Neurodevelopmental **O**utcome after **F**etal **N**eonatal **A**llo**I**mmune **T**hrombocytopenia

NO FNAIT

NEURODEVELOPMENTAL OUTCOME AFTER FNAIT

Protocol ID	NL70410.058.19
Short title	NO FNAIT
EudraCT number	Not applicable
Version	3
Date	20-09-2019
Coordinating investigator	Drs. T.W. de Vos Department of Pediatrics Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden Tel. 071-5262748/06-52665280 t.w.de_vos@lumc.nl
Project leader	Dr. J.M.M. van Klink Department of Pediatrics Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden Tel. 071-5263925 j.m.m.van_klink@lumc.nl
Principal investigators	Prof. Dr. E. Lopriore Department of Pediatrics Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden Tel. 071-5262965 e.lopriore@lumc.nl Prof. Dr. M. de Haas Stichting Sanquin Bloedvoorziening Plesmanlaan 125 1066 CX Amsterdam Tel. 020-5123000 m.dehaas@sanquin.nl

Sponsor	Head of Department of Pediatrics Prof. Dr. E.H.H.M Rings Department of Pediatrics Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden E.H.H.M.Rings@lumc.nl
Subsidising party	Stichting Sanquin Bloedvoorziening Plesmanlaan 125 1066 CX Amsterdam Tel. 020-5123000
Independent expert	Dr. V.E.H.J. Wintjes Department of Pediatrics Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden Tel. 071-5262965 V.E.H.J.Smits-Wintjens@lumc.nl
Laboratory sites	Not applicable
Statistical constulant	Dr. S. Böhringer Department of Biomedical Data Sciences Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden Tel. 071-5269743 S.Boehringer@lumc.nl
Pharmacy	Not applicable

PROTOCOL SIGNATURE SHEET

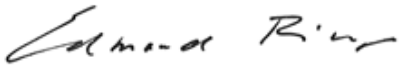
Name	Signature	Date
Head of Department: Head of Department of Pediatrics Prof. Dr. E.H.H.M Rings Department of Pediatrics Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden e.h.h.m.rings@lumc.nl		**20-09-2019**
Principal Investigator: Dr. J.M.M. van Klink Department of Pediatrics Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden Tel. 071-5263925 j.m.m.van_klink@lumc.nl		**20-09-2019**

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	Adverse Event
AR	Adverse Reaction
ASQ	Ages and Stages Questionnaire
BSID	Bayley Scales of Infant and Toddler Development
CARAT	Control of Allergic Rhinitis and Asthma Test
CITO	Centraal Instituut voor Toetsontwikkeling
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
eCRF	Electronic case report form
FNAIT	Fetal and Neonatal Alloimmune Thrombocytopenia
GCP	Good Clinical Practice
GMFCS	Gross Motor Function Classification System
HLA	Human Leukocyte Antigen
HPA	Human Platelet Antigens
HRQoL	Health related Quality of Life
IC	Informed Consent
ICH	Intracranial hemorrhages
IBM	International Business Machines
Ig	Immunoglobulins
IUT	Intrauterine transfusions
IUPT	Intra uterine platelet transfusions
IVIg	Intravenous Immunoglobulin
IQ	Intelligence Quotient
IQR	Interquartile range
LUMC	Leiden University Medical Center
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
NDI	Neurodevelopmental Impairment
(S)AE	(Serious) Adverse Event
SD	Standard deviation

Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
SPSS	Statistical Package for the Social Sciences
WISC	Wechsler Intelligence Scale for Children
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen
WPPSI	Wechsler Preschool and Primary Scale of Intelligence

1. SUMMARY

Rationale: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a disease caused by allo-immunisation during pregnancy. If left untreated, FNAIT can lead to severe fetal intracranial haemorrhage. This complication can be prevented by weekly administration of intravenous immunoglobulin (IVIg) to the mother during pregnancy. Although treatment with IVIg is nowadays considered as the golden standard for the management of FNAIT, the exact mechanism of action of IVIg is still under debate. Most importantly, the long-term effects and safety of IVIg treatment in FNAIT have not yet been evaluated. The use of IVIg during pregnancy for FNAIT is therefore still 'off-label'. The medication leaflet of IVIg continues to state that there is insufficient knowledge on the use of this product during pregnancy, despite all the years of experience and implementation in virtually all international guidelines.

Knowledge on long-term development of FNAIT survivors with or without IVIg treatment is very limited but an important subject in the counselling of parents of newly diagnosed cases. A greater understanding of the long-term outcome and the effects of IVIg on child development is necessary.

Objective: To evaluate the long-term neurodevelopmental outcome in two groups of children with FNAIT. First, children with unanticipated disease 'index cases' that were born without maternal IVIg administration during pregnancy. Second, children with anticipated disease; their mothers were treated with IVIg during pregnancy.

Study design: Observational cohort study.

Study population: The study population consists of two different cohorts of FNAIT children born in the Netherlands between 2002 and 2017.

- Cohort 1: The first cohort of children will consist children with unanticipated disease, 'index cases that were born without maternal IVIg administration during pregnancy.
- Cohort 2: Second cohort will consist of children with anticipated disease; their mothers were treated with IVIg during pregnancy.

Methods: Neurodevelopmental evaluation of the children will be performed, at a minimum age of 24 months. This includes an assessment of neurologic, motor and cognitive development using standardized psychometric tests appropriate for age and parent-report questionnaires on Health Related Quality of Life (HRQoL) and behavioral functioning. Academic trajectories of FNAIT children will be assessed by collecting test scores from the Dutch pupil monitoring system. We will assess allergies and the course of infections by questionnaires.

