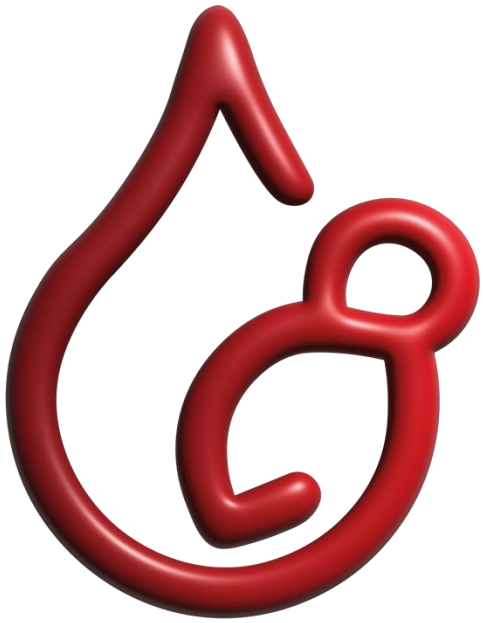
INSPIRE-study

(**I**nternational **N**eonatal tran**S**fusion **P**o**I**nt p**RE**valence)

Research Protocol



PROTOCOL TITLE 'INSPIRE-study: *International Neonatal tranSfusion PoInt pREvalence*'

Protocol ID	G21.207
Short title	INSPIRE-study
EudraCT number	Not applicable
Version	3.0
Date	March 11, 2022
Coordinating investigator/project leader	N.A.M. Houben, PhD student Sanquin Blood Supply Foundation / Leiden University Medical Center Plesmanlaan 125 / Albinusdreef 2 1066 CX Amsterdam / 2333 ZA Leiden, The Netherlands n.a.m.houben@lumc.nl Tel. +31 (0)6-20985851
Principal investigator(s)	Dr. S.F. Fustolo-Gunnink Sanquin Blood Supply Foundation Plesmanlaan 125 1066 CX Amsterdam, The Netherlands s.f.gunnink@amsterdamumc.nl Tel. +31 (0)20-5123000 Prof. Dr. E. Lopriore Department of Paediatrics Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden, The Netherlands e.lopriore@lumc.nl Tel. +31 (0)71-5262965 Prof. Dr. C.J. Fijnvandraat Department of Paediatric Haematology Amsterdam UMC Meibergdreef 9 1105 AZ Amsterdam c.j.fijnvandraat@amsterdamumc.nl Tel. +31 (0)20-5662727 Prof. Dr. J.G. van der Bom Department of Epidemiology Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden, The Netherlands j.g.vanderbom@lumc.nl Tel. +31 (0)71-5685053 Prof. Dr. Christof Dame Department of Neonatology Charité - University Hospital Berlin Augustenburger Platz 1 13353 Berlin, Germany

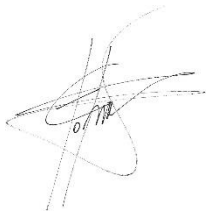
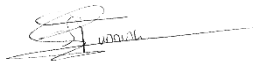
	christof.dame@charite.de Tel. +49 (0)30-450559006 Prof. Charles C Roehr Newborn Services Southmead Hospital, North Bristol Trust Southmead Road, Bristol BS10 5NB Bristol, UK charles.roehr@bristol.ac.uk Dr. Emöke Deschmann Karolinska Institute Solna, Sweden emoke.deschmann@regionstockholm.se Prof. Dr. Simon Stanworth NHS Blood and Transplant (NHSBT) and University of Oxford London, United Kingdom simon.stanworth@nhsbt.nhs.uk Dr. Helen New NHSBT and Imperial College London London, United Kingdom helen.new@nhsbt.nhs.uk
National coordinators	**Austria** Dr. Francesco Cardona Medical University Vienna Vienna, Austria **Bosnia and Herzegovina** Prof. dr. Emina Hadžimuratović University Medical Center Sarajevo Sarajevo, Bosnia and Herzegovina **Croatia** Prof. dr. Ruza Grizelj University Hospital Center Zagreb Zagreb, Croatia **Czech Republic** Dr. Jan Malý University Hospital Hradec Králové Hradec Králové, Czech Republic **Denmark** Christian Heiring Neonatalklinikken Rigshospitalet Copenhagen, Denmark **France** Prof. Dr. Alain Beuchée CHU de Rennes

