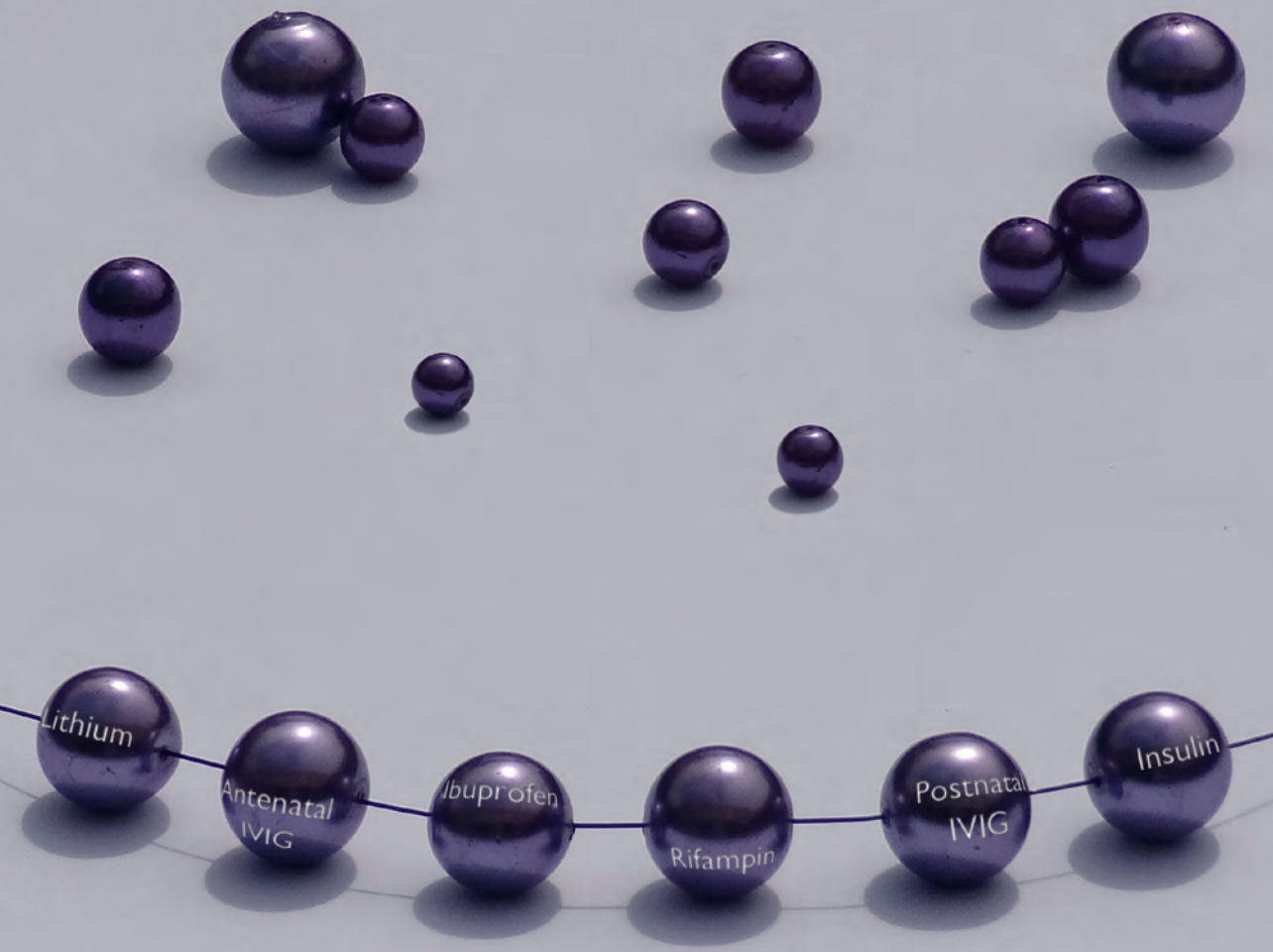
Neonatal Pearls

Safety and efficacy of medication use in fetus and neonate



N. Margreth van der Lugt

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Neeltje Margaretha van der Lugt

Neonatal pearls - Safety and efficacy of medication use in fetus and neonate.

ISBN: 978-90-6464-718-5

Cover design: Margreth van der Lugt.
Lay-out: Ferdinand van Nispen • Citroenvlinder-dtp.nl • DTP & Vormgeving, Bilthoven, The Netherlands.
Printing: GVO drukkers & vormgevers B.V. | Ponsen & Looijen, Ede, The Netherlands.
The research presented in this thesis was performed at the Department of Pediatrics, Leiden University Medical Center, The Netherlands.
Publication of this thesis was financially supported by the Willem-Alexander Children's Hospital Leiden, Chiesi Pharmaceuticals B.V., Friso Kindervoeding B.V., Abbvie B.V..

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Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus Prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 26 november 2013
klokke 10.00 uur

door

Neeltje Margaretha van der Lugt
geboren te Delft in 1986

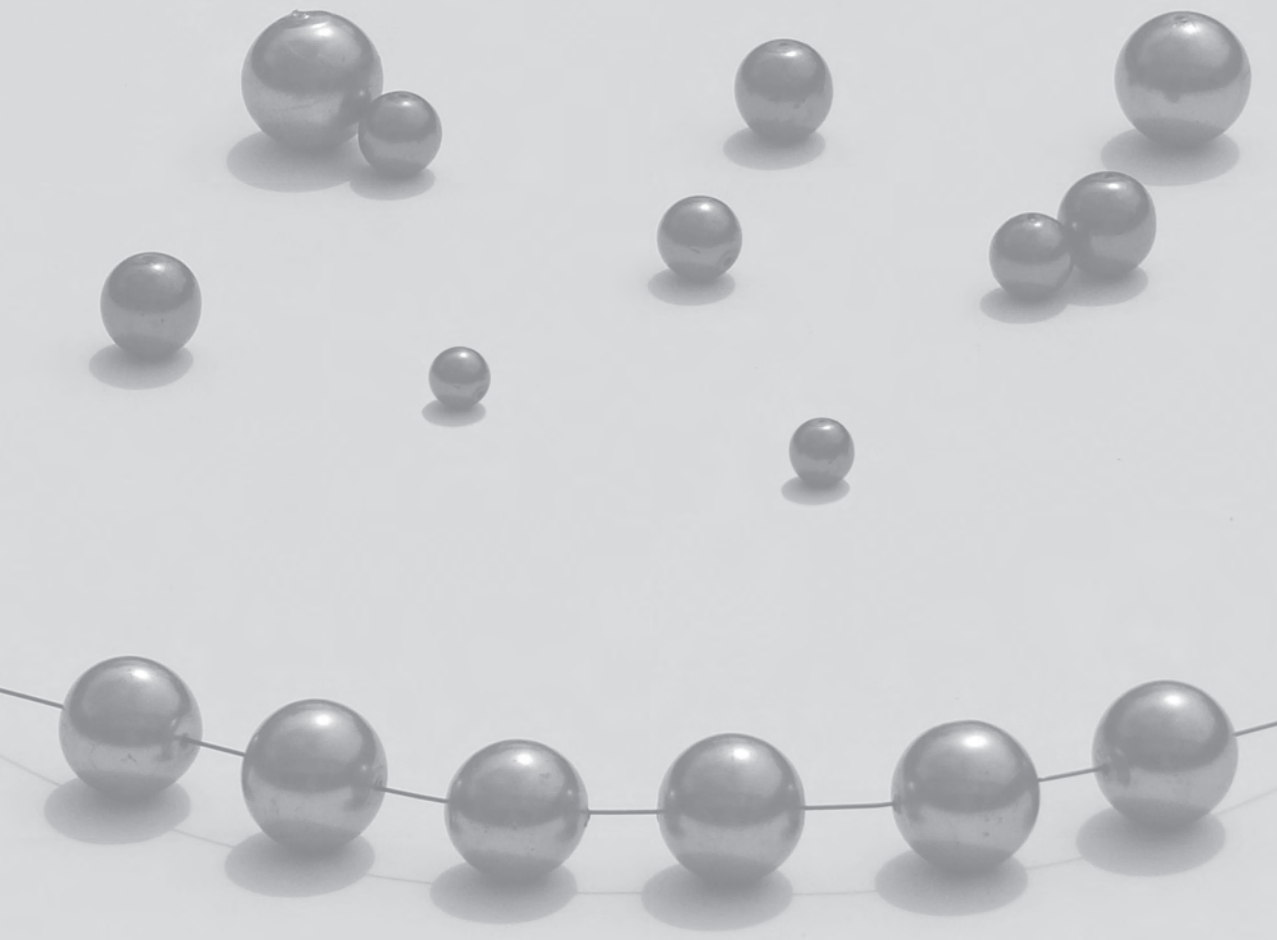
Promotiecommissie

Promotor:	Prof. Dr. F.J. Walther
Co-promotor:	Dr. E. Lopriore
Overige leden:	Prof. dr. H.J. Guchelaar Prof. dr. A. Brand Prof. dr. Allegaert, Universitair Ziekenhuis Leuven (België) Prof. dr. A. Cohen, Center for Human Drug Research Leiden

Contents

Chapter 1

General introduction



General introduction

Pharmacological research in pediatrics

More than 100 years ago the founding father of pediatrics in the United States of America, Dr. Abraham Jacobi, stated "Pediatrics does not deal with miniature men and women, with reduced doses and the same class of disease in smaller bodies, but has its own independent range and horizon".[1] With this statement the need for age-appropriate pharmacological research for children was already emphasized more than a century ago. Several physical aspects with a great influence on distribution and metabolism of drugs change in growing children, such as body composition, motility and pH of the gastro-intestinal tract, perfusion of skeletal muscles, composition of plasma proteins, concentration of CYP-enzymes, glomerular filtration rate etcetera. As this large amount of factors change disproportional, dosing formulas to determine age-specific doses are almost totally unusable, especially for children younger than 8 years.[2] Despite this important argument to develop age-appropriate pharmacological research in children, this kind of research is still limited, particularly in the field of Neonatology. The relatively small pediatric and neonatal population and the need for testing drugs extensively for different age categories make research in children very expensive and therefore less attractive for the pharmaceutical industry. In addition, national law rules, ethical questions and the need for parental consent are other major problems for developing ethical justified clinical trials in children. A direct consequence of the lack of pharmacological research in children is a high amount of unlicensed (not registered in the Netherlands or modified formulations of registered drugs) and off-label (not registered for the used dosage, age, indication and/or route of administration) drug prescriptions.[3,4] These unlicensed and off-label prescriptions are most common in very ill children, especially those admitted on the neonatal intensive care unit (NICU). At least one off-label or unlicensed drug is prescribed to 80-93% of the NICU population.[3] The main reason for being off-label is the lack of age specific dosage regimes. Concerning the prescription of unlicensed and/or off-label drugs, we must consider if we want to deprive children of potential therapeutic benefits of unlicensed drugs instead of exposing them to possible unknown adverse effects.[3] This is a difficult consideration as several clinical conditions could not be treated without unlicensed and off-label prescriptions. Otherwise is usage of unlicensed and off-label drugs associated with an increased risk for adverse effects, urging for careful usage.[4,5]

Until when pharmacological trials in pediatrics become more extensive, pediatricians will have to deal with the limited availability of licensed and on-label drugs and work with alternative sources of evidence concerning safety and efficacy of medicine usage in children.

Retrospective research design

The most appropriate alternative design to evaluate safety and efficacy of medication use in children is a retrospective study. The lack of acquiring reliable evidence from retrospective studies is a common heard argument against this design, as it has a low ranking in the pyramid of evidence. However, an example of a pioneering discovery detected with a case-control study, was the finding of the relationship between smoking and lung cancer, confirming the usefulness of retrospective study design.[6]

When prospective evidence is not available, retrospective studies can be very valuable for evaluation of medical policies. Unlicensed and/or off-label drugs can be analyzed using a cohort of children over a fixed (passed) time period, which received a particular drug. Analysis of baseline characteristics of the population and details about achieving treatment goal, dosage, duration of administration and possible adverse effects, can demonstrate the advantages and disadvantages of the drug, so usage can be fine-tuned.

In addition to unlicensed drugs, the usage of licensed drugs also needs to be evaluated and fine-tuned on a regular basis. When a specific treatment protocol is started after a time-consuming process, it is often considered to be effective and safe enough to be used extensively. However, diversity of diseases, comorbidity and treatment possibilities may change during time. Frequent re-assessments of current management guidelines are thus of paramount importance including evaluation of their efficacy and safety. Nevertheless, evaluations of current management protocols are not frequently performed, since it is probably more "rewarding" or "exiting" to invent completely new treatment strategies. Based on these evaluations, the incidence and associated comorbidity among current policies can be surveyed and compared with other centers and populations (benchmarking). Unexpected outcomes may provide new insights and can be used as guidance for protocol adjustments or provide a basis for new randomized controlled trials. In many cases, these randomized controlled trials are only ethically justified when the advantages of possible new interventions are supported by strong evidence from retrospective cohort studies.

Another great benefit of retrospective studies is that they offer the opportunity to investigate relatively rare diseases, a common problem in the field of Neonatology. In these situations sample sizes are often small and prospective investigation of a large enough number of cases for reliable statistical analysis is sometimes very difficult if not impossible.

Although randomized controlled trials continue to provide the best evidence to guide clinical treatment and retrospective cohort studies may suffer from a various methodological limitations, especially errors due to confounding and bias, the latter play an important role in the evaluation of clinical treatment protocols and continue to be essential in medical research.

Neonatal pearls

In this thesis we present six clinical studies, six 'Neonatal pearls' reflecting our current neonatal practice (using licensed as well as unlicensed medicines). Although topics may seem very diverse at first sight, the common denominator is the evaluation of management guidelines with focus on effectiveness and risk of a particular drug. The problems chosen to investigate are all based on important practical questions with clinical relevance. All described clinical conditions are severe but rare, which results in restricted availability of literature. Our aim is to provide additional evidence with recommendations, directly usable in clinical practice. Due to the lack of literature and the small sample sizes, both caused by the rarity of the studied conditions, a retrospective cohort study was the only appropriate design to evaluate efficacy, safety and/or long term outcome of the current protocols. These six 'Neonatal Pearls' can serve as potential foundations for adjustment of clinical protocols and guidance on future prospective studies and randomized controlled trials.

Outline of the thesis

The general aim of this thesis was to emphasize the importance of retrospective research in Neonatology and the necessity of regular and careful evaluation of already existing treatment policies. We describe six retrospective studies investigating effectiveness, safety and/or long term consequences of various drugs used in neonates.

Chapter 1 provides background information on the choice of the various conditions reported in chapters 2 to 7 and the choice of retrospective cohort studies.

Chapter 2 investigates fetal, neonatal and developmental outcome of children exposed to maternal lithium use during intrauterine life to gain more knowledge about the safety of lithium use during pregnancy. Lithium is the main treatment for women with a bipolar disorder; discontinuation of lithium during pregnancy increases the risk of relapse postpartum. Follow-up was collected prospectively with assessment of growth, neurological examination, cognitive and behavioral assessments.

In **Chapter 3,** we evaluated the efficacy and safety of antenatal treatment of fetal and neonatal allo-immune thrombocytopenia (FNAIT) with weekly maternal IVIG and postnatal matched platelet transfusions (if necessary). Important outcomes were the occurrence of intracranial hemorrhage (ICH), petechiae, hematomas and severe neonatal thrombocytopenia. The postnatal course of neonatal platelet counts was

followed and graphically analyzed.
Chapter 4 investigates the efficacy of repeated courses of ibuprofen for closure of a patent ductus arteriosus (PDA). Ibuprofen is the first treatment of choice for PDA in preterm infants; in most centers failure of a second course is followed by surgical closure. Recent studies suggest that surgical intervention is associated with adverse outcome. The closure rate and safety of a second and third course of ibuprofen in preterm infants was retrospectively evaluated, in order to postpone surgical closure. Risk factors for failure of PDA closure and its clinical consequences were also studied.

Chapter 5 evaluates the combination therapy with rifampin and vancomycin in persistent Coagulase Negative Staphylococcal (CNS) sepsis in neonates. Infection parameters, such as C-reactive protein (CRP) levels before and after start of rifampin therapy, and peak and trough serum levels of vancomycin before the start of rifampin therapy were analyzed for efficacy of this treatment regimen.

In **Chapter 6** we analyzed the outcome of infants of mothers with idiopathic thrombocytopenic purpura (ITP) during pregnancy by scoring the occurrence of intracranial hemorrhage (ICH) and graphical analysis of the postnatal course of neonatal platelet counts. Effectiveness and safety of various postnatal treatment strategies, such as platelet transfusions, IVIG and/or prednisone were evaluated.

Chapter 7 describes a retrospective follow-up study to determine the long term effects of insulin treatment for hyperglycemia on growth and neurodevelopmental outcome of preterm infants. Outcome at two years of age of preterm infants with hyperglycemia and controls, matched for gestational age and birth weight, were compared to estimate the efficacy and safety of insulin therapy for hyperglycemia.

Chapter 8 – In the general discussion the main results of this thesis will be discussed from a broader perspective, focusing on future perspectives. The discussion will end with a short main conclusion, reflecting on the points emphasized in the general introduction.

Chapter 9 – Summary of this thesis.

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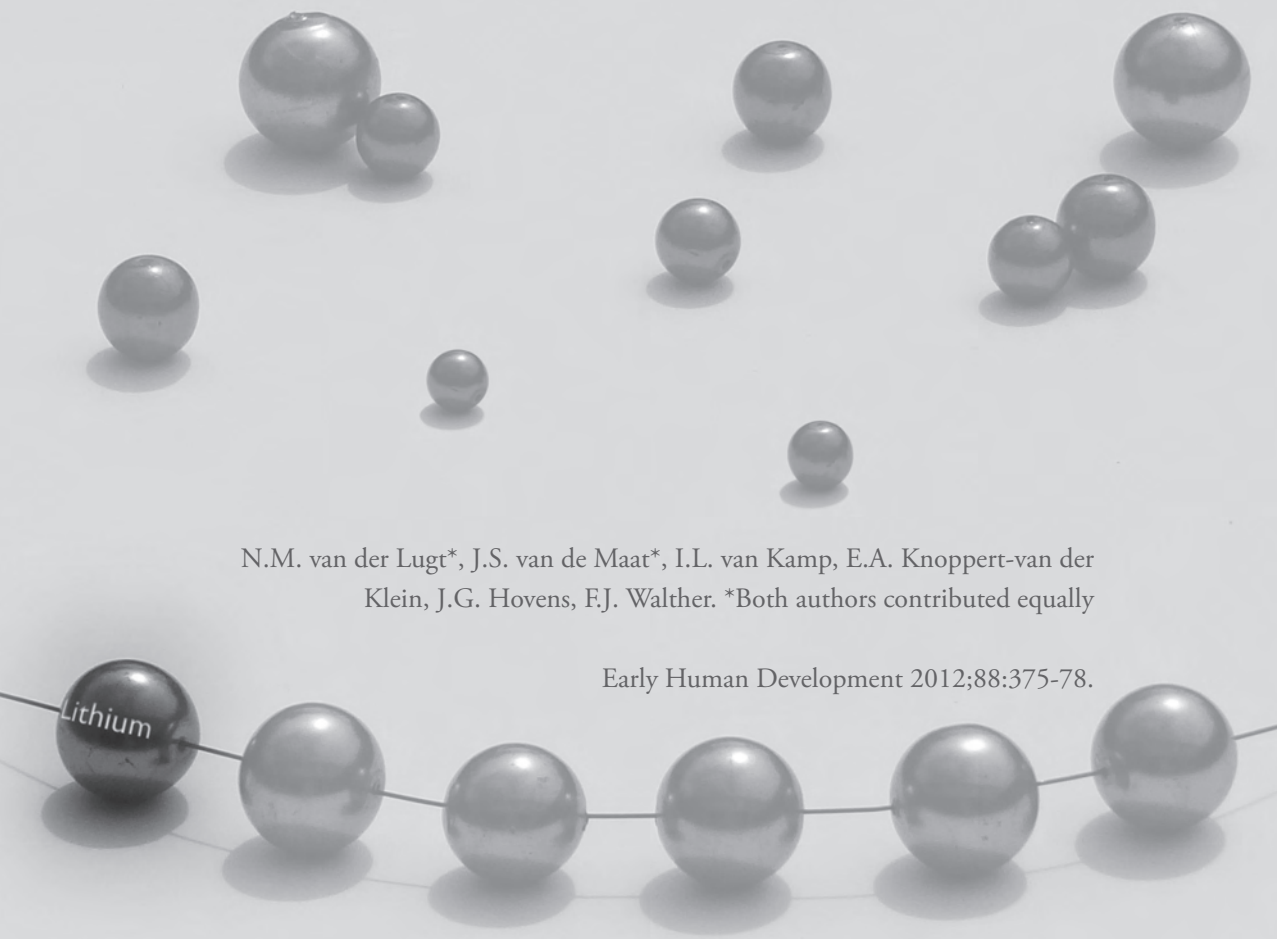
Chapter 2

Fetal, neonatal and developmental outcomes of lithium-exposed pregnancies



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Early Human Development 2012;88:375-78.

Abstract

Background

Many women with a bipolar disorder are of reproductive age and will need to continue lithium treatment during pregnancy. The teratogenic and perinatal effects of lithium are known, but not the long-term effects of lithium on neurodevelopment of the children. This study investigates growth, neurological, cognitive and behavioral development of children exposed to lithium in utero.

Methods

In an observational retrospective cohort study 15 children who were exposed to lithium in utero were investigated at 3–15 years of age. Neurological development was tested using the Hempel or Touwen examination. Cognitive development was assessed with the Bayley Scales of Infant Development III, Wechsler Preschool and Primary Scale of Intelligence or the Wechsler Intelligence Scale for Children. Parents completed the Child Behavior Checklist to assess behavioral development and a standard questionnaire about general development of the child since birth.

Results

One child had signs of a minor neurological dysfunction, but without further clinical implications. The results of the cognitive tests were within normal limits, although most children had lower scores on the performance IQ subtest. Growth, behavior and general development were within the normal range.

Conclusion

Continuing lithium therapy during pregnancy did not cause adverse effects on growth, neurological, cognitive and behavioral development of exposed children.

Introduction

Since the 1950s lithium is the most important drug in the pharmacological treatment of bipolar disorder. Bipolar disorder is common in women in their reproductive age, which raises the question whether intrauterine exposure to lithium affects neurodevelopmental outcome of exposed children.[1] Discontinuation of lithium use during pregnancy is contraindicated as this is associated with a twofold greater risk of recurrence of a new episode of mania or depression.[2,3,4]

Lithium entirely readily crosses the placenta and the fetus receives 100% of the drug during pregnancy. High serum lithium levels at delivery are associated with a higher incidence of neonatal complications and have led to interruption of lithium therapy or use of lower doses of lithium shortly before delivery.[5]

The risk of congenital disorders of children who received lithium in utero has been extensively investigated. A retrospective analysis of data from the Danish Register of Lithium Babies in 1976 suggested a high risk of Ebstein's anomaly (6 out of 225 exposed children versus an incidence of 1 in 20,000 in the general population)[6], but this turned out to be a gross overestimation due to a voluntary reporting bias. The Motherisk Program performed two studies on the potential teratogenic effect of lithium and found a relative risk for all congenital disorders of 1.2 and for cardiac disorders of 1.1 and no association of lithium use with Ebstein's anomaly.[7,8] A review of the epidemiologic data by Cohen et al. concluded that the teratogenic risk of first-trimester lithium exposure is lower than previously suggested.[9]

Many case reports describe neonatal lithium toxicity which often presents as a "floppy infant syndrome" characterized by lethargy, poor sucking, tachypnea, tachycardia, respiratory distress syndrome, cyanosis and hypotonie.[10] Other neonatal problems include structural and functional cardiovascular problems, macrosomia, hyperbilirubinemia, diabetes insipidus, and hypothyreoidism.[10] However, an elevated risk of these adaptation problems and complications has never been significantly proven and most of them are transient and without long-term consequences.[11]

Although lithium does not seem to cause major congenital disorders, there is a realistic possibility of long-term effects on the developing fetal brain during pregnancy.[12,13] The first trimester is the most critical period for drug-induced disorders, but the brain is developing throughout pregnancy and highly vulnerable to the latent cognitive and neurological impact of drugs.[14] Youngs et al. investigated long-term effects of lithium on the developing rat brain and found long-lasting increases in anxiety-like behavior.[15] Except for a follow-up questionnaire reported by Schou, long-term effects of exposure

to lithium in utero have not been studied in humans.[16,17] This lack of knowledge causes fear in women who use lithium and are pregnant, or are planning a pregnancy.[18]
The objective of this study was to investigate growth and neurological, cognitive and behavioral development of children who were exposed to lithium in utero.

Methods

Participants

The Perinatal Center of the Leiden University Medical Center (LUMC) prospectively collected perinatal data on all mothers treated with lithium (target maintenance serum lithium levels 0.6–0.8 mmol/L) for bipolar disorder during pregnancy and their children in the period between 1-1-1994 and 31-12-2007. Mothers with a bipolar disorder without lithium therapy were not identified in the defined period. Twenty-one mothers of 30 children born alive in the LUMC between 1-1-1994 and 31-12-2007 were asked by their psychiatrist or obstetrician for permission to examine their children for follow-up purposes. The psychiatrist or obstetrician called the mothers to inform them about the study and ask them if they wanted to participate. If they agreed, information about the study and follow-up questionnaires were sent and the physical and psychological examination scheduled. The ethics committee of the hospital approved the study and informed consent was obtained from the parents.

Study design

Growth and neurological, cognitive and behavioral outcome of lithium-exposed children were assessed using standard tests, validated in the Dutch population. Data collection was done during the neonatal period and between November 2008 and December 2009. The visit of the children with one of their parents to the hospital lasted about 4 hours.

Assessments

Neonatal assessment

The neonatal database provided 1. data on the medical and obstetric history of the mothers, including use of medications during pregnancy, serum lithium values, method of delivery, and perinatal complications, and 2. data collected during the 24 hour observation of each child after birth, including birth weight; 1 and 5 min Apgar

scores; umbilical cord blood lithium levels; chest X-ray, ECG, and echocardiography (if indicated); blood glucose, serum electrolytes, and thyroid function.

Developmental assessment and growth

Physical and mental development of the child was screened using a questionnaire to be filled in by the mother. Items that were asked were growth, illnesses, behavioral problems, motor problems and developmental milestones. Growth of the children was assessed by measurement of height, weight and head circumference and plotted in the Dutch growth curves.

Neurological examination

Children between 2 and 5 years of age were assessed according to the Hempel examination.[19] Outcome of this test is divided into 3 groups: normal, simple minor neurological dysfunction (MND) when dysfunction is detected in 1 cluster, and complex MND when dysfunction is found in more than 1 cluster. For older children the Touwen protocol for neurological examination was used.[20,21] Outcome for that test is 0 (normal), 1 (simple MND), 2 (complex MND), 3 (Complex Pathology, CP).

Cognitive testing

Cognition was assessed by a child psychologist. Children between 16 and 30 months old were tested with the Bayley Scales of Infant Development (BSID III)[22] and older children were tested with the Wechsler Preschool and Primary Scale of Intelligence or the Wechsler Intelligence Scale for Children (WPPSI/WISC)[23]. The BSID reports a Developmental Score, the WPPSI/WISC consists of a verbal intelligence quotient (VIQ), performance intelligence quotient (PIQ) and total intelligence quotient (TIQ). All tests have a mean score of 100 and a standard deviation of 15.

Behavioral assessment

Behavioral development of the children was tested by having the mothers complete the Child Behavior Checklist (CBCL) for children from 1.5 to 5 or 6–18 years.[24] Outcome of this test consists of T- values on 6 different areas of the DSM-IV: affective problems, anxiety problems, attention deficit/hyperactivity problems, oppositional defiant problems, conduct problems, pervasive problems (1.5–5 year old) or somatic problems (6–18 year old). Results of these scores are divided into 3 categories, with T-values of 50–64.5 being normal, 64.5 to 69.5 subclinical and 69.5–100 clinical.

Statistical analysis

Data are presented as mean ± standard deviation (SD) or as median (range).

Results

None of the 30 lithium-exposed children were born with congenital anomalies known to be associated with maternal lithium use. Mean ± SD birth weight was 3384 ± 510 gram, mean gestational age was 38.0 ± 1.2 weeks and none of the children was asphyxiated (5min Apgar score <7) or needed respiratory support at birth. Seventeen out of 30 children were born by normal vaginal delivery. Ten children (of whom five were seen for follow-up) showed signs of neonatal toxicity: 4 had respiratory symptoms, 4 nausea and vomiting (retching, refusal of nutrition), 2 hypoglycemia, 1 hypotonia and 1 hyperbilirubinemia. Two children had serum lithium values >0.8 mmol/L, of whom one had signs of neonatal toxicity. In addition to lithium, 9 out of 30 children were exposed to other psychotropic medications (4 were exposed to antidepressants, 4 to benzodiazepines and 1 to an antipsychotic drug). One child had a ventricular septal defect with coarctation of the aorta and underwent successful surgical repair. Serum electrolytes, renal function and thyroid hormones were normal in all.

