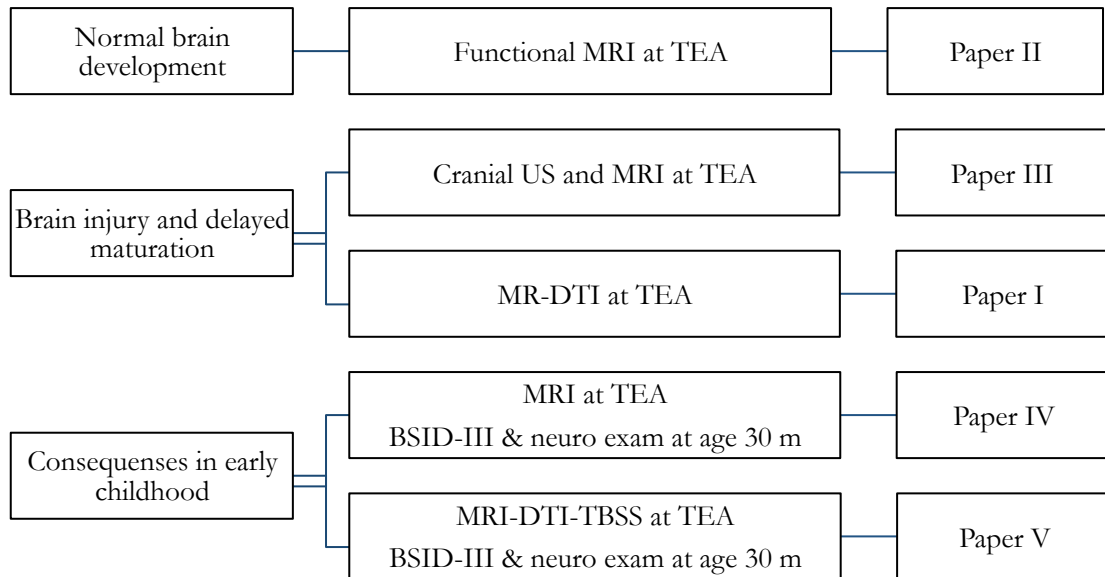
- MRI controls (Study I, V)

For comparison of MRI-DTI data at TEA, a group of healthy term born control infants (n=21) were recruited from the maternity ward. Infants were born after healthy pregnancies and delivered with planned caesarean section. Control infants were scanned at the same post-menstrual age according to the same protocol as the preterm infants, but had no follow-up assessment. Due to movement artefacts, data from 16 control infants could be used for further analysis.

- BSID-III controls (Study IV, V)

For comparison of outcome data, a control group of 85 term born control children, matched for maternal residential area (zip code), age and gender underwent follow-up assessments but did not undergo neonatal MRI.

3.4 METHODS, AN OVERVIEW



3.4.1 Cranial ultrasound (Study III)

3.4.1.1 Protocol

At TEA, immediately prior or after the MRI, infants in Study III were scanned by one of two examiners (Béatrice Skiöld or Sandra Horsch) using the ACUSON Sequoia ultrasound system (Siemens Medical Solutions, Germany) with a multifrequency sector transducer (5-8 MHz). The imaging protocol consisted of the standard projections according to Levene in coronal and sagittal/parasagittal planes through the anterior fontanel [123].

3.4.1.2 Classification

All cUS images were stored digitally for analysis on a later occasion by three independent observers (Béatrice Skiöld, Sandra Horsch and Mats Blennow). The presence of cysts, grey matter abnormalities and gyral folding were scored, and the lateral ventricles, the interhemispheric fissure, the subarachnoidal spaces and the corpus callosum were measured. Cut off values are given in the table below.

	Coronal view	Sagittal or Parasagittal view
Lateral ventricle, frontal horn level of 3 rd ventricle	short axis, normal < 3 mm long axis, normal < 13 mm	normal < 3 mm
Lateral ventricle, midbody		normal < 10 mm
Interhemispheric fissure level of 3 rd ventricle	normal < 3 mm	
Subarachnoidal space sino-cortical width at three levels	normal < 4 mm	
Corpus callosum		normal > 1,5 mm

Images were classified as normal if all the measured and scored items were normal. Severe abnormalities were predefined as single large or multiple smaller cysts, WM loss after periventricular haemorrhagic infarctions and global white and/or grey matter loss without focal lesions usually coinciding with severe ventriculomegaly. All ultrasound findings not fulfilling definitions of “normal” or “severely abnormal” were classified as having mild-moderate abnormalities.

3.4.2 Magnetic Resonance Imaging

3.4.2.1 Conventional MRI (Study I-V)

All infants were scanned at the Astrid Lindgren's Children's Hospital in Stockholm. Noise reduction was accomplished using individually moulded dental putty (10- 20 dB reduction), neonatal "minimuffs" (7 dB) and paediatric headphones (15-32 dB). Moreover, we developed a tailor made sound dampening "hood" tightly attached to the upper half semicircle of the magnet bore, reducing the noise level with up to 24 dB [124]. In the beginning of the study period infants were lightly sedated using chloral hydrate 30 mg/kg orally or rectally. Later on infants were unsedated, including all controls.

MRI data were acquired on a Philips Intera 1.5 Tesla scanner (Philips International, Amsterdam, The Netherlands) using a 6-channel receive-only neonatal head coil. The MRI protocol is shown below.

THE NEO-BIG MRI PROTOCOL	
<ol style="list-style-type: none">1. Survey (00:50)2. Reference scan (00:30)3. Sagittal T2W (02:30)4. Sagittal T1W (02:50)5. Axial T2W (01:00)6. 3D T1W (04:30)7. Axial T1W IR (02:55)8. Axial T2*W (02:10)9. Axial fMRI (10:00)10. Axial DWI (02:40)	
Sagittal T2W TE/TR/Flip = 100ms/5000ms/90deg. Slices = 19 ETL = 16 FOV = 180 Voxels = 0.7mm x 0.7mm x 3.0mm SENSE = 2	Axial T1W IR TE/TR/Flip = 15ms/3500ms/90deg. Slices = 25 FOV = 180 IR = 400ms Voxels = 2.0mm x 2.0mm x 4.0mm SENSE = 1
Sagittal T1W TE/TR/Flip = 9ms/600ms/90deg. Slices = 24 ETL = 3 FOV = 180 Voxels = 0.7mm x 0.7mm x 4.0mm SENSE = 2	Axial T2*W TE/TR/Flip = 23ms/586ms/18deg. Slices = 20 FOV = 180 Voxels = 2.0mm x 2.0mm x 5.0mm SENSE = 1.5
Axial T2W TE/TR/Flip = 100ms/5000ms/90deg. Slices = 22 ETL = 16 FOV = 180 Voxels = 0.7mm x 0.7mm x 4.0mm SENSE = 2	Axial fMRI TE/TR/Flip = 50ms/2000ms ms/80deg. Slices = 28 FOV = 180 Voxels = 2.8mm x 2.8mm x 4.5mm Volumes = 300
3D T1W TE/TR/Flip = 5ms/40ms/30deg. Slices = 22 FOV = 170 Voxels = 2.0mm x 2.0mm x 2.0mm SENSE = 1.8	Axial DWI TE/TR/Flip = 74ms/7500ms ms/90deg. Slices = 28 FOV = 180 Voxels = 1.4mm x 1.4mm x 2.2mm SENSE = 1.5 B = 700 Directions = 15 (OVERPLUS)

3.4.2.2 MRI Scoring system (Study I-V)

The conventional MR images were evaluated according to a scoring system for WM abnormalities [43, 125] judging five separate items: abnormal WM signal, reduction in WM volume, cystic changes, myelination/ thinning of the corpus callosum and ventricular dilatation. According to the calculated composite score infants were divided into groups with 1) No WM abnormalities 2) mild WM abnormalities 3) moderate WM abnormalities or 4) severe WM abnormalities, see Fig 1 and Table 5 in Paper I.