Main study parameters/endpoints: The primary outcome is cognitive development i.e., cognitive test score or Intelligence Quotient (IQ). Secondary outcome is neurodevelopmental impairment (NDI); a composite outcome including cerebral palsy Gross Motor Functioning

Classification System (GMFCS) ≥ II, cognitive and/or motor test score of less than 70 (-2 SD), hearing loss requiring amplification or visual impairment (legally certifiable as blind or partially sighted). Other secondary outcome parameters are HRQoL and behavioral functioning and the academic performance. The course and incidence of infections and allergies will be assessed.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness

The burden of neurodevelopmental assessment tests is minimal (e.g. 120 minutes at 2 to 6 years and 150 minutes at 7 to 17 years follow-up). Neurodevelopmental assessment is generally experienced as enjoyable for children. Parents will complete a HRQoL- and a behavioral questionnaire for their children. Academic performance will be evaluated by scores that were obtained at school. Participation does not result in any direct benefit.

2. INTRODUCTION AND RATIONALE

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is the most common cause of thrombocytopenia in otherwise healthy term-born neonates. [1] FNAIT is a rare disease with an incidence estimated around 1 per 1000 live newborns. [2] During pregnancy alloimmunization can occur due to incompatibility of the Human Platelet Antigens (HPA) on the maternal and fetal platelets. Alloimmunization and maternal production of antibodies directed against the HPA-positive fetal platelets, leads to thrombocytopenia and an increased risk of intracranial hemorrhages (ICH) in the fetus. Clinical presentation can vary from skin bleedings to severe ICH leading to lifelong neurologic sequelae or intrauterine death. [2] [3]

In the past, FNAIT was managed with invasive and high-risk interventions including intrauterine platelet transfusion (IUPT). Since the end of the 20th century, invasive intrauterine transfusions (IUT) were replaced by a new, non-invasive therapy: maternal administration of intravenous immunoglobulin (IVIg). This novel therapy resulted in a significant lower risk of intrauterine fetal death and ICH. [4] Intervention with immune modulation in the semi -allogenic environment of the fetus by administration of immunoglobulins (Ig) is successful, especially in preventing ICH. [5] However antenatal treatment with IVIg has been implemented as standard of care without strong methodological follow-up research of children from mothers treated with IVIg. To date, only two follow-up studies have been published in children with anticipated FNAIT cases. The first study of a FNAIT cohort treated with IVIg was done by Ward et al. in 2006. [6] They concluded that development of children treated for FNAIT was better compared to their non-treated siblings. Their conclusions were based on non-validated questionnaires taken by telephone, assessing the behavioral outcome of the children and were limited by a ~40% lost-to-follow-up rate. [6] A second follow-up study including 39 children was published by a research group from our center in 2004. This research stated that the outcome in children with FNAIT and exposed to maternal IVIg treatment was similar to the normal population. However, this study included a heterogenic group of children with different treatment strategies including IUT, hampering definitive conclusions and substantiating the need for more research. [7]

No long-term standardized follow-up studies were performed on FNAIT cases without antenatal treatment and/or ICH. The natural course of the disease and long-term effects of thrombocytopenia on the developing fetus and newborn are unknown. FNAIT is defined as a disease caused by alloantibodies, resulting in thrombocytopenia and a risk of bleeding in the neonate. In the last years, evidence is increasing that the maternal alloantibodies can also bind to the fetal endothelium and may impair angiogenesis in the developing fetuses [8] [9]. It

is not known at which moment in pregnancy the developing brain is most vulnerable for damage induced by these kind of alloantibodies. The timing in fetal life FNAIT associated ICH ranges from 23 to 42 weeks [10], but small bleeding may not be diagnosed. It may also be that these type of alloantibodies not lead to ICH but to other type of cerebral damage. These lesions can remain subclinical directly after birth but lead to developmental delay on the long term. This knowledge can be of great interest when counseling parents with a risk of FNAIT or in writing guidelines.

For 3 decades a nationwide screening on FNAIT to detect pregnancies with alloantibodies in time and start treatment to prevent bleedings is being discussed. If alloantibodies lead to cerebral damage on the long term also in patients without large ICH this might have large implications in the debate on the introduction of a national screening programme. Therefore we want to underline that more knowledge about the long-term development of FNAIT survivors is required.

The Leiden University Medical Center (LUMC), a national fetal therapy center in The Netherlands, has a close and long-lasting collaboration with Sanquin. This collaboration offers a unique opportunity to evaluate a large and complete cohort of children with FNAIT. LUMC and Sanquin are both nationwide referral centers for FNAIT and committed to improve timely detection of high-risk cases who need intra-uterine therapy. In our opinion we have a duty as a national expertise center to assess long-term outcome in children with FNAIT and describe the natural history of children affected by FNAIT and the long term effects of a given therapy.

3. OBJECTIVES

3.1 Primary objective(s)

The primary objective is to determine the cognitive test score of children diagnosed with FNAIT without and with antenatal treatment. We will assess the neurodevelopmental outcome, with IQ score as outcome measure, of two groups:
Cohort 1: To evaluate the natural history of FNAIT, we will include in this first cohort all FNAIT survivors without antenatal treatment. Our hypothesis is that the cognitive test score in this cohort will be lower compared to the general population.
Cohort 2: To evaluate the safety and benefit of IVIg treatment, the IQ score in all FNAIT survivors treated with antenatal IVIg treatment will be determined. Our hypothesis is that the cognitive test score in this cohort is similar to the general population.

3.2 Secondary Objective(s)

In both groups we will assess the incidence of neurodevelopmental impairment (NDI). NDI is a composite outcome including cerebral palsy (GMFCS ≥ II), cognitive or motor test score of less than 70, hearing loss requiring amplification or visual impairment (legally certifiable as blind or partially sighted). Other secondary outcomes will be HRQoL and behavioral problem score. In both cohorts we will assess academic performance by evaluation of test scores on three domains; arithmetic and spelling performance and reading comprehension. [11-13]

We will also focus on potential long-term side effects of IVIg treatment and determine the prevalence of allergies, eczema, asthma and the course of infections.

3.3 Other study parameters

We will gather the data about the following data of our cases; age and sex of the child, obstetric medical data and neonatal characteristics and morbidity of the study population. Parents will be asked to complete a questionnaire to assess the highest level of education of the parents. We use this data to describe our study population and correct for potential confounding of these factors on neurodevelopmental outcome as described in chapter 11. Study parameters are summarized in Table 1, a list of definitions can be found in Table 3.