	Rennes, France **Germany** Prof. Dr. Christof Dame Charité - University Hospital Berlin Berlin, Germany **Hungary** Dr. Miklós Szabó Semmelweis University Budapest, Hungary **Ireland** Dr. Carmel Maria Moore University College Dublin – The National Maternity Hospital Dublin, Ireland **Italy** Prof. dr. Stefano Ghirardello NICU Fondazione IRCCS Policlinico San Matteo Pavia, Italy **Norway** Dr. Kristin Brække Oslo University Hospital, Ullevål Oslo, Norway **Poland** Dr. Tomasz Szczapa Poznań University of Medical Sciences Poznan, Poland **Portugal** Dr. Sara Domingues Centro Materno Infantil do Norte Porto, Portugal. **Romania** Prof. dr. Gabriela Zaharie University of Medicine and Pharmacy Iuliu Hatieganu Cluj Cluj Napoca, Romania **Slovakia** Dr. Katarina Matasova Martin University Hospital Martin, Slovakia **Slovenia** Dr. Jana Lozar Krivec University Medical Center Ljubljana Ljubljana, Slovenia

	Spain Dr. Marta Aguar Carrascosa La Fe University Hospital Valencia, Spain **Sweden** Dr. Emöke Deschmann Karolinska Institute Solna, Sweden **Switzerland** Dr. Tobias Muehlbacher University Hospital Zurich Zurich, Switzerland **The Netherlands** Prof. Dr. Enrico Lopriore Leiden University Medical Center Leiden, The Netherlands **United Kingdom** Prof. Charles Roehr Southmead Hospital, North Bristol Trust Bristol, UK
Sponsor	Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden, The Netherlands
Subsidizing parties	Sanquin Blood Supply Foundation Clinical Transfusion Research Dept. Plesmanlaan 125 1066 CX Amsterdam, The Netherlands European Society for Pediatric Research (ESPR) Rue de Sablieres 5 1242 Satigny, Switzerland European Blood Alliance (EBA) Plesmanlaan 125 1066 CX Amsterdam, The Netherlands
Advisory board	Dr. O. Karam Division of Pediatric Critical Care Medicine Children's Hospital of Richmond at VCU Richmond, United States oliver.karam@vcuhealth.org Dr. M.E. Nellis Pediatric Critical Care Medicine NY Presbyterian Hospital and Weill Cornell Medicine New York, United States man9026@med.cornell.edu

	Dr. M.C. Sola-Visner Division of Newborn Medicine Boston Children's Hospital and Harvard Medical School Boston, United States martha.sola-visner@childrens.harvard.edu Dr. A. Keir South Australian Health and Medical Research Institute and Adelaide Medical School North Adelaide, Australia amy.keir@adelaide.edu.au Prof. N.L.C. Luban Children's National Hospital and The George Washington University School of Medicine and Health Sciences Washington, United States nluban@childrensnational.org

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Head of Division: Prof. Dr. E. Lopriore Division of Neonatology, Department of Paediatrics Leiden University Medical Center Albinusdreef 2 2333 ZA Leiden, The Netherlands e.lopriore@lumc.nl Tel. +31 (0)71-5262965		*11-03-2022*
Principal Investigator: Dr. S.F. Fustolo-Gunnink Sanquin Blood Supply Foundation Plesmanlaan 125 1066 CX Amsterdam, The Netherlands s.f.gunnink@amsterdamumc.nl Tel. +31 (0)20-5123000		*11-03-2022*
Coordinating Investigator: N.A.M. Houben, PhD student Sanquin Blood Supply Foundation / Leiden University Medical Center Plesmanlaan 125 / Albinusdreef 2 1066 CX Amsterdam / 2333 ZA Leiden, The Netherlands n.a.m.houben@lumc.nl Tel. +31 (0)6-20985851		*11-03-2022*

PROTOCOL AGREEMENT PAGE

I agree to conduct the Clinical Study in accordance with the current protocol and comply with its requirements, subject to ethical and safety considerations.

Institute ……

Local Investigator Name ……

Local Investigator Signature ……

Date ……

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

(S)AE	(Serious) Adverse Event
ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee
CCMO	Central Committee on Research Involving Human Subjects
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
ECMO	Extracorporeal Membrane Oxygenation
EU	European Union
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
IC	Informed Consent
IRB	Institutional Review Board
METC	Medical Research Ethics Committee
NEC	Necrotizing EnteroColitis
NICU	Neonatal Intensive Care Unit
NTN	Neonatal Transfusion Network
RBC	Red Blood Cell
Sponsor	The sponsor is the party that commissions the subsidizing or performance of the research, for example a pharmaceutical company, academic hospital, scientific subsidizing, 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 subsidizing party.

1. SUMMARY

Rationale: Premature neonates are highly transfused patients, though robust evidence supporting neonatal transfusion practice is scarce. Two randomized controlled trials (RCTs) were recently published, indicating no benefit in long-term outcomes when using liberal (high) thresholds for red blood cell (RBC) transfusions. Another RCT, comparing a high and low platelet transfusion threshold, even reported evidence that liberal transfusion treatment (high thresholds) can cause harm. There are no international neonatal transfusion guidelines that have been implemented by Europe as a whole, resulting in significant variation in transfusion practice within Europe. Detailed contemporary data on neonatal transfusion practice in Europe, including neonatal component specifications, are lacking. This point prevalence study will provide a picture of current neonatal transfusion practices within Europe, which can be used to improve practice, promote adherence to evidence-based transfusion guidelines, and inform future randomized controlled trials.