In addition, the grey matter (GM) was assessed for abnormal signal in the cortical or deep GM, enlargement of the subarachnoidal spaces and delayed gyral maturation. Infants were then divided into those with normal or abnormal GM according to the calculated composite GM score.

3.4.2.3 Diffusion Tensor Imaging (Study I, V)

Diffusion weighted MRI data was obtained in 15 directions. DTI post processing generated calculated values for water molecule mean displacement (apparent diffusion coefficient, ADC) and water diffusion directional preference (fractional anisotropy, FA).

- Region of interest analysis (Study I)

ADC and FA were calculated in manually selected regions of interest: the posterior limb of the internal capsule bilaterally (5-8 voxels) and the corpus callosum (the genu 7 voxels, the splenium 7 voxels). The WM was segmented manually at the level of centrum semiovale. See Fig 2A and 2B in Paper I.

We chose to extract small regions of interest in order to achieve precise measures of diffusion in selected anatomical structures. However, all manual selection procedures may be subject to bias, as opposed to the automated analyses used for TBSS.

- TBSS (Study V)

In order to investigate differences in ADC and FA between groups, an automated approach was chosen enabling multisubject comparisons increasing the statistical power. We used the FMRIB's Diffusion Toolbox version 2.0 and Tract-Based Spatial Statistics version 1.2 in FSL version 4.1.4 [74].

3.4.2.4 *Functional MRI (Study II)*

The functional MRI consisted of recording Blood-Oxygen-Level Dependent (BOLD) signal changes during 10 minutes of silent sleep in 12 preterm infants without WM damage. An explorative data driven analysis approach called Independent Component Analysis (ICA) was used to extract anatomically meaningful patterns of low frequency (<0.1 Hz) spontaneous brain activity (i.e. without any overt task performance), so called Resting State Networks [126]. The ICA is a multivariate method with the advantage that it does not require any seed regions specified by the user to measure functional connectivity.

3.4.3 **Neurodevelopmental follow up (Study IV, V)**

3.4.3.1 *Neurological exam*

Infants were examined at age 30 months corrected by a paediatric neurologist (Brigitte Vollmer) judging the quality of movements, posture, reflexes, and muscular tone. Neurological status was categorized into three groups: “normal”, in infants with an entirely normal neurological status or “abnormal” when neurological signs of CP were present as defined by the Surveillance of Cerebral Palsy in Europe, SCPE [127]. The third category comprised infants with “unspecific signs”, such as asymmetry of muscular tone or reflexes, muscular hypotonia, or muscular hypertonia but not fulfilling the SCPE criteria.

3.4.3.2 *Bayley Scales of Infant and Toddler Development –III*

BSID-III was performed on the same day as the neurological examination. The BSID-III [128] is an individually administered instrument for infants and toddlers between 1 and 42 months of age. Its primary purposes are to identify children with developmental delay and to provide information for intervention planning.

Three domains of the BSID-III were used for our studies: cognitive, language (expressed in two subtests, the receptive and the expressive communication) and motor (fine and gross motor). Social-emotional and adaptive functions are two additional domains not part of our research. In order to compare the results from the different domains, a composite score is calculated for each domain (mean 100, SD 15).

3.5 STATISTICAL ANALYSIS

In Paper I the statistical analysis were performed using the Statistica software (StatSoft Inc., Tulsa, OK, USA) and SAS/ STAT Software (SAS Institute Inc., Cary, NC, USA). Student's t-test and Mann-Whitney U-test were used for continuous measures, and χ^2 or Fisher's exact test for dichotomous data. One-way analysis of variance (ANOVA) with Dunnett's post hoc test was performed for multiple groups analysis. Logistic regression was used to identify predictors of DEHSI and WM abnormalities.

In Paper II, thresholded results for activation in each resting-state network was obtained using a data concatenation approach previously described by Beckmann et al. 2005 [129].

In Paper IV and Paper V PASW Statistics[®] software 19.0 (SPSS Inc, Chicago, IL) was used. Student's t-test was used for continuous variables and Pearsons χ^2 for dichotomous data. Non-parametric tests were used when group sizes were unequal. In addition, regression analyses were performed to investigate the contribution of different variables regarding the variance in outcome.

A p-value of <0.05 was chosen as cut-off level for significance in all five papers.

4 RESULTS AND DISCUSSION

4.1 SURVIVAL AND NEONATAL MORBIDITIES

Between January 1, 2004 and March 31, 2007, 192 infants with a gestational age (GA) of <27 weeks + 0 days were born alive (i.e. with a measurable heart rate immediately at birth) in the Stockholm region. Regional recommendations set the age limit for full-scale resuscitation and administration of antenatal corticosteroid to 24 weeks + 0 days early in the study period, but the age limit for possible active intervention was lowered to 23 weeks + 0 days in 2006 [130]. Caesarean section on fetal indication is today performed from 25 weeks + 0 days. At all gestational ages, however, an individual assessment is made for each child with regard to potential risk factors [131].

Our reported survival rates include all live-born infants, and thus also include infants born below the age limit of 23 weeks + 0 days. Sixty-three of the 192 children died before reaching term age (33%), resulting in an overall survival rate in the cohort of 67% ($n = 129$, mean gestational age 25 weeks +4 days $SD \pm 1$ day: mean BW 808 gram $SD \pm 160$ gram). If counting only infants with a GA of 23 weeks+ 0 days to 26 weeks +6 days (intention to treat) the survival rate would rise from 67% to 75%, but we consider it to be of great importance to include also the youngest infants. See Table 1, Paper I.

The well-organized antenatal care, the high rate of antenatal corticosteroids and in hospital centralized deliveries are factors contributing to giving the EPT infants born of this cohort a promising start. In total, 13% of infants were born after multiple pregnancies, 94% of mothers received antenatal steroids and caesarean section was performed in 44% of all deliveries. Male gender was more common than female in the cohort: 56% boys and 44% girls.

The mean time spent on ventilator was 14 days (range: 0-55), and on CPAP 40 days (range: 17-115). BPD (requirement of supplementary oxygen at 36 weeks of age) was diagnosed in 44 % of infants, 30 % underwent surgery for PDA, 6 % developed NEC (Bells grade 2-3) and 19 % had laser treatment of ROP. Nearly half of the infants (44%) had a germinal matrix or IVH of some grade, of which 34% were low-grade bleeds (1-2) and 10% IVH grade 3 or PHI. Only 3% of the EPT infants developed PVL. See Table 3, Paper 1.

The present study cohort comprises a regional subgroup of the EXPRESS study and was very similar to the national cohort with regard to survival rates and gender distribution but had slightly lower rates of multiple pregnancies (Stockholm: 13% versus national: 22%) and caesarean section (Stockholm: 44% versus national: 50%) [1].

The high survival of EPT infants born alive (67%) is an important finding in the present work and is, as said, confirmed by the national study. Survival rates at hospital discharge in other recent population-based studies have been consistently lower [5, 132-135]. There are several possible explanations, mainly originating in the sociomedical structures in Sweden and a very proactive approach to perinatal and neonatal care. However, the EXPRESS study also

demonstrate the prevailing more selective use of perinatal interventions at 22 weeks of gestation.