Possible confounding factors for allergies are the family history for asthma, allergies or eczema. For evaluation of the infections it is important to assess smoking at home and day-care visit as possible confounding factors.

Table 1: Study parameters

Study parameters		
Obstetric data	Maternal characteristics	Age mother at delivery
		Obstetric history
		Medication usage during pregnancy
	Pregnancy characteristics	Gravidity/Parity
		Pregnancy induced hypertension
		Pre-eclampsia
		Signs of bleeding in the fetus at ultrasound
	FNAIT related therapy	IVIg treatment
		Intra-uterine transfusions
		Treatment with corticosteroids
	Labour characteristics	Mode parturition
		Gestational age at delivery
	Placental features	Placenta weight
Neonatal data	Birth data	Gestational age at birth
		Birthweight
		Apgar score
	Severe neonatal morbidity	Respiratory distress syndrome
		Perinatal asphyxia
		Proven early onset neonatal sepsis
		Retinopathy of prematurity
		Necrotizing enterocolitis
	Neonatal neurologic sequelae	Intracranial haemorrhage
		Cystic periventricular leukomalacia
		Ventricular dilatation
		Porencephalic or parenchymal cysts
		Convulsions
		Any cerebral lesions associated with adverse neurologic outcome
	Signs of bleeding	Skin manifestations
		Intracerebral bleeding
		Organ bleeding
		Increased bleeding tendency, reported by the caregiver
	Therapy	Platelet transfusions
		IVIg treatment

Table 1: Study parameters

Study parameters		
Laboratory data	HPA	HPA incompatibility
		HPA genotyping
		Anti-HPA-titre
	Platelet count	Directly after birth
		Nadir
	Other	Presence of HLA class I antibodies
Outcome	Neurodevelopmental scores	Cognitive test score
		Motor test score
		Cerebral palsy
	Neurodevelopmental impairment	Impaired cognitive or motor development
		Impaired functioning in communication
		Bilateral blindness
		Bilateral deafness
	Quality of life	Health related quality of life score
	Behavior	Behavioural test score
	Academic performance	Arithmetic test score at primary school
		Spelling test score at primary school
		Reading comprehension test score at primary school
	Immunology	Prevalence of allergies
		Patient reported anaphylaxis
		Poor control of allergic rhinitis
		Poor control of asthma
		Patient reported eczema
		Course and incidence of infections
Potential confounders	Demographic characteristics	Age and gender of the child
		Family history (first degree) of allergy, astma or eczema
		Highest level of education of the parents
		Smoking at home
		Day-care visit
		Ethnic origin of the parents