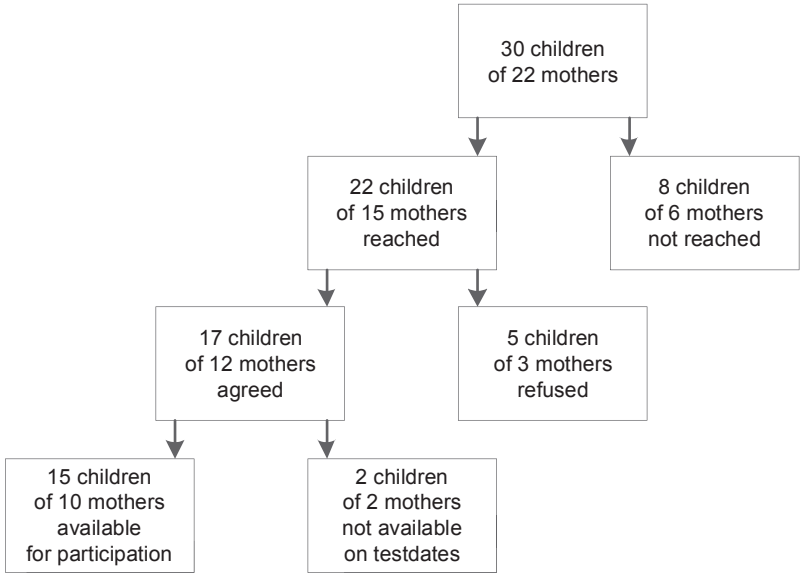

Figure 1. Flowchart of included infants.

Six mothers (8 children) could not be reached for follow-up because they had moved without leaving a forwarding address or moved out of the country, 3 mothers (5 children) refused participation and 2 mothers (2 children) were not available on test dates. Ten mothers were left, with a total of 15 children, to participate in the study. In figure 1 a flowchart can be seen of participating children and mothers.

Table 1 shows the baseline characteristics of the 15 children who participated in the follow-up study. No significant differences were found in baseline characteristics comparing participating and non-participating children in the follow-up study.

Results of the neurological and physical examinations are presented in table 2. One child had a minor neurological dysfunction (MND), but this result was without further clinical implications. In all other cases no neurologic abnormalities were found. Growth measurements were all within the normal range.

Table 2 also reports the results of the psychological examinations. Testing of cognition with WISC-III-NL, WPPSI-R and Bayley tests did not demonstrate any abnormality. One child had a low VIQ and TIQ, but the PIQ was normal. All other data were within the normal range. Most children had a lower score on the performance tests, especially on the subtest Block patterns, but this was not a significant deprivation. The child tested with the WPPSI-R had above average scores, so did one child tested with the Bayley test.

The results of the CBCL-questionnaire showed no abnormalities in the areas of Affective Problems, ADH, Conduct Problems and Pervasive Disorders and all scores were within the normal range. In the area of Anxiety Problems two children scored in the subclinical range, one of whom also scored in the subclinical range in the area of Somatic Problems. One child was in the subclinical range in the area of Oppositional Problems.

Developmental milestones were normal in all children. Parents mentioned hyperactivity and concentration problems in 3 children. Medical histories frequently reported viral upper airway infections, eczema in 4 children and allergies in 2. Two children received physical therapy when they were 1.5 to 4 years old to improve their gross motor skills. One child had transient motor problems at the age of 7.

Table 1. Baseline characteristics of children who participated in the follow-up study.

Patient #	Gravida/ Para	Age of mother (years)	Other psychotropic medication	Gestational age (weeks)	Delivery [a]	Gender (Male, Female)	Apgar Scores		Lithium cord blood level	Neonatal toxicity
							1 min	5 min	>0.8 mmol/L	
1	G1P0	34		39	VE	F	8	9		
2	G2P1	37		39	VE	F	9	10		X
3	G1P0	34		39	VE	M	9	10		
4	G2P1	36		38	V	F	10	10		X
5	G1P0	37		38	V	M	8	9		X
6	G2P1	28	fluoxetine	37	V	F	8	9		
7	G1P0	36		37	VE	M	8	9		
8	G1P0	29		40	VE	M	5	8		
9	G2P1	40		40	CS	F	8	9		
10	G1P0	32		40	CS	M	8	9		X
11	G2P0	35	haloperidol	39	VE	F	8	10		
12	G2P1	31		38	V	F	10	10		
13	G3P1	38	nortriptyline	36	V	F	7	9		
14	G2P1	36	lorazepam	39	CS	F	9	9	X	
15	G1P0	35		37	V	F	9	10	X	X

[a] V: vaginal delivery; VE: vacuum extraction; CS: cesarean section.

Table 2. Neurological and growth assessments and psychological examinations at follow-up.

Patient #	Age (years)	Neurological and growth assessment				Psychological examination				
		Neurological outcome [a]	Height (cm)	Weight (kg)	Head circumference (cm)	WISC-III-NL and WPPSI-R [b]			BSID-III[c]	
						VIQ	PIQ	TIQ	DS	95% CI
1	15	Normal	183.7	62.8	55	122	105	116		
2	13	Normal	172	49.3	54	108	99	105		
3	11	Normal	154	42.0	57	115	96	107		
4	10	Normal	147.5	36.2	55	122	118	123		
5	9	Normal	128.7	24.9	52	103	91	97		
6	9	Normal	133.5	26.8	51	83	88	84		
7	7	Simple MND	132.8	26.5	53	107	107	108		
8	8	Normal	132	27	53	106	86	96		
9	7	Normal	125.2	22.5	51	105	88	96		
10	6	Normal	124	24.8	54	122	110	119		
11	6	Normal	119	24.3	51	107	99	104		
12	6	Normal	118	22	50	126	121	128		
13	4	Normal	98	15.5	49	109	137	126		
14	3	Normal	96.5	15.5	50				139	126-139
15	3	Normal	99.4	16.4	48.6				105	97-113

[a] Patients 1-9: Touwen exam; patients 10-15: Hempel exam.
[b] WISC-III-NL: patients 1-12;, WPPSI-R: patient 13; BSID-III: patient 14 and 15
[c] DS: developmental score; 95% CI: 95% confidence interval.

Discussion

This study reports the long-term outcome of 15 children who were exposed to lithium in utero and were not breastfed. Neurological screening and growth measurements did not show significant abnormalities in the children, all were well within the normal range. Intelligence tests detected lower scores in the performance tests, especially in the Block pattern subtest, in nearly all children, but the difference with a control general population was not significant. Motor and behavioral development showed no significant abnormalities, based on the CBCL and developmental questionnaire.

The only study on follow-up of lithium exposed children was done by Schou in 1976.[16] In this follow-up study a questionnaire was sent to the psychiatrist or the general practitioner of the mothers of 60 children who had been exposed to lithium in utero. These doctors were requested to ask the mothers if any problems in physical or mental development had occurred in the lithium-exposed children and their non-lithium-exposed siblings who served as a control group. No significant differences were found between both groups. However, this study was based on the subjective report of the mothers, never a study was done where a pediatrician and a psychologist examined the children. Our study comprised growth measurements and formal testing of neurological, cognitive and behavioral outcome in 15 children 3–15 years of age and shows that outcome of these children is indeed within normal limits.

Limitations of this study are the relatively small sample size, lack of a suitable control group, and use of other psychotropic medications. Because of the observational character of the study, no specific hypothesis was formulated in advance. Small differences may be difficult to detect due to the small group of children tested and the intrinsic limitations of the standard screening instruments for behavioral development.[25] A possible negative finding may be the lower scores on the performance IQ (PIQ) tests, but further and more specific research needs to be done on this subject. As the lower scores of PIQ were not statistically significant, it was not necessary to investigate the role of potential confounding factors on cognitive development (maternal IQ, socioeconomic status, use of alcohol and tobacco). No suitable control group could be found, because it is almost impossible to identify children who were raised in the same situation and family, but without in utero exposure to lithium. This type of controls was not available to us. However, normal values of tests for psychological and behavioral development are based on a large cohort of children from the general population, which functions like a control group.

Based on our results we conclude that the children are developing normally after

being exposed to lithium in utero and that no major developmental problems have evolved. This supports the thesis that continuing lithium therapy during pregnancy does not adversely affect the development of a child, and that it is rather safe to do so. This information may provide the counseling doctor and the bipolar woman more confidence in planning a safe pregnancy and continuing lithium treatment.

Acknowledgments

The authors thank Drs. Sylvia Veen and Monique Rijken for their assistance with the neurological and physical examinations and Tanja Kooij for the supervision of the psychological tests.

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Chapter 3

Neonatal outcome in allo-immune thrombocytopenia after maternal treatment with antenatal intravenous immunoglobulin

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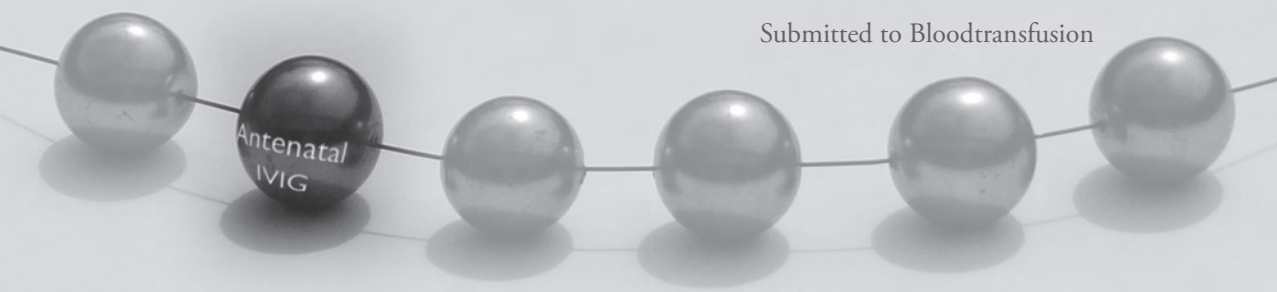
Submitted to Bloodtransfusion



Abstract

Background and objectives

Weekly maternal intravenous immunoglobulin (IVIG) is the cornerstone in antenatal treatment of fetal and neonatal allo-immune thrombocytopenia (FNAIT). The aim of this study was to describe the neonatal outcome and management in neonates with FNAIT treated antenatally with IVIG.

Materials and methods

All neonates treated antenatally and delivered at our center between 2006 and 2012 were included in the study. We assessed the neonatal outcome and management, including the occurrence of intracranial hemorrhage (ICH), platelet count at birth and need for postnatal platelet transfusions or postnatal IVIG treatment.

Results

A total of 23 neonates were included of which 12 (52%) had severe thrombocytopenia at birth (platelet count $\leq 50 \times 10^9$/L). Most neonates (75%, 9/12) with severe thrombocytopenia received a platelet transfusion after birth. None of the neonates required postnatal treatment with IVIG. Three neonates had petechiae and hematomas, without clinical consequences. One neonate suffered from ICH, which was detected just before the planned start of antenatal IVIG at 28 weeks' gestation.

Discussion

Our results suggest that antenatal maternal IVIG and, if necessary, postnatal matched platelet transfusions, are effective and safe for treatment of FNAIT.

Introduction

Fetal and neonatal allo-immune thrombocytopenia (FNAIT) is the most common cause of isolated severe thrombocytopenia in the fetus and neonate.[1-3] It is caused by maternal allo-antibodies against antigens of paternal origin on fetal platelets, resulting in platelet destruction and severe fetal and neonatal thrombocytopenia.[4] In 80-95% of the affected cases, FNAIT is caused by fetomaternal incompatibility for human platelet antigen 1a (HPA 1a).[5-8] Approximately 2% of the Caucasian women are HPA-1a-negative (HPA-1bb), of which only 8-12% will become immunized and produce allo-antibodies.[9-14] The most feared complication of severe neonatal thrombocytopenia is intracranial hemorrhage (ICH), with an incidence of 14-20% in untreated first pregnancies affected with FNAIT.[5,15-17] The majority of ICH occurs at the end of the second trimester and clinical outcome are devastating for most cases.[18,19]

The main treatment goal in FNAIT is prevention of (intrauterine) ICH. The optimal antenatal management is controversial and includes an invasive strategy with repeated fetal blood sampling and intrauterine platelet transfusions (IUPT) or a non-invasive strategy with weekly maternal antenatal IVIG.[6,20] Our center, the Leiden University Medical Center (LUMC), is the national referral center for FNAIT-cases in the Netherlands. Our management changed over time from an invasive to a non-invasive strategy.[6] Although antenatal IVIG administration has become the cornerstone of current treatment, questions remain about the optimal dose, best gestational age to start and the exact mechanisms of action.[21] In addition, little is known about the optimal postnatal management. Treatment of first choice after birth is transfusion of matched platelets, but in case of emergency without immediate availability, random platelets are also given. Postnatal IVIG is effective in increasing platelet counts, but the response is much slower compared to platelet transfusions and the risk of destruction of transfused random platelets exists.[15,16,22-24] The aim of this study was to evaluate the neonatal outcome and management in all FNAIT-cases treated antenatally with IVIG during a 6-year period.

Materials and methods

Study population

All infants with FNAIT, treated with antenatal maternal IVIG (Nanogam®) at our center between January 2006 and January 2012, were included in this study. All

these cases were already known due to a previously affected pregnancy and were treated according to a completely non-invasive protocol.[6] Pregnancies were divided in standard and high risk groups, based on the presence or absence of a previous sibling with ICH. In the standard risk group (no sibling with ICH), antenatal IVIG was started at a gestational age of 28 weeks. In the high risk group (previous sibling with ICH), IVIG treatment was started earlier, at 16-18 weeks' gestation. Between 2005 and 2008 the LUMC participated in the NOICH-trial, in which mothers were randomized for a dosage of 0.5 or 1 g/kg maternal weight. The trial was prematurely stopped in 2008 due to shortage of inclusions, however all FNAIT cases are still prospectively collected in an international web-based registry (www.medscinet.com/noich/).
Since 2008, dosage of IVIG in patients with standard risk pregnancies varies between 0.5 g/kg and 1 g/kg maternal weight. Dosage of IVIG in high risk pregnancies is 1 g/kg maternal weight.
At birth, platelets were determined from the umbilical cord using a standardized flow cytometric method, in case of thrombocytopenia <100 x 10^9/L, platelet count determination was repeated manually. Neonates were examined to rule out the presence of hematomas and petechiae. A cranial ultrasound examination was performed in all neonates within 24 hours postpartum. Matched platelet transfusions were administered if platelet count was ≤ 50 x 10^9/L in bleeding neonates or < 30 x 10^9/L in non-bleeding neonates. In 2010, the transfusion trigger for non-bleeding neonates was lowered to 20 x 10^9/L. HPA-1bb/5aa-typed platelet concentrates were available 24 hours a day. In case of emergency (i.e. clinical bleeding) and no immediate availability of matched platelets, random platelets were transfused. When multiple matched platelet transfusions did not result in sufficient rise of platelet count, treatment with IVIG was considered. No strict criterion for the start of IVIG was maintained. Platelet counts were determined at least daily during the first days of life, until a spontaneous rise or stable level was observed. The postnatal protocol was independent of inclusion in the NOICH-study.

Data collection

Data were collected retrospectively and entered in a database, which included also data about previous pregnancies. Antenatal and postnatal baseline characteristics were collected and included HPA incompatibility type, dosage of IVIG, gestational age at start of IVIG, ICH in previous pregnancy, mode of delivery, gestational age at birth, birth weight and Apgar score ≤7 at 5 minutes. Outcome measures were platelet count at birth, presence of hematomas and/or petechiae, occurrence of ICH, number of needed postnatal platelet transfusions

(matched or random), postnatal use of IVIG, course of platelet counts over time and neonatal outcome.

Results

During the 6-year study period, 23 neonates with FNAIT treated with antenatal IVIG were included. Two pregnancies (9%) were considered high risk due to a sibling with ICH, IVIG dosage was set at 1 g/kg starting at 16 weeks' gestation. Twenty-one (91%) pregnancies were considered as standard risk, of which 20 were treated from 28 weeks' gestation onward with 0.5 g/kg or 1 g/kg. Eight pregnancies were included in the NOICH trial and were randomized to receive either 1 g/kg (n=2) or 0.5 g/kg (n=6). One standard risk pregnancy was treated with 1 g/kg maternal weight antenatal IVIG at 35 weeks' gestation, because of a delayed retrospective diagnosis of FNAIT in the previous pregnancy. Another pregnancy in the standard risk population started with 1 g/kg IVIG at 28 weeks' gestation, because of a detected antenatal ICH. No adverse effects of maternal IVIG therapy were reported. A flowchart of all included patients can be seen in figure 1. Baseline characteristics of the included patients are depicted in table 1.

Table 1. Baseline characteristics of included patients (n=23).

		N = 23
HPA type	1a, n (%)	19 (83)
	5b, n (%)	2 (9)
	15a, n (%)	1 (4)
	1a and 5b, n (%)	1 (4)
ICH in previous pregnancy, n (%)		2 (9)
Gestational age at start of IVIG	16 weeks, n (%)	2 (9)
	28 weeks, n (%)	20 (87)
	35 weeks, n (%)	1 (4)
Dosage IVIG	0.5 g/kg, n (%)	17 (74)
	1 g/kg, n (%)	6 (26)
Caesarean delivery, n (%)		8 (35)
Neonates of mothers with miscarriages/spontaneous abortions, n (%)[b]		3 (13)
Gravidity		2.5 ± 1.1 (2-6)
Gestational age at birth, weeks [a]		37.3 ± 1.6 (33-39)
Birth weight, gram [a]		2922 ± 526 (1855-3730)
Apgar score <7 at 5 minutes, n (%)		0 (0)

[a] Value given as mean ± SD (range)
[b] Two included neonates were siblings, so in total two mothers in the population had miscarriages or spontaneous abortions.

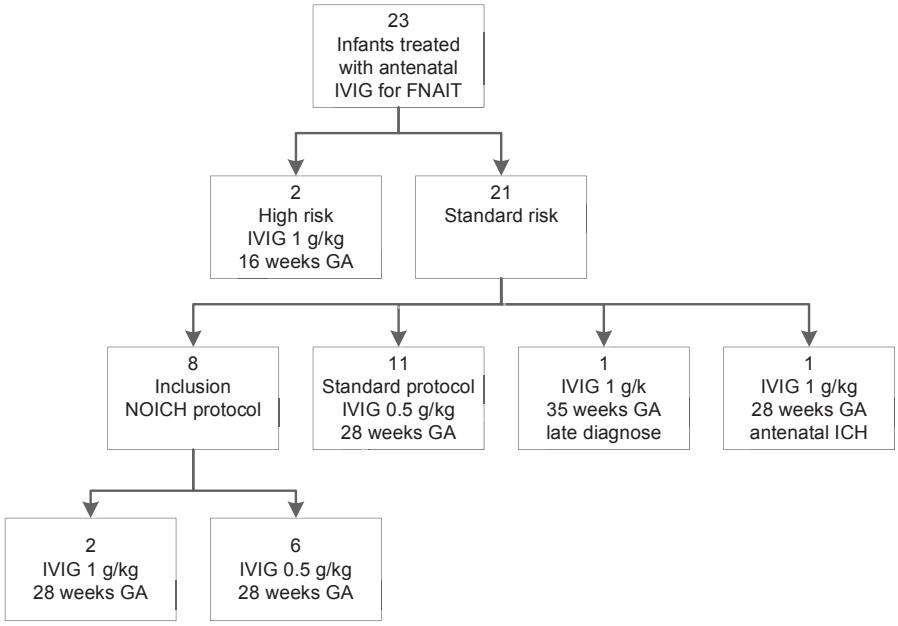


Figure 1. Flowchart of included patients.

Abbreviation:
GA: Gestational age.

Mean platelet count at birth was 91 ± 88 x 10^9/L (range: 6-277 x 10^9/L) and severe thrombocytopenia (platelet count ≤ 50 x 10^9/L) was detected in 12 (52%) neonates. Mean platelet count at birth was 104 ± 89 x 10^9/L in neonates which received 0.5 g/kg IVIG antenatally and 55 ± 83 x 10^9/L in those who received 1 g/kg IVIG. In the group with severe thrombocytopenia, eight neonates required 1 matched platelet transfusion and one neonate needed 2 platelet transfusions, whereas in three neonates the platelet count increased spontaneously. None of the neonates received postnatal IVIG. Mean age at the time platelet count achieved a safe level above 150 x 10^9/L was 3.0 ± 1.6 (1-7) days. Five infants were discharged with a stable platelet count between 100 and 150 x 10^9/L. Individual course of platelet counts of treated neonates is graphically shown in figure 2.

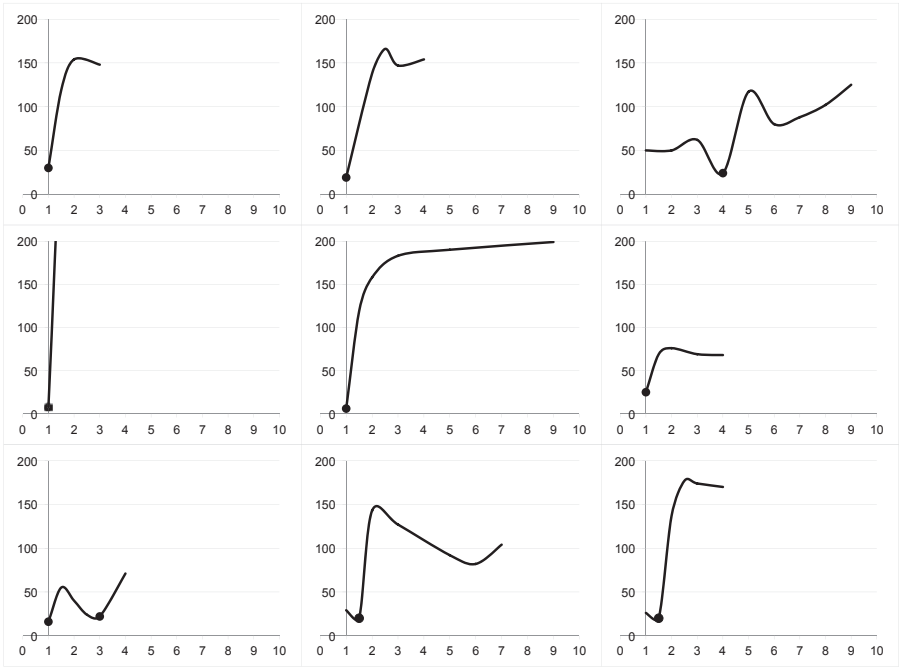


Figure 2. Individual course of platelet count per day.

Legend: Dots indicate the time of platelet transfusions.

Routine cranial ultrasound was performed in all neonates. One neonate had an ICH, which was detected by coincidence during a routine fetal ultrasound at 27 weeks' gestation, just 1 day before the planned start of IVIG treatment. Platelet count at birth was 6 x 10^9/L and the neonate had several petechiae and hematomas. One matched platelet transfusion was administered at birth with good increment. On the second day of life a MRI of the brain showed a left occipital porencephalic cyst, remnant of the antenatal ICH (figure 3). At 2 years of age, a physical and neurodevelopment assessment (using the Bayley Scales of Infant Development III)[25] was performed showing a normal growth and neurodevelopment outcome. Petechiae were detected in two other neonates with platelets of 29 x 10^9/L and 26 x 10^9/L at birth, both had a good response with 1 matched platelet transfusion.

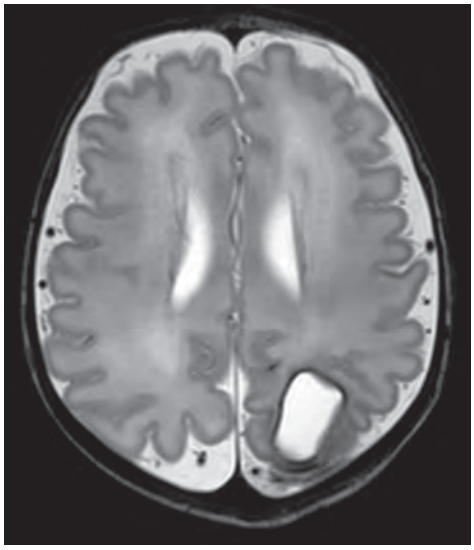


Figure 3. Neonatal cerebral MRI showing a large porencephalic cyst in the left occipital lobe. The cyst is a remnant of a large antenatal ICH, detected at 27 weeks' of gestation.

Five neonates with severe thrombocytopenia were born after 2010, when platelet transfusion trigger in non-bleeding neonates was lowered from 30 x 10^9/L to 20 x 10^9/L platelets. One of them (with a platelet count of 22 x 10^9/L without clinical bleeding) had a spontaneous rise and did not receive a platelet transfusion according to the new protocol. The other 4 infants had platelets <20 x 10^9/L (2) or petechiae or hematomas (n=2) as indication for a platelet transfusion. Table 2 provides an overview of the neonatal outcome.

Table 2. Neonatal outcome.

	N = 23
Platelet count at birth, x 10^9/L[a]	91.1 ± 88.1 (6-277)
Platelet count ≤ 150 x 10^9/L, n (%)	16 (70)
Platelet count ≤ 50 x 10^9/L, n (%)	12 (52)
Platelet count ≤ 30 x 10^9/L, n (%)	9 (39)
Lowest platelet count, days [a]	1.5 ± 0.9 (1-4)
Petechiae and/or hematomas, n (%)	3 (13)
ICH, n (%)	1 (4)
Neonates receiving postnatal platelet transfusions, n (%)	9 (39)
Postnatal age when receiving platelet transfusions, days [a]	1.2 ± 0.7 (1-3)
Postnatal age PC > 150x109/L, days [a]	3.0 ± 1.6 (1-7)

[a] Value given as mean ± SD (range)

Discussion

This cohort study shows a favorable neonatal outcome in FNAIT after non-invasive antenatal maternal treatment with IVIG. Approximately one third (8/23) of the neonates required matched platelet transfusions at birth with a rise in platelets within a few hours and postnatal IVIG was not necessary. An antenatal ICH was detected in only 1 infant at 27 weeks of gestation, just before the start of antenatal IVIG. Despite the cerebral injury, the infant showed a normal neurobehavioral outcome at 2 years of age. Non-invasive management of FNAIT with antenatal maternal IVIG and postnatal matched platelet transfusions seems to be effective and safe.

In this study, approximately one half (12/23) of the neonates had a platelet count at birth ≤ 50 x 10^9/L. The incidence of severe thrombocytopenia found in this study is similar to the incidence reported in several other cohorts (range 34% to 61%).[16,26,27] Berkowitz et al reported a lower incidence of severe thrombocytopenia (14%), but they included only infants without siblings with severe thrombocytopenia or ICH.[28] A sibling with ICH or severe thrombocytopenia is one of the most important risk factors for recurrence of severe thrombocytopenia.[29] Besides neonates with HPA1a-incompatability, we also included cases with HPA5b-incompatibility (2) and HPA15a-incompatibility (1). The incidence of severe thrombocytopenia reported in this study may be influenced by the fact that HPA5b incompatibility is associated with less severe thrombocytopenia.[18,30]

The incidence of ICH in our study was 4% (1/23) and concurrent with the incidence reported by others (range 0-10%).[5-7,16,26-28,31] The only infant with ICH in our study did not have a sibling with ICH and was therefore planned to start with IVIG at 28 weeks of gestation (standard risk group). However an ICH was detected just 1 day before starting IVIG. Whether starting IVIG before 28 weeks of gestation could have prevented of the development of ICH is not known. Consensus on the optimal timing of starting the treatment with IVIG is currently lacking. In our study, severe thrombocytopenia at birth was not associated with an increased rate of ICH. As previously suggested, our study confirms the possible protecting effect of IVIG for ICH even without an increase in fetal platelet counts.[27,32,33] In addition, all infants had an adequate and quick response on postnatal matched platelet transfusions and postnatal IVIG was not necessary. This positive effect of antenatal IVIG in combination with postnatal matched platelet transfusions was also reported in earlier studies.[16,22,24] In contrast to our observations in human beings, reduction of bleeding complications in mouse studies with IVIG was accompanied by an increase of platelet counts.[34]

Several questions on the optimal IVIG treatment remain unanswered, including the optimal dose (0.5 or 1 g/kg), schedule (weekly or more frequently) and gestational age to initiate antenatal IVIG. Our study was not designed nor powered to analyze these issues and was primarily focused on postnatal management. Currently, a large international web-based registry of all FNAIT cases (prospective continuation after the NOICH-trial) may shed more light on this subject in the near future. Data on timing of antenatal intracranial hemorrhages with suggestions for gestational age to initiate antenatal IVIG have already been published.[19]

Prevention of ICH in unknown first cases of FNAIT may also be achieved using antenatal screening on HPA1a negative genotypes in all pregnant women. Still more studies suggest the level of maternal antibody titer during pregnancy as a possible predicting factor for severe thrombocytopenia, although the diagnostic value is not clear as reliable cut off levels were not repeatedly demonstrated yet.[9,13,16] Kjeldsen et al suggested a policy of antenatal screening for immunized HPA1a negative pregnant women and performing a cesarean section at a gestational age of 36-38 weeks. Their incidence of ICH was 2 out of 170 immunized HPA1a negative women, although with lacking control group with at term vaginal delivery no strict conclusions can be drawn from this study.[35] More research is needed to identify women at greatest risk in order to investigate the advantages of offering antenatal treatment with maternal IVIG in a more targeted way.