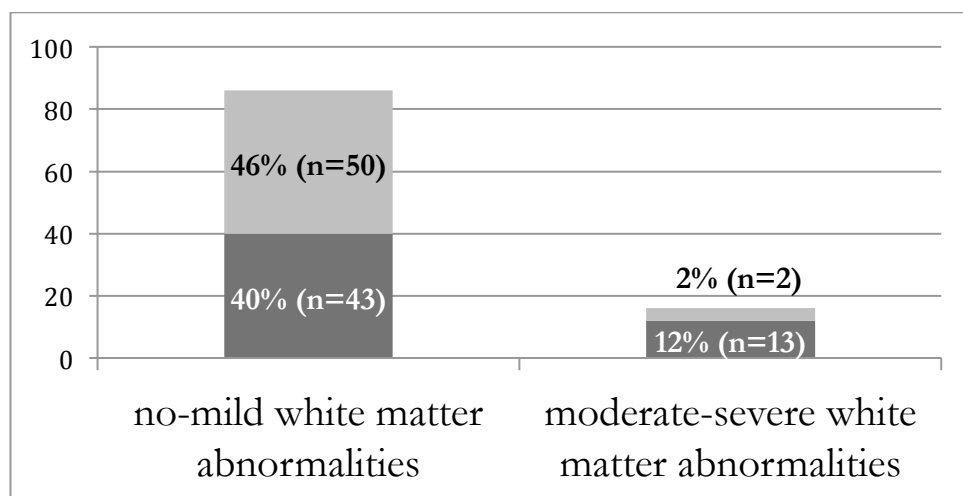
As emphasized previously, survival rates must be interpreted in the light of neonatal morbidities associated with increased risks of adverse long-term outcomes. In the present study and national data from the EXPRESS study, almost half (45%) of EPT survivors were free of major neonatal morbidity (PVL, severe IVH, severe ROP or severe BPD). In a comparable EPT cohort in Canada, rates were very similar (49%) [25] whereas yet others report somewhat lower impairment-free survival (38%) in addition to considerably lower survival rates [5]. In Norway however, 79% of infant <28 weeks were free of major neonatal morbidity [133] and with the exception of ROP, the morbidity rates were not higher at the lowest gestational ages. Surely, there is no controversy regarding the absolute need of long-term follow-up of these EPT cohorts.

4.2 BRAIN IMAGING AT TERM EQUIVALENT AGE

4.2.1 Conventional MRI findings (Paper I)

Paper I provides an accurate report of the incidence of WM abnormalities of EPT infants born 2004-2007 in the Stockholm region, in addition to brain injuries diagnosed with cUS during the hospital stay (Table 3, Paper I). A third of infants had extensive WM signal changes, 13% had severe WM reduction, 3% had clearly delayed myelination and a thin corpus callosum, 5% had severe ventricular dilatation and 4% had large single or multiple small focal cysts (Table 5, Paper I). This way of reporting imaging findings adds information to the standard way of reporting diagnose specific incidences of e.g. PVL or post haemorrhagic ventricular dilatation.

Similar to other published data reporting DEHSI in a majority of EPT infants at term corrected age [16, 17], we found DEHSI in 56% of infants. When analysing the composite WM score, only 14% had moderate or severe WM abnormalities on MRI at term equivalent age (below and Table 4, Paper I). Comparable incidences were described by Inder et al [125], however in a more mature cohort (mean GA 28 weeks + 4 days versus 25 weeks + 4 days). Similarly, although using another scoring system, Miller et al [39] studied 89 preterm infants with a median GA of 28 weeks and found more than a third having moderate-severe brain abnormalities. Possibly by consequence of the lower neonatal morbidity rates in Sweden discussed above, we suggest that the results in Paper I reflect a true difference regarding the incidence of brain injuries in the youngest infants.



Incidences of white matter abnormalities on conventional MRI in the Stockholm cohort.

4.2.2 Risk factors of WM abnormalities

In addition to describing the brain damage panorama in the Stockholm cohort, we aimed at identifying risk factors of WM abnormalities. The need for surgical ligation of patent ductus arteriosus (PDA) was found to be a significant risk factor for DEHSI with an OR of 3.0 (CI: 1.1–8.0), $p = 0.03$, when adjusting for the following possible confounders: GA, BW, gender, maternal signs of infection, inotropic support, NEC, postnatal sepsis, number of days on CPAP and / or ventilator, severe BPD and IVH. In contrast, no individual predictors of moderate or severe WM abnormalities were found.

The finding that infants who had surgically ligated (but not medically treated) PDA are at greater risk of DEHSI has not been reported in other studies. PDA as a risk factor for moderate-severe WM abnormalities is not new [125] but no relation to DEHSI has previously been described. The cerebral circulatory changes during medical and surgical treatment of PDA are not fully understood [136, 137], hence the present results underline the importance of studying effects of PDA surgery on cerebral haemodynamics.

Interestingly, and contrary to others [138, 139], we found no effect of infection (maternal fever, PROM, neonatal sepsis) on WM abnormalities or DEHSI in the present study. These controversies may possibly be due to difficulties in correctly identifying mothers with chorioamnionitis and infants with sepsis in our study. Most certainly, the role of inflammation and infection in the pathogenesis of WM damage needs further investigation.

4.2.3 Scoring Systems for conventional MR images

The intention of a scoring system is to simplify and compile the highly subjective interpretation of images, facilitating comparisons to other studies and providing tools for prediction of outcome. For all Papers I-V we used the scoring system for WM abnormalities described by Woodward et al [43], based on the original Inder et al scoring [125].

Composite scores of the present scoring have been correlated to outcome at age 2 years corrected using the BSID-II in 167 infants born <30 weeks GA. Moderate-severe WM abnormalities at TEA were strongly predictive of motor delay (OR 10.3; 95 % CI: 3.5 - 30.8) and cerebral palsy (OR 9.6; 95 % CI: 3.2 - 28.3) but less predictive of cognitive delay (OR 3.6; 95 % CI: 1.5 - 8.7) and neurosensory impairment (OR 4.2; 95 % CI: 1.6 -11.3). Grey matter abnormalities were also, but less strongly, associated with cognitive delay (OR 3.00; 95 % CI: 1.20–7.13), motor delay (OR 3.83; 95 % CI: 1.19–12.31), and cerebral palsy (OR 3.77; 95 % CI: 1.17–12.09) but not neurosensory impairment [43].

Visual inspection of MR images will always be subjective, and interpretations will differ between observers. Scoring models are constructed with good intentions but all will have shortcomings, and so also the present one. Ramenghi et al have presented an alternative simplified MRI scoring system ([140] preliminary report) focusing on abnormalities in the periventricular white matter (DEHSI, punctate and cystic lesions), basal ganglia, thalami and posterior limb of internal capsule. The proposed scoring was compared to the Inder-Woodward score and yet another scoring mainly used for assessment of brain maturation [141] and showed higher correlation to outcome measures (Griffiths Mental Developmental Scale) at age 3 years corrected, however these data are not published yet.

A simple scoring, highly predictive of both motor and cognitive outcome is indeed desired in the field of MR imaging of perinatal brain injury but may never replace the individual assessment of MR images.

4.2.4 Diffusion Tensor Imaging findings (Paper I)

In Paper I, were calculated ADC and FA in regions of interest to assess brain microstructure integrity.

Centrum semiovale

In the WM of centrum semiovale, all infants with WM abnormalities (both mild, moderate, severe) and DEHSI had altered diffusion. In infants with moderate-severe WM abnormalities, mean FA was lower, and mean ADC was higher in this region compared to preterms with milder forms of WM abnormalities (fig 3A, Paper I). In addition, when analysing infants with DEHSI separately, excluding the infants with moderate-severe WM abnormalities, mean FA was lower, and mean ADC was higher compared to both full-term controls and EPT infants without DEHSI. No differences were seen when comparing infants without DEHSI with controls.