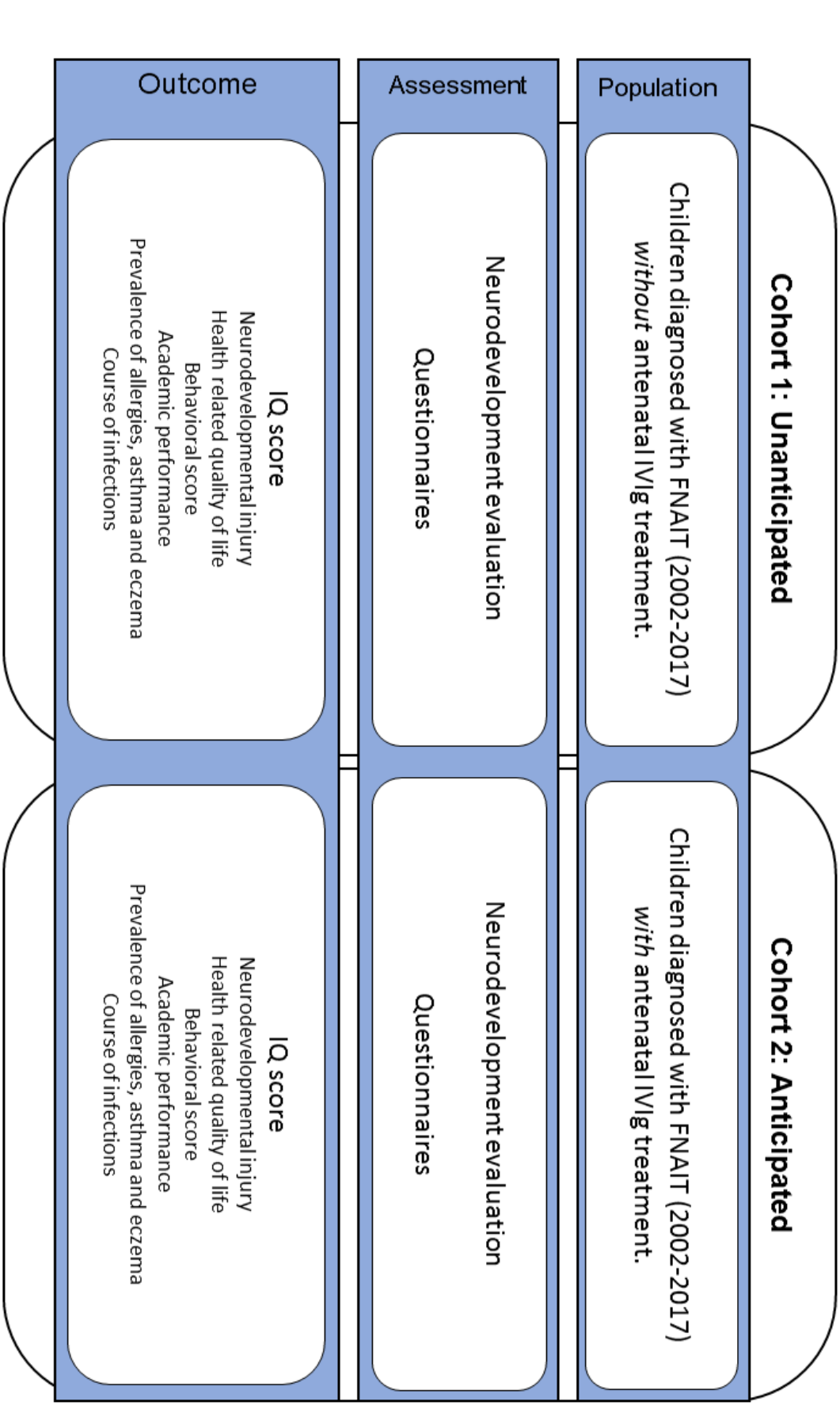
4. STUDY DESIGN

We will perform an observational cohort study. The long-term neurodevelopmental outcome of children affected by FNAIT will be evaluated. All children born between 2002 and 2017 and diagnosed with FNAIT are eligible for follow-up assessment and will be invited for an assessment at our outpatient clinic. The FNAIT survivors will be collected in two cohorts; cohort 1 will consist of FNAIT survivors without antenatal treatment, cohort 2 will consist of FNAIT survivors that were antenatally anticipated and therefore IVIg treatment to the mother was given. A summary of our study design is presented in Figure 1.

Enrollment in our study will take place via the LUMC and Stichting Sanquin Bloedvoorziening. The LUMC is national referral center for intrauterine therapy and Sanquin is national reference laboratory to diagnose FNAIT. Retrospectively FNAIT cases will be collected and asked for permission directly or via referring specialist according to the procedures described in paragraph 12.2.

After informed consent, child cognitive functioning will be assessed with a formal psychological test of cognitive functioning described in table 2, chapter 9. According to age, the parents will complete a standardized behavioral and HRQoL questionnaire. Academic performance will be assessed by collecting the most recent CITO test scores from the Dutch Pupil monitoring system developed by the National Institute for Educational Measurement [14]. Assessment of the prevalence of possible late effects of IVIg on the immune system will be assessed by questionnaires about the prevalence of allergies, astma, eczema and course of infections by questionnaires. Parents and children, when 12 years old or older, are asked for consent to request the medical letters from the maternity or neonatology ward to obtain perinatal and neonatal data.

Figure 1: Study design



5. STUDY POPULATION

5.1 Population (base)

All children born between 2002 and 2017 and diagnosed with FNAIT are eligible for this study. They will be invited for an assessment at our outpatient clinic. FNAIT cases that were not antenatally treated with IVIg will be eligible for the study (cohort 1), as well as FNAIT cases which were anticipated antenatally by materal IVIg administration (cohort 2).

The retrospective study design might bias the results (e.g. survival and recall bias). However in our opinion this is the only feasible way to perform a long term development study in this disease. Between 2002 and 2017 the NOICH trail was performed, after this trial the dosage regime of IVIg treatment was reduced from 1.0 g/kg/week to 0.5 gr/kg/week. We do not think this will have important effects on the results of our study but will verify this effect by description of the maternal treatment dosage.

5.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Children diagnosed with FNAIT during pregnancy or postnatal, at moment of inclusion 2 to 16 years of age.
- Children living in the Netherlands.
- Parents or guardian aged ≥ 18 years old, with parental authority.
- Written informed consent form both parents with, form being approved by Ethic Committee.

5.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Children born with congenital and/or chromosomal abnormalities.
- Children that passed away before inclusion.

5.4 Sample size calculation

Of note, performing sample size calculations in the context of this rare disease is difficult since literature is scarce and heterogeneous.

We performed two different sample size calculations for the two cohorts.
Cohort 1: Using the literature to date we estimated that the IQ score of FNAIT survivors without antenatal treatment is lower, that is 92 versus average (100) in the normal population. To show a difference in IQ score between FNAIT survivors without IVIg treatment compared to a fixed average of 100, we performed a sample size calculation for a one-sample t-test. We calculated that a minimum group size of 39 was needed to show a mean difference of 7 IQ points with a power of 0.8 and a significance of 0.05. We assume a standard deviation (SD) of 15 for the IQ score.

Cohort 2: In the FNAIT survivors with antenatal treatment we expect no difference in IQ score between cases and the national average. To show that there is no difference IQ score in anticipated IVIg treatment compared to the Dutch average we perform a non-inferiority calculation by a one-sample t-test. In this case we need a sample size of 39 children with a non-inferiority margin of 7 IQ points, with a power of 0.8 and a significance level of 0.025. We assume a standard deviation (SD) of 15 for the IQ score.

6. TREATMENT OF SUBJECTS

Not applicable.

7. INVESTIGATIONAL PRODUCT

Not applicable.

8. NON-INVESTIGATIONAL PRODUCT

Not applicable.

9. METHODS

9.1 Study parameters/endpoints

9.1.1 Main study parameter/endpoint

To determine the cognitive and motor development score in children diagnosed with FNAIT without and with antenatal treatment. This will be evaluated using formal neurologic examination [15] and Bayley scales of Infant and Toddler Development third edition (Bayley III) at 2-3 years,[16] Wechsler Preschool and Primary Scale of Intelligence third edition (WPPSI III) at 4 to 6 years,[17] and Wechsler Intelligence Scale for Children fifth edition (WISC V) at 7 to 16 years [18].

9.1.2 Secondary study parameters/endpoints

NDI is a composite outcome of:

- Cerebral palsy ≥ grade II according to Gross Motor Classification System (GMFCS) [19]
- Impaired cognitive, language and/or motor development (test scores <70)
- Bilateral blindness and/or bilateral deafness
- Bilateral deafness requiring amplification

The Health Related Quality of life will be assessed with PedsQL and behavioral problem score will be assessed using the Child Behavior Checklist [20, 21].

Academic performance will be evaluated by obtaining test scores from the Dutch Pupil monitoring system. [14] The latest test scores from primary school will be requested. If children are in high school grades from grade 6 in primary school will be used in our evaluation to obtain uniform test scores. Three academic domains will be assessed; arithmetic and spelling performance and measurements on reading comprehension. Of these three domains the following scores will be obtained from the monitoring system; the raw test scores, standardized test scores and the level of the child.