Main objective: To describe the prevalence, indications, adverse effects, and component specifications of RBC, platelet, and plasma transfusions in preterm neonates. Additionally, to describe the use of local or national guidelines and the evidence-basedness of transfusion practices in preterm neonates.

Study design: Prospective, European, multicenter, observational point prevalence study.

Study population: Neonates with a gestational age of less than 32 weeks at birth who are admitted to a participating tertiary level Neonatal Intensive Care Unit (NICU).

Expected results: This study will identify current neonatal transfusion practices that can be improved, and areas with substantial clinical variation which can be targeted in future clinical trials. The resulting data may help reduce unnecessary transfusions through increased awareness of the proper use of transfusions in this vulnerable patient population. This may eventually lead to a reduction in the number of adverse events, lower costs, optimal allocation of donor blood, and ultimately, better long-term neonatal outcomes.

2. INTRODUCTION AND RATIONALE

Every year, approximately 66,000 very premature babies in Europe receive one or more blood transfusions. (1) Despite the high frequency of transfusions in this population, the efficacy and safety of many of these transfusions are not known, as the number of appropriate trials in this population is limited. The blood components most frequently transfused to babies are red blood cells (RBCs), platelets and plasma. Four randomized controlled trials assessing RBC transfusion thresholds have been published, of which two were published in 2020. (2-5) Two trials assessed platelet transfusions and only one trial assessed fresh frozen plasma (FFP) transfusions. (1, 6-8) As a broad generalization, the findings of these studies do not support the use of liberal transfusion policies, but data on optimal thresholds, dosing and product specifications are still lacking.

Recent studies have shown that many neonatal transfusions may be redundant. Redundant transfusions are transfusions given at thresholds or indications that are not supported by existing evidence and are therefore not expected to convey benefit to the neonate. Redundant transfusions are problematic because they expose babies to unnecessary risks, lead to increased costs and undue burden on health care facilities and donor blood availability. The ETNNO-trial ('Effects of Liberal vs Restrictive Transfusion Thresholds on Survival and Neurocognitive Outcomes in Extremely Low-Birth-Weight Infants') and TOP-trial ('Transfusion Of Prematures'), assessing liberal versus restrictive RBC transfusion thresholds, both found that restrictive thresholds were non-inferior to liberal thresholds. The PlaNeT-2/MATISSE platelet transfusion trial ('Platelets for Neonatal Transfusion - study 2 / MAnaging Thrombocytopenia in a Special Subgroup: nEonates') showed that a $25x10^9$/L platelet count threshold was superior to a $50x10^9$/L threshold in preterm infants. (2, 3) In short, all three studies support restrictive transfusion strategies. Despite these findings, our recent European neonatal transfusion survey, which included 341 NICU's from 18 countries, showed 53% of platelet transfusions given at a threshold $>25x10^9$/L The interquartile ranges (IQR) for haemoglobin thresholds in different clinical scenarios varied between 6-20 g/L. (9)

Recent studies have also shown that neonatal transfusions may cause side effects or even direct harm. Transfusion-associated side effects in neonates are poorly defined, and therefore likely to be under-recognized. Despite this likely under-recognition, data from national hemovigilance systems in the Netherlands and the UK suggest that neonates are at much higher risk of transfusion-associated adverse events compared to children or adults. (10) More importantly, there is evidence that transfusions may cause direct harm. The PlaNeT-2/MATISSE trial, comparing a high ($50x10^9$/L) and low ($25x10^9$/L) platelet transfusion threshold, showed unexpected higher rates of mortality and/or severe bleeding, in the higher threshold (liberal transfusion) study group. (7) The mechanisms of this apparent platelet transfusion-associated harm have yet to be elucidated in detail but indicate that the potential benefits of transfusions must be carefully weighed against the risk of transfusion-related harm.

There are no neonatal transfusion guidelines that have been implemented by Europe as a whole, and there is significant variation in transfusion practice within Europe. Two national guidelines specific to neonatal transfusions have been published in peer-reviewed journals, but whether their recommendations apply to the whole of Europe remains to be determined. (11, 12) It is unknown whether other countries or individual NICU's have national or local guidelines and what these guidelines recommend. To describe neonatal transfusion practices in Europe, we have recently performed a large European neonatal transfusion survey in which 18 countries participated. We analysed data from 341 NICU's. The results of this survey suggest that a substantial number of transfusions may be redundant, as described previously. The results also showed wide variation in thresholds, even within relatively well-defined patient groups. For example, the interquartile range for red

blood cell transfusion haemoglobin thresholds for stable preterm infants of less than 32 weeks gestational age in the first week of life was 80-100 g/L, with 25% of centers using more extreme thresholds ranging from 50-140 g/L. Platelet transfusion thresholds for stable, non-bleeding infants with a gestational age of less than 28 weeks at birth, varied between no prophylaxis up to transfusion at a platelet count of $100x10^9$/L, with an IQR of 20-$47x10^9$/L. Volumes and rates of transfusion also varied substantially. These results are in line with older surveys and a recent report from the USA showing similarly variable transfusion patterns. (13-16)