Visual assessment of DEHSI is difficult [142] and it has been a matter of debate whether DEHSI represents true WM pathology or not. The results in Paper I are in agreement with previously published data, reporting altered diffusion in the WM of infants with DEHSI compared to preterms with normal WM [78] and controls [98]. Although these findings support the theories of DEHSI as an entity of diffuse white matter disease of prematurity, a recent large DTI study found no differences in ADC values at TEA in preterm infants with DEHSI when compared to preterms without DEHSI [143]. Clearly the controversies persist, and the possible clinical relevance of DEHSI needs to be evaluated in follow-up studies (see Paper IV).

Corpus callosum

In the WM of the corpus callosum (CC) we found altered diffusion in all the preterm groups, even when studying only preterms with normal WM compared to control infants (fig 3B, Paper I). Our findings are in agreement with others reporting an adverse effect of prematurity on CC development [144]. These findings may be ominous, as morphological changes in CC have been linked to adverse motor and cognitive outcome [95, 145]. There are also an increasing number of DTI-studies investigating the microstructural development of the CC and possible relations to outcome at different ages [101, 102].

However, later investigations have encouragingly indicated possible catch up growth of the CC during adolescence in preterms in contrast to controls [146]. The CC plays important roles for diverse aspects of brain functioning, and certainly deserves close attention both regarding imaging findings and outcome measures.

Posterior limb of the internal capsule

DTI analyses in the PLIC revealed no significant differences in FA or ADC within the preterm groups or between preterms and full term controls (Fig 3C, Paper I). This was an unexpected finding as other studies have demonstrated that WM abnormalities influence diffusion parameters in the PLIC at TEA [97, 98]. However, our results in Paper I are now supported by a large DTI-tractography study where no association was found between ADC or FA and the degree of WM injury in the PLIC or CC [147].

In order to further assess diffusion parameters in the PLIC, we selectively analysed FA and ADC in a subgroup of preterms with clearly delayed myelination on visual inspection of conventional MR images (n=14/54). In these infants, the delayed myelination could be objectified by altered diffusion. An association between altered diffusion in PLIC at TEA and adverse motor outcome have been demonstrated in several studies [118, 120]. In Paper IV the present findings are put in relation to outcome, and do in fact indicate a risk of unfavourable prognosis.

In summary, Paper I provides important additional information regarding microstructural correlates of WM injury in our EPT cohort. Even though prematurity per se seems to have an unfavorable role on the maturation of the CC, our findings from DTI in centrum semiovale and PLIC support the theories of Bonifacio et al [148], that ETP infants have a normal capacity for brain development.

4.2.5 Findings on functional MRI (Paper II)

In 12 lightly sedated EPT infants with normal conventional MR imaging, Blood-Oxygen-Level-Dependent (BOLD) signal changes were recorded during 10 minutes of silent sleep. Five resting-state networks (RSN) were consistently observed in all infants (Fig 1A-E, Paper II): in the medial section of the occipital lobe (Fig. 1A), bilaterally in the somatomotor cortex (Fig. 1B), bilaterally in the posterior temporal cortex (Fig. 1C), in the posterior medial and lateral parts of the parietal cortex (Fig. 1D), and in the anterior prefrontal cortex (Fig. 1E).

In adults at least 10 RSN have been described, and there are both resemblances and differences between the infant and adult RS-patterns [149]. The adult RS networks are mainly lateralized and show predominantly anterior-posterior connectivity [150]. In infants however, we found stronger functional correlation across the brain hemispheres, rather than anterior-posteriorly. This is likely due to an earlier maturation of transcallosal WM tracts.

The patterns found in the visual [151, 152], sensorimotor [126, 153] and auditory regions [150, 154] show significant similarities with patterns demonstrated in adults. Moreover, the RSN in prefrontal regions bilaterally resembles networks described in adults [129, 150]. On the contrary, the RSN of the bilateral superior parietal cortex, precuneus and lateral aspects of the cerebellum (Fig. 1D, Paper II) have, to our knowledge, no certain correspondence in adults. Interestingly though, if one collapses the two networks in parts of the parietal cortex and in the anterior prefrontal cortex (Fig. 1D+E, Paper II) the result resembles the adult “default-mode network” (DMN).

The default modes of brain function are regions that routinely decrease their activity during task performance, and are more active during rest than during task. The DMN in adults comprises the medial aspects of the prefrontal cortex, precuneus/posterior cingulate cortex, bilateral parietal cortex, and the lateral and medial temporal cortex. DMN mediates processes thought to be important for the resting state, and it has been suggested that these regions are involved in social cognition and self-projection [155]. Considering this, we speculate that the above described “collapsed” infant networks may be a DMN ‘progenitor’.

This theory is now supported by both another group [156] and our own results in a group of 19 healthy control neonates studied in natural sleep [157]. In these infants, a sixth RSN was found in the basal ganglia, not previously shown in neonates, but in adults [158]. The reasons why this basal ganglia RSN was not identified in the preterms at TEA is unclear, but may be due to the smaller sample size. It cannot be ruled out, however, that prematurity plays a role, and future fMRI studies in preterms with WM injuries may shed light on these matters.

We believe that exploring RSN will enhance our understanding of fundamental brain functioning, and may in the future also be used in the study of plasticity and reorganization after neuronal injury. This is particularly interesting considering the improvements in neurodevelopmental outcomes discussed elsewhere in this thesis, and the difficult task of pinpointing the neuroanatomical correlates of specific impairments. An important question is whether this is linked to differences in adaptive abilities, as recently indicated by some [159].

As mentioned above, ex-preterms are at increased risk of autism and ADHD. Interestingly, studies have shown abnormal intrinsic activity in the DMN in adults [160] and adolescents [161] with autism spectrum disorders, and decreased functional connectivity in children with ADHD [162]. At present, Paper II adds novel knowledge to basic developmental neuroscience.

4.2.6 Findings on cUS in relation to conventional MRI (Paper III)

In Paper III a subgroup of the cohort was studied with cUS on the same day as the MRI. Conventional MR images were scored as previously described and infants were categorized according to the composite WM and GM score; 31/72 (43%) had no WM abnormalities, 29/72 (40%) had mild WM abnormalities, 9/72 (13%) had moderate, 3/72 (4%) had severe WM abnormalities and 8/72 (11%) had abnormal grey matter. In addition, small cerebellar haemorrhages were found on MRI in four infants. These incidences did not differ significantly from the entire cohort of 109 infants included in Paper I.

On cUS, severe abnormalities were identified in 3 infants (4%) whereas 28/72 (39%) of infants had an entirely normal scan. The remaining 41/72 infants (57%) had mild to moderate abnormalities.

The foremost aim of Paper III was to compare the two imaging modalities. MRI is considered to provide anatomically superior images compared to cUS, but is far more expensive and not as easily available as cUS. Other advantages/disadvantages and safety aspects have been discussed previously. Importantly, all three infants with severe WM abnormalities on MRI were identified as having severely abnormal cUS. Reassuringly, no infant with a normal cUS was found to have moderate or severe WM abnormalities on MRI, or abnormal GM. Inversely, of infants with a normal cUS at TEA, 36% were scored as having mild WM abnormalities, and none of the four infants with cerebellar haemorrhages were identified with cUS.

Similar comparative cUS-MRI studies have been conducted previously. There is little controversy that major destructive lesions are readily identified with both techniques, as shown in Paper III and by others [163]. However, our findings are also in agreement with the

conclusion by Miller et al 2003 [164], that cUS is an insensitive predictor of the milder WM abnormalities that may be identified with MRI. The use of the anterior fontanel only, not combined with mastoid/ posterior views, may explain why the small cerebellar haemorrhages were overlooked [165]. This is an important lesson, as the role of the cerebellum in neurodevelopmental disability after prematurity is becoming increasingly recognized [166].