To assess long-term effects of maternal IVIg treatment on the immune system of the child we will assess two outcome parameters:

- The prevalence of allergies, asthma and eczema will be evaluated by a questionnaire composed in collaboration with an allergist. The severity and control of the allergic rhinitis will be assessed by the CARATkids questionnaire [22].

- The prevalence of children with an abnormal incidence or frequency of infections will be assessed by a questionnaire based on the guideline; ‘Richtlijn diagnostiek naar onderliggende aandoeningen bij kinderen met recidiverende luchtweginfecties’ [23] and the 10 warning signs of PI of the Jeffrey Model Foundation. [24]

9.1.3 Other study parameters

An inventory will be made of the following confounders gender, obstetrical and neonatological complications and education level of the parents according to the International Classification of Education. [25] We will assess the family history of allergies asthma and eczema to obtain information about possible confounding factors in our study groups. Also smoking and day-care visit will be questioned.

Table 2: Overview assessments

Study assessments			
Age group	**Name assessment tool**	**Abbreviation**	**Outcome**
Cognitive development			
2-3 years	Bayley scales of infant and toddler development third edition.	Bayley III	IQ score
4-6 years	Wechsler Preschool and Primary Scale of Intelligence third edition.	WPPSI III	IQ score
7-16 years	Wechsler Intelligence Scale for Children fifth edition	WISC V	IQ score
Motor development			
2-3 years	Bayley scales of infant and toddler development third edition	Bayley III	Motor test score
2-18 years	Neurologic Examination According to Touwen.	Touwen	Gross Motor Function Classification System
Quality of life			
5-7 years	Pediatric Quality of Life Inventory	PedsQoL	Health related quality of life score
8-12 years	Pediatric Quality of Life Inventory	PedsQoL	Health related quality of life score
Behaviour			
1.5-5 years	*Child Behavior Checklist*	CBCL	Behaviour test score
6-18 years	*Child Behavior Checklist*	CBCL	Behaviour test score
Course of infections			
2-18 years	Questionnaire based on '*Richtlijn diagnostiek naar onderliggende aandoeningen bij kinderen met recidiverende luchtweginfecties'*		Indication for diagnostic evaluation by immunologist; Yes/No.

Table 2: Overview assessments

Study assessments			
Age group	**Name assessment tool**	**Abbreviation**	**Outcome**
Atopic constitution			
2-18 years	Questionnaire composited in collaboration with allergist (Dr. H. de Groot).		(Parent reported) Allergy Anaphylaxis Asthma Eczema
Severity of asthma and hay fever			
6-18 years	Control of allergic rhinitis and asthma test.	CARATkids	CARATkids score

Table 3: Definitions of outcome

Definitions of outcome (1/3)	
Outcome parameter	Definition
Obstetric medical data	
Pregnancy induced hypertension	De novo hypertension (blood pressure above 140/90 mmHg) after gestational week 20.[26]
Pre-eclampsia	De novo hypertension after gestational week 20 and new onset of one of the following; proteinuria (>300 mg/day), renal insufficiency, liver disease, neurological problems, hematological disturbances or fetal growth restriction. [26]
Signs of bleeding in the fetus at ultrasound	For instance intraventricular hemorrhages or ventricular dilatation.
Antentatal IVIg-treatment	Administration during pregnancy from 28 weeks gestational age or 16-18 weeks gestational age in high risk pregnancies according to the protocol of the LUMC.[27]
Intra-uterine transfusions	Any platelet transfusions of the fetus during pregnancy.
Treatment with corticosteroids	Maternal treatment with corticosteroids to prevent burden or worsening of alloantigen production.
Mode parturition	Type of parturition (e.g. caesarean section and instrumental delivery).
Gestational age at birth	Gestational age: completed weeks and additional days since the first day of the last menstruation period of the mother.
Placenta weight	Weight of placenta after birth in grams.
Neonatal data (part 1)	
Respiratory distress syndrome	Requiring mechanical ventilation and/or surfactant.
Perinatal asphyxia	One of the following criteria; Apgar score < 7 and/or umbilical cord pH ≤ 7.0.
Proven early onset neonatal sepsis	Positive blood culture within 72 hours postpartum and clinical suspicion of a neonatal sepsis.
Retinopathy of prematurity	Staging according to ICROP [28]
Neonatal morbidity	Scored positive if one of the following conditions were present: Respiratory distress syndrome, Perinatal asphyxia, Proven early onset neonatal sepsis, NEC, Retinopathy of prematurity.

Definitions of outcome (2/3) Outcome parameter	Definition
Neonatal data (part 2)	
Necrotizing enterocolitis	Stage 2 or higher [29]
Intracranial hemorrhage	Any cranial hemorrhage
Intraventricular hemorrhage	Grading according to Volpe [30]
Cystic periventricular leukomalacia	Grade 2 or higher [31]
Ventricular dilatation	Above 97th percentile [32]
Porencephalic or parenchymal cysts	Presence of porencephalic or parenchymal cysts. Porencephalic cyst: focal area of encephalomalacia that communicates with the ventricular system and/or the subarachnoid space. Parenchymal cyst: smooth rounded borders and minimal-to-no surrounding signal intensity abnormality.
Convulsions	Any paroxysmal, repetitive or stereotypical events interpreted as neonatal convulsions by a pediatrician.
Signs of bleeding	
Intracerebral bleeding	Any bleeding in the cerebral cavity.
Organ bleeding	Bleeding in any organ located in the thorax or abdomen.
Postpone bleeding	Postpone bleeding that was noted as sign of bleeding by the caretaker.
Skin manifestations	Petechiae, purpura or hematoma diagnosed by the caretaker at neonatal period (gynecologist, pediatrician or midwife).
Neonatal IVIG treatment	Any administration of IVIG after birth.
Platelet transfusion	Any platelet transfusion.
Neurodevelopmental impairment	
Cerebral Palsy	Spastic bilateral, spastic unilateral or mixed Classification by European CP Network [19].
Impaired cognitive or motor development	Score < 70 (2 SD below the mean) as assessed by Bayley Scales of Infant and Toddler Development version 3 (BSID III) [16]
Bilateral blindness	Blind or partially sighted.
Bilateral deafness	Needing hearing aids.