The retrospective design of this study is a limiting factor; we tried to minimize possible

bias by using strict definitions and cut off values. Another limitation is the relatively small sample size, due to the rarity of this disease. However, sufficient retrospective evidence is needed to design and perform ethical justified randomized controlled trials. In conclusion, our study results suggest that non-invasive antenatal treatment of FNAIT with weekly maternal IVIG and, if necessary, postnatal transfusion of matched platelets is safe and effective. We recommend the use of matched platelets to non-bleeding neonates with a platelet count of < 20 x 10^9/L and to bleeding neonates with a platelet count < 50 x 10^9/L. In general, postnatal IVIG administration can be omitted. More research is needed to optimize dose and schedule of antenatal IVIG treatment and to detect more predicting factors of severe fetal or neonatal thrombocytopenia.

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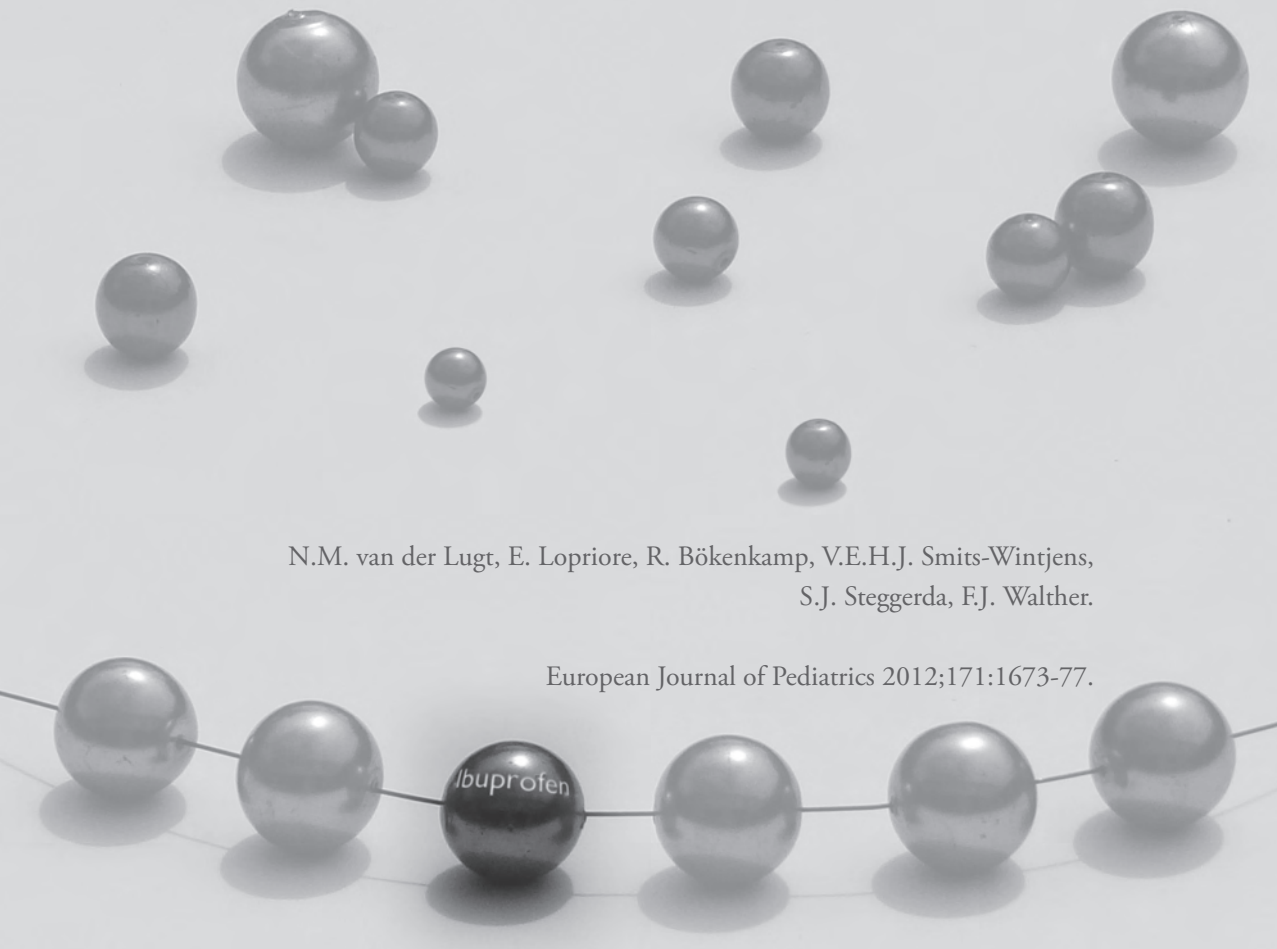
Chapter 4

Repeated courses of ibuprofen are effective in closure of a patent ductus arteriosus



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European Journal of Pediatrics 2012;171:1673-77.

Abstract

Patent ductus arteriosus (PDA) is a frequent complication in preterm infants. Ibuprofen and indomethacin (both COX-inhibitors) are used for pharmacological closure of PDA. In most centers a failed second course of COX-inhibitors is followed by surgical closure.

Our aim was to estimate the closure rate of clinically significant PDA after second and third courses of ibuprofen and record possible side effects.

A study population, consisting of 164 preterm infants (<32 weeks' gestational age) with PDA admitted at our tertiary care center between November 2005 and September 2011, was retrospectively analyzed. Primary outcome was the closure rate after repeated courses of ibuprofen. The closure rate was similar after the first (109/164), second (24/43) an d third (6/11) course of ibuprofen ($X^2 = 2.1$, $p = 0.350$). Late start of the first course of ibuprofen was a predictive factor for increased need of a second course ($X^2 = 4.4$, $p = 0.036$). No additional side effects of multiple courses of ibuprofen were detected.

Conclusion

Repeated courses of ibuprofen are an effective and safe alternative for surgical closure and should be considered after failure of the first course of ibuprofen.

Introduction

Patent ductus arteriosus (PDA) is a frequent complication in preterm infants, with a reported incidence of 28% in preterm infants with a gestational age <32 weeks up to 60-70% in those with a gestational age of <29 weeks.[1,2] PDA is associated with increased neonatal mortality, chronic lung disease and necrotizing enterocolitis. The optimal management of PDA is still not clear as the evidence for and against treatment remains controversial.[1,3-7]

Indomethacin and ibuprofen (both COX-inhibitors) are commonly used for pharmacological closure of PDA and appear to be equally effective.[7-9] However, ibuprofen has shown fewer side effects than indomethacin.[7,10] One course of COX-inhibitors resulted in a closure rate of 68-88% for indomethacin and 45-91% for ibuprofen. A second course led to a lower closure rate of 44-47% for indomethacin and 40-45% for ibuprofen.[10-13]

In most centers, closure failure after a second course of COX-inhibitors is followed by surgical closure. However, surgical closure has been associated with an increased risk of chronic lung disease, retinopathy of prematurity and neurodevelopmental impairment, compared to indomethacin therapy.[14,15] A comparison of morbidity and neurodevelopmental outcome after surgical closure and pharmacological closure with ibuprofen is not available.

Only few studies report on the efficacy and safety of three courses of COX-inhibitors. Two small studies showed a PDA closure rate varying from 16 (3/19) to 43% (10/23) after a third course of indomethacin.[10,16] The effect of a third course of ibuprofen has only been studied in two small studies with a closure rate varying from 19% (7/37) to 66% (2/3), and was associated with more renal complications than first and second courses.[10,13]

The aim of this retrospective study was to estimate the effectiveness and safety of second and third courses of ibuprofen on PDA closure and its potential, as an alternative for invasive and possible harmful surgical closure of the PDA. In addition, possible predicting factors for unsuccessful closure were investigated.

Methods

Study population

We included all preterm infants (gestational age at birth <32 weeks) admitted to the Neonatal Intensive Care Unit (NICU) of the Leiden University Medical Center

between November 2005 and September 2011 with a PDA treated with ibuprofen. We excluded infants with congenital heart disease and infants referred to our center for surgical closure of the PDA. The Medical Ethics Committee of the Leiden University Medical Center did not require approval of this study because it consisted of retrospective chart review, nor did the medical ethics committee require written consent by the parents for their infant's information to be (anonymously) stored in the hospital database and used for research.

Diagnosis of PDA was reached and confirmed using echocardiography (Aloka α10, Biomedic Nederland B.V., Almere, The Netherlands). First echocardiography was made when PDA was suspected, due to clinical symptoms such as widened pulse pressures, cardiac murmur, bounding pulses and/or significant respiratory disease. A PDA was considered clinically significant and requiring treatment, when the ductus was moderate to large in size (>1.5 mm) with evidence of left to right ductal shunting and an increased left atrium to aortic ratio.

Each ibuprofen course was prescribed as 10 mg/kg for the first dose followed by two additional doses of 5 mg/kg on consecutive days. Doses were administered intravenously with a 24 hour interval between each dose. Control echocardiography was routinely performed on the first day after the third dose of ibuprofen. A closed or hemodynamic insignificant ductus was scored as closed. No infants received prophylactic treatment with ibuprofen.

Indications for immediate surgical closure after one or two courses of ibuprofen were congestive heart failure and respiratory instability due to the PDA.

Data collection

Data were collected prospectively throughout the study period and entered in a dedicated database. Data for demographic and perinatal characteristics, as well as postnatal clinical conditions included birth weight, gestational age at birth, gender, intrauterine growth restriction, multiple gestation, pre-eclampsia, chorioamnionitis (defined as smelly amniotic fluid, maternal fever or signs of infection at birth), neonatal sepsis (defined as presence of clinical signs of infection with a positive blood culture), respiratory distress syndrome (RDS)[17], duration of mechanical ventilation, need for postnatal steroids, bronchopulmonary dysplasia (need for oxygen therapy at a gestational age > 36 weeks)[18], hypotension requiring inotropics, necrotizing enterocolitis (NEC)[19], cystic periventricular leukomalacia[20] and intraventricular hemorrhage[21,22], duration of admission (until transfer to another secondary center) and neonatal mortality.

Primary outcome data were closure of the PDA after the first and (if necessary), second or third course of ibuprofen or after surgical ligation. We studied potential risk factors for failure of closure, including general characteristics (gestational age, birth weight), postnatal characteristics possibly pathophysiologic associated with PDA (RDS, duration of mechanical ventilation, NEC, neonatal sepsis) and age at start of ibuprofen for each individual course. Bronchopulmonary dysplasia, duration of admission and mortality were studied as possible consequences of failure of closure. We scored the following potential adverse effects of ibuprofen: oliguria (urine production <1.0 ml/kg/hour) with or without renal failure (serum creatinine level >133 μmol/L[23]), severe thrombocytopenia (platelet count < 50 x 10^9/L after ibuprofen with platelet count > 150 x 10^9/L before).

Statistical analysis

Data are reported as median values and ranges. Statistical analyses were performed with SPSS Version 18.0 (SPSS Inc., Chicago, IL). Data were not normally distributed and therefore analyzed using a Fisher's Exact test. A p-value of <0.05 was considered significant. Study size was determined by the total number of exposed infants in the defined period.

Potential confounders in this study were all demographic and postnatal characteristics, mentioned previously. Confounding was minimized by using regression analysis. Infants with missing characteristics were excluded from regression analyses.

Results

Between November 2005 and September 2011 931 preterm infants with a gestational age <32 weeks were admitted to our NICU. The incidence of PDA was 18% (170/931). Six infants were excluded from analysis because of referral to our center for primary surgical closure of PDA (n=6). A flowchart of the study population can be seen in figure 1. An overview of characteristics of the study population of preterm infants with PDA is shown in table 1.

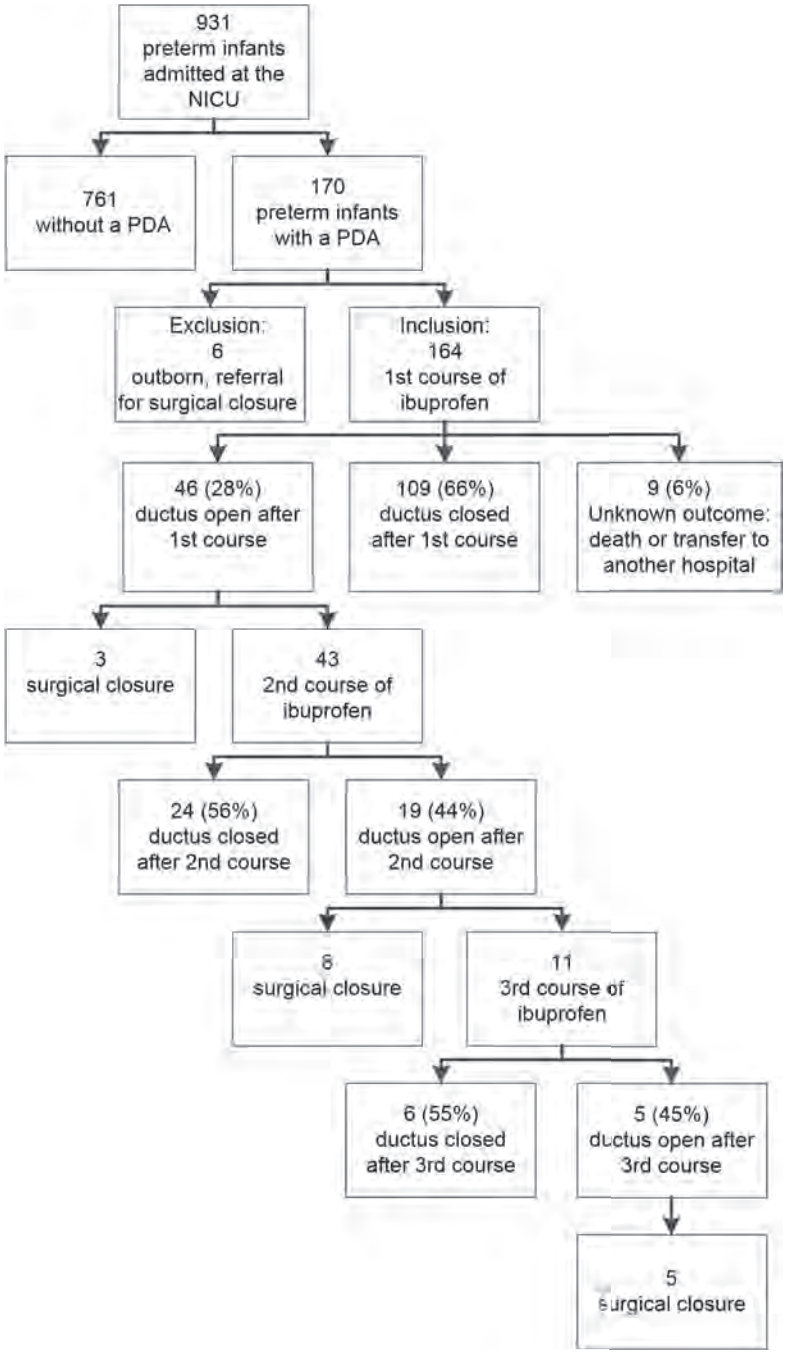

Figure 1. Flowchart of included infants.

Table 1. Characteristics of the studied population.

	N = 164
Gestational age at birth (weeks) [a]	27.0 (23-31)
Birth weight (grams) [a]	995 (525-2041)
Gender (male) – n (%)	81 (49)
Multiple gestation – n (%)	60 (37)
Chorioamnionitis – n (%)	6 (4)
Pre-eclampsia – n (%)	36 (22)
IUGR – n (%)	16 (10)
Respiratory distress syndrome – n (%)	125 (76)
Duration of mechanical ventilation (days) [a]	8 (0-36)
Postnatal steroids – n (%)	42 (26)
Bronchopulmonary dysplasia – n (%)	13 (8)
Hypotension – n (%)	64 (39)
Necrotizing enterocolitis – n (%)	12 (7)
Neonatal sepsis – n (%)	64 (39)
Periventricular leukomalacia (≥ grade 2) – n (%)	6 (4)
Intraventricular hemorrhage (≥ grade 3) – n (%)	15 (9)
Duration of admission (days) [a]	27 (3-138)
Mortality – n (%)	14 (9)

[a] Value given as median (range)

PDA closure rate was similar after the first (109/164), second (24/43) and third (6/11) course of ibuprofen ($X^2 = 2.1$, $p = 0.350$) (table 2).

Table 2. Closure rate of ductus arteriosus after ibuprofen or ligation.

	Courses of ibuprofen		
	1	2	3
Number of infants	164	43	11
Closed ductus after ibuprofen	109	24	6
Closed ductus after ligation	3	8	5
Unknown outcome	9		

We studied several general characteristics, postnatal characteristics and age at start of ibuprofen for possible associations with failure of closure (as potential risk factors or consequences). Analysis of each ibuprofen course separately, showed a positive

correlation between postnatal age at start of the first dose of ibuprofen and the need for a second course of ibuprofen: the older the neonate (in terms of postnatal age at start of ibuprofen), the lower the chance of definitive PDA closure after one course of ibuprofen. The median age at the start of the first course of ibuprofen in the group with successful closure after one course was 4 days (range 2-24), compared to 5 days (range 2-19) in the group with unsuccessful closure after one course. Closure rate after the first course of ibuprofen significantly increased if ibuprofen treatment was started prior to postnatal day 5: 64/84 when started before day 5 versus 43/71 when started on day 5 or later ($X^2 = 4.4$, $p = 0.036$).

Oliguria was observed in 10 infants (9 during the first, 1 during the second course of ibuprofen). One infant had an increased serum creatinine level >133 μmol/L during the first course of ibuprofen without oliguria. Four infants developed severe thrombocytopenia (platelet count < 50 x 10^9/L) during the first course of ibuprofen, without clinical signs of bleeding. All reported side effects were transient and resolved spontaneously. No relationship was found between adverse effects and the number of courses of ibuprofen.

Discussion

This study shows that the closure rate of PDA after a second or third course of ibuprofen was similar to the closure rate after the first course and that multiple courses of ibuprofen were not associated with an increase in adverse effects.

Recently, two small studies reported on the PDA closure rate in a population of premature infants receiving multiple courses of ibuprofen. In a study in 182 preterm infants, Kushnir et al found closure rates for the first, second and third course of 92% , 54% and 19%, respectively[10], whereas PDA closure rates in a study from Richards et al were 45%, 40% and 66%, respectively[13]. In comparison, the closure rates in our study cohort were 66%, 56% and 55%. The cumulative closure rate after all courses of ibuprofen was 71% (130/183) in the study by Kushnir et al[10] and 69% (110/160) in the study by Richards et al[13] versus 85% (139/164) in this study. Differences in closure rates between the studies are probably due to differences in study designs and methodology. The preterm infants in the study of Richards et al[13] had a lower mean gestational age and birth weight (25.6 weeks, 757 gram) than the infants included in our study. In the study of Kushnir et al[10] mean gestational age and birth weight were comparable (27.8 weeks, 1083 gram).

Kushnir et al[10] reported more adverse effects, i.e. an increase of serum creatinine levels and decrease of urine output in infants receiving more than one course of ibuprofen. We found no additional adverse effects during second and third courses of ibuprofen in our study, similar to the findings of Richards et al[13]. The most remarkable difference between the patient populations was the duration between the first and second course of ibuprofen (1 day in Kushnir et al, 6 days in Richards et al versus 3 days in our study population) and the second and third course of ibuprofen (1 day in Kushnir et al versus 5 days in our study).[10,13] The relatively short interval between the treatment courses in the Kushnir study might explain the increased risk of adverse effects.

The value of our study lies in the meticulous analysis of a large cohort, the focus on timely start of ibuprofen therapy and evaluation of current experience with repeated courses of COX-inhibitors versus surgical closure. The importance of this type of information is supported by the web-based questionnaire survey by Amin et al which evaluated the use of repeated courses of indomethacin in neonatal intensive care programs in the USA. Those centers using more than two courses of indomethacin, based their usage of a third course on local experience, because of lack of evidence on repeated courses in clinical literature.[24] The reported harmful effects of surgical ductal closure, such as chronic lung disease, retinopathy and neurodevelopmental impairment, warrant in depth investigation of a pharmacological alternative without major side effects.[14,15]

Several factors have been associated with a higher closure rate after pharmacological treatment, including earlier age and a large ductal diameter (>2 mm) at start of the first course of ibuprofen.[25] Our findings confirm the association between advanced postnatal age at the start of the first course and failure of ductal closure after the first course of ibuprofen. Closure rate after the first course of ibuprofen significantly increased if ibuprofen treatment was started prior to postnatal day 5. Similarly, in a study using indomethacin, re-opening of the ductus arteriosus with echographic luminal flow after the first course of indomethacin was more common in infants who received their indomethacin course at a higher postnatal age.[26] None of these studies showed a relationship between closure rate and starting age of the second and third course of ibuprofen or indomethacin.

The higher closure rate after the first course of ibuprofen, if started at a lower postnatal age, maybe due to the pharmacokinetic characteristics of ibuprofen. Ibuprofen is metabolized by the cytochrome P450 complex, especially the CYP2C9 and CYP2C8 enzymes. Directly after birth these enzymes are absent in the serum, during the first week of life they increase to 33% of the adult serum level, independent of gestational

age. Therefore, the available concentration of ibuprofen decreases during the first week of life due to increased metabolism, resulting in less bio-availability for closure of the ductus.[27,28] The reason why the starting age of the second and third course of ibuprofen does not influence the closure rate is not clear. Hypothetically, it could be expected that the fast increase in serum level of the CYP enzymes during the first week of life will flatten to a stable level in the next weeks, without influencing closure rate anymore.

The results of this study may have important consequences for clinical practice, especially when a second course of ibuprofen fails to close the PDA. Our data suggest that a third course of ibuprofen may be an effective alternative to surgical closure, without additional risks for pharmacological side effects. As shown in this study, closure rates of a second and third course of ibuprofen are independent of postnatal age at the start of the consecutive course. Therefore, advanced postnatal age may not be an argument in favor of surgical ligation instead of a second or third course of ibuprofen.

For many years PDA closure was the main goal in ductal management. More recently, various studies have raised important questions concerning the optimal management of PDA and the potential benefits of ductal closure.[4,29,30] The management of PDA at our institution is to date still based on ductal closure. This will remain our standard of care unless new evidence is provided showing that ductal closure is associated with more disadvantages compared to conservative management.

Although our results add to the understanding of efficacy and safety of repeated courses of ibuprofen on PDA closure, we recognize there are several potential limitations. The retrospective design of this study is the first limiting factor. We tried to minimize possible bias by using strict definitions and cut-off values. A second limitation is the relatively small size of the study population receiving multiple ibuprofen courses. Nevertheless, retrospective and small studies are necessary to design future randomized controlled trials. Such a trial is urgently required to determine whether short and long-term outcome of pharmacological management of PDA with multiple courses of ibuprofen is better or worse than surgical closure. In addition, a larger prospective study using a third course of ibuprofen is needed to determine its pharmacological effects and side-effects.

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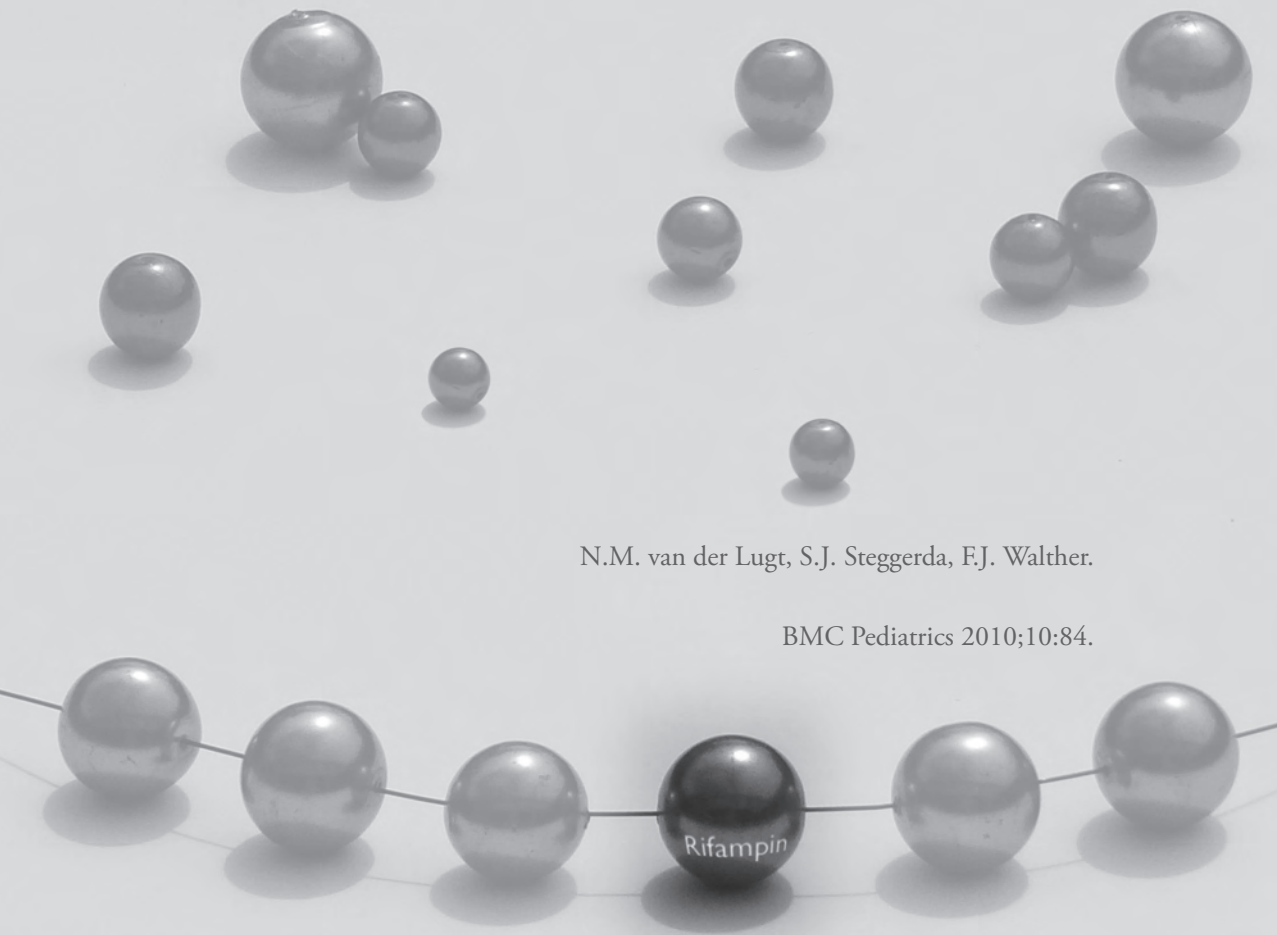
Chapter 5

Use of rifampin in persistent coagulase negative staphylococcal bacteremia in neonates



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BMC Pediatrics 2010;10:84.

Abstract

Background

Coagulase negative staphylococci (CoNS) are the most common cause of neonatal sepsis in the Neonatal Intensive Care Unit (NICU). A minority of neonates does not respond to vancomycin therapy and develops persistent bacteremia, which may be treated with rifampin. We evaluated the use of rifampin in persistent CoNS bacteremia.

Methods

Retrospective study of 137 neonates with CoNS bacteremia during admission to a tertiary NICU between July 2006 and July 2009. Main outcome measures were total duration of bacteremia and the adequacy of vancomycin and rifampin therapy.

Results

137/1696 (8.0%) neonates developed a CoNS bacteremia. Eighteen were treated with rifampin because of persistent bacteremia (3 positive blood cultures at least 48 hours apart with clinical symptoms) or (a serious suspicion of) an intravascular thrombus. Duration of bacteremia prior to rifampin therapy (8.0 ± 3.6 days) was positively correlated ($p<0.001$) to the total duration of bacteremia (10.3 ± 3.7 days). After starting rifampin therapy C-reactive protein (CRP) levels of all neonates declined and blood cultures became sterile after 2.3 ± 1.6 days. Vancomycin levels were not consistently measured in all neonates, resulting in late detection of subtherapeutic trough levels.