In relation to neurodevelopmental outcome, early studies demonstrated a greater predictive value of neonatal cUS compared with MRI performed at TEA, whereas more recent ones have reported the opposite [106, 167]. In the prediction of later cerebral palsy, de Vries et al [64] reported high sensitivity/specificity 79%/95% using serial cUS scans including a scan at TEA. Others have reported lower both sensitivity and specificity, and found MRI superior in the prediction of CP, but with methodological weaknesses of fewer cUS scans, and no cUS scan at TEA [43, 167].

It is important to emphasize that cUS is user dependent and our study was performed under ideal conditions with only two trained examiners and a careful protocol for evaluation of the images, which is not done in clinical practice. Under these specific conditions, we were able to identify all children with moderate-severe WM abnormalities on conventional MRI. One key question, however, is what possible impacts the milder forms of brain abnormalities, only identified by MRI, will have on later outcome.

4.3 OUTCOME AT 30 MONTHS CORRECTED AGE

4.3.1 Follow-up rate

At TEA, 117 EPT survivors entered the study and 91 children attended the research follow-up program (78%). For all children not participating in the research follow-up, information was retrieved from clinical charts ($n=26$, mean corrected age at clinical assessment: 26 months ± 8.3 , range 11-36 months). The drop out-group was similar to the EPT group with regard to neonatal characteristics (Table 1, Paper IV).

The follow-up rate in the present study is similar to the large EPIPAGE study where 77% (1817/2357) of the very preterm children were eligible for follow-up at age 5 years [28]. Others have reported follow-up rates of 92% at age 20 and 30 months respectively [19, 21]. Yet another example is The Victorian Infant Collaborative Study Group in Australia, who has completed long-term follow-up of several preterm cohorts achieving high retainment rates all the way into late adolescence [36, 168-170]. However, one strength of the present work, similar to the EPICure, is that clinical data was collected for 100% of children not attending the formal research assessments.

4.3.2 Neurological examination (Paper IV)

We used the SCPE definitions of cerebral palsy [127] and in total six children ($6/91=7\%$) fulfilled the criteria. The majority of the EPT children had an entirely normal neurological status; $60/91=65\%$ whereas almost a third had “unspecific neurological signs”; $26/91=28\%$. No child in the drop out-group had CP according to the clinical charts, while the proportion of children with normal neurological status or “unspecific neurological signs” were similar between groups.

The rates of CP are often used as a marker of quality of the neonatal care and an indicator of “at what cost” improved survival rates are obtained. In the study by Wilson-Costello et al 2007, including infants with $BW < 1000g$, three time periods were compared: period I: 1982–1989, Period II: 1990–1999 and Period III: 2000–2002. The rate of CP increased from 8% in period I to 13% in period II, but decreased to 5% in period III ($p < 0.008$) [21].

However, comparing incidences of CP in different cohorts is not easy. In the above study for example, only patients with moderate-to-severe CP according to definitions by Bax M, 1964 [171] were reported. In contrast, in Paper IV we report all children fulfilling the SCPE criteria. Importantly, four of the six children had only mild forms of CP (Gross Motor Function Classification System, GMFCS=1), one had moderate (GMFCS=4) and one severe (GMFCS=5). Additional information is shown in Table 3, Paper IV.

Compared to similar EPT cohorts born in the late 1990s, i.e. the EPIPAGE study ($GA < 27$ weeks) as well as the EPICure data ($GA < 26$ weeks) where 18% of children had CP [19, 28], we conclude that the rate of severe motor impairments is low in the present very high-risk population. In addition, the trend of a decline in CP rates was previously more marked for more mature preterms [172] while our results indicate that this is now true also for the most immature infants [173].

4.3.3 BSID-III (Paper IV)

The BSID-III were used to assess cognitive, motor and language performances at age 30 months corrected. The overall mean scores of the EPT children were within the normal range for age on the all three BSID scales. Compared to a full term control group, however, the preterms scored significantly lower (Table 2, Paper IV).

Cognitive performance

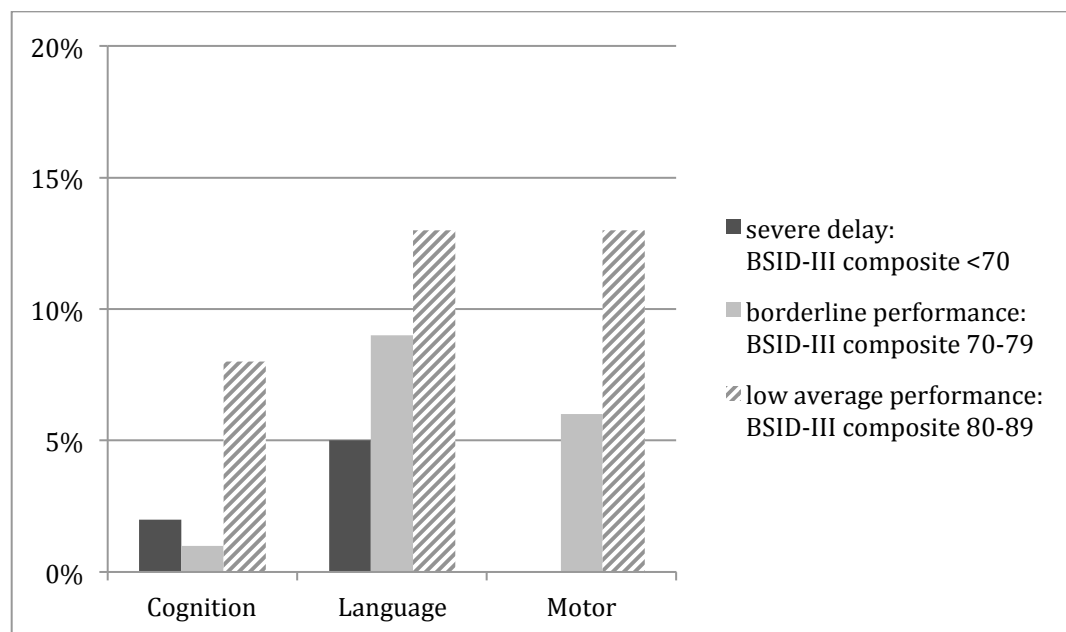
Complete cognitive BSID-III data was obtained for 86 children. Two children (2%) scored <70 thus having a severe cognitive delay. One child (1%) had "borderline" cognitive performance (mean cognitive composite 70-79) while seven children (8%) had "low average" cognitive scores (80-89). Five children had incomplete assessments, and information was instead collected from the clinical charts. In the drop out group, 3 children had a cognitive delay verified and tested in a clinical setting.

Language performance

Complete language BSID-III data was obtained for 85 children. Four EPT children scored <70 on the BSID-III, thus having a severe language delay (5%). Eight children (9%) had "borderline" language performance (mean language composite 70-79) while eleven children (13%) had "low average" language scores (80-89). In addition five children in the drop out group had very few words or no speech at the time of follow-up.

Motor performance

Complete motor BSID-III data was obtained for 84 children. All six children with CP attended research follow-up but for two children motor assessments were incomplete, see Table 3, Paper IV. No EPT child scored <70 on the BSID-III, five children (6%) had "borderline" motor performance (mean motor composite 70-79) while eleven children (13%) had "low average" motor scores (80-89).



Proportion of EPT infants with suboptimal scores on the BSID-III (test standard mean 100, SD 15)

In order to correctly interpret our results and make fair comparisons to other cohorts, the chosen assessment tool must be commented upon; the third edition of the Bayley Scales of Infant and Toddler Development [128].