Definitions of outcome (3/3)	
Outcome parameter	Definition
Secondary outcomes	
Abnormal course or incidence of infections	Need to refer to an immunologist or the need to preform diagnostics based on history taking according to the Dutch guidelines. [23]
Prevalence of allergies	Parent reported allergy.
Patient reported anaphylaxis	Serious allergic reaction, requiring urgent medical treatment with epinephrine.
Poor control of allergic rhinitis	CARAT score of upper airways < 8.[22, 33]
Poor control of asthma	CARAT score of lower airways < 16.[22, 33]
Prevalence of eczema	Parent reported eczema.
Demographic characteristics	
Ethnic origin of the parents	Ethnic origin of mother and father.
Educational level of the parents	Highest level of graduation of the parents.
Smoking	Any person living in the household of the child smoking one or more cigarettes a day.
Day-care visit	When child is in day care at least one day a week.
Family history (first degree) of allergy, asthma or eczema	Positive when 1st degree of family members receive daily treatment for asthma or eczema. Or when a family member wears an epinephrine pencil because of a severe allergy.

9.2 Randomisation, blinding and treatment allocation

Not applicable.

9.3 Study procedures

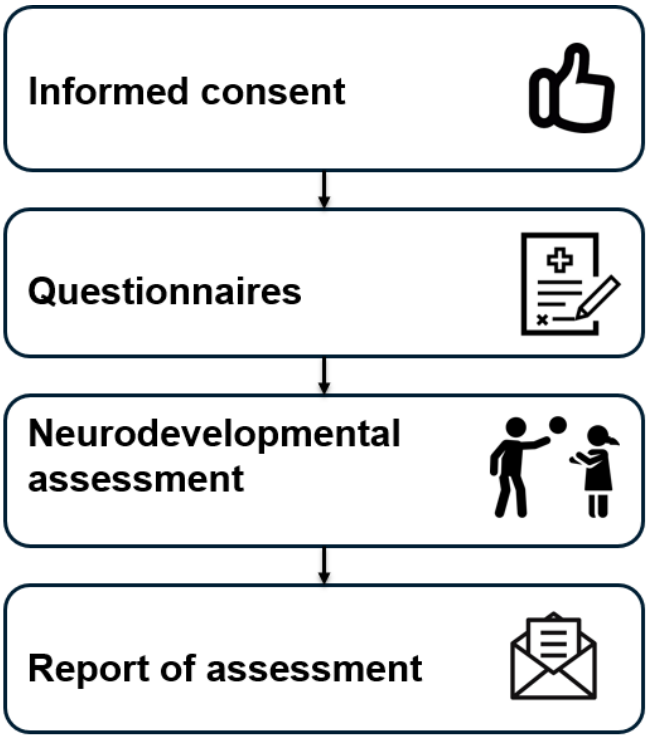
After informed consent is obtained, assessment will be planned at the outpatient clinic of the LUMC. Questionnaires considering HRQoL, behaviour, allergies or infections will be send home with the request to fill in the forms and bring them at the planned visit. We will

provide parents with a letter to request the most recent academic test scores from the teacher.

When parents visit the outpatient clinic with their children, the assessment will take place. No laboratory tests will be performed in this study, however data of the laboratory tests that were performed at timepoint of diagnosing FNAIT will be involved in this study. To gather important patient data from the pregnancy and neonatal period parents will be asked permission to obtain the medical letters from referral hospital of the child. Secondly mothers will be asked for informed consent to obtain the medical letter from the gynaecologist.

After assessment a report will be made from the observations during psychological testing.

Figure 2: Study procedures



9.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons during the assessment.

9.4.1 Specific criteria for withdrawal

Not applicable

9.4.2 Replacement of individual subjects after withdrawal

Not applicable

9.4.3 Follow-up of subjects withdrawn from treatment

Not applicable

9.5 Premature termination of the study

In case of premature termination of the study the project leader will notify the accredited METC within 15 days, including the reasons for the premature termination.

10. SAFETY REPORTING

10.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

10.2 AEs, SAEs and SUSARs

10.2.1 Adverse events (AEs)

The risk on adverse events in this study negligible, therefore AEs will not be reported. If parents notify psychological burden of the assessments and notify this burden to the researchers in writing, this will be recorded and reported to the METC.

10.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the METC without undue delay after obtaining knowledge of the events.

The investigator will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a

period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

10.3 Follow-up of adverse events

The risk of AE in this study is negligible, therefore follow-up of AEs is not applicable. Unexpected findings or study results that might be of importance for the development or health of our subjects will be reported to the general physician.

10.4 Data Safety Monitoring Board (DSMB)

An independent Data Safety Monitoring Board (DSMB) will not be installed for this study.

11. STATISTICAL ANALYSIS

Continuous parametric data will be presented with mean ± standard deviation (SD) or median with interquartile range (IQR) as appropriate. Categorical data will be presented as counts and percentages within each study group.

The cognitive test score of both cohorts will be compared with the Dutch average by using a lineair regression model. The following potential confounders for cognitive development (IQ score) identified in earlier research will be used in the analysis;

- Sex
- Gestational age at birth
- Small for gestational age
- Neonatal morbidity
- Level of education of the parents

In second cohort; the FNAIT survivors with IVIg treatment atopic constitution and the course of infections will be assessed in a secondary analysis.

In the assessment of course of infections the following confounders will be used in the analysis by using a lineair regression model;

- Day-care visit
- Smoking in a household
- Time of the year that the questionnaire was filled in

In the analysis of allergies and asthma the following confounders will be used in the analysis by using a lineair regression model;

- Presence of a first degree family member with asthma eczema or allergy
- Smoking in a household

All data is analysed with IBM SPSS Statistics version 24 (IBM Software, NY, USA, 2016).