Conclusion

Rifampin may be effective in the treatment of persistent CoNS infections in neonates. Outcome may be improved by adequate monitoring of vancomycin trough levels.

Introduction

Sepsis due to coagulase negative staphylococci (CoNS) is common in the neonatal intensive care unit (NICU). The incidence of CoNS sepsis varies between 1.3 and 19.9%, depending on birth weight and gestational age.[1-5] Most of these infections respond well to vancomycin, the first drug of choice. A minority of neonates develops a persistent staphylococcal bacteremia, which does not respond to vancomycin. For these neonates rifampin may be a safe and effective additive treatment to vancomycin.[1,6-8] Interaction between vancomycin and rifampin in treatment of staphylococcal infections is ambiguous, as some studies demonstrate antagonism and others synergism or indifference.[9-12] High concentrations of rifampin may result in antagonism.[13,14] Rifampin is only effective as combination therapy, because resistance develops when rifampin is used as monotherapy.[15]

Through its highly lipophilic character, rifampin molecules can easily cross biological membranes, resulting in a wide tissue distribution.[6,16] The efficacy of rifampin in persistent staphylococcal bacteremia is due to its abilities to enhance serum bactericidal activity and to penetrate phagocytic leukocytes for intraleukocytic killing of staphylococci.[17]

Pharmacokinetic research has demonstrated a positive correlation between the duration of rifampin therapy and its clearance, the equilibrium clearance is achieved after one to two weeks. The increase in clearance and decrease in half-life are probably due to auto-induction of the metabolism of rifampin and require caution to maintain serum levels within the therapeutic range by adjusting the dose of rifampin, when necessary.[6,16,18]

Although CoNS bacteremia is common in NICUs and the treatment of persistent CoNS bacteremia with rifampin seems successful, previous studies were only small case reports or studies focusing on pharmacokinetics. The aim of this study was to evaluate the existing local guidelines for the use of rifampin therapy in persistent CoNS infection, checking the current indications to start rifampin therapy and estimating its efficacy.

Methods

Study population

The study population of this retrospective chart review consisted of all neonates admitted to the neonatology department of the Leiden University Medical Center (LUMC) between July 2006 and July 2009. The Medical Ethics Committee of the

LUMC did not require approval of this study because it consisted of retrospective chart review, nor did the medical ethics committee require written consent by the parents for their infant's information to be stored in the hospital database and used for research. Approval by the ethics committee and informed consent was not necessary as the patient data were analyzed anonymously.
Inclusion criterion was the presence of a positive blood culture for CoNS. Persistent CoNS bacteremia was defined as 3 positive blood cultures, spaced at least 48 hours apart, in combination with clinical symptoms of sepsis. The indication to start rifampin treatment was persistent CoNS bacteremia despite treatment with vancomycin and removal of indwelling catheters, or a non-persistent CoNS bacteremia in combination with a proven intravascular thrombus. Starting dose of rifampin was 10 mg/kg/day intravenously.

Data collection

Data on demographic, perinatal and postnatal clinical characteristics were collected to provide an overview of baseline characteristics and included birth weight, gestational age, gender, exposure to prenatal and postnatal steroids, presence of chorioamnionitis, hyperglycemia, prolonged rupture of membranes (PROM), asphyxia, respiratory distress syndrome (RDS)[19], bronchopulmonary dysplasia (BPD)[20], necrotizing enterocolitis (NEC)[21], cystic periventricular leukomalacia (PVL)[22] and intraventricular hemorrhage (IVH)[23,24]. These data were collected from the neonatal charts and used to compare neonates with non-persistent and persistent CoNS bacteremia.
Primary outcome measures were the total duration of bacteremia and the adequacy of vancomycin treatment, estimated by following trough levels obtained after the initiation of vancomycin therapy until the tenth day of rifampin therapy. The desired range of trough levels of vancomycin was 5-10 mg/L, trough levels <5 mg/L were considered to be subtherapeutic and the desired range for peak levels was 20-30 mg/L. Other variables studied included plasma urea and creatinine levels and duration of vancomycin therapy.
Main outcomes for analysis of the group of rifampin treated neonates were total duration of bacteremia and rapidity of sterilization of blood cultures after the start of rifampin. Age at start of infection, CRP levels from the first day of CoNS positive blood culture until the tenth day of rifampin treatment, and duration and dose of rifampin treatment were additional variables among rifampin treated neonates.
Identification of CoNS isolates was performed by the microbiology department using Bactec Peds Plus bottles (Becton and Dickinson, Franklin Lakes, NJ USA). Blood cultures, complete blood count and CRP were drawn upon clinical suspicion of sepsis.

CRP levels were determined daily during therapy with antibiotics. Vancomycin serum samples were drawn just before the third dose and 1 hour after administration of the third dose. When the dosage of vancomycin was changed, another serum sample was drawn around the second dose after the change. CRP levels were measured using a immunoturbidimetric assay (imCRP, detection limit ≥3 mg/L[25]) and serum vancomycin levels by a fluorescence polarization assay[26].
CoNS bacteremia was an indication for removal of central venous lines and sonography for a remaining vascular thrombus.

Statistical analyses

Data are reported as mean values ± standard deviation, minimum and maximum, numerical values or categories. Analyses were performed with SPSS Version 16.0 (SPSS Inc., Chicago, IL). Numerical data were analyzed by bivariate Pearson correlation and unpaired T-tests, categorical data were analyzed using a chi-squared test. To correct for potential confounding effects, logistic regression analysis was done.

Results

In the period between July 2006 and July 2009 1696 neonates were admitted to the NICU with a mean birth weight of 1271 ± 663 gram and a gestational age of 29.2 ± 3.2 weeks. The incidence of CoNS bacteremia was 137/1696 (8%), 17 (12%) of these neonates developed a persistent CoNS bacteremia and in 3 of them an intra-vascular thrombus was identified. One neonate with a CoNS sepsis also had a S. aureus sepsis.
A flowchart of the included patients can be seen in figure 1.
Baseline characteristics of the included patients are listed in table 1.
Newborn infants with persistent CoNS bacteremia had lower birth weights (p = 0.008) and, independent of birth weight, more often hyperglycemia (p = 0.007), than infants with non-persistent CoNS bacteremia. Subtherapeutic vancomycin trough levels were equally divided among the groups with persistent and non-persistent CoNS bacteremia (p = 0.712).
Eighteen of the 137 neonates received rifampin treatment, started after 8.0 ± 3.6 days of CoNS bacteremia. 13/18 neonates had persistent CoNS bacteremia (in three of them an intravascular thrombus was found), 3/18 had an intravascular thrombus with a non-persistent CoNS bacteremia, 2/18 received rifampin because of increasing CRP

levels during vancomycin therapy in combination with severe thrombocytopenia and a serious suspicion of an intravascular thrombus.
Figure 2 shows the course of the CRP levels before and after the first CoNS positive blood culture, for both infants treated with and without rifampin (start of rifampin is marked, dotted lines represent infants without rifampin therapy).

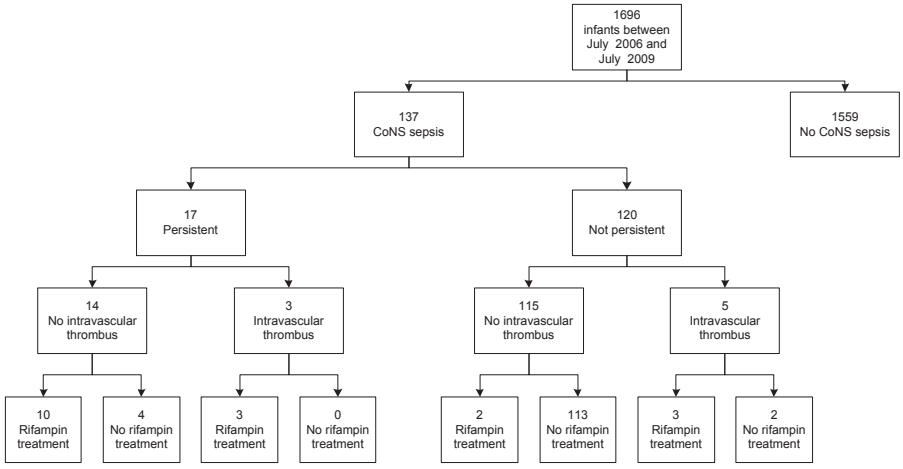

Figure 1. Flowchart of included infants

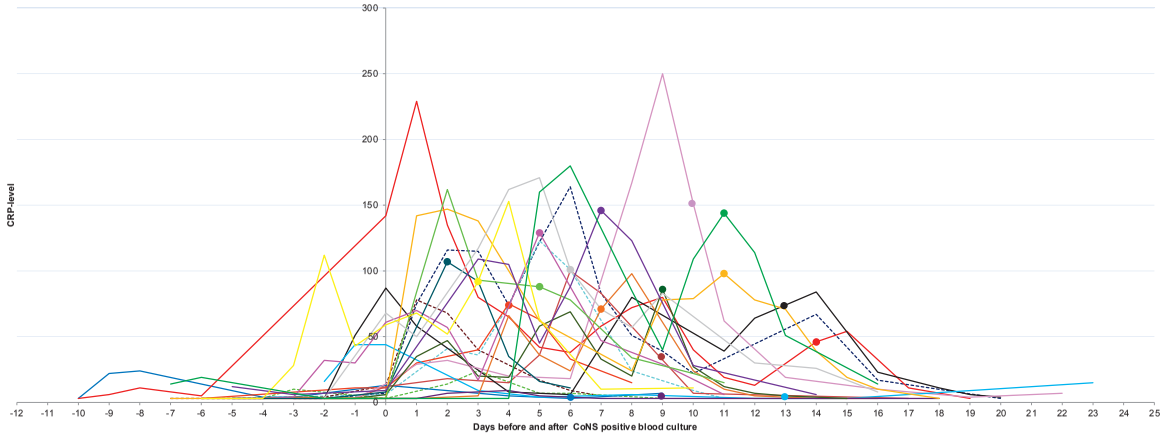

Figure 2. CRP levels (mg/L) from 10 days before positive blood culture until maximum of 10 days after negative blood culture.

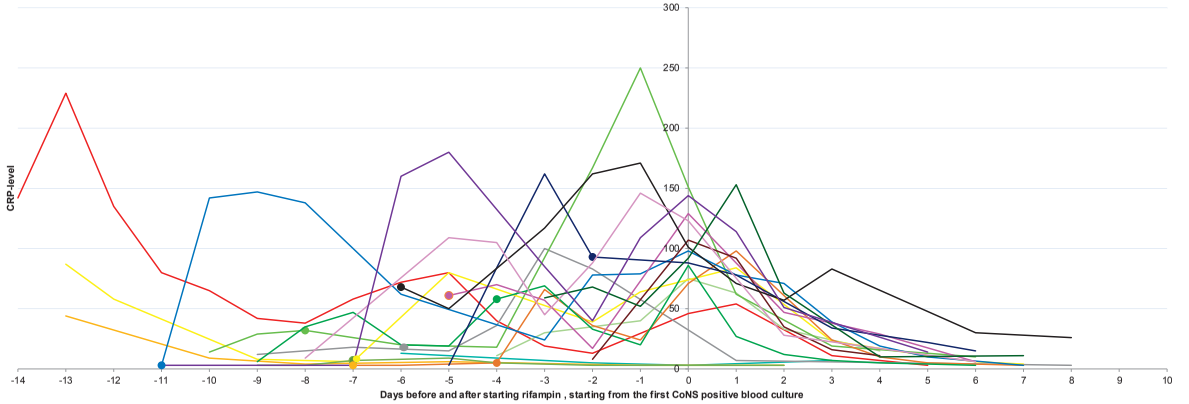

Figure 3. CRP levels (mg/L) from the first day of CoNS-positive blood culture until 10th day after starting rifampin treatment.

Table 1. Baseline characteristics of all included patients.

		Non-persistent CoNS bacteremia N = 120	Persistent CoNS bacteremia N = 17	p-value
Prenatal steroids – n (%)				0.668
	0 doses	50 (43.1)	9(52.9)	
	1 dose	47 (40.5)	5 (29.4)	
	2 doses	19 (16.4)	3 (17.6)	
Chorioamnionitis – n (%) [b]		9 (7.8)	1 (5.9)	0.784
PROM – n (%) [c]		14 (12.0)	2 (11.8)	0.981
Asphyxia – n (%) [d]		7 (5.8)	0 (0)	0.307
Gestational age (weeks) [a]		29.4 ± 3.3	28.0 ± 2.3	0.093
Birth weight (grams) [a]		1327 ± 686	874 ± 204	*0.008*
Gender (male) – n (%)		71 (59.2)	10 (58.8)	0.979
				Multivariate p-value (corrected for birth weight)
Hyperglycemia – n (%) [e]		8 (6.7)	8 (47.1)	*0.007*
IVH grade 3/4 – n (%)		9 (7.5)	0 (0.0)	0.999
Cystic PVL – n (%)		2 (1.7)	1 (5.9)	0.640
NEC grade 2/3 – n (%)		4 (3.3)	2 (11.8)	0.766
RDS grade 3/4 – n (%)		23 (19.2)	7 (41.2)	0.788
BDP – n (%) [f]		26 (21.7)	9 (52.9)	0.771
Postnatal steroids – n (%)		7 (5.8)	4 (23.5)	0.652
Died during admission – n (%)		3 (2.5)	1 (5.9)	0.754

[a] Value given as mean ± standard deviation
[b] Smelly amniotic fluid, maternal fever or signs of infection at birth
[c] Rupture of membranes >24 hours
[d] Presence of minimal 3 criteria:
1) Decelerative CTG or meconium containing amniotic fluid
2) Umbilical cord pH <7.10
3) Apgar score <5 after 5 minutes
4) Spontaneous respiratory depression >5 minutes after birth
5) Multiple organ failure
[e] Glucose levels of >10 mmol/L during >12 hours, treated with insulin >12 hours
[f] Need for oxygen-therapy at a gestational age of 36 weeks or at discharge

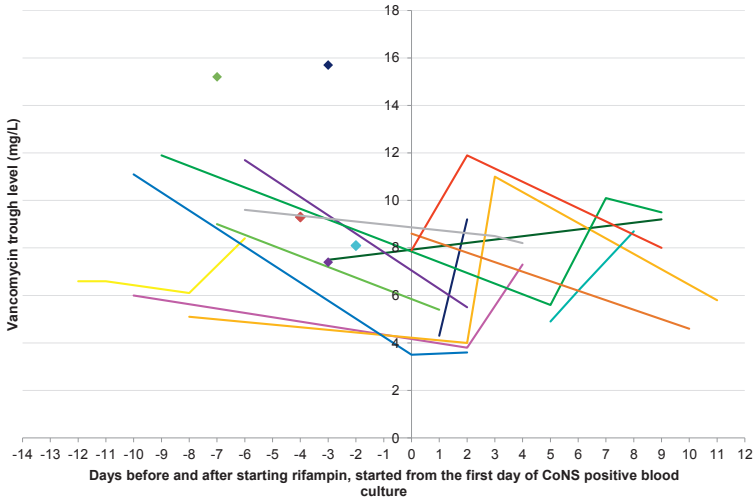

Figure 4. Vancomycin trough levels (mg/L) of rifampin treated infants from the first day of CoNS-positive blood culture until 10th day after starting rifampin treatment.

Table 2. Infection- and pharmacokinetic parameters of neonates treated with rifampin.

N = 18	Mean	SD	Min	Max
Age at start of bacteremia (days)	5.4	2.7	1	11
Days of bacteremia before start of rifampin	8.0	3.6	2	14
Days of vancomycin/ceftazidim therapy before start of rifampin	8.9	4.5	2	20
Maximum peak level vancomycin (µg/ml)	26.7	3.5	20.0	32.0
Minimum peak level vancomycin (µg/ml)	20.3	4.7	12.8	28.0
Maximum trough level vancomycin (µg/ml)	10.8	2.6	7.3	15.9
Minimum trough level vancomycin (µg/ml)	7.3	3.6	3.5	15.9
CRP at start of rifampin (mg/l)	77.5	44.8	3	146
Maximum CRP during bacteremia (mg/l)	120.3	64.4	9	250
Duration of rifampin therapy (hour)	147.2	52.5	52	264
Dose of rifampin (mg/kg/day)	10.4	3.0	6.0	17.0
Rapidity of sterilization of blood culture after start of rifampin (days)	2.3	1.6	0	6
Total duration of bacteremia (days)	10.3	3.7	4	15

CRP levels from the first day of a CoNS positive blood culture until the tenth day of rifampin therapy demonstrate a serious decline in CRP levels after starting rifampin therapy (figure 3). Indwelling catheters were removed before the first CoNS positive blood culture (6 times), on the day of the first CoNS positive blood culture (3 times) or after the first CoNS blood culture (6 times, although in all these infants maximum CRP was achieved after removal of the catheter). The most important decline in CRP

occurred during the first 3 days after the start of rifampin. In these first 3 days the blood culture of most neonates became sterile, with a mean duration of 2.3 ± 1.6 days. Values of other infection and pharmacokinetic parameters of the neonates treated with rifampin are listed in table 2.

Before the start of rifampin all neonates received vancomycin as monotherapy (n = 1) or in combination with ceftazidim (until definitive identification and antimicrobial susceptibility testing of gram-positive cocci in clusters) (n = 17). The duration of vancomycin therapy was 8.9 ± 4.5 days. Ten neonates had adequate initial vancomycin levels, in 8 infants vancomycin dosage had to be readjusted. Vancomycin trough levels between the first day of CoNS positive blood culture and the tenth day of rifampin treatment are presented in figure 4. In contrast to vancomycin levels, rifampin levels were never obtained.

The presence of an intravascular thrombus did not correlate with the total duration of CoNS bacteremia or with the rapidity of sterilization of blood cultures after the start of rifampin treatment.

Discussion

Comparing our incidence of CoNS bacteremia (8%) with other studies is difficult, as the composition of study populations vary. Most studies report a lower incidence, probably due to higher birth weights and gestational ages in these populations.[2-4] One study reported an incidence of 19.9%, but neonates in this study had a lower gestational age.[5] Effectiveness of rifampin treatment in persistent staphylococcal bacteremia in neonates has been demonstrated in several case reports and pharmacokinetic studies, in which speed of sterilization of the blood culture was the main outcome.[1,6-8] Our data also show a substantial decline in CRP during the first days of rifampin treatment. To our knowledge, studies evaluating the treatment of CoNS bacteremia, focusing on the adequacy of monitoring and responding to vancomycin trough levels and the compliance with starting rifampin after 3 positive blood cultures with an interval of 48 hours, have not been reported earlier.

This retrospective study has several limitations. The most important one is the small size of the study population (18 patients) available for evaluation of rifampin treatment. As most neonates with a CoNS bacteremia respond well to vancomycin, rifampin is given only incidentally. Another limitation is the absence of an appropriate control group. As 4 patients with a persistent CoNS bacteremia did not receive rifampin, this group was too small for comparison purposes. Comparison of the occurrence of

vancomycin levels below the therapeutic margin between persistent and non persistent bacteremia appeared difficult, as vancomycin levels were not regularly assessed in all neonates, especially in neonates with a non-persistent bacteremia.
Comparing the groups with and without persistent CoNS bacteremia, significant differences were seen for birth weight and the presence of hyperglycemia. Hyperglycemia is caused by relative insulin deficiency and resistance, due to high levels of circulating cytokines and inflammatory markers during sepsis.[27] Persistent bacteremia may increase the risk for developing co-morbidity such as hyperglycemia. No clear statements can be made about the possible influence of adequate monitoring and the response to vancomycin trough levels on the risk of developing a persistent CoNS bacteremia. Because vancomycin levels were not consistently obtained in all neonates (especially in those without persistent CoNS-bacteremia), an accurate comparison of the occurrence of subtherapeutic vancomycin trough levels of infants with and without a persistent CoNS bacteremia was not possible.

Conclusion

Our results suggest that the treatment strategy for persistent staphylococcal bacteremia with rifampin may be effective, but can be optimized by improving the monitoring of vancomycin trough levels and minimizing the delay in starting rifampin treatment. If, in spite of adequate vancomycin levels, CoNS bacteremia becomes persistent, rifampin therapy may be started after 6 days of bacteremia (3 positive blood cultures with a 48 hours interval after each).

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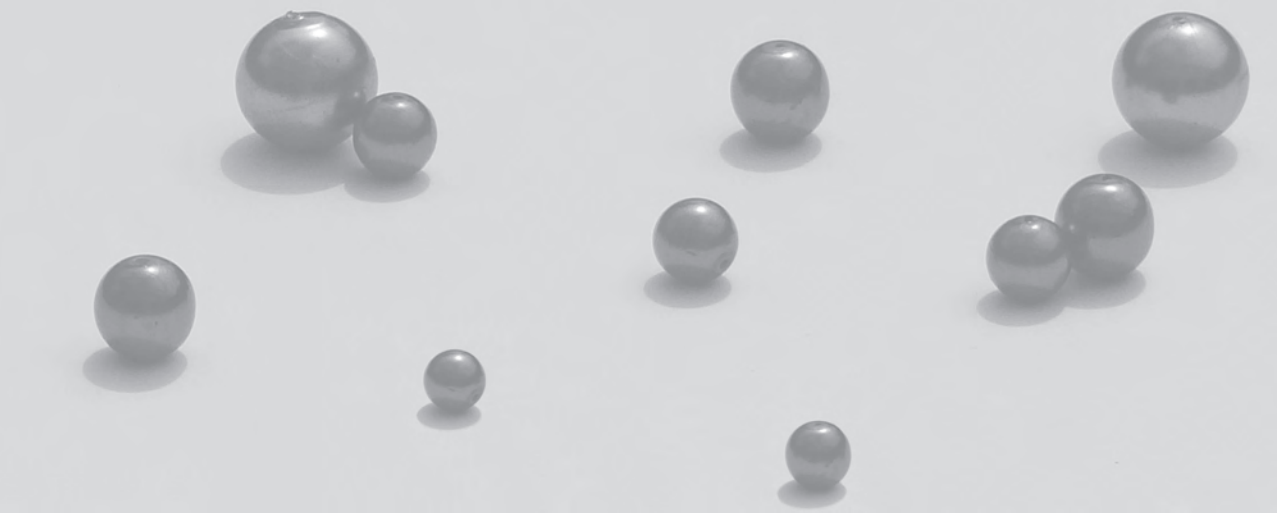
Chapter 6

Outcome and management in neonatal thrombocytopenia due to maternal idiopathic thrombocytopenic purpura



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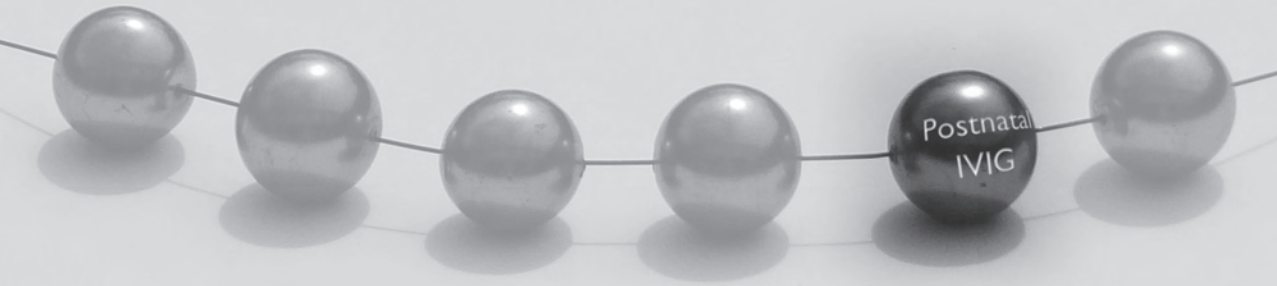
Vox Sanguinis 2013;105:236-43.



Abstract

Background and objectives

Pregnant women with Idiopathic Thrombocytopenic Purpura (ITP) can deliver neonates with severe thrombocytopenia. Clear evidence declaring the pathophysiological cause of this neonatal thrombocytopenia is lacking, as anti-platelet antibodies are not always detectable in maternal serum. Severe neonatal thrombocytopenia below 50 x 10^9/L is reported in 8-13 % of the neonates from mothers with ITP and, intracranial hemorrhage (ICH) in 0-2.9%. Evidence about the optimal postnatal treatment is scarce. Our objective was to evaluate the outcome and management in neonates with passive ITP.

Materials and methods

All neonates from mothers with ITP born between 1980 and 2011 were included. Platelet counts during the first 10 days, presence of ICH and postnatal treatment were recorded. Maternal characteristics were analysed as possible risk factors for severe neonatal thrombocytopenia.

Results

Sixty-seven neonates were included. Severe thrombocytopenia (<50 x 10^9/L) occurred in 20/67 (29.9%) neonates. In 3 neonates platelet count rose spontaneously, 18 neonates were treated (1 with persistent moderate thrombocytopenia) with: platelet transfusions (3), prednisone (2), IVIG (1), platelet transfusions and IVIG (11), platelet transfusions and prednisone (1). Recurrence of low platelet counts after transfusions were commonly seen. Risk factors for severe neonatal thrombocytopenia were a previous sibling with severe thrombocytopenia and low maternal platelet nadir during pregnancy.

Conclusion

In this cohort severe neonatal thrombocytopenia occurs more frequently than previously reported. To maintain a platelet count above 50 x 10^9/L often multiple transfusions and IVIG are required. Multiple transfusions may be avoided by starting IVIG, when platelet count falls below 50 x 10^9/L after the first platelet transfusion.

Introduction

Idiopathic thrombocytopenic purpura (ITP) has an incidence of 1-10 in 10.000 pregnant women; in one-third ITP presents during pregnancy.[1,2] ITP is considered to be caused by auto-antibodies (IgG) against non-polymorphic platelet antigens, although antibodies cannot be detected in all women with ITP. Hypothetically these IgG antibodies can be transported through the placenta and can cause destruction of fetal platelets, but evidence for this hypothesis lacks.[3,4] Severe thrombocytopenia (<50 x 10^9/L) in infants of mothers with ITP is however present in only 8-13% of the neonates. As opposed to fetal and neonatal allo-immune thrombocytopenia (FNAIT), severe bleeding complications such as intracranial hemorrhage (ICH) are more rare, with a reported incidence of 0-2.9%.[1,2,5-12]

Several risk indicators to predict severe neonatal thrombocytopenia have been explored. Most consistent are a previous sibling with thrombocytopenia[2,6,9,10] and maternal splenectomy for therapy resistant ITP.[1,6,11,13,14] Other factors such as maternal platelet counts (during pregnancy and delivery), presence of detectable anti-platelet antibodies in maternal serum and maternal treatment with corticosteroids and/or intravenous immunoglobulin (IVIG) do not correlate with neonatal platelet count at birth.[1,5,6,8,10,11,13,15] A small randomized controlled trial comparing maternal betamethasone with placebo showed no difference in neonatal platelet count.[16]

The natural course of platelet counts in neonates of mothers with ITP describes a platelet nadir at postnatal day 3-5, after which platelets will stabilize or rise spontaneously.[1,3,12,17,18] However, the optimal postnatal treatment for severe neonatal thrombocytopenia is not evident and includes IVIG, platelet transfusions and/or prednisone. [2,4,6,7,15,19-21] Evidence on the neonatal outcome, risk factors and optimal management is scarce due to lack of studies. The aim of our study is to describe a relative large cohort of neonates, exposed to maternal ITP during pregnancy, with the focus on postnatal management and outcome.

Materials and methods

Study population

Neonates born between April 1980 and October 2011 from mothers with ITP, controlled during pregnancy by the hematology department of the Leiden University Medical Center were included in this study. Criteria for maternal diagnosis of ITP

were isolated thrombocytopenia for which other causes were excluded, normal or hypermegakaryocyte production in bone marrow, normal white and red blood cell counts. Mothers had been treated prior to pregnancy with corticosteroids, intravenous immunoglobuline (IVIG), platelet transfusions, cyclokapron, cyclosporin and/or splenectomy. Maternal autoantibodies were detected by direct platelet Immunofluorescence using anti-IgG and autologous platelets (if above 30 x 10^9/L). Serum antibodies were evaluated by ELISA distinguishing HLA class I antibodies and antibodies against platelet GP IIb/IIIa and Ib/IX. Detection of maternal anti-platelet antibodies against autologous platelets or in serum was not systematically performed as this was not required for diagnosis of ITP, nor to distinguish gestational thrombocytopenia from ITP in mothers presenting with thrombocytopenia in pregnancy.[22]

At birth, platelets were determined from the umbilical cord or capillary as soon as possible on the first day of life; the neonates were examined physically for hematomas and petechiae. Since 1998 cranial ultrasound was routinely made in each neonate with a platelet count of < 50 x 10^9/L. Management protocols changed over time, so different postnatal treatment approaches were followed during the past decades. In most cases indication for platelet transfusion was a platelet count < 50x 10^9/L. Platelet counts were determined at least daily during the first 5 days of life or longer until a spontaneous rise or stable level was observed.