A prospective point prevalence study is timely and will provide crucial data for new randomized trials and implementation projects. The neonatal transfusion survey was a crucial first step to improve neonatal transfusion practices but needs to be followed up by a prospective study for several reasons. The survey targeted only one neonatologist per NICU, which did not allow for assessment of within unit variation in practice. More importantly, we need exact data about transfusion prevalence, indications, and specifications to be able to set up new randomized controlled trials and develop effective implementation strategies. We did not collect data on component specification or on use of local or national transfusion guidelines. And lastly, a known limitation of surveys is that reported behaviour may differ from actual behaviour, whether intentionally or unintentionally. Therefore, a prospective international observational study is warranted.

The INSPIRE will be performed by the Neonatal Transfusion Network (NTN), an international, interdisciplinary neonatal transfusion research network. The NTN aims to improve current practices and generate more evidence for neonatal transfusion medicine. As of January 2021, approximately 100 participants from 31 countries are represented in this network. The European Blood Alliance (EBA), the European Society for Pediatric Research (ESPR), the International Haemovigilance Network (IHN) and the European Foundation for the Care of the Newborn Infant (EFCNI) have endorsed the NTN. As all stakeholders, including neonatologists, haematologists, epidemiologists, as well as parent representatives and blood bank agents, collaborate within our international NTN, the results of this study can directly be translated into practice change and may thus substantially impact the quality of neonatal transfusion medicine in Europe and worldwide.

To summarize, neonates receive blood transfusions even though they might not be effective or could be harmful, as current transfusion guidelines are not supported by sufficient evidence and existing evidence has not yet been incorporated into clinical practice. Whilst the neonatal population may seem small, over the next 20 years, approximately 1 million infants in Europe will receive a transfusion while being a premature newborn. The potential short- and long-term effects of these transfusions should therefore not be underestimated. Recent consensus meetings and scientific reviews underline the need for high quality, global epidemiologic data as a first step towards improving neonatal transfusion medicine. (1, 17-19) These data will help to improve practice, develop research protocols, and inform guideline writing. **We therefore aim to perform a European point prevalence study (INSPIRE), which will provide high quality multinational epidemiologic data that can be used to improve neonatal transfusion medicine in Europe.**

3. OBJECTIVES

To describe the prevalence, indications, adverse effects, and component specifications of transfusions among preterm neonates admitted to tertiary level NICUs in Europe. Additionally, to describe the use of guidelines and the evidence-basedness of transfusion practices in very preterm neonates.

3.1 Primary Objective

To describe the prevalence of RBC, platelet, and plasma (FFP and cryoprecipitate) transfusions in very preterm neonates with a gestational age less than 32 weeks at birth admitted to a tertiary level NICU.

3.2 Secondary Objectives

To describe the variations in prevalence, the indications for transfusion, duration, volume, rate, adverse effects and component specifications of the prescribed RBC, platelet, or plasma transfusions. Additionally, to assess the number of transfusions per transfused neonate, the proportion of neonates who received at least one transfusion, the incidence of receiving at least one transfusion. Furthermore, to describe use of guidelines and the evidence-basedness of transfusion practices in preterm neonates with a gestational age less than 32 weeks at birth. Lastly, to assess the use of transfusions with blood products other than RBC, platelet, and plasma or agents that promote or reduce coagulation.

4. STUDY DESIGN

4.1 Study design

International, multicenter, prospective, observational point prevalence study.

4.2 Duration

Data will be collected over a one-year period. During this year, each participating NICU will collect data during a six-week period in which they will screen all 7 days of each week.

4.3 Setting

Data collection will take place in tertiary level NICUs in Europe, a minimum of 61 NICUs must be recruited to reach our sample size (see section 5.4). A tertiary level NICU is defined as a hospital NICU organized with personnel and equipment to provide continuous life support and comprehensive care for extremely high-risk newborn infants with a gestational age of less than 32 weeks, and those with complex and critical illness. (20)

5. STUDY POPULATION

5.1 Population (base)

Approximately 700,000 neonates are born prematurely each year in Europe, of whom about 16% are born very premature at a gestational age of less than 32 weeks. (21) Within this population, neonates will be included based on inclusion and exclusion criteria defined below.

5.2 Inclusion criteria

A potential subject who meets both of the following criteria will be included in this study:

1. Admission to a participating tertiary level NICU (including outborn neonates or neonates readmitted to the NICU)
2. Gestational age at birth below 32 weeks.
   Note: gestational age at admission can be >32 weeks, however postnatal age at inclusion cannot exceed 30 days after estimated due date.