12. ETHICAL CONSIDERATIONS

12.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013; version 2013; www.wma.net) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

12.2 Recruitment and consent

The investigator will identify potential subjects according to the aforementioned criteria. Eligible patients are informed about the study and are asked for their participation. Enrolment in will take place in two ways;

- First directly via the LUMC, the LUMC is national referral center for intrauterine therapy. Parents of children that survived FNAIT will be invited to participate by sending a patient information letter and information leaflet for children. In this way, the parents will be informed that they will be contacted by phone approximately two weeks after sending the information letter.
- Secondly patients will be asked via Stichting Sanquin Bloedvoorziening, Sanquin is the national reference laboratory to diagnose FNAIT. Retrospectively all FNAIT cases are collected in the Laboratory of platelet and leukocyte serology of Sanquin. Referring specialists (mostly pediatricians) will be asked to obtain permission from the parents to inform them about our study by letter enclosed by the patient information leaflet. If parents do not object to be informed they will be contacted by our research team approximately two weeks after sending this letter.

When parents wish not to be approached by our research team they can indicate this by responding by e-mail, phone or reply letter. Informed consent is obtained from all patients who are willing to participate. In case of minors both parents or their guardian will be asked for participation and informed consent. The patient information letter and informed consent form will be attached as a separate document. If informed consent is given, assessment will be planned. At most, 3 attempts will be made to contact parents by phone. It is expected that enrollment for this study will cover a period of one year.

12.3 Objection by minors or incapacitated subjects

Neuropediatric examination and neurodevelopmental tests are performed by trained personnel who are in close contact with the child and parents to detect any resistance. All tests have age related items and can be performed in a playful way to avoid stress.

The investigators will follow the guidelines of the Dutch pediatric society concerning resistance or anxiety in minors during investigation procedures and the CCMO code of conduct concerning research with minors.

12.4 Benefits and risks assessment, group relatedness

Participation in the study does not result in individual benefits. There is no risk associated with study participation.

Our department has extensive experience in conducting neurodevelopmental assessment of children. The main part of the neurodevelopmental assessment and the completion of questionnaires are part of routine care for preterm children born with a gestational age <30 weeks and/or birth weight <1000 grams or <P10, for children treated with fetal therapy for children with documented severe neonatal brain injury. The examinations are performed according to the guidelines of the Dutch Neonatal follow-up (LNF) Study Group. Parents and children will receive a report of the test results and, if necessary, advice for further support and interventions.

12.5 Compensation for injury

A dispensation for the liability insurance was accredited by the METC Leiden Den Haag Delft.

12.6 Incentives

Children will receive a small present (to be determined, for example a stuffed animal) after the follow-up assessments. Parents receive a parking fee and travel allowance.

13. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

13.1 Handling and storage of data and documents

Data management will be implemented according to Good Clinical Practice (GCP)-guidelines. Patient data will be entered by way of an eCRF in a central GCP proof internet-based database to facilitate on-site data-entry. Castor was chosen as our data management system. Data will be stored at the LUMC network. Security is guaranteed with login names, login codes and encrypted data transfer. The researchers will maintain the database and check the information in the database for completeness, consistency and plausibility with consultancy of the department advanced data management.

The data of all subjects will be coded and this coding will not be retraceable to the individual patient. The key to this coding is safeguarded by the investigator. A limited number of people have access to the source data. These are the principal investigators, investigating personnel. Personal data are only processed by the researchers or by those who fall directly under their authority. In addition, the study monitor, quality assurance auditor, employees from the Health Care Inspectorate of the Ministry of Health, welfare and Sport (Nederlandse Inspectie voor de Gezondheidszorg) have access to the source data. All are subject to the pledge of confidentiality. Data will be stored for 15 years strictly confidential.

Consent for our study or storage of personal data can be withdrawn at any time during our study. Research data that was collected till withdraw of permission will be used in our current study. The board of directors of the LUMC is responsible for processing data. The data protection officer, *functionaris gegevensbescherming,* of the LUMC or/and Sanquin can be contacted by participants when they have questions about the protection of their privacy.

13.2 Monitoring and Quality Assurance

The study will be monitored by the internal monitoring pool of the LUMC throughout its duration by means of personal visits to the investigator's facilities and through other communications (e.g., telephone calls, written correspondence). Monitoring visits will be scheduled, conform to the risk classification, once throughout the study and at frequency deemed appropriate for the study. For details we refer to the monitoring plan of the study.

This visit will be conducted to evaluate the progress of the study, ensure the rights and wellbeing of the subjects are protected, check that the reported clinical study data are

accurate, complete and verifiable from source documents, and the conduct of the study is in compliance with the approved protocol and amendments, GCP and applicable national regulatory requirements. A monitoring visit will include a review of the essential clinical study documents (regulatory documents, CRFs, source documents, subject informed consent forms, etc.) as well as discussion on the conduct of the study with the Investigator and staff. The Investigator and staff should be available during these visits to facilitate the review of the clinical study records and resolve/document any discrepancies found during the visit.

13.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

13.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

13.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

In case the final study report will not be available within one year, another term should be defined including the reasons.

13.6 Public disclosure and publication policy

The final publication of the study results will be written by the study coordinator(s). A draft manuscript will be submitted to co-authors for review. After revision the manuscript will be sent to a peer reviewed scientific journal. Publication will be conform CCMO publication policy. [34]

14. STRUCTURED RISK ANALYSIS

Not applicable.