The first version of the Bayley test was published in 1969 to assess the maturation of abilities in cognitive and motor development of infants between 2 and 30 months of age. The first revision of the BSID in 1993 was designed to update the normative data (now based on a sample of 1700 infants in 1988) and to expand the age range to 1 to 42 months. Several other adjustments were made to improve the predictive validity. A revision of the norms on the BSID was needed due to the upward drift of approximately 10 points on both the Mental and the Motor Scale. A similar pattern has been demonstrated in other cognitive assessments for children and is sometimes referred to as the Flynn effect [174]. It has been attributed to reflect improvements in general health such as fewer early infections, better nutrition, environmental conditions, and family relations.

The latest version of the BSID is normed on 1700 infants born in the US 2000 and claims improved representativeness of this normative sample. Comparative tests, conducted in the process of developing the third version, reported that the BSID-III was consistent with the results of the WPPSI-III, the PLS-4, and the Peabody Developmental Motor Scales, Second Edition but also that Bayley-III scores were approximately 7 points higher than the BSID-II Mental Development Index and Psychomotor Index scores (the Bayley-III Technical Manual) [128].

It has previously been reported that using published normative data as reference may result in an underestimation of impairment, however the magnitude differ [32, 175, 176]. A recent independent comparison between the second and third version of the BSID addresses serious scepticism toward the BSID-III as the proportion of children with developmental delay was grossly underestimated using the reference values [169]. These findings are in agreement with the present study and emphasize the importance of including an appropriate control group when designing outcome studies. Moreover, this suggests that higher cut off values may be needed when using the BSID-III, in order to identify children “at risk”.

Aware of the above reasoning, fair comparisons to other cohorts using the BSID-II or other assessment tools are difficult. In the Paper IV, only two infants (2%) scored <70 on the cognitive BSID-III and in total ten infants scored <90 (12%). In the EPIPAGE study, using the Kaufman assessment battery for children (K-ABC), a considerably larger group of infants born <27 weeks had cognitive difficulties (17% had a mental processing composite below 70 and 40% below 85) [28].

From the results in Paper IV, we conclude that strikingly few EPT infants were identified as having a severe cognitive delay using the BSID-III. However, as a group they performed 8 points (0.53 SD) below the term controls on the cognitive composite score. In addition, it should be kept in mind that the BSID-III test is a measure of development, rather than a pure IQ test, and consequently it may be in the interest of the individual to consider all infants with cognitive composite scores <90 (12% of our cohort) as infants “at risk”.

Consequently 27% of the EPT infants in our cohort are “at risk” vis-a-vis their language development, and 5% had a severe language delay according to scores on the BSID-III. Compared to the control group, the mean language composite score of the EPT infants was 11 points (0.73 SD) below the term controls. Hence, the language development in the cohort needs to be closely monitored and if persistently delayed at later follow-up ages, our study implies the need for early interventions.

In conclusion, the rates of severe impairments detected at toddler age are low in the present study population, both compared to historical Swedish data, and recent international reports. However, the 6-year follow-up of the Stockholm cohort is ongoing, and will hopefully clarify the impact of the present results, as well as providing important additional information regarding behavioural aspects and early school performances.

4.3.4 Gestational age and outcome

Twelve born infants born at week 23 had an MRI at TEA. Compared to infants born at weeks 24-26, they had a higher incidence of moderate-severe WM abnormalities (3/12= 25% vs 6/96=6%, $p=0.02$). Interestingly, when considering infants born at 23-24 weeks together, no significant differences were seen compared to 24-25 weekers.

Seven 23-weekers attended research follow-up. None had CP, however, a larger proportion had unspecific neurological signs compared to the entire cohort ($p=0.02$). Moreover, they had lower cognitive ($p=0.01$) and language scores ($p=0.004$) than the entire cohort (see Fig 5, Paper IV). These differences in outcomes were not explained by higher incidences of morphological brain injuries, as differences remained also when excluding infants with moderate-severe WM abnormalities on neonatal MRI.

Regression analysis demonstrated significant correlations between GA and performances on both the cognitive ($p=0.033$) and language scales ($p=0.003$). Motor performance was not influenced by GA. An obvious limitation to the statistical power is the small number of 23-weekers. Thus, we also stratified infants in larger groups; when considering infants born at week 23 and 24 together ($n=23$), no significant differences were found compared to infants born at week 25-26 ($n=64$).

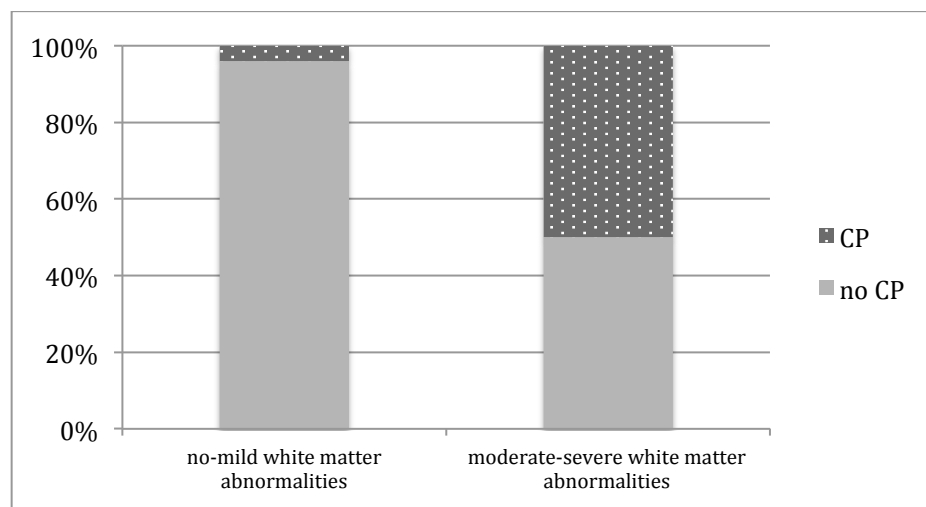
The 23-weekers are no doubt a high-risk population with higher rates of WM abnormalities, and lower cognitive and language scores. None, however, had CP or severe cognitive delay. In Sweden a tradition of proactive perinatal management has been practiced during the last decade resulting in improved survival rates also for the youngest preterms [1]. However a more restrictive use of perinatal interventions remain for infants born at week 22 [131]. Whether survival is in the best interest of the infant and family, is a delicate question that must be individually assessed. Importantly, our results show that survival without major disability is likely even at extremely low gestational ages.

4.4 MR-FINDINGS AND OUTCOME

The incidences of moderate-severe WM abnormalities on MRI at TEA, as well as major impairments at 30 months corrected age, were low. However, many infants had DEHSI, and many had suboptimal performances on the BSID-III scales and unspecific neurological signs on follow-up.

4.4.1 WM abnormalities on MRI and later cerebral palsy

Although the crude number of infants with moderate or severe WM abnormalities was low, 50% developed CP compared to 2,5% of infants with no or mild WM abnormalities on MRI at term equivalent age ($p<0.001$).



White matter abnormalities on MRI at TEA and later CP.

There was a high negative predictive value (97,5%) of a normal MRI at TEA in the present study. This is confirmed by a recent meta-analysis of comparable international studies [63]. Inversely, the positive predictive value of moderate–severe WM abnormalities was 50%, yielding a sensitivity of 60% and specificity of 96%. For comparison, Woodward et al [43] reported that moderate–severe WM abnormalities predicted CP with similar sensitivity (65%) and somewhat lower specificity (84%). The available studies point to the need of more specific diagnostic tools, possibly by the combination of different MRI modalities.