5.3 Exclusion criteria

No exclusion criteria.

5.4 Sample size calculation

5.4.1 Sample size calculation

There are several ways to describe transfusion practices, such as describing the proportion of neonates receiving any number of transfusions, the number of neonates receiving a first transfusion (incidence) or describing the total number of transfusions given per patient-time period (prevalence rate). We have chosen the prevalence rate as our primary outcome, because this takes into account variations in duration of follow up and variations in the total number of transfusions neonates receive.

We will calculate the prevalence rates by dividing the number of transfusions of the respective transfusion type by the total sum of neonate study days. We performed sample size calculations for RBC, platelet, and plasma transfusion prevalence rates, estimating the sample size necessary to ensure that the confidence interval for a prevalence is of a predetermined width. Based on data from previously conducted studies, we expect the approximate rates of RBC, platelet, and plasma transfusions to be 2.0 (1.3-2.6), 0.9 (0.6-0.12) and 0.4 (0.2-0.6) transfusions per 100 study days, respectively. (2, 3, 7, 16, 22-24) We aim to estimate the prevalence rates with sufficient certainty, which we have defined as rates within +/-0.5 for RBC, +/- 0.2 for platelet, and +/-0.1 for plasma transfusions per 100 study days. This corresponds to half of the width of the confidence interval for each transfusion type found in the literature. To achieve this level of certainty, we have to obtain data on 3152, 6833, and 15366 study days for RBC, platelet and plasma transfusions, respectively.

We expect that each participating NICU will contribute on average 252 patient days. This number is based on the length of the data collection period (42 days), the estimated percentage of eligible NICU admissions with gestational age <32 weeks (30%) and the estimated number of beds per unit (20, unpublished data obtained within the NTN). We therefore expect we need to recruit at least 61 (15366/252) NICUs to reach our sample size. As we will collect data in a large geographical area, we aim to recruit NICUs in at least 10 countries and recruit at least 10% of NICUs within each country or at least 2 centers per country, whichever is greater, even if this will result in a sample size higher than 61. The

number of centers included per country will be proportional to the population size of the country compared to that of all other included countries.

Details of the interim analysis will be included in the statistical analysis plan prior to the interim analysis. If needed, results of the interim analysis can lead to implementation one of two measures: extending de data collection period or recruiting additional centers. Any decisions following the interim analysis will be made in close consultation with the national coordinators.

6. METHODS

6.1 Main study endpoint

The primary outcome of this study is the prevalence of RBC, platelet, and plasma (FFP and cryoprecipitate) transfusions in neonates with a gestational age of less than 32 weeks at birth, admitted to a tertiary level NICU. The prevalence is calculated by dividing the number of transfusions of the respective transfusion type by the total sum of neonate study days.

A transfusion is registered if administered to a neonate during the study period. Transfusions issued by the blood bank but not administered will not be registered.

To calculate the total sum of neonate study days, each center will register date and time of start and end of study of all eligible neonates during the center's data collection period. For neonates already admitted at the start of the study, the start of study will be the moment the data collection period starts. For all other infants, start of study equals time of admission. These data will allow us to calculate the exact number of study days for each individual neonate in the study.

To describe the study population, we will collect NICU characteristics (e.g. unit size) and neonatal baseline characteristics (e.g. gestational age).

6.2 Secondary study endpoints

6.2.1 Number of transfusions per transfused neonate
We will assess the number of transfusions per neonate for RBC transfused neonates, platelet transfused neonates and plasma transfused neonates.

6.2.2 Proportion of neonates who received at least one transfusion
We will calculate the proportion of neonates who received at least one transfusion during the study period, per transfusion type.

6.2.3 Incidence of receiving at least one transfusion
We will calculate the incidence of receiving at least one transfusion, both for any transfusion as per transfusion type. The incidence is calculated by neonates receiving at least one transfusion by the total sum of neonate study days.

6.2.4 Variations in prevalence
We will describe the variations in prevalence rates of RBC, platelet, and plasma transfusions. Data analysis will be predominantly descriptive, comparing both between participating countries and between different type of NICUs. Types of NICU's will be defined based on unit size and whether surgical procedures are being performed.

6.2.5 Indication for transfusion
We will collect the primary indication for which the transfusion is prescribed by the treating physician. If applicable, we will also register the secondary and tertiary indication, as clinicians may consider multiple factors when deciding to give a transfusion. For each transfusion, the prescribing physician must choose from a list of predefined indications (up to three options can be selected, ranked primary to tertiary indication). The primary indication is the indication that has the most weight in the decision to prescribe a transfusion. If the indication for transfusion is registered by someone other than the prescribing physician, the prescribing physician must confirm the correctness of these data.