Postnatal neonatal treatment

Platelet transfusions were prepared from bloodgroup O-Rh-D negative donors by apheresis; platelets were leukocyte reduced by filtration prior to storage and volume was reduced prior to transfusion. The administered dose was approximately 20 x 10^9/kg recipient body weight. For newborns with a gestational age below 32 weeks platelets were irradiated (25Gy).

Intravenous immunoglobulin (Immunoglobulin IV/Nanogam, Sanquin, Amsterdam, The Netherlands) was administered in standard dosage of 0.4 g/kg/day. Treatment was continued for 3-5 days, dependent on the severity of neonatal thrombocytopenia and response (i.e. a rising platelet count above 50 x 10^9/L during a couple of successive days).

Prednisone was started with a dosage of 2 mg/kg/day, which was decreased when platelet count increased.

Data collection

Data were collected retrospectively and entered in a database. Antenatal and postnatal baseline characteristics were collected and included maternal diagnosis of ITP before or during pregnancy, maternal splenectomy before pregnancy, maternal medical treatment during pregnancy, nadir of maternal platelet count during pregnancy and last maternal platelet count before delivery, detectable maternal anti-platelet antibodies, mode of delivery, a previous neonate and platelet count of a previous sibling, gestational age and birth weight. Outcome measures were platelet count at birth, presence of hematomas and/or petechiae, occurrence of ICH, need for postnatal treatment, nature of postnatal treatment, duration of thrombocytopenia, interval to reach a platelet count > 50 x 10^9/L and course of platelet counts over time. A subsequent fall or recurrence was defined as a platelet count of > 50 x 10^9/L directly after platelet transfusion, with a subsequent decline below 50 x 10^9/L within 24 hours after the platelet transfusion.

Statistical analysis

Data are reported as median (range), numerical values or categories. Statistical analyses were performed with SPSS Version 18.0 (SPSS Inc., Chicago, IL). As data were not normally distributed, data were analysed with non-parametric test such as Mann-Whitney test (numerical data) and Fisher's Exact test (categorical data). Correlation was analysed using Spearman's correlation. Confounding was prevented using regression analysis. Infants with missing baseline characteristics were excluded from regression analyses.

Results

A total of 83 neonates from 47 mothers with ITP were identified and deemed eligible for our study. Sixteen neonates from 13 mothers were excluded, because of missing data (including platelet counts at birth or platelet counts of the first neonatal days). Sixty-seven neonates of 41 mothers (including 3 twins) were included; for 18 mothers more than 1 pregnancy was analyzed. Seven mothers were excluded for one pregnancy, but included for another pregnancy. Splenectomy was performed before pregnancy in 16 mothers. Maternal anti-platelet antibodies were tested in 38/64 pregnancies and detected in 23 of them (60.5%). ITP was diagnosed prior to pregnancy in 53 pregnancies (82.8%). Antenatal

maternal treatment consisted of IVIG (n=3), prednisone (n=8), cyclokapron (n=2), a combination of prednisone and IVIG (n=4), a combination of IVIG and platelet transfusions (n=2), a combination of IVIG and cyclosporin (n=1) a combination of prednisone, IVIG and splenectomy in the second trimester (n=1). Median nadir of maternal platelet count during pregnancy was 75 x 10^9/L (range 5-347), the last maternal platelet count before delivery was 105 x 10^9/L (range 22-399). Baseline characteristics of the included pregnancies are depicted in table 1. None of the 5 preterm born infants had severe thrombocytopenia. Only one of the neonates born from a mother with ITP diagnosed prior to pregnancy and pre-eclampsia had severe thrombocytopenia. In our study population the incidence of caesarean section was higher compared to the normal Dutch incidence (approximately 15%[23]). The reason for the high caesarean section incidence is not known. The indications for caesarean sections in our study population were not specifically scored; maternal ITP was not per se a contra-indication for vaginal delivery.

Median neonatal platelet count at birth was 202 x 10^9/L (range 4-378). Thirty-four neonates had thrombocytopenia (< 150 x 10^9/L). Severe thrombocytopenia (platelet count < 50 x 10^9/L) was detected in 20 (29.9%) neonates in the first postnatal days, of which 8 (10.6%) had a platelet count of < 20 x 10^9/L. Only 5 neonates had severe thrombocytopenia at birth, whereas the majority developed severe thrombocytopenia within a few days postpartum. Two infants, which developed severe thrombocytopenia in the first 4 days of life, had a platelet count of >150 x 10^9/L at birth. Median age at the time of platelet nadir was 3.0 days (range 1-7) in all neonates.

Routine cranial ultrasound showed no ICH in 14 neonates with severe thrombocytopenia. One neonate showed unilateral (left sided) polymicrogyria on cranial ultrasound and cerebral MRI (magnetic resonance imaging). In 6 neonates cranial ultrasound was not routinely performed, because they were born before 1998, since when the policy of routine cranial ultrasound for neonates with a platelet count below 50 x 10^9/L was introduced. These six neonates showed no clinical neurological signs of intracerebral bleeding. Petechiae were seen in 2 neonates: one had a platelet count of 84 x 10^9/L at birth and a nadir of 54 x 10^9/L on day 7; another had a platelet count of 42 x 10^9/L at birth and 5 x 10^9/L as nadir on day 6.

Table 1. Baseline characteristics of included pregnancies (n=64).

		N = 64
Diagnosis of ITP before pregnancy, n (%)		53 (82.8)
Number of pregnancies a mother, n (%)	1	23 (56.1), 1 twin
	2	14 (34.1), 2 twins
	3	3 (7.3)
	4	1 (2.4)
Maternal age (year)[a]		32.1 (22-40)
Maternal anti-platelet antibodies, n (%)	Tested	38 (59.4)
	Positive	23 (60.5)
Splenectomy before pregnancy, n (%)		16 (25.0)
Maternal treatment during pregnancy, n (%)	No treatment	43 (67.1)
	Prednisone	8 (12.5)
	IVIG	2 (3.1)
	Platelet transfusion	1 (1.6)
	Cyclokapron	2 (3.1)
	Prednisone and IVIG	4 (6.3)
	IVIG and platelet transfusions	2 (3.1)
	Prednisone, IVIG and splenectomy	1 (1.6)
	IVIG and cyclosporin	1 (1.6)
Nadir of platelet count during pregnancy × 10^9/L[a]		75 (5-347)
Last platelet count before delivery × 10^9/L[a]		105 (22-399)
Maternal complications, n (%)	ITP related[b]	3 (4.7)
	Pregnancy complications[c]	7 (10.9)
Cesarean section, n (%)		19 (29.7)
Included infants (including 3 twins)		**N = 67**
Gender (male), n (%)		32 (47.8)
Gestational age (weeks)[a]		39.0 (31-42)
Birth weight (gram)[a]		3240 (1345-4635)

[a] median (range)
[b] Bruising, petechiae, gingival bleeding
[c] Pre-eclampsia, HELLP or hypertension

Table 2. Postnatal outcome and management in 67 neonates born after maternal ITP.

		N = 67
Platelet count at birth x 10^9/L[a]		202 (4-378)
Thrombocytopenia during first week of life, n (%)	101-150 x 10^9/L	34 (50.7)
	51-100 x 10^9/L	25 (37.3)
	21-50 x 10^9/L	20 (29.9)
	< 20 x 10^9/L	8 (11.9)
Platelet nadir x 10^9/L[a]		129 (4-315)
Age at platelet nadir (days)[a]		3 (1-7)
Petechiae, n (%)		2 (3.0)
Postnatal treatment (divided for infants with and without severe thrombocytopenia)		
	> 50 x 10^9/L (n=47)	≤ 50 x 10^9/L (n=20)
No treatment	46 (68.6)	3 (4.5)
IVI		1 (1.5)
Platelet transfusion	1 (1.5)	2 (3.0)
Prednison		2 (3.0)
Platelet transfusion and IVIG		11 (16.4)
Platelet transfusion and prednisone		1 (1.5)

[a] median (range)

Postnatal treatment of infants with severe thrombocytopenia consisted of IVIG (n=1), prednisone (n=2), platelet transfusions (n=2), platelet transfusions combined with IVIG (n=11) and platelet transfusions combined with prednisone (n=1). IVIG was administered during median 5 days (range 3-5). Three children of one mother were treated with prednisone for a period of 11 days to 6 months, exact details of these neonates were not traceable (age of start was unknown in 1 case; exact duration of prednisone therapy given because of prolonged thrombocytopenia was unknown in all 3 cases), due to aged data sources. Three neonates had a quick spontaneous rise in platelet count and did not receive any treatment. Another neonate, without severe thrombocytopenia, but with a persisting platelet count between 50-60 x 10^9/L and petechiae, received 1 platelet transfusion. A general overview of neonatal outcome and management is given in table 2.

Fifteen neonates received median 3 platelet transfusions (range 1-6) per neonate, in 12 neonates combined with IVIG (n=11) or prednisone (n=1). A subsequent fall to a platelet count of <50 x 10^9/L after the platelet transfusion occurred for 9/15 (60.0%) after the first, 8/9 (88.9%) after the second and 6/8 (75.0%) after the third

platelet transfusion. Seven out of 8 infants who had a fall of platelets below 50 x 10^9/L after the first platelet transfusion without IVIG, subsequently received IVIG, and platelets recovered after 2-6 platelet transfusions. IVIG was started at median 3 days (range 1-14) after the first determination of a platelet count < 50 x 10^9/L, median duration until IVIG treatment gained an increasing platelet count above 50 x 10^9/L was 1 day (range 0-8). Table 3 depicts a more detailed overview of the outcome of infants treated with IVIG and/or platelet transfusions.The course of platelet counts of severe thrombocytopenic neonates, receiving treatment for thrombocytopenia, is graphically shown in figure 1.

Table 3. Characteristics of neonates treated with platelet transfusions and/or IVIG

		N = 16
Number of platelet transfusions a neonate[a]		3 (1-6)
Fall of platelet count after platelet transfusion, n (%)[b]	1st	9/15 (60.0)
	2nd	8/9 (88.9)
	3rd	6/8 (75.0)
	4th	5/6 (83.3)
	5th	2/5 (40.0)
	6th	0/3 (0.0)
Increment after first platelet transfusion x 10^9/L [a]		52 (3-147)
Decline of platelet count < 24 hours after platelet transfusion x 10^9/L [a]		66 (15-163)
Interval of start IVIG, after first platelet count < 50 x 10^9/L (days)[a]		3 (1-14)
Duration until platelets count > 50 x 10^9/L and increasing after start IVIG (days)[a]		1 (0-8)

[a] median (range)
[b] Fall of platelet count to < 50 x 10^9/L after platelet transfusion

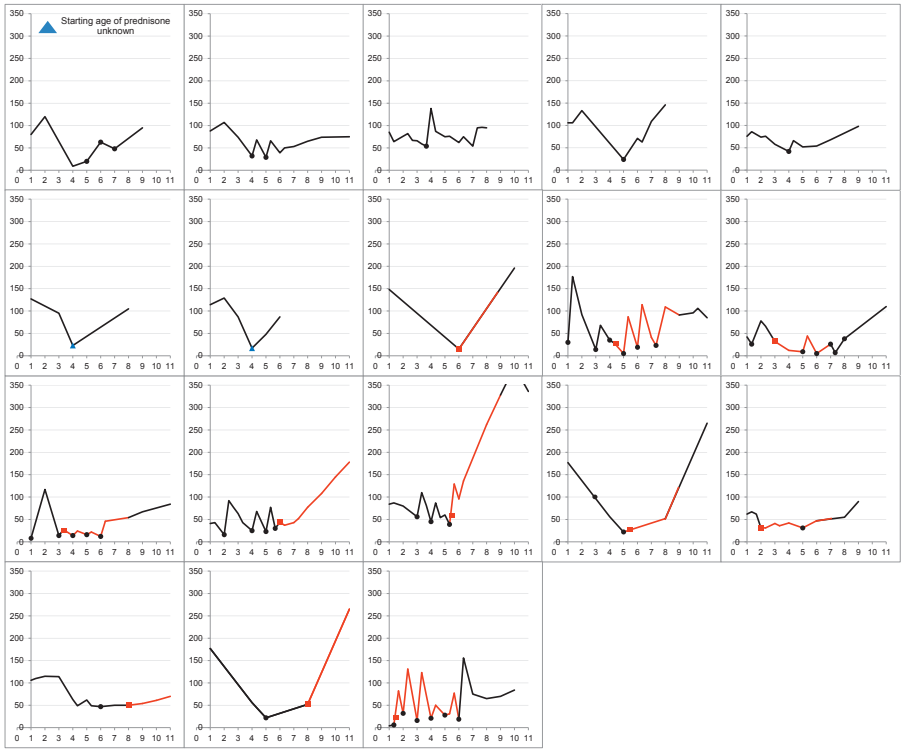


Figure 1. Course of neonatal platelets of all treated neonates per day (n=18).

Legend: Each line indicates one neonate. **Black** dots indicate the timing of platelet transfusions. **Red** squares and line indicate the start and stop of IVIG therapy. **Blue** triangles indicate start of prednisone therapy.

Figure 1 shows that none of the infants receiving treatment for severe thrombocytopenia had a stable and/or rising platelet count above 50 x 10^9/L before the third day of life. Only one of the three neonates with spontaneous rise of platelet counts (without treatment), had this rise already on the second day of life (the other two neonates had their nadir below 50 x 10^9/L on day 6).

Median duration of thrombocytopenia (in all neonates with platelet counts < 150 x 10^9/L) was 11 days (range 3-58). Thirteen of the thirty-four neonates with thrombocytopenia were not followed until a platelet count of >150 x 10^9/L was achieved, but had a stable or rising platelet count between 50-150 x 10^9/L (n=13). In neonates with severe thrombocytopenia platelet counts achieved a safe level above 50 x 10^9/L within median 2.5 days (range 1-17). One neonate received an additional course of IVIG at the age of three weeks, because of a late relapse, with an adequate increment.

Two maternal risk factors for severe neonatal thrombocytopenia were identified, including having an earlier neonate with severe thrombocytopenia (p = 0.032) and a low maternal platelet nadir during pregnancy (p = 0.031). Three mothers had 3 infants and 2 mothers had 2 infants with severe neonatal thrombocytopenia. Platelets at birth and platelet nadir of the first and second sibling were highly correlated (p = 0.001 in Spearman's correlation) in 18 mothers with more than 1 pregnancy (total of 43 neonates), this correlation can be seen in figure 2. We didn't found a relationship between detectable circulating maternal anti-platelet antibodies and severe neonatal thrombocytopenia. An overview of the risk factor analysis can be seen in table 4.

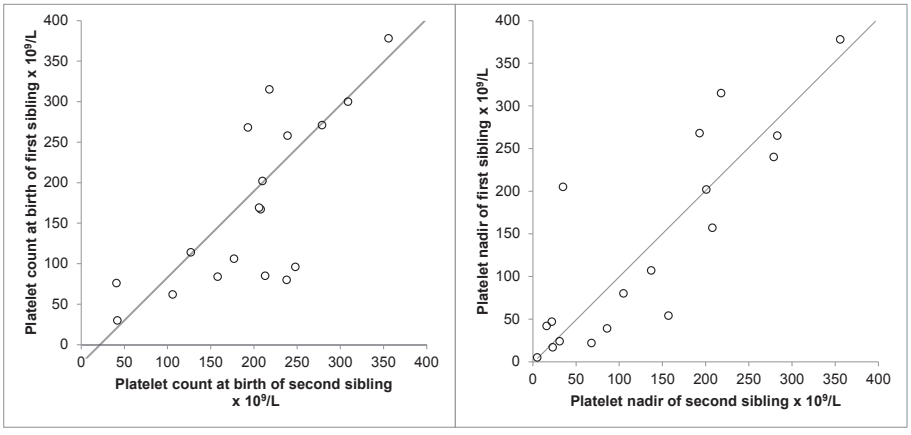


Figure 2. Correlation between platelet counts at birth of the first two siblings of mothers with ITP during pregnancy.

Table 4. Analysis of possible risk factors associated with severe neonatal thrombocytopenia (<50 x 10^9/L).

	Univariate p-value N = 67	Multivariate p-value N = 61
Nadir of maternal platelet count during pregnancy	*0.002*	*0.015*
Last maternal platelet count before delivery	*0.038*	0.084
Splenectomy	0.530	
Diagnosis of ITP before current pregnancy	0.151	
Mode of delivery	0.384	
Detectable maternal anti-platelet antibodies	0.271	
Severe neonatal thrombocytopenia in sibling	*0.003*	*0.016*

Discussion

In this retrospective study the incidence of severe neonatal thrombocytopenia due to maternal ITP is high (29%) with a low incidence of bleeding complications. We report on 20 cases of severe thrombocytopenia, with only 2 cases of symptomatic thrombocytopenia with transient petechiae. A variety of postnatal treatment was used reflecting the lack of international consensus on optimal initial management. The effect of platelet transfusions was often short with frequent recurrence to a low platelet count, requiring additional transfusions and/or IVIG treatment. Our study is one of the larger reporting and analysing the effect of different postnatal treatment strategies. Especially analysing increments after platelet transfusions (currently only recommended for severe bleeding in several guidelines) and reporting individual courses of platelet counts was not performed earlier.[21,24]

Our incidence of severe neonatal thrombocytopenia seems high (29%), but we described all neonates with severe neonatal thrombocytopenia in the first postnatal days, in contrary to the majority of other studies reporting only severe thrombocytopenia at birth.[2,5,7] The incidence of severe thrombocytopenia at birth was only 6% (4/66) in this study, which is comparable with reported incidences by others (8-13%).[2,5-8] The high incidence could also be due to selection bias, because the more complicated cases were more likely to be referred to our tertiary care center (such as mothers with an earlier neonate with severe thrombocytopenia). As platelet counts of excluded neonates were not known, reporting bias caused by their exclusion can not be ruled out. One neonate in our study had severe cerebral injury of antenatal origin (unilateral polymicrogyria). Polymicrogyria is associated with prenatal events including congenital infections, chorioamnionitis, genetic syndromes, metabolic disorders and vascular events. Hypothetically, polymicrogyria in this infant may have resulted after an antenatal ICH due to thrombocytopenia. However, as other causes of polymicrogyria could not be ruled out and bleeding sequels could not be visualized, the occurrence of an antenatal ICH is uncertain. Follow-up at the age of 2 year showed hemiplegia on the right side.[25] In agreement with other studies, we found severe neonatal thrombocytopenia in a sibling a risk factor for severe neonatal thrombocytopenia.[6,9,10] We also observed an association with a low maternal platelet count during pregnancy.[4,7] Evidence about associations between neonatal platelet counts and several maternal factors is conflicting, as most large studies show a lack of association with maternal platelets, antibodies and timing of diagnosis, whereas more severe neonatal thrombocytopenia in mothers which underwent splenectomy was quite frequently described.[1,5,6,9,13]

In the analysis of neonatal course of platelet counts, the effect of postnatal platelet transfusions was frequently short. Only 6/15 infants did not have a subsequent fall after their first platelet transfusion, although 3 of them were already treated with IVIG. Also after the second, third and fourth platelet transfusion recurrence of low platelet counts were common. With a median duration of 1 day (range 0-8) after starting IVIG until an increasing platelet count above 50 x 10^9/L was achieved, these results may indicate that IVIG should be started earlier than was done in this population (median 3 days after the first platelet count of < 50 x 10^9/L), to avoid multiple platelet transfusions. Otherwise, as 3 patients showed spontaneous improvement and 3 patients came to a persistent safe platelet level after one platelet transfusion, IVIG can be withheld in a substantial proportion of infants. Platelet transfusions can be considered in particular for neonates with a very low platelet count at birth, with the expectation that in the majority platelets will further decrease after birth, to tide over the period before IVIG will become effective. Only 3 patients received prednisone, not allowing conclusions to be drawn from our data.

We confirm the greatest risk of neonatal passive ITP to occur postpartum with the lowest platelet count at 3 days after birth. Lacking, apart from the neonatal platelet count of a previous sibling, risk indicators for delivering a severely thrombocytopenic neonate, focus should be turned to postnatal management.[9,11] In this respect this retrospective report is of clinical importance as only two studies could be found that reported on neonates receiving postnatal platelet transfusions, IVIG or prednisone. One study described the effect of postnatal IVIG in 11 neonates, in some cases combined with steroids or platelet transfusions. No significant increments after platelet transfusions as single therapy were reported, but they did not analyze this in detail and individually. IVIG (eventually combined with steroids) had a success rate of 75%.[26] Another study described a series of 6 neonates with disappointing increments after 5 days of IVIG, a subsequent 5 days of prednisone therapy resulted in platelet counts of >150 x 10^9/L in all 6 infants.[27] This study suggested preferring prednisone above IVIG as postnatal therapy, but the adequate platelet increase after 5 days of prednisone could also be a combined effect of IVIG and prednisone. We were the first study analyzing the individual platelet response of neonates with severe thrombocytopenia on platelet transfusions and IVIG.

This study has several limitations, of which the retrospective character is the first one. The sample size was relatively large, compared to other reported populations, however 66 cases is still not a number on which very reliable statistical analyzes are applicable. In regression analysis for multiple variables with such a small sample size, a p-value of

< 0.01 would be more appropriate and preferable. Missing data was another limiting factor, which was caused by the need to collect cases from 30 years ago to have a sample size as large as possible and led to the exclusion of 16 neonates from 13 mothers. All three limitations can create considerable biases, but are the direct consequence of the rarity of the described condition, which makes it really difficult to collect enough cases to apply statistics. With strict cut-off values and definitions we tried to reduce the risk for possible bias as much as possible. Confirmation of our observations in a larger prospective cohorts in future studies is needed trough multicenter international collaboration reporting on all newborns. As it may take weeks before platelet counts are completely recovered, it would be very valuable to look for long term beneficial and adverse effects of different postnatal treatment strategies.

We can conclude that despite severe neonatal thrombocytopenia due to maternal ITP during pregnancy was fairly high in our series, severe bleeding manifestations were rare. As platelet nadir is seen within the first 7 days postpartum, bleeding risks are the highest in the first week, and daily platelet count control is necessary until a stable or rising safe platelet count above 50 x 10^9/L is observed (as advised in guidelines).[20,24,28] In addition should each individual case be evaluated precisely, to determine if daily platelet count measurement is really necessary or once in two days will suffice.

When a platelet transfusion fails to result in a stable increase of platelet counts, further platelet transfusions are not advised without IVIG treatment. Especially, because in a substantial number of neonates it takes some days until IVIG becomes effective, early start is preferable to achieve a safe platelet count of > 50 x 10^9/L more quickly.

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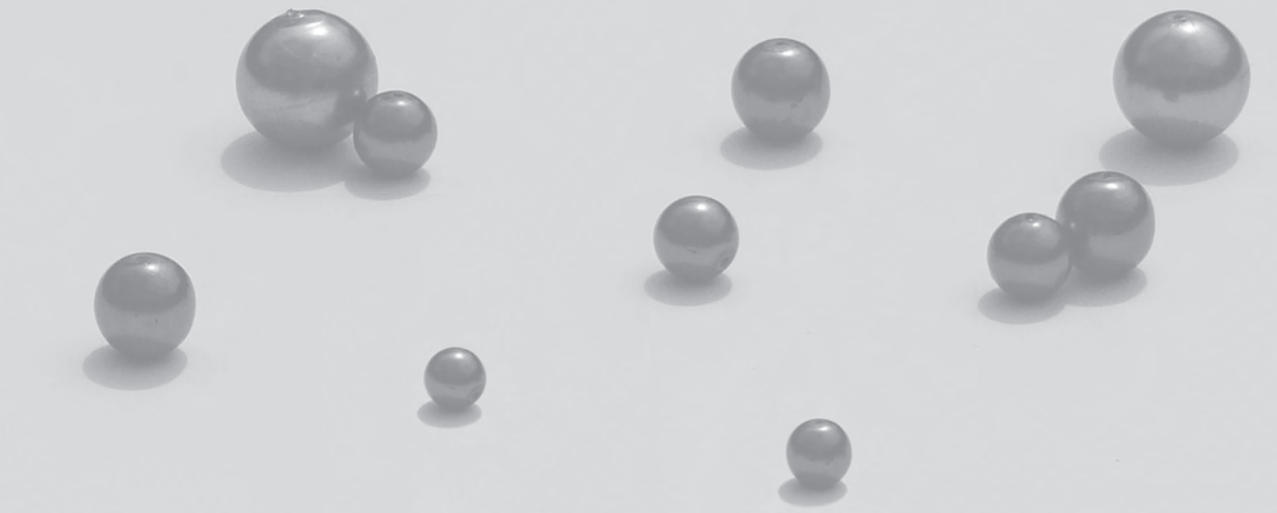
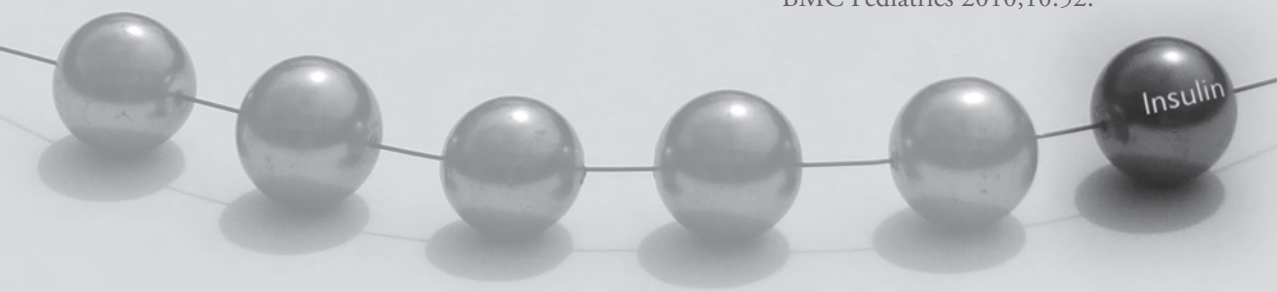
Chapter 7

Short and long term outcome of neonatal hyperglycemia in very preterm infants: a retrospective follow-up study



N.M. van der Lugt, V.E.H.J. Smits-Wintjens, P.H.T. van Zwieten, F.J. Walther.

BMC Pediatrics 2010;10:52.



Abstract

Background

Hyperglycemia in premature infants is associated with increased morbidity and mortality, but data on long-term outcome are limited. We investigated the effects of neonatal hyperglycemia (blood glucose ≥10 mmol/l, treated with insulin for ≥12 hours) on growth and neurobehavioural outcome at 2 years of age.

Methods

Retrospective follow-up study at 2 years of age among 859 infants ≤32 weeks of gestation admitted to a tertiary neonatal center between January 2002 and December 2006. Thirty-three survivors treated with insulin for hyperglycemia and 63 matched controls without hyperglycemia were evaluated at a corrected age of 2 years. Outcome measures consisted of growth (weight, length, and head circumference) and neurological and behavioural development.

Results

66/859 (8%) infants ≤32 weeks of gestation developed hyperglycemia. Mortality during admission was 27/66 (41%) in the hyperglycemia group versus 62/793 (8%) in those without hyperglycemia ($p<0.001$). Mortality was higher in infants with hyperglycemia with a birth weight ≤1000 gram ($p = 0.005$) and/or gestational age of 24-28 weeks ($p = 0.009$) than in control infants without hyperglycemia. Sepsis was more prominent in infants with hyperglycemia and a birth weight of >1000 gram ($p = 0.002$) and/or gestational age of 29-32 weeks ($p = 0.009$) than in control infants without hyperglycemia. Growth at 2 years of age was similar, but neurological and behavioural development was more frequently abnormal among those with neonatal hyperglycemia ($p = 0.036$ and 0.021 respectively).

Conclusion

Mortality was higher in very preterm infants with hyperglycemia treated with insulin during the neonatal period. At 2 years of age survivors showed normal growth, but a higher incidence of neurological and behavioural problems. Better strategies to manage hyperglycemia may improve outcome of very preterm infants.