To investigate these associations further, the scoring items were studied individually; cystic changes ($p=0.003$), delayed myelination ($p=0.01$) and severe WM reduction ($p<0.001$) were found to be significantly associated with CP. These findings are in agreement with others showing specific neuroanatomical correlates of later CP both at TEA [113] and at later ages [177], especially decreased volume of WM in the periventricular regions [178].

In contrast, dilatation of the lateral ventricles or enlargement of the subarachnoidal space was not significantly associated with CP in the present setting. Others have demonstrated relationships between the size of the lateral ventricles at TEA and adverse outcome [179]. Interestingly, our data indicate that it is the WM damage and subsequent WM reduction per se that is of concern, rather than enlarged lateral ventricles or subarachnoidal spaces, which

can occur either isolated or secondary to WM injury. Our findings have recently been supported by a Finnish study showing that isolated ventricular dilatation did not increase the risk for developmental impairments whereas ventricular dilatation with additional brain pathology was significantly associated with both CP and cognitive delay [108].

4.4.2 WM abnormalities on MRI and later BSID-III performance

Overall, the EPT infants had poorer performances on all the BSID-III scales compared to term born controls (Table 2, Paper IV). In addition, infants with moderate-severe WM abnormalities on MRI had lower performance on cognitive and language scales compared to preterm with no or only mild WM abnormalities. The same was true for fine motor performance, but not for gross motor and therefore there was no significant difference in the composite motor score (Fig 3, Paper IV).

In contrast, DEHSI showed no relation to later cognitive, language and motor performances (Fig 4, Paper IV). Although DEHSI is a very common imaging appearance in preterm infants, few studies have demonstrated a relation to outcome [17, 119]. From the present study we conclude that DEHSI had no effect on any of the investigated outcomes at this age. However, it may be too early to rule out possible long-term effects, since subtle impairments may not become evident until later in childhood.

When analysing separate scoring items, we found severe WM reduction ($p=0.006$) and cysts ($p=0.034$) to be significantly associated with lower cognitive composite scores. Global thinning of the CC, delayed myelination and abnormal GM also correlated to lower cognitive scores, but these results should to be interpreted with care as the numbers were small.

The results in Paper IV illustrate the usefulness of MRI for the prediction of cognitive impairments, although to a lesser degree than for severe motor impairments such as CP. These results are in agreement with Woodward et al [43], who found moderate-severe WM abnormalities at TEA to be predictive of severe cognitive delay (OR 3.6; 95% CI: 1.5-8.7) as well as neurosensory impairments (OR 4.2; 95% CI: 1.6-11.3). However, the prediction of language impairments, commonly seen in EPT children, proves specifically difficult.

4.4.3 Gender, brain structure and outcome (Paper V)

At age 30 months corrected, EPT boys were found to have significantly poorer cognitive ($p=0.004$) and language ($p=0.018$) performances compared to EPT girls. Their motor performance also tended to be poorer. This was consistently true when infants with focal brain lesions or moderate-severe WM abnormalities on neonatal MRI were excluded. Moreover, there were no differences between genders with regard to GA, BW or other perinatal characteristics. The EPT boys spent longer time on CPAP but were similar to girls with regard to baseline characteristics and neonatal morbidities, see Table I, Paper V.

Similar to the present findings, other outcome studies have noted a male disadvantage. In the EPIPAGE study, very preterm boys had slightly lower developmental quotient than girls

at 2 years [180]. More pronounced differences were found in the EPICure study both at ages 30 months [19] and 6 years [176]. For example, 6-year old EPT boys scored 10 points below girls on the overall cognitive score and CP was present in 25% vs 14% of the EPT girls [176].

Preterm birth, neonatal mortality and perinatal morbidity are rather consistently found to be more common in male infants [40, 181, 182]. A range of adverse perinatal factors has been associated to male gender and brain injury has been the focus of several studies. EPT boys have higher incidences of intraventricular haemorrhages and periventricular leukomalacia [183, 184]. Moreover, McCarthy et al recently investigated cerebellar haemorrhages on routine cUS in infants of ≤ 32 weeks gestation [185]. In 672 infants, nine cases of cerebellar haemorrhages were identified, all in boys, and all of whom died.

These higher incidences of perinatal brain damages may explain some of the differences in childhood outcomes, although male gender has in fact been found to be an independent predictor for CP and adverse cognitive outcome at 30 months [19]. Similarly, in another large study including more than 2500 infants born < 28 weeks, gender differences were investigated with regard to both neonatal morbidities and outcomes at 18-22 months [41]. Preterm boys had poorer outcomes compared to girls; moderate-severe CP: 10.7% vs 7.3% and Mental Development Index < 70 (BSID II): 41.9% vs 27.1%. When adjusting for perinatal risk factors and the higher incidences of morbidities, preterm boys still had poorer outcomes, and the authors speculate that preterm boys appear to have an inherently greater baseline risk.

Despite the fact that EPT boys and girls were similar with regard to overt brain injuries and DEHSI on conventional MRI, outcomes differed at 30 months. Consequently we sought to investigate possible underlying mechanisms further. In Paper V, we used whole brain analysis of TBSS and regional analyses of DTI in order to evaluate possible subtle differences in WM integrity. In contrast to others [186] we found no explanation to the gender differences in outcomes. This may be due to our more conservative statistical methods or differences between the study populations.

In conclusion, the results in Paper V support the theories of a male disadvantage, independent of perinatal brain damages, with regard to outcome after preterm birth. In support of this, Gimnenez et al investigated baseline brain diffusion differences in healthy preterm boys and girls and found no significant differences in the FA maps at TEA [187]. Although we could not relate outcome differences to WM structure in the neonatal period, they may well be related to later WM development, not studied yet in the present cohort.

5 GENERAL DISCUSSION AND FUTURE DIRECTIONS

The advances in neonatal neuroimaging have dramatically increased our understanding of the diverse effects that prematurity may have on the developing brain. Technical improvements of both cUS and MRI will continue, resulting in higher quality data at lower costs. This will surely facilitate future research, and be of great clinical importance. From the present studies we conclude that MRI is a valuable tool for assessments of the neonatal brain, providing more detailed information than cUS. In the clinical setting however, cUS performed by an experienced examiner (serially during the hospital stay and at TEA) is clearly a very good ‘second best’.

Increasing attention is given the potentials for plasticity of the newborn brain. Our fMRI studies demonstrate that many of the active networks at rest are related to sensory-motor activity. This implies that sensory-motor information can be processed at a cortical level already at birth, and speculatively provides a basis for future treatments. Indeed, several neuroprotective therapies are currently under investigation, and necessitate proxy biomarkers of reorganisation after neuronal damage. Hopefully, advanced MR techniques will prove valuable in evaluating and implementing these interventions.

Predicting future impairment is certainly the foremost aim and the central challenge of many current studies. The MR scoring system used in the present studies is helpful to describe the brain damages in the cohort, making comparisons to other centres easy. However, for the prediction of outcome in the individual child, there is need for more specific diagnostic tools. This may possibly be achieved by the combination of different MRI modalities, focusing on MR findings in specific brain structures in relation to specific impairments. In all cases, a unique evaluation must be very carefully made for every individual infant. Clearly, many other perinatal and environmental factors influence outcomes in addition to brain lesions, and long-term follow-up is essential.