15. REFERENCES

1. Dreyfus, M., et al., *Frequency of immune thrombocytopenia in newborns: a prospective study. Immune Thrombocytopenia Working Group.* Blood, 1997. **89**(12): p. 4402-6.
2. Williamson, L.M., et al., *The natural history of fetomaternal alloimmunization to the platelet-specific antigen HPA-1a (PlA1, Zwa) as determined by antenatal screening.* Blood, 1998. **92**(7): p. 2280-7.
3. Winkelhorst, D., et al., *Perinatal Outcome and Long-Term Neurodevelopment after Intracranial Haemorrhage due to Fetal and Neonatal Alloimmune Thrombocytopenia.* Fetal Diagn Ther, 2018: p. 1-8.
4. Bussel, J.B., et al., *Antenatal management of alloimmune thrombocytopenia with intravenous gamma-globulin: a randomized trial of the addition of low-dose steroid to intravenous gamma-globulin.* Am J Obstet Gynecol, 1996. **174**(5): p. 1414-23.
5. Winkelhorst, D., et al., *Antenatal management in fetal and neonatal alloimmune thrombocytopenia: a systematic review.* Blood, 2017. **129**(11): p. 1538-1547.
6. Ward, M.J., et al., *Long-term effects of fetal and neonatal alloimmune thrombocytopenia and its antenatal treatment on the medical and developmental outcomes of affected children.* Am J Perinatol, 2006. **23**(8): p. 487-92.
7. Radder, C.M., et al., *Follow up of children after antenatal treatment for alloimmune thrombocytopenia.* Early Hum Dev, 2004. **80**(1): p. 65-76.
8. Yougbare, I., et al., *Maternal anti-platelet beta3 integrins impair angiogenesis and cause intracranial hemorrhage.* J Clin Invest, 2015. **125**(4): p. 1545-56.
9. van Gils, J.M., et al., *HPA-1a alloantibodies reduce endothelial cell spreading and monolayer integrity.* Mol Immunol, 2009. **46**(3): p. 406-15.
10. Tiller, H., et al., *Fetal intracranial haemorrhages caused by fetal and neonatal alloimmune thrombocytopenia: an observational cohort study of 43 cases from an international multicentre registry.* BMJ Open, 2013. **3**(3).
11. Janssen J, V.N., Engelen R, et al., *Wetenschappelijke verantwoording van de toetsen LOVS rekenen-wiskunde voor groep 3 tot en met 8. [Scientific justification of the mathematics test for grade 1 until grade 6].* 2010, Cito: Arnhem.
12. Mols A, K.F., *Wetenschappelijke verantwoording van de toetsen Spelling nietwerkwoorden voor groep 7 en 8. [Scientific justification of the spelling test for grade 5 and 6].* . 2010, Cito: Arnhem.
13. Weekers A, G.I., Kleintjes F, et al., *Wetenschappelijke verantwoording papieren toetsen Begrijpend lezen voor groep 7 en 8. [Scientific justification of the reading comprehension test for grade 5 and 6].* 2011, Cito: Arnhem.
14. Gilijns P, V.L., *Het CITO leerlingvolgsysteem: Met het oog op de praktijk [The CITO pupil monitoring system: focus on practice].* Pedagogische Studiën 1992. **86:291-6**.
15. Touwen, B.C., M.S. Hempel, and L.C. Westra, *The development of crawling between 18 months and four years.* Dev. Med. Child Neurol, 1992. **34**(5): p. 410-416.
16. Bayley, N., *Bayley scales of infant and toddler development–Third edition*. 2006: San Antonio, TX: Pearson Education, Inc.
17. Wechsler, D., *Wechsler Preschool and Primary Scale of Intelligence - Third Edition (WPPSI-III-NL)*. 2002: TX, The Psychological Corporation.
18. Wechsler, D., *Wechsler Intelligence Scale for Children (5th ed.)*. 2014a, San Antonio, TX: NCS Pearson.
19. Palisano, R.J., et al., *Development and reliability of a system to classify gross motor function in children with cerebral palsy.* Dev. Med. Child Neurol, 1997. **39**(4): p. 214-232.
20. Verhulst, F.C., J. van der Ende, and H.M. Koot,, *Child Behavior Checklist (CBCL)/4-18 manual*. 1996: Rotterdam: Afdeling Kinder- en Jeugdpsychiatrie, Sophia Kinderziekenhuis/Academisch Ziekenhuis Rotterdam/ Erasmus Universiteit Rotterdam.
21. Varni, J.W., M. Seid, and P.S. Kurtin, *PedsQL 4.0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations.* Med Care, 2001. **39**(8): p. 800-12.

22. Emons, J.A., et al., *Use of the Control of Allergic Rhinitis and Asthma Test (CARATkids) in children and adolescents: Validation in Dutch.* Pediatr Allergy Immunol, 2017. **28**(2): p. 185-190.
23. Driessen, G.J., *Richtlijn diagnostiek naar onderliggende aandoeningen bij kinderen met recidiverende luchtweginfecties.* 2016.
24. Foundation, J.M. *10 Warning signs of primary immunodeficiency*. 2016 [cited 2019 31-05].
25. UNESCO, *Institute for Statistics. International standard classification of education: ISCED 2011*. 2012, UNESCO Institute for Statistics, : Montreal.
26. Brown, M.A., et al., *The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP).* Hypertens Pregnancy, 2001. **20**(1): p. IX-XIV.
27. Winkelhorst, D., D. Oepkes, and E. Lopriore, *Fetal and neonatal alloimmune thrombocytopenia: evidence based antenatal and postnatal management strategies.* Expert Rev Hematol, 2017. **10**(8): p. 729-737.
28. *An international classification of retinopathy of prematurity.* Pediatrics, 1984. **74**(1): p. 127-33.
29. Bell, M.J., et al., *Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging.* Ann Surg, 1978. **187**(1): p. 1-7.
30. Volpe, J.J., *Intraventricular hemorrhage and brain injury in the premature infant. Diagnosis, prognosis, and prevention.* Clin Perinatol, 1989. **16**(2): p. 387-411.
31. de Vries, L.S., P. Eken, and L.M. Dubowitz, *The spectrum of leukomalacia using cranial ultrasound.* Behav Brain Res, 1992. **49**(1): p. 1-6.
32. Levene, M.I., *Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound.* Arch Dis Child, 1981. **56**(12): p. 900-4.
33. Fonseca, J.A., et al., *Validation of a questionnaire (CARAT10) to assess rhinitis and asthma in patients with asthma.* Allergy, 2010. **65**(8): p. 1042-8.
34. CCMO, *CCMO Notitie publicatiebeleid*. 2002, CCMO: Den Haag. p. 2.