Introduction

Hyperglycemia is a common problem in very preterm infants. Among extremely low birth weight infants the incidence of neonatal hyperglycemia is estimated to be between 45% and 80%.[1-3] This is possibly an under-estimation as 50% of the abnormalities in glucose levels are not detected by standard intermittent sampling and the incidence is also influenced by the use of different definitions.[4,5] Accurate monitoring of blood glucose levels is clinically important, because abnormalities in glucose homeostasis can have serious short term consequences. Hyperglycemia is associated with increased mortality, which is significantly related to the duration of the hyperglycemia.[6,7] A higher incidence of retinopathy of prematurity and intraventricular hemorrhage (IVH) grade 3 and 4 has also been reported.[7,8]

Prematurity is an important risk factor in the complex pathogenesis of hyperglycemia. Insulin resistance develops due to a high circulating level of inflammatory markers, cytokines and catecholamines. Consequently, glucose production in the liver is not inhibited. The pancreas needs to produce insulin to compensate, but is probably unable to do so due to immature beta-cells, leading to relative insulin deficiency.[7,9,10] Since twenty-five years continuous insulin infusion is used, nevertheless in many centers restriction of glucose intake is still the first step in treatment of hyperglycemia.[5] Comparison of these interventions has demonstrated the efficacy of continuous insulin infusion in glucose control. Infants treated with insulin had a higher glucose intake, a higher weight gain, less sepsis and an increased endogenous insulin production.[11,12] The benefit of early elective insulin therapy in the prevention and treatment of hyperglycemia has been demonstrated in critically ill adults, but is questionable in premature infants. Although early elective insulin therapy decreases hyperglycemia, it significantly increases the incidence of hypoglycemia and mortality by 28 days.[13-15] Whereas the short term effects of neonatal hyperglycemia in very preterm infants have been reported, less is known about the effects of hyperglycemia on growth and neurobehavioural outcome. The aim of this retrospective follow-up study was to determine the possible effects of hyperglycemia on growth and neurobehavioral development by comparing very preterm infants with and without a history of neonatal hyperglycemia at the corrected age of two years.

Methods

Study population

The study population consisted of 859 very preterm infants (≤32 weeks) admitted to the Neonatal Intensive Care Unit (NICU) of the Leiden University Medical Center between January 2002 and December 2006. Neonatal data and data on growth and neurobehavioural outcome at the corrected age of 2 years were collected from the charts in the tertiary neonatal center and the regional hospitals where these infants were regularly seen for follow-up. The Medical Ethics Committee of the Leiden University Medical Center did not require approval of this study because it consisted of retrospective chart review, nor did the medical ethics committee require written consent by the parents for their infant's information to be stored in the hospital database and used for research.

Hyperglycemia was defined as at least 2 blood glucose levels of ≥10.0 mmol/L (180 mg/dL) during a 12-hour period. Insulin treatment was started at 0.05 U/kg/h[14] when hyperglycemia persisted after these 12 hours, despite reduction of glucose intake to 5-6 mg/kg/min. The insulin dosage was tapered after blood glucose levels dropped to <10 mmol/L. The cohort of exposed infants consisted of all preterm infants with neonatal hyperglycemia and the unexposed cohort was a matched selection of preterm infants admitted during the same timeframe, but without hyperglycemia (and without short-lived hyperglycemia <12 hours) and insulin therapy. The infants were matched by birth weight (± 0.1 kg), gestational age (± 1 week), gender, and date of admission (a period of ± 1 year was preferred). Each exposed infant was matched to 2 unexposed controls. The exclusion criteria for both exposed and unexposed infants were major congenital anomalies and chromosomal abnormalities. The study size was determined by the number of exposed infants during the study period and the availability of matches.

Data collection

Blood glucose levels were measured in whole blood using the glucose oxidase method (Siemens RapidPoint 400/405 system, Siemens Healthcare Diagnostics B.V., Breda, The Netherlands). Frequency of glucose level measurements was based on clinical and laboratory findings. During the first week after birth blood glucose levels were measured at least 6 times a day, thereafter glucose levels were measured at least 3 times a day. In the acute phase of hyperglycemia, glucose levels were measured regularly with intervals of approximately 1-2 hours.

Data for demographic and perinatal characteristics as well as postnatal clinical conditions of all infants were collected from the charts and included birth weight, gestational age, length of stay (as an indicator of severity of illness), gender, exposure to prenatal and postnatal steroids, presence of chorioamnionitis, sepsis, prolonged rupture of membranes (PROM), respiratory distress syndrome (RDS)[16], bronchopulmonary dysplasia (BPD)[17], necrotizing enterocolitis (NEC)[18], cystic periventricular leukomalacia (PVL)[19] and intraventricular hemorrhage (IVH)[20,21].
For the exposed infants the mean, minimum and maximum glucose levels, glucose intake and insulin infusion rate were calculated for the first 5 days after the onset of the hyperglycemia episode or until the end of the hyperglycemia episode if the episode lasted longer than 5 days. Additionally, duration of hyperglycemia, frequency of hypoglycemic periods, duration of insulin infusion, mean serum sodium concentration, maximum daily percentage of inhaled oxygen and maximum infusion rate of inotropic drugs (dobutamine and dopamine), were recorded.
Outcome data collected at the corrected age of 2 years (± 3 months) included weight (kg), length (cm), head circumference (cm), and neurobehavioral development. Neurological outcome was classified into 3 groups: normal, mildly abnormal (detectable but not disabling abnormalities of tone and reflexes, presence of abnormal movements or asymmetry) and severely abnormal (disabling abnormalities) according to the neurological examination by Hempel.[22] Behavioural outcome was scored normal or inadequate based on the Child Behavior Checklist/2-3 (CBCL/2-3)[23] completed by the parents or orally by the clinic pediatrician.

Statistical analysis

Data are reported as mean values ± standard deviation, numerical values or categories. Statistical analyses were performed with SPSS Version 16.0 (SPSS Inc., Chicago, IL). Numerical growth parameters were analyzed with an unpaired T-test. The categorical data for behavioural and neurological outcome were analyzed using a chi-squared test. Subgroup analyses were performed for subgroups in gestational age (24-28 and 29-32 weeks) and birth weight (≤1000 gram and >1000 gram). Potential confounders in this study were gestational age, gender, birth weight, length of stay, exposure to prenatal and postnatal steroids, presence of severe RDS, sepsis, PROM, chorioamnionitis, NEC, BPD, PVL and IVH. Confounding was prevented by matching and using multivariable regression analysis for analyzing mortality and morbidity, as well as growth, neurological and behavioural development. Infants with missing baseline characteristics were excluded from regression analyses.

Results

Between January 2002 and December 2006 a total of 859 very preterm infants with a gestational age less than 33 weeks were admitted for neonatal intensive care. Mean gestational age was 29.4 ± 2.0 weeks and birth weight 1323 ± 410 gram. Fifty-six percent was male. Prenatal steroids were administered to 63% of the mothers. Chorioamnionitis and PROM were present in 13 and 27% respectively. Twenty-nine percent of the infants developed sepsis, 6% IVH grade 3 or 4, 3% cystic PVL, 2% NEC grade 2 or 3, 19% RDS grade 3 or 4 and 14% BPD at 36 weeks postmenstrual age.

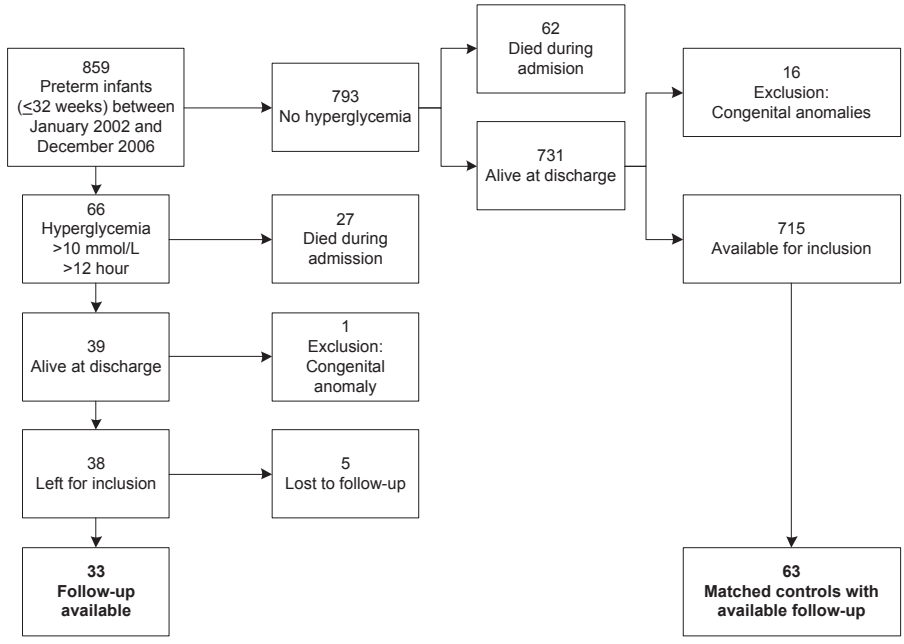


Figure 1. Flowchart of included patients.

Postnatal steroids were administered to 10% of the infants. Hyperglycemia occurred in 66 (8%) infants of whom 53 had a birth weight of ≤1000 gram, the incidence in this subgroup was 25%. The mean age at onset of the hyperglycemia episode was 3.2 ± 3.7 days. Twenty-seven out of 66 infants with hyperglycemia (41%) died during admission versus 62/793 (8%) infants without hyperglycemia. Six of the remaining 39 infants with hyperglycemia were excluded from this follow-up study because of major congenital anomalies (1) and loss to follow-up (5). Emigration (1), placement in an unknown foster home (1) and no appearance at follow-up appointments (3) were the reasons for the loss to follow-up. These infants were excluded from

the analysis together with their unexposed matches. For 3 exposed infants only 1 unexposed match was available. The complete follow-up population consisted of 96 infants; a flowchart can be seen in figure 1.

Table 1. Baseline characteristics of included patients in the follow-up study.

Demographic and perinatal data	Exposed (N = 33)	Unexposed (N = 63)	p-value
Gestational age (week) [a]	28.2 ± 2.2	28.2 ± 2.2	1.000
Birth weight (gram) [a]	962 ± 316.4	1008 ± 292	0.478
Length of stay (day)	47.2 ± 32.5	25.7 ± 45.1	*0.017*
Gender (male) - n (%)	21 (64)	39 (62)	1.000
Prenatal steroids			0.721
0 doses	12 (36)	21 (35)	
1 dose	13 (39)	21 (33)	
>1 doses	8 (24)	20 (32)	
Chorioamnionitis - n (%) [b]	2 (6)	6 (10)	0.711
PROM - n (%) [c]	9 (27)	14 (22)	0.620
Postnatal clinical conditions			
Sepsis - n (%) [d]	21 (64)	37 (59)	0.667
IVH grade 3/4 - n (%)	3 (9)	3 (5)	0.411
RDS grade 3/4 - n (%)	10 (33)	19 (30)	0.818
Cystic PVL - n (%)	0 (0)	2 (3)	0.544
NEC grade 2/3 - n (%)	1 (3)	0 (0)	0.334
BPD - n (%) [e]	20 (61)	25 (40)	0.056
Postnatal steroid exposure - n (%)	10 (30)	13 (21)	0.321

[a] Value given as mean ± standard deviation
[b] Chorioamnionitis was defined as smelly amniotic fluid, maternal fever or signs of infection at birth.
[c] PROM was defined as rupture of membranes >24 hours.
[d] Sepsis was defined as a positive blood culture.
[e] Need for oxygen-therapy at a gestational age of 36 weeks or at discharge

An overview of the baseline characteristics of included patients is given in table 1. The exposed group had a significantly longer length of stay than the unexposed group, reason why statistical correction for confounders by regression analysis was done.
Table 2 shows growth and development at 2 years of corrected age. Weight, length, head circumference were not different in the exposed cohort compared to the unexposed cohort. The incidence of an abnormal neurological and behavioural outcome was higher in the exposed group (p = 0.036 and 0.021 respectively).

Table 2. Growth and development at 2 years of corrected age.

Growth and outcome measures		Exposed (N = 33)	Unexposed (N = 63)	Multivariate p-value
Head circumference (cm)		47.8 ± 1.9	48.0 ± 1.8	0.613
Length (cm)		85.7 ± 4.0	85.0 ± 3.9	0.410
Weight (kg)		11.0 ± 1.5	11.0 ± 1.3	1.000
Neurological outcome - n (%)				*0.036*
	Normal	16 (48)	47 (75)	
	Mild abnormal	12 (36)	12 (19)	
	Severe abnormal	5 (15)	4 (6)	
Behavioural outcome - n (%)				*0.021*
	Normal	17 (52)	48 (76)	
	Delayed	16 (48)	15 (24)	

Analyses of the data on the hyperglycemia episodes (table 3) showed some significant relations. Mean and maximum glucose levels on the third and fourth day of the hyperglycemia episode correlated with mortality (mean: p = 0.046, maximum: p = 0.041). Infants with a mean glucose level >8.0 mmol/L (144 mg/L) or a maximum glucose level >9.5 mmol/L (171 mg/L) on the third and fourth day had a higher mortality rate (p = 0.042 and p = 0.030, respectively). All hypoglycemia episodes were brief events which were corrected quickly. Neurobehavioural outcome, growth or mortality were not influenced by the frequency of hypoglycemia periods and the duration of the hyperglycemia episode and correction for these potential confounders was therefore not necessary.

Table 3. Characteristics of the hyperglycemia episode in all exposed infants.

N = 66 [a]	Mean	Minimum	Maximum	SD
Age at onset of hyperglycemia (days)	3.2	1	19	3.7
Number of hypoglycemia episodes	0.3	0	6	0.9
Mean glucose level (mmol/L)	12.9	10.1	21.9	2.3
Mean glucose intake (mg/kg/min)	6.3	3.4	11.0	1.3
Mean insulin infusion rate (U/kg/h)	0.06	0.01	0.34	0.06
Duration of hyperglycemia (hours)	34	12	169	25
Duration of insulin infusion (hours)	129	3	754	132

[a] Including deaths and infants lost to follow-up

A multivariable regression analysis on all very preterm infants admitted between January 2002 and December 2006 (n=798 infants with complete baseline characteristics), confirmed a significant increase in mortality in the exposed cohort (p = 0.001) (Table 4).
Subgroup analysis demonstrated that infants with a birth weight ≤1000 gram (mean gestational age 27.3 ± 1.8 weeks) and/or a gestational age of 24-28 weeks (mean birth weight 972 ± 229 gram) also had a significant relation between hyperglycemia and mortality. In the subgroup of infants with a birth weight >1000 gram (mean gestational age 30.1 ± 1.6 weeks) hyperglycemia was associated with an increased incidence of IVH (p = 0.025), severe RDS (p = 0.012) and sepsis (p = 0.002). Sepsis was also more prominent in a subgroup of infants with a gestational age of 29-32 weeks (p = 0.009, mean birth weight 1503 ± 362 gram). In the regression analyses for morbidity and mortality statistical correction was done for gestational age, gender, birth weight, exposure to prenatal and postnatal steroids, presence of severe RDS, sepsis, PROM, chorioamnionitis, NEC, BPD, PVL and IVH.

Table 4. Regression analysis for mortality in preterm infants.

		Multivariate p-value for hyperglycemia
Total population (N = 798[a])		*0.000*
Gestational age subgroups		
	24-28 weeks (N = 265)	*0.009*
	29-32 weeks (N = 533)	0.899
Birth weight subgroups		
	≤1,000 gram (N = 188)	*0.005*
	>1,000 gram (N = 610)	0.402

[a] 61 of the 859 very preterm infants had missing baseline characteristics and were excluded from the model

Discussion

The findings in this study indicate that hyperglycemia has no effect on long term growth. However, in addition to the already known short term effects, hyperglycemia has a distinct negative influence on neurological and behavioural outcome at 2 years of age. The observation by other investigators that hyperglycemia is a risk factor for early mortality in very preterm infants was confirmed. We found a firm association

between neonatal mortality and the mean and maximum glucose levels on days 3 and 4 of the hyperglycemia episode. This association suggests that increased mortality is probably due to poorly controlled hyperglycemia. No association between mortality and duration of hyperglycemia was found. In subgroup-analyses we found relations between hyperglycemia and morbidity. Sepsis, severe RDS and IVH were more common in infants with hyperglycemia and a birth weight of >1000 gram (mean gestational age 30.1 ± 1.6 weeks).

Hays et al. demonstrated that blood glucose concentration had significant effects on both early death and the occurrence of severe IVH.[7] To our knowledge, adverse neurological and behavioural outcome secondary to neonatal hyperglycemia has not been reported yet.

This retrospective study has several limitations. An observer-bias cannot be excluded because several physicians did the follow-up consults at the age of 2 year. To reduce the observer-bias as much as possible strict cut off points were made and the follow-up information was interpreted by only one person. In the future it would be interesting to do a prospective study, in which Bayley scores will be collected from all participants. Furthermore, it was quite difficult to match unexposed controls for date of admission within 1 year to reduce the influence of changing policies. The composition of the unexposed control group was randomly chosen and purely based on the in advance defined matching criteria. Nonetheless, unintentional selection cannot be excluded and the matched controls may not 100% represent the complete population of very preterm infants admitted to the nursery during the study period. An inevitable complicating factor in this study was the concomitant occurrence of hyperglycemia and insulin therapy, through which the individual influence of hyperglycemia or insulin therapy cannot be demonstrated. Although this study does not have the strength of a prospective follow-up study, the results will give some support and direction for the prognosis of the development of very preterm infants with hyperglycemia.

Treatment with insulin is not unequivocally associated with better outcome. The first study in adults by van den Berghe showed very promising results.[15] However, it proved to be difficult to repeat the positive results in later studies in adults, and some studies even showed negative effects. Early treatment with insulin in preterm infants (without hyperglycemia) even showed higher mortality at 28 days in the early-insulin group than in the control group.[14] The less favourable outcome in the insulin-treated group in this study could therefore also be due to the insulin therapy.

Several follow-up studies in infants with diabetes mellitus type 1 may explain our findings concerning neurobehavioural outcome. Infants with longer episodes of

hyperglycemia seem to have an impaired cognitive development, though the specific impaired elements of cognitive development vary between the studies. Compared with healthy siblings, infants with hyperglycemia had a lower verbal intelligence.[24] A follow-up study conducted 2 years after the onset of type 1 diabetes suggests that hyperglycemia is associated with compromised learning- and consolidation capacities and organizational strategies. However, this follow-up study was repeated after another 4 years, at which time this relationship could not be confirmed.[25,26]

Conclusion

The results of this retrospective follow-up study suggest that hyperglycemia and insulin therapy are not only associated with increased mortality and short term morbidity, but also exert long term effects on development. Given the definition of hyperglycemia, including the use of insulin for >12 hours, one can not assume that the association is due to poorly controlled hyperglycemia in this design, but may be the effect of insulin.

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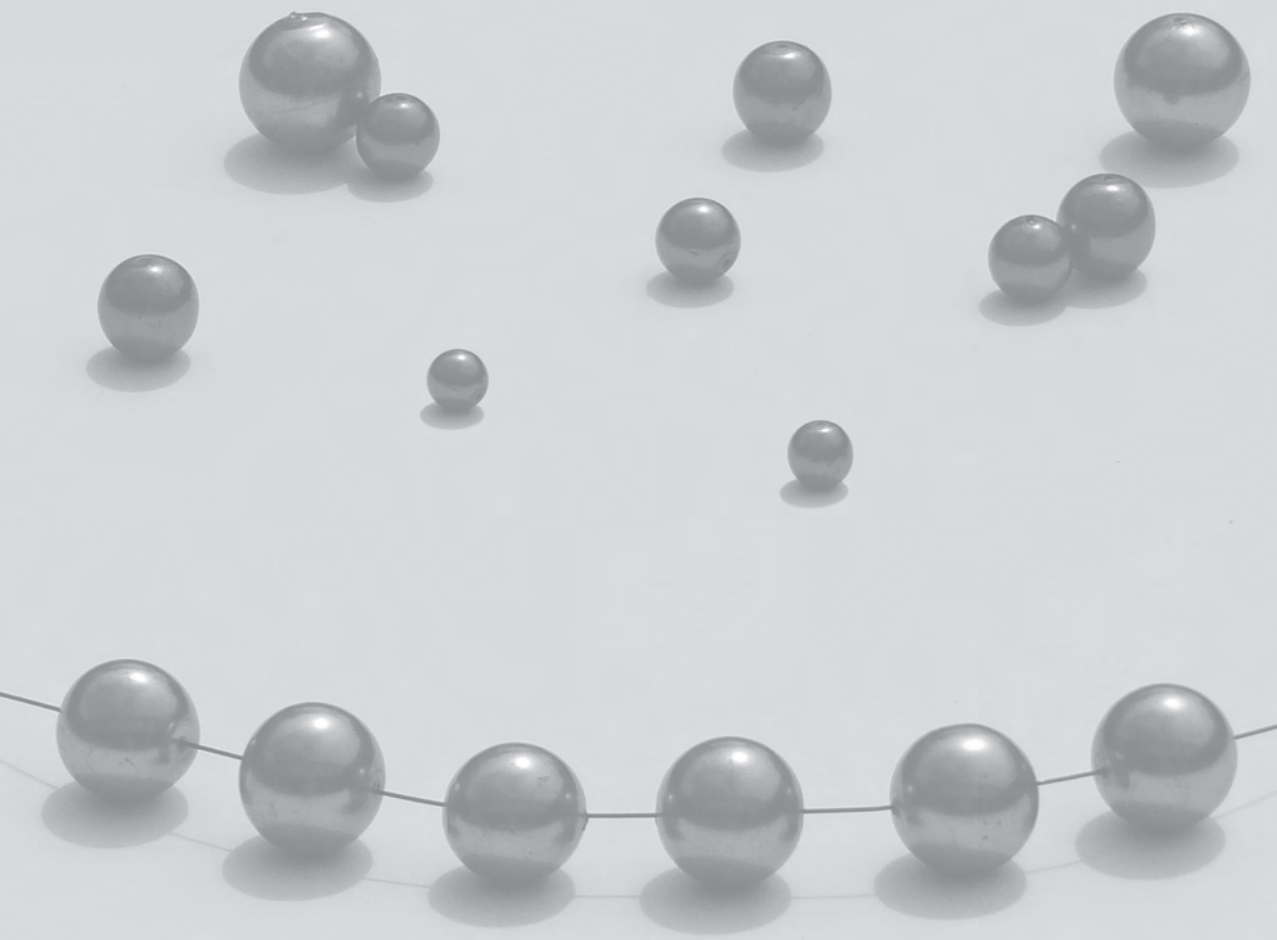
Chapter 8

General discussion



Introduction

In this chapter we will discuss each "pearl" separately, provide short recommendations for best practice and future perspectives.

Lithium: Fetal, neonatal and developmental outcomes of lithium-exposed pregnancies

Since 1970 lithium is registered by the Food and Drug Administration for treatment of acute manic episodes and as maintenance treatment for patients with bipolar disorders. Question rose about the safety of lithium during pregnancy for the developing fetus, as bipolar disorders are common in women of childbearing age.[1] An international register of lithium babies, consisting of 225 infants exposed to lithium during pregnancy, reported an extremely increased risk (500-fold) for Ebstein's anomaly. Ebstein's anomaly is a very rare cardiac malformation with an incidence of 1:20.000 in normal pregnancies.[2] Since that reported outcome, lithium was considered teratogenic and usage during pregnancy was highly dissuaded. Later case-control and cohort studies showed the 500-fold increased risk to be overestimated, due to a voluntary reporting bias of the lithium baby register. A causal relationship between intra-uterine lithium exposure and Ebstein's anomaly could not be ruled out, as a general incidence of 1:20.000 would require a very large study population, but other studies showed the expected increased risk to be maximal 28-fold.[3-5]

Slowly, lithium usage during pregnancy was reintroduced as the benefits of lithium continuation for maternal wellbeing became clear: discontinuation of lithium during pregnancy resulted in a 2-fold greater risk of recurrence of a new episode.[6,7] In addition, prenatal and postpartum maternal illness can have negative consequences for later neurobehavioral outcome of the infant.[8] Considering the small risk of teratogenic effects and the high risk of relapse after discontinuation of lithium during pregnancy, a balanced decision about optimal management should be made for each bipolar woman individually. Crucial information on the long term neurobehavioral outcome of intra-uterine lithium exposure is scarce. Only one other study reported the follow-up of 60 infants, but this was performed with questionnaires, which created a considerable bias.[9] The study in this thesis is the first follow-up study of lithium-babies performed by professionals using verified developmental scales. We found a normal neurobehavioral outcome in 15 infants exposed to lithium in utero. The small group size and the lack of an appropriate

control group are limitations and a much larger study population is necessary to draw more definite conclusions. While we are awaiting larger studies, these data can give counseling doctors and bipolar women more confidence in planning a safe pregnancy.

Recommendation

Teratogenicity of lithium is relatively limited and to date no evidence of impaired neurobehavioral outcome of offspring after intra-uterine lithium exposure has been reported. Therefore, we recommend continuation of lithium therapy during pregnancy in bipolar women who have until then benefited here from. Advantages and disadvantages of continuing lithium therapy during pregnancy should be precisely balanced for each bipolar woman individually.

Future perspectives

As lithium usage during pregnancy has slowly increased since the reports of less increased risk of teratogenicity from Jacobson and Zalstein, there must be large numbers of intra-uterine exposed infants.[3,4] Psychiatric departments are still performing studies to determine the most optimal treatment strategy of bipolar women during pregnancy and include large numbers of mothers and infants.[6,7,10] To gain more knowledge about the long term effects of intra-uterine lithium exposure it would be very helpful to add neonatal and long term follow-up evaluations to such relatively large psychiatric studies. As these mothers already consented for their own postpartum evaluation, they are probably also interested to consent for follow-up evaluation of their infant.
Importantly, future studies should include an adequate control group of infants from bipolar mothers who were not treated with lithium. As postpartum maternal illness can have influence on long term neurobehavioral development, comparison with control infants of non-bipolar mothers will not be reliable enough to demonstrate possible long term effects of intra-uterine lithium exposure.

8 Antenatal IVIG: Neonatal outcome in allo-immune thrombocytopenia treated with antenatal intravenous immunoglobulin

The most common cause of severe thrombocytopenia in fetus and neonate is fetal and neonatal thrombocytopenia (FNAIT).[11-13]
Maternal immunization to paternally inherited antigens on fetal platelets causes

destruction of fetal platelets and severe fetal and neonatal thrombocytopenia. This disorder is comparable with red cell allo-immunization, but in contrast to that condition, FNAIT can also affect first pregnancies. Screening for FNAIT has not been realized yet, so the first child is frequently severely affected with severe thrombocytopenia and intracranial hemorrhage (ICH), which often occurs antenatally.[14-16]

Cornerstone for the management of pregnant women, allo-immunized in a previous pregnancy, is antenatal treatment with intravenous immunoglobulins (IVIG) or, in some centers, with additional steroids. There is a rising trend towards a more non-invasive approach with minimization of fetal blood sampling, because the complication rate of this procedure (4.4-14% per pregnancy) is similar to or even higher than the risk for ICH in antenatally treated infants (0-10%).[17-24] Effectiveness and safety of a (non-invasive) policy with maternal IVIG were confirmed by studies in our center by van den Akker et al. (2007) and in the study outlined in this thesis.[21]

Questions about the optimal dosage and the best age to start IVIG remain and require further investigation. In only one study dosage was varied from 1 g/kg to 2 g/kg maternal body weight, without clear benefits for one specific dose.[23] Most studies about FNAIT report small groups and even smaller subgroups (sometimes varying in more than one study variable), making it difficult to draw firm conclusions. Because of this paucity of data, it is important to report all affected cases, as this may lead to a general international point of view and management can be based on a larger cohort of infants. Currently, a large international web-based registry of all FNAIT cases is active with antenatal IVIG dosages of 0.5 g/kg or 1.0 g/kg maternal body weight. This registry may shed more light on the optimal antenatal management in the near future.

In addition to optimizing therapy for already known affected cases of FNAIT, prevention of complications in first affected pregnancies is another important research topic. As HPA 1a incompatibility is responsible for 80-95% of the affected cases, screening for HPA 1a negative genotypes in all pregnant women is the first step in this process.[17,18,21,23,25-27] However, only 8-12% of these HPA 1a negative women with fetal incompatibility will become immunized and produce allo-antibodies.[27-32] Producing allo-antibodies is highly dependent on HLA type, e.g. HLA DRB3*0101 positivity is a poor predictor of becoming immunized, but has a high negative predictive value of 96-100%.[27,28,32] Since there are still no clear factors to predict which immunized HPA 1a negative women are at greatest risk for delivering a severely affected neonate, antenatal screening is not practical yet. Some studies suggest use of the level of maternal antibody titer during pregnancy as a possible predicting factor, but data on its predictive value are conflicting and the diagnostic value is not clear.[24,28,29]

Recommendation

We recommend treatment of FNAIT according to a non-invasive protocol, with administration of antenatal IVIG starting at 16-18 weeks' gestation in high risk pregnancies (i.e. those with ICH in an earlier sibling) and at 28 weeks' gestation in standard risk pregnancies. Our small study does not allow us to make a statement about an optimal dosage of IVIGs and gestational age to start, although in our cohort a dose of 0.5 g/kg in standard risk pregnancies and 1 g/kg maternal body weight in high risk pregnancies seemed effective in preventing ICH.