6.2.6. Duration, volume, and transfusion rate
We will assess the duration of transfusion, transfusion volume, and transfusion rate of the prescribed RBC, platelet, and plasma transfusions. We will describe the variations in duration of transfusion, transfusion volume, and transfusion rate.

6.2.7 Guideline use
We will collect data on whether centers have guidelines in place regarding RBC, platelet, and plasma transfusion for neonates. Following the implementation model by Wensing and Grol, we aim to gather information on the implementation of transfusion guidelines. (25) Additionally, we will ask centers with established guidelines to provide us with the guidelines in place at the start of data collection. We will analyse how many of the RBC and platelet guidelines have already incorporated the results of the recent clinical trials. We will categorize the existing RBC guidelines into “TOP and/or ETTNO incorporated” and “TOP and/or ETTNO not incorporated”.(2, 3) We will categorize the existing platelet guidelines into “PlaNeT-2/MATISSE incorporated” and “PlaNeT-2/MATISSE not incorporated”. We will assess to what extent centers follow their own local and/or national guidelines. (7)

6.2.8 Evidence-basedness of practice
Before the start of data collection, we will collaborate with various experts to define what we view as high quality evidence regarding RBC and platelet transfusion practices in neonates, using the best available evidence including the TOP, ETTNO, and PlaNeT-2/MATISSE trials. (2, 3, 7) We will assess if the RBC and platelet transfusions were prescribed following the best available evidence, by categorizing the prescribed transfusions into different levels of certainty in evidence. We will assign a panel review by three ‘blinded’ experts to discuss cases where there may be ambiguity on the certainty of evidence to support transfusion practice. Transfusion events in clinical scenarios that were not addressed in randomized trials will not be assessed.

6.2.9 Transfusion-associated adverse effects
In the absence of clear definitions of transfusion-associated side effects in neonates, we will ask participating centers to register any perceived transfusion-associated adverse effects if the local investigators consider the adverse event to be potentially associated with the preceding transfusion. With this we hope to gain insight into what adverse effects neonatologists identify in clinical practice, despite the lack of well-defined descriptions.

6.2.10 Component specifications
We will perform an online survey among transfusion experts in which we explore variations in transfusion component characteristics. We chose this strategy because neonatologists may not be aware of all relevant component specifications. The survey will be sent to transfusion experts in all countries participating in this study during the data collection period. We will record the blood banks from which each participating NICU receives their blood products, which will allow us to link the NICU clinical data to the component specifications reported by the transfusion specialists. We aim to include all blood banks that provide blood products to participating NICUs in our survey.

6.2.11 Transfusion with blood products other than RBC, platelet, and plasma or agents that promote or reduce coagulation.
We will collect data on transfusions of blood product other than RBC, platelet, and plasma transfusions or agents that promote or reduce coagulation, such as erythropoietin. We will describe these treatments and the indications for which they were prescribed.

6.3 Study procedures

Participating sites
The study aims to include a broad range of European countries. Each country will be represented by a national coordinator for this study, who will be responsible for recruiting centers and coordinating ethical procedures in collaboration with the study coordinator. Based on the number of European countries participating, we will distribute the required 61 NICUs proportionally to the population size of the country, determining the minimum number of centers each country has to include. In cooperation with the national coordinators, we aim to achieve, where possible, a representative sample for their country, based on one or more of the following factors: unit size, non-academic vs academic centers, surgical vs non-surgical centers. Each site will have a local investigator who is responsible for the study in their NICU, including the coordination of the ethical procedure and data collection.

Pilot
We aim to perform a pilot study in 5 centers to check feasibility of and the workload for our study protocol. This can inform us on the workload on collecting and entering patient data for the study. Additionally, it can help identify possible problems that participating centers may encounter during the entry. There will be no data sharing of patient data in the pilot. If needed, modifications to the protocol and/or database will be made based on the results of the pilot study.

Parental involvement
The Neonatal Transfusion Network strives to achieve parental involvement in neonatal transfusion medicine studies. Preceding the finalization of the database, the NTN will establish an international parental advisory board, in collaboration with the European Foundation for the Care of Newborn Infants (EFCNI). This board will be invited to provide input for the study protocol. If needed, modifications to the protocol and/or database will be made based on this feedback.

Allocation of data collection periods
Data will be collected over a one-year period to account. Study sites will have the opportunity to choose a preferred start date from a limited number of options, aiming to start within 4 months after ethical approval by their local institutional review board and signing of the data sharing agreement (DSA), allowing centers to select the option that is most convenient for them. We aim to start data collection in the spring of 2022.

Data collection
Each site will collect data during a six-week period. As not to bias against sites with differing working days, the centers will screen all 7 days of the assigned weeks, using an online database. Data will be collected by study personnel, which may include research nurses, data managers, medical students, and PhD students, under supervision of the local investigator. We will provide online data entry training opportunities. Data will be collected from the hospital's written or electronic patient record files, recorded imaging reports, hospital blood bank records, and nurses' records. Study data will be entered into the electronic data capture tool Castor. Data collection ends at the end of the six-week period.