Science never solves a problem without creating ten more - GEORGE BERNARD SHAW

Several projects are still on-going:

- An outcome study comparing the predictive value of cUS and MRI is underway
- Functional MRI data is being investigated in preterm infants with brain damage, to investigate the role of white matter injury in the maturation of resting state networks
- General Movements have been performed in a subgroup of the cohort and will be analysed in relation to MRI data at term and outcome at age 30 months
- The development of an improved MR scoring system will continue

*Learn from yesterday, live for today, hope for tomorrow.
The important thing is not to stop questioning.*

ALBERT EINSTEIN

6 CONCLUSIONS

Brain imaging at term equivalent age

- The rates of major brain injury were low in this 3-year Stockholm cohort of extremely preterm infants; only 14% had moderate or severe white matter abnormalities on MRI at term equivalent age.
- Subtle white matter changes, so called DEHSI, were found in 56% of infants and were verified as changes on DTI, indicating possible alterations in white matter microstructure.
- No individual perinatal predictors of moderate or severe white matter abnormalities were identified. Surgical ligation of patent ductus arteriosus was found to be a significant risk factor for DEHSI.
- Cranial US detected all infants with moderate or severe white matter abnormalities on MRI, when imaging was performed on the same day. One third of infants with mild white matter abnormalities and four infants with small cerebellar haemorrhages on MRI were overlooked with cUS.
- In extremely preterm infants with normal conventional MR findings, we found five unique resting state networks using functional MRI.

Outcomes at 30 months corrected age

- The rates of severe impairments were low in this very high-risk population. Seven percent of the children had cerebral palsy (CP). Mean performances of the extremely preterm children were within the normal range for age on all three BSID scales. Compared to a full term control group, however, the preterms scored 0.5-1.0 SD lower on the BSID-III.
- In the absence of overt brain damage, extremely preterm boys were found to have poorer cognitive and language performances compared to girls.

MRI-findings and outcome at age 30 months

- Of infants with moderate or severe white matter abnormalities, 50% developed CP compared to 2,5% of infants with no or mild white matter abnormalities on MRI at term equivalent age.
- Infants with moderate or severe white matter abnormalities on MRI had lower performance on BSID-III cognitive and language scales compared to preterm with no or only mild white matter abnormalities.
- Severe white matter reduction and cysts were the individual MR-findings most strongly related to adverse motor and cognitive outcome.
- DEHSI showed no relation to later cognitive, language or motor performances.

7 SVENSK SAMMANFATTNING

Utvecklingen inom neonatologin under de senaste decennierna har gjort att allt fler extremt för tidigt födda barn överlever. Dessa barn har ofta en intensivvårdskrävande nyföddhetsperiod och hjärnan utsätts för stora påfrestningar under en känslig utvecklingstid. Det övergripande syftet med denna avhandling är att studera hjärnskador och utvecklingsavvikelser bland extremt för tidigt födda barn med hjälp av flera olika avbildningstekniker, samt relatera fynden till barnens utveckling under småbarnsåren.

Idag undersöks alla extremt för tidigt födda barn med ultraljud (UL) av hjärnan under vårdtiden, för att upptäcka blödningar och större strukturella avvikelser. Det senaste decenniet har magnetresonanstomografi (MRT) börjat användas i ökande utsträckning. MRT ger en anatomiskt överlägsen bild av den nyfödda hjärnan, där även subtila morfologiska förändringar kan bedömas. Diffusion Tensor Imaging (DTI) är en vidareutveckling av konventionell MRT som ger detaljerad information om den vita substansen, nervbanornas utbredning och hjärnvävnadens mognadsgrad. Funktionell MR (fMRI) är en av de nyaste metoderna att avbilda hjärnan; genom att mäta spontana blodflödesfluktuationer eller svaret på olika stimuli, kan man se vilka delar av hjärnan som är aktiva vid ett givet tillfälle.

I avhandlingen ingår fem studier av barn födda före gestationsvecka 27+0 dagar i Stockholms län mellan 2004 och 2007. Den totala överlevnaden i kohorten var 67%. Sammanlagt genomgick 109 för tidigt födda barn MRT vid motsvarande fullgången ålder, samt 16 friska kontrollbarn.

I Delarbete I beskrivs hjärnskadepanoramata i kohorten: endast 14% uppvisade måttliga eller svåra förändringar i hjärnans vita substans. Däremot hade många barn diffust ökad signalintensitet, sk DEHSI. I dessa områden påvisades ändrade diffusionsegenskaper motsvarande en förändrad mikrostruktur.

Syftet med Delarbete II var att studera vilka nätverk som är aktiva i vila hos underburna barn. Tolv extremt för tidigt födda barn undersöktes därför med fMRI under 10 minuters sömn och vi fann fem unika sk. Resting State Networks jämfört med den vuxne individens tio. Studien tillför därmed kunskap i en ny dimension kring hur barnets hjärna mognar och utvecklas.

I Delstudie III utfördes UL och MRT i en del av prematurkohorten ($n=72$). Förutsatt att både UL och MRT utfördes på samma dag vid motsvarande fullgången tid, upptäcktes alla barn med svåra avvikelser med båda metoderna. Barn med normalfynd vid ultraljudsundersökningen hade en normal MRT eller milda förändringar i hjärnans vita substans, däremot förbisågs samtliga fyra barn med små lillhjärneblödningar med UL.

Syftet med Delarbete IV var att studera konsekvenserna av extrem prematuritet vid 30 månaders ålder, samt att undersöka hur MRT i fullgången tid korrelerar till dessa uppföljningsdata. De för tidigt födda barnen, samt en grupp friska kontrollbarn ($n=85$) undersöktes av barnneurolog samt barnpsykiolog som utförde Bayley Scales of Infant Development III.

Av Stockholmskohorten medverkade 78% i uppföljningen. Sju procent av barnen uppfyllde kriterierna för cerebral pares (CP). Extremprematurerna presterade som grupp inom ramen för det normala på de tre Bayley-skalorna: kognition ($96 \text{ SD} \pm 9.5$), språk ($97 \text{ SD} \pm 14$) och motorik ($103 \text{ SD} \pm 15$). Jämfört med kontrollerna däremot var resultaten signifikant lägre för alla tre delskalor, även när MRT var normal. Måttliga-svåra förändringar i hjärnans vita substans på MRT var förknippat med högre förekomst av CP, samt lägre prestation på Bayleytesterna avseende både

språklig och kognitiv förmåga. Däremot presterade för tidigt födda barn med DEHSI lika bra som de utan DEHSI, och de hade inte CP i större utsträckning. De yngsta prematurerna, födda i gestationsvecka 23 hade måttliga-svåra förändringar i hjärnans vita substans i högre grad än övriga, och presterade sämre på samtliga tre skalor. Dock var fynden endast statistiskt signifikanta för språkförmåga när man tog hänsyn till avvikelser på MRT. Sammanfattningsvis tillhandahåller Delarbete IV utförliga uppföljningsdata på tre årskullar extremprematurer i Stockholm och visar att överlevnad utan grava funktionsnedsättningar är sannolik även i de lägsta gestationsåldrarna.

Slutligen, påvisades i Delarbete V att extremt för tidigt födda pojkar presterade sämre avseende kognition och språk jämfört med extremt för tidigt födda flickor vid 30 månaders ålder. Dessa skillnader kunde inte förklaras av någon enskilda perinatal variabel eller en högre förekomst av hjärnskador på MRT. Diffusionsegenskaperna i den vita substansen analyserades med Tract-Based Spatial Statistics och befanns vara lika mellan könen. Prematurfödda pojkar är möjligen biologiskt skörare än flickor och anledningarna till detta är sannolikt multifaktoriell.

Denna avhandling visar att förekomsten av hjärnskador och senare funktionshinder till följd av extrem prematuritet, är jämförelsevis låg bland barn födda i Stockholm. Avvikelser påvisade med hjälp av neonatal neuroradiologi är kopplat till sämre prognos, men att förutspå framtida funktionshinder är fortfarande svårt i det enskilda fallet. Långtidsuppföljning av extremt underburna barn är absolut nödvändig.

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