A matched platelet transfusion should be given if the platelet value at birth is <20 x 10^9/L in non-bleeding and <50 x 10^9/L in bleeding infants. In case of an emergency (i.e. clinical bleeding) and no immediate availability of matched platelets, random platelets may be life-saving and recommended. When multiple matched platelet transfusions do not result in a sufficient rise in platelet value, treatment with IVIG should be considered. Neonatal platelet values need to be determined at least daily during the first days of life, until a spontaneous rise or stable level is observed.

Future perspectives

Future research should focus on finding usable predictive factors for a neonate with FNAIT in the first pregnancy, to prevent overtreatment of all immunized HPA 1a negative women and to treat only those pregnancies at highest risk for severe FNAIT. Only when useful predictive factors have been identified, antenatal screening and selective antenatal treatment may contribute to a reduction in long term neurodevelopmental morbidity due to ICH and will become a cost-effective intervention. More knowledge about optimal gestational age to start and dosing regimen of antenatal IVIG would be very valuable to optimize antenatal treatment of already known to be affected neonates with FNAIT. Addition of long term follow-up assessments to future studies, can give more insight in possible effects of antenatal IVIG on long term neurobehavioral outcome.

Ibuprofen: Repeated courses of ibuprofen are effective in closure of a patent ductus arteriosus.

Closure of the ductus arteriosus in term infants normally occurs within 48-72 hours after birth, stimulated by an increase of arterial pO2 and decrease of circulating vasodilating prostaglandins. In preterm infants the ductus arteriosus frequently fails

to close or remain closed, with an incidence of 55-70% in those with a gestational age of <29 weeks or a birth weight of <1000 gram. The premature ductus arteriosus has a higher sensitivity for circulating vasodilating factors, such as nitric oxide and prostaglandins, and limits vasoconstriction, hypoxia, ischemia and the process of changing into a non-contractile ligament.[33-36]

Patency of the ductus arteriosus (PDA) was associated with increased morbidity and mortality and this led in the mid 1970s to a search for adequate treatment opportunities. Based on a few small randomized controlled trials, reporting improvement of lung compliance, shorter duration of mechanical ventilation and shorter need for oxygen after surgical or pharmacological ductal closure, the main goal of management became closure of a hemodynamic significant PDA.[37-43] Since that time, a multitude of studies to detect the most optimal treatment strategy for PDA closure have been performed: fluid restriction, non-steroidal anti-inflammatory drugs (NSAIDs) which selectively inhibit cyclooxygenase and the formation of prostanoids from arachidonic acid (COX inhibitors such as indomethacin and ibuprofen), indomethacin versus ibuprofen, prophylactic versus symptomatic approach, pharmacological versus surgical closure, oral versus intravenous ibuprofen and studies to determine the most optimal dosing regimen for pharmacological closure.

However, in contrast to the short term effects on lung function, effects of ductal closure on general neonatal morbidity and neurodevelopmental outcome were only sporadically investigated. Associations of PDA with NEC and chronic lung disease were confirmed in recent studies, but causality was never proven and these complications occurred despite ductal closure.[44-49] Although a delay of surgical closure of >21 days results in a longer duration of mechanical ventilation and higher oxygen need, long term outcome such as chronic lung disease has not been reported.[50] A prophylactic indomethacin protocol prevented severe IVH, but did not influence neurodevelopmental outcome at follow-up.[51] Mortality is the only concrete outcome measure in which the benefit of ductal closure seems clear, with an odds ratio of 8 for preterm infants with failure of ductal closure.[52]

Since neonatal health care developed and improved drastically in the last 30 years (including the introduction of antenatal steroids and surfactant usage), some authors doubt the advantages of ductal closure and treatment preferences of PDA are again a hot topic. The fundamental idea that closure of a hemodynamic significant PDA is preferable is based on evidence of more than three decades ago, but it is questionable if our current population of preterm infants is comparable with the nursery population of

30 years ago. Vanhaesebrouck et al investigated a conservative approach with increase in mechanical ventilation conditions (PEEP) and decrease in daily fluid intake and found a high rate of spontaneous ductal closure. However, this study lacks data on the duration of spontaneous ductal closure, long term morbidity and consequences of a possible longer duration until ductal closure. [53] Others suggest to investigate symptomatic treatment of PDA, instead of focusing on ductal closure.[54]

A main problem in the quest to prove the possible disadvantages of ductal closure (or confirm the earlier reported benefits) is the difficulty to design an ethically justified randomized controlled trial, in which half of the PDA's will be closed and the other half will remain open. Such a RCT should include long term follow-up to assess neurodevelopmental outcome and long term consequences of neonatal morbidities such as chronic lung disease, NEC, and ROP.

Until there is evidence to demonstrate that it is better to leave a PDA untreated, studies striving for optimization of ductal closure, like the study reported in this thesis, remain necessary. We found an acceptable closure rate of PDA after a second and even third course of ibuprofen, without additional adverse effects. Based on these results we prefer a third course of ibuprofen, after a failed second course, above possible harmful surgical closure.

Recommendation

Our current treatment policy is still focused on closure of the hemodynamic significant PDA because no disadvantages of closure have been demonstrated. Our retrospective study demonstrated that second and third courses of ibuprofen, in an attempt to close the ductus, were effective and safe. We therefore prefer multiple courses of ibuprofen above possible harmful surgical closure.

Future perspectives

Partially based on our results, the most realistic design to gain more knowledge about the advantages and disadvantages of ductal closure (without performing a RCT) is a large scale prospective study. In this study all preterm infants with a hemodynamic significant PDA should be initially treated with ibuprofen, up to three courses if necessary. Data about mortality, morbidity, long term neurobehavioral development and long term consequences of neonatal morbidities should be collected prospectively. These outcomes should then be correlated with the duration of the PDA. Recently, two case-series of preterm infants treated with paracetamol for PDA (because of COX-inhibitor resistance or contra-indications) were published, with surprising closure

rates. Larger studies of paracetamol treated infants are needed to analyze effects and side effects of this treatment.[55,56] Another upcoming issue is the usage of oral instead of intravenous ibuprofen as cheap alternative. Also for this treatment approach more large studies are necessary to confirm the efficacy and especially the safety of oral ibuprofen, before considering this as treatment of first choice.[57-59]

Rifampin: Use of rifampin in persistent coagulase negative staphylococcal bacteremia in neonates

Late onset sepsis (i.e. >72 hours after birth) is a common problem in Neonatal Intensive Care Units and is mainly caused by coagulase negative staphylococci (CoNS), which are considered to be minimal virulent pathogens. CoNS are skin commensals and are therefore often seen as contaminants in blood cultures. However, their presence on the skin facilitates entry into the body through indwelling catheters, which are an important risk factor for CoNS bacteremia and may present with serious illness with clinical sepsis and instability.[83-87]

Among infants with CoNS bacteremia 12-40% develops persistent CoNS bacteremia, which is in most studies defined as at least 3 positive blood cultures with the same species despite antimicrobial treatment drawn at intervals of at least 48 hours.[88-91] Risk factors for persistent CoNS bacteremia are low birth weight, low gestational age, low absolute neutrophil count (<1000 cells/μL), parenteral feeding and receiving artificial instead of breast feeding. A relationship with the persistence and presence of indwelling catheters remains doubtful, as some studies confirm and others deny this relationship.[88,90-93] Short term outcome of persistent CoNS bacteremia includes an increased incidence of neonatal hyperglycemia, endocarditis and higher creatinine levels (possibly due to longer duration of vancomycin treatment), but neonatal mortality does not increase due to persistence.[89-93] In the long term, persistent CoNS bacteremia is associated with longer hospitalization and chronic lung disease (O2 need at 36 weeks postmenstrual age).[93]

The incidence of late onset sepsis in preterm infants is increasing, possibly due to increased survival of very preterm infants and increased usage of indwelling catheters.[83,84] The incidence of persistent CoNS bacteremia is also increasing, with higher minimal inhibitory concentrations (MIC) of vancomycin to provide adequate treatment of CoNS bacteremia as well.[91,93] The cause of this increased incidence

and decreased susceptibility for vancomycin is not totally clear yet. Most likely it's a complicated process of changes in virulence of the CoNS species, colonization of the NICUs and the admission of more extreme preterm infants. An important bacterial virulence factor is biofilm production, which is more common in persistent compared to non-persistent CoNS isolates. These biofilm producing CoNS isolates (especially S. epidermidis) have increased MIC values and resistance to many antimicrobials. Another consequence of biofilm production is a reduced access of antimicrobials through indwelling catheters.[90,94-96] Hypothetically, an increase in biofilm producing CoNS species that colonize our NICU's could be explain the changes in epidemiology. However, the studies reporting an increased incidence of persistent CoNS bacteremia do not report the biofilm production of their isolates. In addition, some studies have shown that bacteremia persists after removal of indwelling catheters, suggesting that other factors than biofilm are also responsible for persistence.[89,91]

Although the complete causal pathway leading to an increasing incidence of persistent CoNS bacteremia is not clear yet, we have to deal with this serious health problem in neonatal health care. Several studies in adults have confirmed the additional value of rifampin to antimicrobial treatment.[97-101] In neonates only 16 clinical cases (and 21 cases in a pharmacological analysis) were reported before we studied a series of 18 neonates, outlined in this thesis. We confirmed the additive effect of rifampin to vancomycin treatment for persistent CoNS bacteremia, with sterilization of blood culture within 2.3 ± 1.6 days and a considerable decline of C-reactive protein (CRP), especially in the first 3 days of rifampin treatment. However, special attention should be paid to optimization of vancomycin treatment by monitoring blood levels.

Recommendation

Based on current evidence we recommend vancomycin monotherapy when CoNS, susceptible for vancomycin, are isolated from a blood culture. Vancomycin trough and peak serum levels should be precisely monitored to optimize treatment. Indwelling catheters should be removed when blood culture becomes positive, but only if the clinical condition of the neonate does allow this. When bacteremia persists for at least 6 days, with 3 positive blood cultures (with 48 hours intervals), we recommend adding rifampin therapy to vancomycin. In addition, echocardiography and an ultrasound of the large abdominal vessels should be done to exclude an intravascular thrombus as cause of the persisting bacteremia.

Future perspectives

To gain more knowledge about the changing epidemiology of late onset sepsis caused by CoNS, we need a prospective study with a large cohort of infants with non-persistent and persistent CoNS bacteremia, describing clinical as well as microbial and pharmacological details. In addition, it would be very valuable to compare duration of bacteremia with and without rifampin addition, in a placebo-controlled double blind randomized controlled trial. As persistent CoNS bacteremia is associated with neonatal morbidity on short term and chronic lung disease on long term, investigation of neurobehavioral outcome on long term is desirable.

Postnatal IVIG: Outcome and management in neonatal thrombocytopenia due to maternal ITP

The incidence of idiopathic thrombocytopenic purpura (ITP), also known as auto-immune thrombocytopenia, in pregnant women is 1-10:10.000. Maternal anti-platelet antibodies, especially against GPIIb/IIIa platelet proteins on the cell membranes of all platelets, cause maternal thrombocytopenia and can pass via the placenta to the fetus.[60-63] Surprisingly, severe neonatal thrombocytopenia (<50x10^9/L) is present in only 8-13% of the neonates. Unlike the earlier in this thesis described condition of fetal and neonatal allo-immune thrombocytopenia, severe bleeding complications (i.e. ICH) are very rare in neonates born from mothers with ITP with an incidence of 0-2.9%.[64-72] The nadir of neonatal platelets is mostly seen around postnatal days 3-5; an explanation for this relatively late nadir is not clear yet.[61,69,73-75] Koyama et al reported an increased risk for severe neonatal thrombocytopenia in neonates delivered vaginally, suggesting an additional antibody boost passing the placenta due to uterine contractions. Hypothetically, this could be the reason for the relatively late nadir, but the late nadir for infants born by elective cesarean section can not be declared with this hypothesis. As the nadir of neonatal platelets is postpartum instead of antenatal, risk of intrauterine or peripartum ICH is less prominent. This is confirmed by a total reported rate of intrauterine ICH of 5/23 (22%) of all reported ICHs in neonates from mothers with ITP.[70,76] As peripartum ICH has never been reported, the method of delivery is nowadays only determined by obstetrical indications.[70,72]

Several retrospective studies describe possible predicting factors for severe neonatal thrombocytopenia, only maternal splenectomy and delivery of a previous infant with severe thrombocytopenia were associated with an increased risk for

thrombocytopenia.[65,66,69-72,77,78] Evidence is conflicting, but in most large studies maternal platelet counts (during pregnancy and delivery), maternal antibody state and maternal ITP treatment were not associated with severe neonatal thrombocytopenia.[64,64,66,68,69,71,72,77,79] As antenatal treatment with IVIG seems to prevent ICH in FNAIT without an associated increase in neonatal platelets (chapter 3 in this thesis), this mechanism is perhaps also active in maternal IVIG treatment for ITP. However, this is difficult to prove, because the incidence of ICH is very low in neonates from mothers with ITP during pregnancy.

Considering the extremely small risk of intrauterine or peripartum ICH, one may wonder about the clinical importance of determining risk factors for severe neonatal thrombocytopenia. As the neonatal platelet nadir is on postnatal day 3-5, the greatest bleeding risk is at that moment. Therefore, it makes more sense to focus on optimization of postnatal management, instead of determining possible risk factors. The majority of retrospective studies only appoint the number of infants which receive the different kinds of treatment, without analyzing the effectiveness of each separate treatment, possible due to the small amounts of severely affected infants.[62,65-67,79,80] Only 2 studies described a relatively large cohort of infants recommending one specific treatment: Ballin et al described 11 infants receiving postnatal IVIGs after platelet transfusions and Ovali et al described 6 neonates receiving prednisone after failed postnatal IVIGs. Ballin et al, however, did not analyze the increments after platelet transfusions and IVIG in detail; the effect of prednisone in the study of Ovali et al is dubious as all infants received IVIG prior to prednisone, which is known to take time before becoming effective.[81,82] The study described in this thesis is the first to analyze the course of postnatal platelet values and treatment effects of platelet transfusions and IVIG of each neonate with severe thrombocytopenia individually. We found a substantial relapse rate after multiple platelet transfusions without IVIG, but IVIG did not rule out relapse either. When relapse was occurring during IVIG treatment, this was most likely caused by the fact that IVIG needs a couple of days before becoming effective.

Recommendation

We recommend checking platelet values from umbilical cord blood, followed by daily platelet counts until postnatal day 5 or until a spontaneous rise (or stable level) is observed. If platelets are $<50 \times 10^9$/L, a cranial ultrasound scan should be made to rule out ICH. Treatment with platelet transfusions is indicated in case of severe thrombocytopenia ($<50 \times 10^9$/L); in case of relapse after the first platelet transfusion

we recommend to add IVIG. When thrombocytopenia persists and does not resolve after treatment with IVIG and multiple platelet transfusions, prednisone therapy can be considered.

Future perspectives

A randomized controlled trial for optimization of postnatal management of severe neonatal thrombocytopenia due to maternal ITP was never done and would be extremely difficult to perform because of the rarity of the condition. An international prospective registry of all infants with severe neonatal thrombocytopenia due to maternal ITP, containing details about their treatment and platelet course over time, would be very valuable to gain more knowledge for optimization of postnatal therapy. Such prospective registry can be completed with a follow-up assessment on long term, to consider possible effects of antenatal and postnatal IVIG and/or prednisone on long term neurobehavioral outcome.

Insulin: Short and long term outcome of neonatal hyperglycemia in very preterm infants

Hyperglycemia in preterm infants is caused by a complicated pathway including a combination of insulin resistance and relative insulin deficiency. Due to high circulating levels of inflammatory markers, cytokines and catecholamines resistance to insulin develops, with minimal glucose absorption for storage and no inhibition of gluconeogenesis by the liver. The immature pancreatic β-cells aren't able to compensate for these high glucose levels, as they can almost only produce pro-insulin, which is a 10-fold less active precursor of insulin, leading to relative insulin deficiency.[102-104]
Reported incidence of hyperglycemia in preterm infants is variable, as gestational ages differ between studies and there's still no consensus about a strict cut-off value for a widely used definition of hyperglycemia. In neonates with a birth weight of <1500 grams incidence of hyperglycemia varies between 36 and 68%.[105-108]
Hyperglycemia is associated with increased neonatal mortality.[107-111] ROP, NEC and cerebral white matter damage are also more common in preterm infants with hyperglycemia.[108,109,111-114] A relationship between hyperglycemia and IVH was only established in subgroup-analyses in hyperglycemic preterm infants and in an old study not correcting for confounding factors. Another frequently cited article for the relationship between IVH and hyperglycemia, of Finberg et al, only suggested

a relationship based on the pathophysiological pathway of hyperosmolality leading to intracranial hemorrhage, but didn't describe a population of preterm infants with evidence for this relationship.[110,115-117]

In many centers restriction of glucose intake is the first step in treatment of hyperglycemia. However, adequate caloric intake and weight gain in the first weeks of life are essential for later growth and neurobehavioral development.[118-120] Therefore, restriction of glucose intake must be limited to ensure a minimum intake to avoid malnutrition. Since the early eighties continuous insulin infusion is used to control excessive glucose levels. Advantages of insulin compared to the earlier used glucose restriction policy were less sepsis, higher glucose intake, better growth and more daily weight gain. [121-124]

In pediatric and adult intensive care units tight glucose control seems to reduce morbidity and mortality. This positive effect of tight glucose control in the older intensive care population, led to the hypothesis that this might also be applicable for preterm infants with hyperglycemia.[125-127] Beardsall et al studied an early elective insulin strategy (with prophylactic insulin from the first day of life), leading to a better energy intake, increased lower leg length and less episodes of hyperglycemia. However, mortality at postnatal day 28 and the incidence of hypoglycemic episodes were significantly higher with this early elective insulin policy.[105,128] The use of tight glycemic control, without prophylactic insulin, was also associated with an increased risk for hypoglycemia.[129] Recurrent or prolonged episodes of hypoglycemia are associated with cerebral damage, especially in parietal and occipital white matter and cortex, leading to an impaired neurodevelopmental outcome.[130-132] The advantages of insulin treatment for hyperglycemia need therefore to be balanced against the risks of hypoglycemia.

The only study on long term outcome of hyperglycemia in preterm infants was retrospective and performed by ourselves (outlined in this thesis). We found an impairment of both neurological and behavioral outcome in preterm infants with hyperglycemia, compared to those without. However, all infants received insulin treatment, which obfuscates the possible beneficial effects of insulin for neurobehavioral outcome. Did our infants have an impaired neurobehavioral outcome because of hyperglycemia induced brain damage or was this due to insulin usage with the possibility of undetected episodes of hypoglycemia?[110,133] Unfortunately, above mentioned associations of hyperglycemia with increased mortality and short term morbidity were only reported in study groups who also received insulin.

We concluded that hyperglycemia in preterm infants is associated with neonatal

mortality, short term morbidities and long term impaired neurobehavioral outcome. Despite the ability of insulin to lower serum glucose levels, beneficial effects in reduction of mortality, morbidity and impairment of neurobehavioral outcome have never been shown.

Recommendation

The real benefits of insulin treatment for hyperglycemia in preterm infants are not clear yet. This is due to the lack of comparisons of mortality, short term morbidity and long term outcomes in infants treated with and without insulin. Until specific advantages or disadvantages are proven, we recommend using insulin to control extreme glucose values. It is pivotal to strictly monitor glucose levels to prevent episodes of hypoglycemia during insulin administration.

Future perspectives

Comparison of prospectively collected data about mortality, short term morbidities and long term neurobehavioral outcome of preterm infants with hyperglycemia, treated with and without insulin, is necessary to detect the real advantages (or disadvantages) of insulin use. It would be helpful to obtain a uniform glucose cut-off value beyond which hyperglycemia has harmful effects and insulin treatment should be started.

Main conclusion

The aim of this thesis was to emphasize the clinical importance of retrospective research and the necessity of regular evaluations of currently used treatment protocols. All neonatal pearls described in this thesis were based on practical and clinical questions about currently used protocols; each study contains clinically relevant and usable recommendations for daily medical practice. Despite their retrospective character, which is not the design to gain the strongest evidence, these pearls are very important for neonatal medical health care.

Rare diseases will always be there, health care opportunities will be expanding and populations will continue to change: for these reasons we should continue to reflect on our medical handling and adapt and improve our policies. The chain of neonatal pearls should therefore remain open for new reflecting pearls, so beading can proceed in future.

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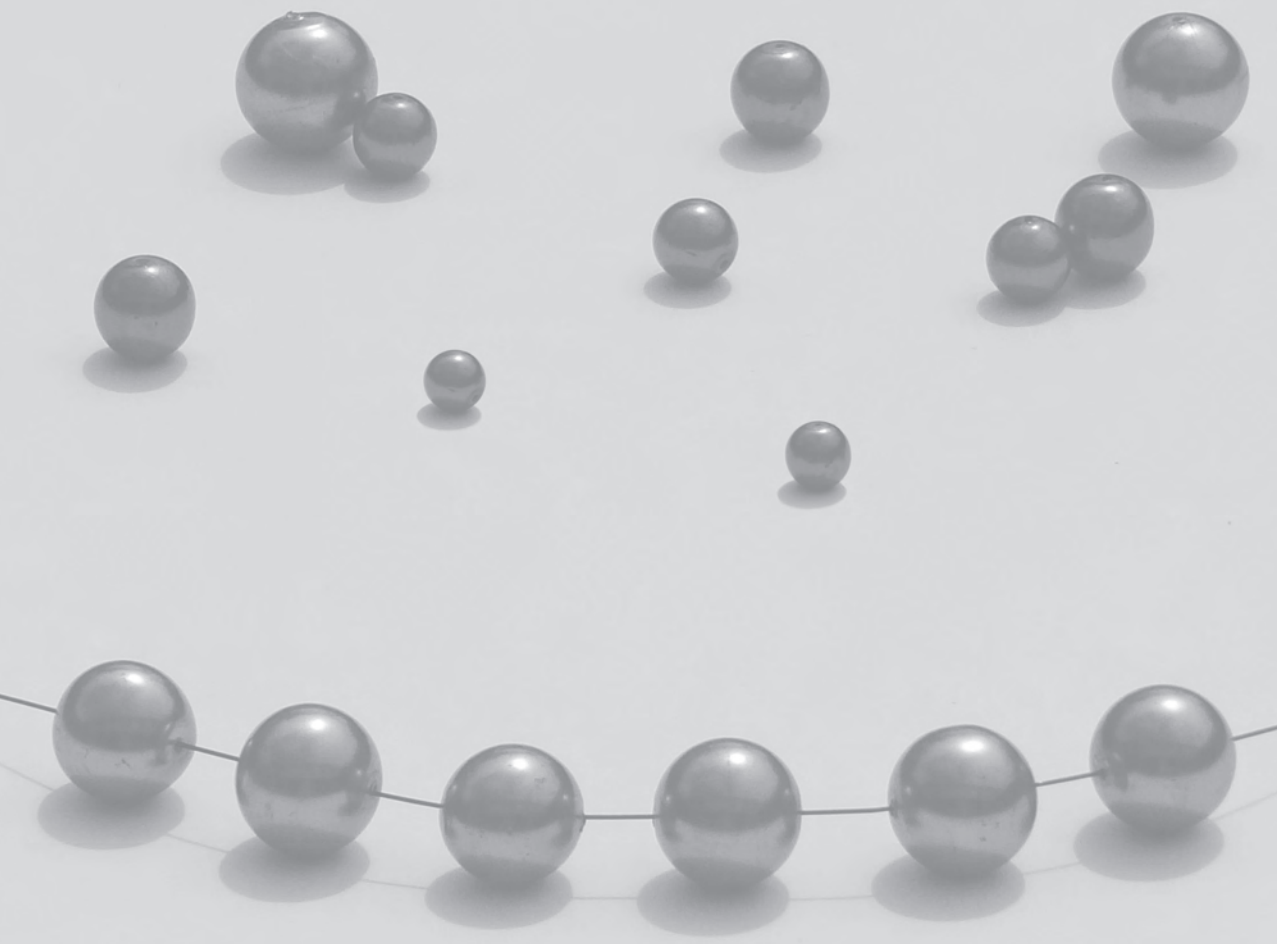
Chapter 9

Summary



Summary

Chapter 1 - General introduction.

Neonatal health care is provided with medication and protocols for almost all morbidities. Before the use of these medicines is allowed, they are extensively studied and tested for efficacy and safety. As patient population and knowledge on specific diseases changes with time, repeated evaluation of efficacy and safety of current used policies is of paramount importance. The most suitable study design for such evaluations is a retrospective overview, through which incidence and accompanying comorbidity can be surveyed. Results can possibly lead to adjustments of the protocol or be an incentive for new randomized controlled trials (RCT). RCTs are, however, only ethically justified once retrospective evidence is sufficient to suggest advantages of a possible new intervention.
Another great benefit of retrospective studies is their usefulness in investigation of rare diseases, which are common in neonatology. Designing prospective studies for possible new interventions in rare conditions, is extremely difficult.
Both above named reasons make retrospective studies indispensable in medical research.
In this thesis six 'Neonatal Pearls' are presented: six relatively rare clinical conditions, of which a retrospective study evaluates the efficacy, safety and/or long term consequences of the current protocol. Despite their retrospective design and relatively small sample size, they are all of significant value and may serve as potential foundations for future protocol adjustments and randomized controlled trials.

The general aim of this thesis was to emphasize the importance retrospective studies and evaluation of already existing protocols. Six retrospective studies investigating effectiveness, safety and/or long term consequences of different medicines in neonates are described.

Chapter 2 – Fetal, neonatal and developmental outcomes of lithium-exposed pregnancies.

Many women with a bipolar disorder are of reproductive age and will need to continue lithium treatment during pregnancy, as risks for relapses are high when discontinuing

lithium. The teratogenic and perinatal effects of lithium are slightly known, in contrary to the long-term effects of lithium on neurodevelopmental outcome of these children. In chapter 2 we investigated growth, neurological, cognitive and behavioral development of children exposed to lithium in utero in an observational retrospective cohort study. Of the 30 infants who were exposed to lithium in utero, 15 were available for follow-up and were investigated at 3-15 years of age. Only one child had signs of a minor neurological dysfunction, but without further clinical implications. Cognitive tests scores were within normal limits, although most children had lower scores on the performance IQ subtest. Growth, behavior and general development were all within the normal range. According to our results continuing lithium therapy during pregnancy seems not to cause adverse effects on growth, neurological, cognitive and behavioral development of exposed children.

Chapter 3 – Favorable neonatal outcome in allo-immune thrombocytopenia treated with antenatal intravenous immunoglobulin.

Fetal and neonatal allo-immune thrombocytopenia (FNAIT) is the most common cause of severe thrombocytopenia in neonates. Weekly maternal intravenous immunoglobulins (IVIG) is the cornerstone in antenatal treatment for already known cases of FNAIT. Nowadays, most centers prefer a non-invasive approach without fetal blood sampling (FBS) and intra-uterine platelet transfusions (IUPT). In chapter 3 we described a retrospective overview of 23 neonates treated antenatally between January 2006 and January 2012 with weekly maternal administration of IVIG. Twelve neonates (52%) had platelet counts <50 x 10^9/L, of which 3 had spontaneous rise, 8 received 1 matched platelet transfusion and 1 needed 2 matched transfusions. Three neonates had petechiae and hematomas, without clinical consequences. Only 1 neonate, without a sibling with intracranial hemorrhage (standard risk), had an intracranial (ICH) just before the start of antenatal IVIG at 28 weeks. Neurodevelopmental follow-up at two years of age was normal. The results of this study suggest that antenatal treatment with weekly maternal IVIG and postnatal matched platelet transfusion are effective and safe for the management of FNAIT.

Chapter 4 – Repeated courses of ibuprofen are effective in closure of a patent ductus arteriosus.

Patent ductus arteriosus (PDA) is a frequent complication in preterm infants. Ibuprofen and indomethacin (both COX-inhibitors) are used for pharmacological closure of PDA. In most centers a failed second course of COX-inhibitors is followed by possible harmful surgical closure. In chapter 4 we described a retrospective study to estimate the closure rate of clinically significant PDA after second and third courses of ibuprofen and record possible side effects. A total of 164 preterm infants, admitted between November 2005 and September 2011, with PDA were included. The closure rate was similar after the first (109/164), second (24/43) and third (6/11) course of ibuprofen (X^2=2.1, p=0.350). Late start of the first course of ibuprofen was a predictive factor for increased need of a second course (X^2=4.4, p=0.036). No additional side effects of multiple courses of ibuprofen were detected. Based on this study we conclude that repeated courses of ibuprofen are an effective and safe alternative for surgical closure and should be considered after failure of the first course of ibuprofen

Chapter 5 – Use of rifampin in persistent coagulase negative staphylococcal bacteremia in neonates.