Outborn or readmitted infants
Infants that are outborn or readmitted to the unit will be included in the study. Readmitted neonates will not receive a new study number, but data collection will continue under the study number they received during their first admission. Data collection for outborn infants will be identical to data collection for inborn infants, as we will not collect data from the period before admission.

7. SAFETY REPORTING

The International Neonatal Transfusion Point Prevalence Study is an observational study, we therefore consider reporting of adverse events or serious adverse events not applicable for this study.

8 STATISTICAL ANALYSIS

A full data analysis plan will be drawn up before the start of the data analysis. Data analysis will be predominantly descriptive, with primary results provided of all countries combined, as well as data on individual countries. We will not publish non-anonymised data of individual centers. To calculate the overall prevalence rate for each transfusion type, we will first determine the prevalence rate per country, as some countries may include more than the required number of NICU's based on their population size. The prevalence rate per country will be calculated by dividing the number of transfusions of the respective transfusion type prescribed in the country by the total sum of neonate study days of the country. Thereafter, the overall prevalence will be determined by calculating the average in proportion to population size.
We will perform relevant subgroup and sensitivity analyses, including subgroup analyses for gestational age, birthweight, neonates with congenital malformations, neonates who underwent surgery, and subgroups based on center characteristics. Sensitivity analyses will at a minimum include an analysis excluding neonates with congenital malformations and neonates with gestational age <24 weeks. Missing data will be handled using simple imputation or multiple imputations where appropriate. Dependent on the distribution of the variables, the results will be described as proportions (confidence intervals), as means (standard deviations (SD)) or as medians (interquartile range (IQR)). All tests will be two-sided, with an α level of 0.05.

9. ETHICAL CONSIDERATIONS

9.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, October 2013) and the General Data Protection Regulation (GDPR).

9.2 Consent

We will submit the study protocol to the institutional review boards of the coordinating and participating centers for ethical approval. Our application will be handled according to the local and/or national rules and procedures that apply in the participating foreign centers. Each site will be responsible for obtaining ethical consent if required by their institution, in collaboration with the study coordinator.

We will request waiver of consent, which means parents do not have to give consent for their child's data to be used in this study. The decision to apply for waiver of consent for the INSPIRE-study was made considering the trade-offs, where we feel that these advantages are considered to outweigh the disadvantages of approaching all parents individually for consent. Our rationale is that we aim to avoid more burden for parents with a waiver of consent and that the validity and generalisability of our study would be threatened by selection bias if waiver of consent is not granted.

A. Avoiding selection bias

Consent bias, a type of selection bias, can occur when neonates whose parents do not give consent differ from neonates whose parents do. A recent study by Weiss et al. assessed the parental factors associated with their participation in neonatal research. (26) They found that both parents with lower socioeconomic status and Black parents more frequently declined consent for their infant's participation. Previous studies have shown that neonatal outcomes of both infants from parents with lower incomes as well as Black infants are worse. (27-33) Additionally, two studies comparing the population representation in neonatal clinical research found higher (severe) morbidity and mortality rates among the non-enrolled preterm infants compared to enrolled preterm infants. (34, 35) High transfusion rates have previously been described in premature neonates with severe comorbidity. (36) If informed consent is required, certain groups that are expected to receive a substantial proportion of transfusions would thus be systematically underrepresented in our study. This will have important implications for the generalisability of the findings of our study to the wider neonatal population. More importantly, it will perpetuate existing disparities in clinical outcomes between these groups as they do not benefit from the research. As we will use our findings to inform future studies, the biased results would make it impossible to provide meaningful guidance for the design of randomized controlled trials and quality improvements projects. Consent bias thus has serious implications for future research, as these studies should benefit all premature neonates. In conclusion, consent bias threatens the validity, relevance, and generalizability of our study.

B. Avoiding more burden for parents

For many parents, the first days of their newborn's stay in the NICU are very stressful and they may feel overwhelmed by the huge amount of information they receive. However, as most neonates receive blood transfusions during the first week of life, it is important to be able to start data collection immediately after birth to avoid missing transfusion events. (22, 37, 38) During these early days, the additional information that parents may receive through an informed consent procedure for their child's participation in clinical research can contribute to this overload. The available