Coagulase negative staphylococci (CoNS) are the most common cause of late onset sepsis in the Neonatal Intensive Care Unit (NICU). A minority of neonates does not respond to vancomycin therapy and develops persistent bacteremia, which may be treated with rifampin (originally an antibiotic against the tubercle bacillus). In chapter 5 we evaluated the use of rifampin in persistent CoNS bacteremia with a retrospective study of 137 neonates with CoNS bacteremia. Eighteen of these neonates were treated with rifampin, because of persistent bacteremia (3 positive blood cultures at least 48 hours apart with clinical symptoms) or (suspected) intravascular thrombus. Duration of bacteremia prior to rifampin therapy (8.0 ± 3.6 days) was positively correlated to the total duration of bacteremia (10.3 ± 3.7 days). The earlier rifampin was started, the earlier the blood culture became sterile. After starting rifampin therapy C-reactive protein (CRP) levels of all neonates declined and blood cultures became sterile after 2.3 ± 1.6 days. Vancomycin levels were not consistently measured in all neonates, sometimes resulting in late detection of sub-therapeutic trough levels. The results of this study indicate that rifampin is effective in the treatment of persistent CoNS

infections in neonates, but outcome may be more improved by adequate monitoring of vancomycin trough levels.

Chapter 6 – Outcome and management in neonatal thrombocytopenia due to maternal ITP.

Neonatal thrombocytopenia due to maternal idiopathic thrombocytopenic purpura (ITP) is not uncommon, but ICH is very rare(<1%). Postnatal neonatal treatment consists of platelet transfusions, IVIG and/or prednisone; however evidence about the preferred postnatal treatment is scarce. In chapter 6 we described a retrospective analysis of the 67 neonates born from 41 mothers with ITP during pregnancy. Severe thrombocytopenia occurred in 20/67 (29.9%) neonates; in one neonate unilateral polymycrogyria was detected on cranial imaging. In 3 neonates platelet count rose spontaneously, whereas 18 neonates received treatment (of which 1 due to persistent moderate thrombocytopenia). Postnatal treatment consisted of: platelet transfusions (n=3), prednisone (n=2), IVIG (n=1), platelet transfusions and IVIG (n=11), platelet transfusion and prednisone (n=1). Relapses of platelet counts after platelet transfusions were commonly seen. Risk factors for severe neonatal thrombocytopenia were delivery of a previous neonate with severe thrombocytopenia and low maternal platelet nadir during pregnancy.
The results of this study suggest severe thrombocytopenia in neonates from mothers with ITP occurs more frequently than previously reported. Treatment with multiple platelet transfusions and IVIG is often required to reach a platelet count above $50x10^9$/L. We propose starting IVIG, when platelet count falls quickly below 50 x 10^9/L after the first platelet transfusion.

Chapter 7 – Short and long term outcome of neonatal hyperglycemia in very preterm infants.

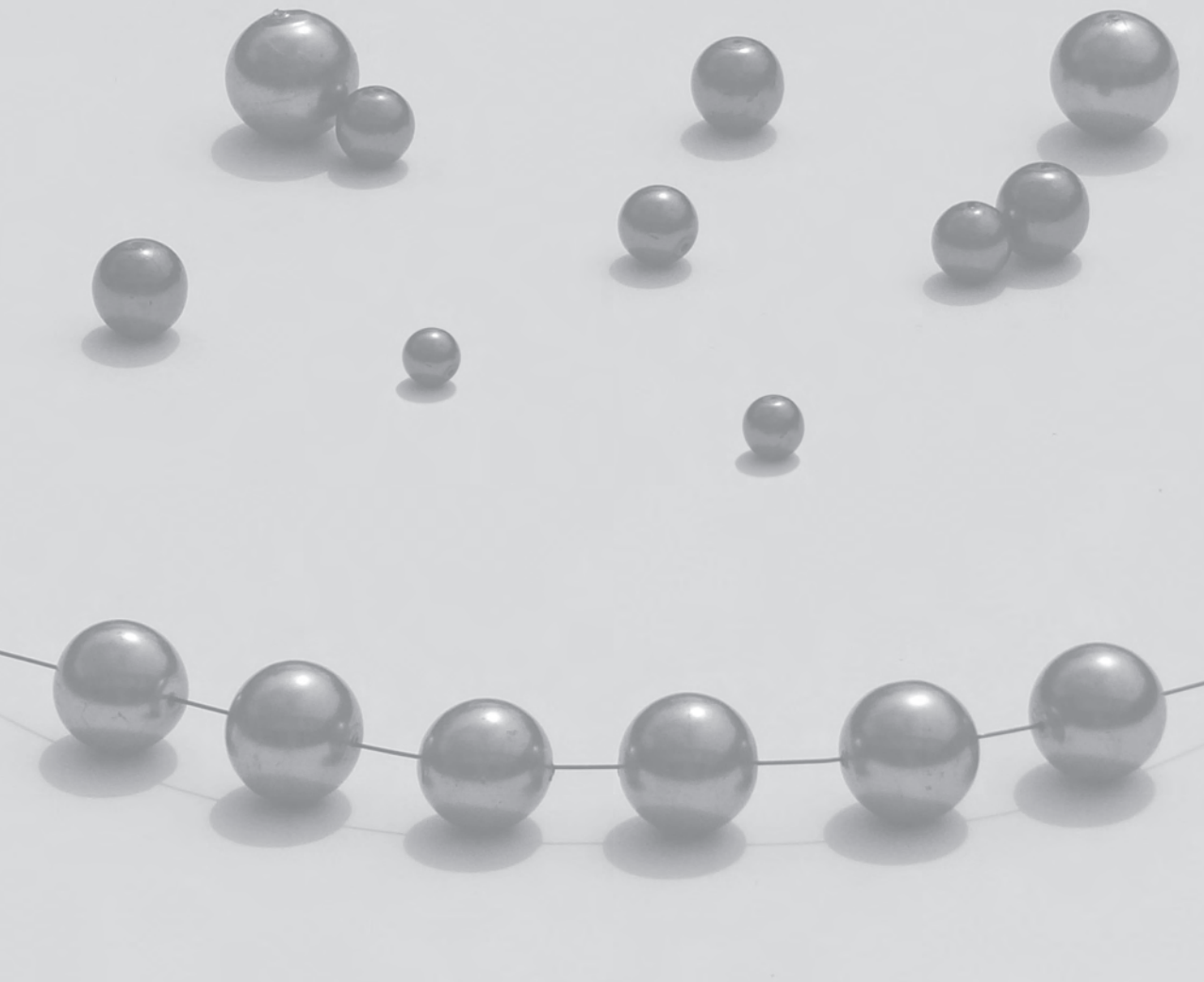
Hyperglycemia in preterm infants is associated with increased morbidity and mortality, but data on long-term outcome are limited. In chapter 7 we investigated the effects of neonatal hyperglycemia (blood glucose >10 mmol/l, treated with insulin for >12 hours) on growth and neurobehavioral outcome at 2 years of age in a retrospective follow-up study. Between January 2002 and December 2006 859 preterm infants (<32 weeks) were admitted, of which 66 (8%) developed hyperglycemia.

Hyperglycemia was significantly correlated to mortality, with 27/66 (41%) in the hyperglycemia group versus 62/793 (8%) in those without hyperglycemia. Mortality was predominantly observed in hyperglycemic infants with mean glucose values > 8.0 mmol/L or maximum glucose values > 9.5 mmol/L on days 3-4 after the diagnosis of hyperglycemia. Morbidity was also more common in infants with hyperglycemia and a birth weight of <1000 gram or a gestational age below 28 weeks.
Thirty-three survivors treated with insulin for hyperglycemia and 63 controls (matched for gestational age, birth weight, gender and year of admission) without hyperglycemia were evaluated for follow-up at a corrected age of 2 years. Growth was similar, but behavioral and neurological development were more frequently abnormal among those with hyperglycemia. We concluded that despite treatment with insulin, hyperglycemia has negative effects on mortality and neurobehavioral outcome. Therefore, more research to the pathophysiology of hyperglycemia-induced brain injury should be performed and better strategies to manage hyperglycemia are urgently required.

Chapter 8 – General discussion.

In the general discussion, the main results of this thesis were discussed for each chapter separately. A summary on 'what is already known' of each topic was given, underlining the weaknesses and contradictions in current evidence. Suggestions for further research were done, to improve our knowledge for optimization of treatment policies. Practical recommendations based on the current evidence, in combination with the results of the studies described in this thesis are proposed. We conclude by stating that evaluation and adjustment of medical interventions in neonates is a continuous process, which requires increased awareness from the medical community and well-designed studies.

Appendix



Nederlandse Samenvatting

Hoofdstuk 1 – Algemene introductie.

De neonatale gezondheidszorg is vandaag de dag voorzien van medicatie en protocollen voor bijna alle ziektes. Voordat deze medicijnen officieel gebruikt mogen worden, worden ze uitgebreid onderzocht op effectiviteit en veiligheid. Aangezien patiënt populaties en kennis over specifieke ziektes veranderen in de loop van de tijd, is het erg belangrijk effectiviteit en veiligheid te blijven evalueren. Een retrospectief overzicht is het meest geschikt voor zulke evaluaties, waardoor incidentie en co-morbiditeit duidelijk wordt. Uitkomsten kunnen daarna leidend zijn voor aanpassingen van bestaande protocollen of nieuwe gerandomiseerde trials. Deze gerandomiseerde trials zijn echter alleen ethisch verantwoord, als er voldoende retrospectieve aanwijzingen zijn om de voordelen van de nieuwe interventie te bewijzen.
Een ander groot voordeel van retrospectieve studies, is de bruikbaarheid voor het onderzoeken van zeldzame aandoeningen, die relatief veel voorkomen in de neonatologie. Voor deze aandoeningen is het heel moeilijk prospectieve studies uit te voeren, waardoor het extra belangrijk is alle aangedane cases te rapporteren.
Beide bovengenoemde toepassingen maken retrospectieve studies onmisbaar in de medische wetenschap. In dit proefschrift worden zes 'Neonatale parels' gepresenteerd: zes relatief zeldzame klinische condities, waarvan met behulp van een retrospectieve studie effectiviteit, veiligheid en/of lange termijn gevolgen van het huidige medicamenteuze protocol worden geëvalueerd. Ondanks hun retrospectieve karakter en de relatief kleine onderzoeksgroepen, zijn al deze zes parels van grote waarde als basis voor toekomstige protocolverbeteringen of grotere gerandomiseerde studies.

Het belangrijkste doel van dit proefschrift was het belang van retrospectieve studies en evaluatie van huidige gebruikte protocollen te benadrukken. Zes retrospectieve studies beschrijven de evaluatie van veiligheid, effectiviteit en/of lange termijn uitkomst van medicatie voor foetus en neonaat.

Hoofdstuk 2 – Korte en lange termijn uitkomsten van kinderen, blootgesteld aan lithium tijdens de zwangerschap.

Veel vrouwen met een bipolaire stoornis zijn in de vruchtbare periode van hun leven en moeten de behandeling met lithium continueren tijdens de zwangerschap, omdat het risico op een terugval direct na de zwangerschap bij staken erg groot is. De

teratogene en perinatale effecten van lithium op de foetus en neonaat zijn enigszins bekend, in tegenstelling tot de lange termijn effecten van intra-uteriene lithium blootstelling. In hoofdstuk 2 onderzochten we de groei, neurologische, cognitieve en gedragsmatige ontwikkeling van kinderen die tijdens de zwangerschap blootgesteld werden aan lithium, in een observationele retrospectieve cohort studie. Vijftien van de 30 (50%) kinderen, die blootgesteld werden aan lithium tijdens de zwangerschap, waren beschikbaar voor follow-up onderzoek op een leeftijd van 3-15 jaar. Slechts 1 kind had tekenen van milde neurologische afwijkingen, maar zonder verdere klinische gevolgen. Cognitieve testen waren binnen de normaalwaarden, wél hadden de meeste kinderen lagere scores op performance IQ testen. Groei, gedragsmatige- en algemene ontwikkeling waren binnen de normaalwaarden. Op basis van deze resultaten lijkt het continueren van lithium gedurende de zwangerschap geen duidelijke nadelige effecten te hebben op groei, neurologische, cognitieve en gedragsmatige ontwikkeling van intra-uterien blootgestelde kinderen.

Hoofdstuk 3 – Goede neonatale uitkomst in allo-immune thrombocytopenie, behandeld met antenatale intraveneuze immunoglobulines.

Foetale en neonatale allo-immuun trombocytopenie (FNAIT) is de meest voorkomende oorzaak van ernstige trombocytopenie bij neonaten. Wekelijkse maternale behandeling met intraveneuze immunoglobulines (IVIG) is de basis van de behandeling van bekende gevallen van FNAIT. De meeste centra prefereren een niet-invasieve benadering zonder foetale bloedafname en intra-uteriene trombocytentransfusies. In hoofdstuk 3 beschreven we een retrospectieve studie van 23 antenataal behandelde neonaten tussen januari 2006 en januari 2012. Twaalf neonaten (52%) hadden trombocyten van $<50 \times 10^9$/L bij de geboorte, waarvan er 3 een spontane stijging hadden. Acht neonaten kregen 1 gematchte trombocytentransfusie, 1 neonaat had 2 gematchte trombocytentransfusies nodig voor een adequate stijging in trombocytengetal. Drie neonaten hadden petechiën en hematomen, zonder klinische consequenties. Slechts 1 neonaat, zonder broertje of zusje met een intracraniële bloeding (standaard risico zwangerschap), had een intracraniële bloeding vlak voor de start van antenatale IVIG bij een amenorroeduur van 28 weken. Follow-up op de leeftijd van 2 jaar was niet afwijkend. De resultaten van deze studie suggereren dat antenatale behandeling met wekelijks maternale IVIG en postnatale behandeling met gematchte trombocytentransfusies effectief en veilig zijn in de behandeling van FNAIT.

Hoofdstuk 4 – Meerdere ibuprofen kuren zijn effectief voor het sluiten van een persisterende ductus arteriosus.

Een persisterende ductus arteriosus (PDA) is een veel voorkomende complicatie bij prematuren. Ibuprofen en indomethacin (beiden COX-remmers) worden gebruikt voor farmacologische sluiting van de ductus arteriosus. In de meeste centra wordt een falende tweede kuur COX-remmers gevolgd door chirurgische sluiting, wat geassocieerd is met morbiditeit en een slechtere ontwikkeling. In hoofdstuk 4 beschreven we een retrospectieve studie die het sluitingspercentage van een tweede en derde ibuprofen kuur en de mogelijke bijwerkingen rapporteerde. In totaal waren er tussen november 2005 en september 2011 164 prematuren met een PDA opgenomen. Het sluitingspercentage was gelijk (X^2=2.1, p=0.350) voor de eerste (66%, 109/164) , tweede (56%, 24/43) en derde (55%, 6/11) kuur ibuprofen. Laat starten met de eerste kuur ibuprofen was een voorspellende factor voor de behoefte aan een tweede kuur ((X^2=4.4, p=0.036). Er werden geen extra bijwerkingen van meerdere ibuprofenkuren gerapporteerd. Op basis van deze studie wordt geconcludeerd dat meerdere kuren ibuprofen effectief en veilig lijken. Er wordt daarom geadviseerd een tweede of derde ibuprofenkuur te prefereren boven mogelijk schadelijke chirurgische sluiting, indien een eerste of tweede ibuprofenkuur gefaald heeft.

Hoofdstuk 5 – Gebruik van rifampicine voor persisterende coagulase negatieve staphylococcen sepsis bij neonaten.

Coagulase negatieve staphylococcen (CNS) zijn de meest voorkomende oorzaak van late sepsis in de Neonatale Intensive Care Unit (NICU). Slechts een klein deel van de neonaten reageert niet op vancomycine en ontwikkelt een persisterende bacteriemie, die behandeld kan worden met rifampicine (van oorsprong een antimicrobieel middel tegen de tuberkel bacil). In hoofdstuk 5 evalueerden we het gebruik van rifampicine voor persisterende CNS bacteriemie met een retrospectieve studie van 137 neonaten met CNS bacteriemie. Achttien van deze kinderen werden behandeld met rifampicine voor een persisterende CNS bacteriemie (3 positieve bloedkweken met 48 uur ertussen en klinische symptomen) of (een serieuze verdenking op) een intravasculaire trombus. De duur van de bacteriemie voor de start van rifampicine (8.0 ± 3.6 dagen) was positief gecorreleerd aan de totale duur van de bacteriemie (10.3 ± 3.7 dagen), dus hoe eerder rifampicine gestart werd, des te sneller de bloedkweek negatief werd. Na het starten van rifampicine daalden C-reactive protein (CRP) waarden snel en werden bloedkweken

negatief binnen 2.3 ± 1.6 dagen. Vancomycine spiegels werden niet structureel gemeten bij alle neonaten, waardoor in enkele gevallen subtherapeutische dalspiegels laat ontdekt werden. De resultaten van deze studie suggereren dat rifampicine effectief is in het behandelen van persisterende CNS bacteriemie bij neonaten, waarbij de uitkomst nog verbeterd kan worden door vancomycine dalspiegels goed te monitoren.

Hoofdstuk 6 – Uitkomst en behandeling van neonatale thrombocytopenie als gevolg van maternale ITP tijdens de zwangerschap.

Ernstige neonatale trombocytopenie door maternale idiopathische trombocytopenische purpura is niet veel voorkomend, maar intracraniële bloedingen zijn zeer zeldzaam (<1%). Postnatale neonatale behandeling bestaat uit trombocytentransfusies, IVIG en/of prednison; maar bewijs over de postnatale behandeling van voorkeur is schaars. In hoofdstuk 6 rapporteerden we een retrospectieve analyse van 67 neonaten, geboren uit 41 moeders met ITP tijdens de zwangerschap. Ernstige neonatale trombocytopenie kwam voor bij 20/67 (29.9%) van de neonaten, bij één van hen werd unilaterale polymicrogyria waargenomen op een echo en MRI van het cerebrum. De ernstige trombocytopenie herstelde spontaan bij 3 neonaten; 18 neonaten werden behandeld met trombocytentransfusies, IVIG, prednison of combinaties daarvan (waarvan 1 neonaat met persisterende matige trombocytopenie). Het terugvallen van trombocytengetal < 50 x 10^9/L na trombocytentransfusies werd vaak gezien. Ernstige neonatale trombocytopenie in een vorige zwangerschap en een lage laagste maternale trombocytenwaarde tijdens de zwangerschap leken risicofactoren voor het krijgen van een neonaat met ernstige trombocytopenie. De resultaten van deze studie laten zien dat het postnatale neonatale trombocytengetal een grillig beloop kan hebben, met meerdere terugvallen na trombocytentransfusies. Dit suggereert dat behandeling met IVIG niet te lang uitgesteld moet worden en gestart dient te worden na een terugval na de 1^e trombocytentransfusie.

Hoofdstuk 7 – Korte en lange termijn uitkomsten van neonatale hyperglycemie in extreme prematuren, behandeld met insuline.

Hyperglycemie bij prematuren is geassocieerd met een toename in morbiditeit en mortaliteit, maar data over de gevolgen op lange termijn zijn beperkt. In hoofdstuk

7 onderzochten we de effecten van neonatale hyperglycemie (bloed glucose > 10 mmol/l, behandeld met insuline > 12 uur) op groei, neurologische en gedragsmatige ontwikkeling op de leeftijd van 2 jaar in een retrospectieve follow-up studie. Tussen januari 2002 en december 2006 werden er 859 prematuren (< 32 weken) opgenomen, waarvan er 66 (8%) hyperglycemie ontwikkelden. Hyperglycemie was significant gerelateerd aan mortaliteit, met 27/66 (41%) in de hyperglycemische groep versus 62/793 (8%) in de groep zonder hyperglycemie. Mortaliteit werd vooral gezien in hyperglycemische kinderen met gemiddelde glucosewaardes van > 8.0 mmol/L of maximum glucosewaardes > 9.5 mmol/L op dag 3-4 na het begin van de episode. Morbiditeit werd voornamelijk gezien bij kinderen met hyperglycemie én een geboortegewicht van < 1000 gram of amenorroeduur onder de 28 weken.
Drieëndertig overlevende kinderen met hyperglycemie, behandeld met insuline, en 63 controles (gematched voor amenorroeduur, geboortegewicht, geslacht en jaar van opname) zonder hyperglycemie werden geëvalueerd voor follow-up op de gecorrigeerde leeftijd van 2 jaar. Groei was vergelijkbaar, maar neurologische en gedragsmatige ontwikkeling waren significant vaker afwijkend bij kinderen met neonatale hyperglycemie in de voorgeschiedenis. We concludeerden dat, ondanks behandeling met insuline, hyperglycemie negatieve effecten op mortaliteit en neurologische en gedragsmatige ontwikkeling heeft. Meer onderzoek naar de pathofysiologie van hyperglycemie geïnduceerde breinschade zou waardevol zijn, naast verbetering van de huidige behandelingsstrategieën van neonatale hyperglycemie.

Hoofdstuk 8 – Algemene discussie.

In de algemene discussie werden de resultaten, beschreven in dit proefschrift, voor elk hoofdstuk apart besproken. Er werd een samenvatting gegeven van wat al bekend is over elk onderwerp, waarbij de zwaktepunten, tegenstellingen en vraagtekens in de huidige literatuur aangestipt werden. Suggesties voor toekomstig onderzoek werden gedaan, voor het verbeteren van onze kennis en het optimaliseren van huidige protocollen. Per hoofdstuk werd een praktische toepasbare aanbeveling gedaan, gebaseerd op het huidige bewijs en de resultaten van de studies, beschreven in dit proefschrift. De algemene discussie eindigt met de boodschap dat evaluatie van beleid en aanpassing van protocollen voor neonaten een continue proces is, wat een toegenomen bewustzijn van de medische wereld en goed ontworpen studies vereist.

List of abbreviations

ADH	Attention deficit/hyperactivity
BPD	Bronchopulmonary dysplasia
BSID	Bayley scales of infant development
CBCL	Child behaviour checklist
CoNS	Coagulase negative staphylococci
COX	Cyclo-oxygenase
CRP	C-reactive protein
CTG	Cardio tocography
CYP	Cytochrome P
DSM	Diagnostic and statistical manual of mental disorder
ECG	Electric cardiogram
FBS	Fetal blood sampling
FNAIT	Fetal- and neonatal allo-immune thrombocytopenia
GA	Gestational age
HPA	Human platelet antigen
ICH	Intracranial haemorrhage
IgG	Immunoglobulin, subclass G
ITP	Idiopathic thrombocytopenic purpura
IUGR	Intra uterine growth restriction
IUPT	Intra uterine platelet transfusion
IVH	Intraventricular haemorrhage
IVIG	Intravenous immunoglobulin
MND	Minor neurological dysfunction
MRI	Magnetic resonance imaging
NEC	Necrotizing enterocolitis
PDA	Patent ductus arteriosus
PEEP	Positive end expiratory pressure
PIQ	Performance intelligence quotient
PROM	Prolonged rupture of membranes
PVL	Periventricular leukomalacia
RDS	Respiratory distress syndrome.
SD	Standard deviation
TIQ	Total intelligence quotient
VIQ	Verbal intelligence quotient
WPSSI/WISC	Wechsler intelligence scale for children

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GGZ Rijnstreek, Rivierduinen, Alphen aan de Rijn
Elise A.M. Knoppert-van der Klein

Dankwoord (acknowledgements)

In de zomer van 2009 maakte ik tijdens mijn wetenschapsstage kennis met wetenschappelijk onderzoek op de afdeling Neonatologie van het LUMC. Alle studenten schreven meteen een echt wetenschappelijk artikel in het Engels, waar ik dus ook een verwoede poging toe deed. Ruim vier jaar later ben ik nog geen twee jaar afgestudeerd én mag ik hier dit proefschrift verdedigen, iets wat ik in mijn stoutste dromen niet had durven dromen en waar ik veel mensen heel dankbaar voor ben.

Prof. F.J. Walther, beste Frans, heel erg bedankt voor de geboden mogelijkheid om het resultaat van mijn wetenschapsstage en aanvullende losse onderzoeken tot dit proefschrift te mogen vormen. Vanaf het begin maakten uw enthousiasme, opbouwende kritiek en laagdrempelige benaderbaarheid de samenwerking heel prettig, waardoor ik heel veel geleerd heb en ben aangestoken met het wetenschapsvirus.
Dr. E. Lopriore, beste Enrico, bedankt voor je vele kritische blikken en altijd enthousiaste en motiverende reacties. Het was ontzettend fijn om zo laagdrempelig vragen te kunnen stellen en daarop heel snel antwoorden terug te krijgen, ook op zon- en feestdagen.

Zonder vele co-auteurs hadden de artikelen in dit proefschrift niet tot stand kunnen komen, daarnaast hebben Anouk Figee en Aukje van Kampen fantastisch geholpen met het verzamelen van de data: allemaal bedankt daarvoor!

Ook gaat veel dank uit naar de vakgroep kindergeneeskunde en opleiders van het Reinier de Graaf Gasthuis in Delft. Bedankt voor jullie vertrouwen in mij als klinische dokter, voor het geboden voorstel om het schrijven van dit proefschrift eerst zo ver mogelijk af te maken, met een super leuke baan als ANIOS in het vooruitzicht. Natuurlijk mogen mijn assistentencollega's hier niet ontbreken: dank voor jullie roosterflexibiliteit voor deze week, promotie-tips & trucks, stelling-ideeën en veel gezelligheid op de assistentenkamer.

Blaaskwintet Kwintvlaag: Marijke, Maaike, Léon en Jurjen, heel erg bedankt voor de vele gezellige avonden, lay-out technische adviezen, muzikaliteit en luisterende oren: naar muziek, naar proefschrift-gerelateerde en persoonlijke zaken. We zijn in drie jaar tijd een hecht vijftal geworden en ik hoop dat we nog heel veel jaar in deze samenstelling van ons kunnen laten horen.

Claire, Marleen, Ingrid en Joyce, bedankt voor jullie blijvende interesse in mijn onderzoek en alle gezellige 'oreo-avonden'. In het bijzonder wil ik daarbij Joyce en Ingrid bedanken, mijn paranimfen. Als drie naïeve meisjes van 20 vertrokken we in 2007 naar Nepal en nu, zes jaar later, zijn we alweer ruim een jaar echte dokters. Ontzettend bedankt voor onze vriendschap van de afgelopen 8 jaar en voor jullie inspanningen om deze dag tot een feestje te maken.

Lieve Marieke, mijn kleinste en tegelijkertijd ook grootste zus, na jouw middelbare school eindexamen in 2011 en mijn afstuderen in 2012 was jij eigenlijk weer aan de beurt om in het middelpunt van de belangstelling te staan. Ik ga nu voor m'n beurt en dat voelt oneerlijk, maar ondanks dat moet je weten dat ik echt super trots op je ben! Lieve pap en mam, bedankt voor jullie steun in mijn keuzes, jullie eeuwige interesse in alles waar ik mee bezig ben, de aaien over m'n bol (soms ook telefonisch) en de Naaldwijkse deur die altijd voor me open staat. Van jullie kreeg ik de Westlandse harde werkers mentaliteit met de paplepel ingegoten, iets waar ik heel blij mee ben, want we zijn liever moe dan lui. Op naar de volgende uitdaging, want stilzitten is best moeilijk!

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Curriculum Vitae

Margreth van der Lugt was born on the 6th of December 1986 in Delft, the Netherlands. She grew up in Naaldwijk and went to Interconfessionele Scholengemeenschap Westland for secondary school, she graduated in 2005. In September of the same year she started to study Medicine at the Leiden University Medical Center. The optional study-space in the second year she filled with a clinical round in Scheer Memorial Hospital in Nepal, where she was for five weeks with her friends Joyce and Ingrid. For three years (from 2007 until 2009), Margreth combined her Medicine study with participating in a minor study flute at the Royal Conservatory in The Hague. She also joined the student orchestra 'Krashna Musika' in Delft, where she's still an active member in the orchestra and several committees. When lectures were finished, in the fourth year of Medical school, Margreth started doing research at the Neonatology department guided by dr. Enrico Lopriore and Prof. dr. Frans Walther. After the obligatory research practice of five months, she started with her clinical internships in February 2010, but continued doing research. In February 2012 she graduated cum laude as a Doctor of Medicine; afterwards she used half a year to almost finish this thesis. Since September 2012 Margreth is working as a pediatric registrar at the Reinier de Graaf Gasthuis in Delft. In January 2014 she will start her clinical training in pediatrics at the LUMC.