research assessing this burden in the NICU setting is very limited to our knowledge, so we have also identified several pivotal studies in a similar setting, the Pediatric Intensive Care Unit (PICU). A study by Hodson et al. found that 52% of the parents of children in the PICU felt overwhelmed when approached for their consent to non- or minimal risk observational studies. (39) Two other studies assessing consent for participation in clinical research in the PICU both found that parents most commonly described feeling too stressed or overwhelmed to consider participation as the reason for withholding consent. (40, 41) As parents of severely ill neonates are likely to be the most stressed and therefore less likely to consent, this may also contribute to selection bias described above. Moreover, a study by Rich and Katheria, examining parents' perceptions of a waiver of consent for participation in a neonatal clinical trial, found that the majority of the parents felt positive or strongly positive about their infant's participation in the study. (42) Given the emotional burden experienced by parents during their child's NICU stay and that there are no or minimal risks associated with participation in our study, we feel that waiver of consent is the most fitting approach.
We do, however, feel that it is important to inform parents about the participation of their child in the study. Together with the European Foundation for the Care of Newborn Infants (EFCNI), we have drafted an information letter to inform parents about the study and provide them the opportunity to receive the study results. This letter can be given to the parents at a time when it is more convenient for them and they no longer feel overwhelmed. Additionally, the EFCNI has expressed its support for our request for a waiver of consent (see document '*K6.2 Support letter EFCNI 5-10-2021*').

If a waiver is not granted, we will ask for an opt-out possibility as an alternative or otherwise follow the regular consent procedure. With opt-out, parents are given an opportunity to object to their child's data being used for scientific research. The regular consent procedure requires the consent of the infant's parents before participation in the study. Each site will be responsible for obtaining ethical approval if required by their institution, in collaboration with the study coordinator. We will prioritize centers where a waiver of consent has been granted over centers where an opt-out or regular consent is required to reduce consent-related bias, as described previously.

10. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

10.1 Handling and storage of data and documents

Data will be collected, stored, and processed in accordance with the ICH E6 Good Clinical Practise (GCP)- Guidelines. Electronic records will be held on a secure network requiring user ID and password access. Neonates enrolled in the INSPIRE study will be automatically assigned to a study number to encode all data directly after they are included in the study. The anonymization key will be safeguarded and kept by the local investigator of each participating center. Regulations between the participating centers and the initiating center about the intended use, confidentiality, security and sharing of the data, and potential financial costs will be arranged in a data sharing agreement (DSA).

Study data will be entered into Castor, a certified electronic database for collection and analysis. Castor complies with all applicable laws and regulations, including ICH E6 Good Clinical Practice (GCP) and by using Castor, researchers are enabled to comply with these laws and regulations (www.castoredc.com). Coded data will be stored in a Leiden University Medical Center ProMISe Datasafe. ProMISe meets the requirements for data safety and privacy set by international law. The ProMISe system facilitates the availability, integrity, and confidentiality of study data, according to the security conditions demanded by GCP. All data is coded and stored on Datasafes, that are exclusively accessible for the involved researchers. The ProMISe Datasafe automatically makes back-ups twice a day. Data will be stored for the length of the study and 15 years afterwards.

10.2 Monitoring and Quality Assurance

Data collection procedures will be designed in such a manner as to minimize data entry errors, and we will perform a pilot study to assess the quality of the database. We will monitor the incoming data during the data collection period for inconsistencies, data entry errors, and missing data. Data acquired by this study will be available for inspection by the respective institutional review boards of the participating centers, representatives of national and local health authorities, if applicable, upon request. Given the neglectable risk associated with this observational study, no data safety monitoring board will be installed.

10.3 Amendments

Not applicable since amendments to the protocol of non-WMO studies do not have to be submitted to the METC.

10.4 End of study report

Not applicable.

10.5 Public disclosure and publication policy

The study will be registered on the website of the Dutch National Competent Authority, the ‘Centrale Commissie Mensgebonden Onderzoek’ (CCMO) and the public ClinicalTrials.gov study registry. The results from the International Neonatal Point Prevalence Study will be analysed and published as soon as possible in open access peer-reviewed international scientific journals and presented at scientific meetings unless the study was terminated prematurely and did not yield sufficient data for a publication. The responsibility for presentations and/or publications belongs to the investigators. No restriction regarding the public disclosure and publication of the research data have been or will be made by the funding agencies. All papers written as a part of this study will list the funding agencies. The final publication of the study results will be written by the principal investigators and the co-investigators. National coordinators will be named as co-authors on any publications resulting from this study, local coordinating investigators will be named under the INSPIRE-group authorship (max. 2 investigators per participating center). A draft manuscript will be submitted for review to all co-authors. Results will also be published in a PhD-thesis.

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AMENDMENT PROTOCOL

August 2nd, 2022

As described in Section 6.3 of the protocol (page 20), we have established a parental advisory board for the study, in collaboration with the European Foundation for the Care of the Newborn Infants (EFCNI). The board was invited to provide input for the study. Their feedback indicated that, as parents, they are very concerned about iatrogenic blood loss as a result of blood testing in extremely premature infants. In response to their feedback, we included an additional section on blood testing in the CRF. In this section, we collect the number of blood tests determined in neonates born with a gestational age below 28 weeks. This data is only collect during the first 28 postnatal days or part thereof, depending on the length of hospitalisation/study period.