INSPIRE

STATISTICAL ANALYSIS PLAN

Platelet Transfusion Paper

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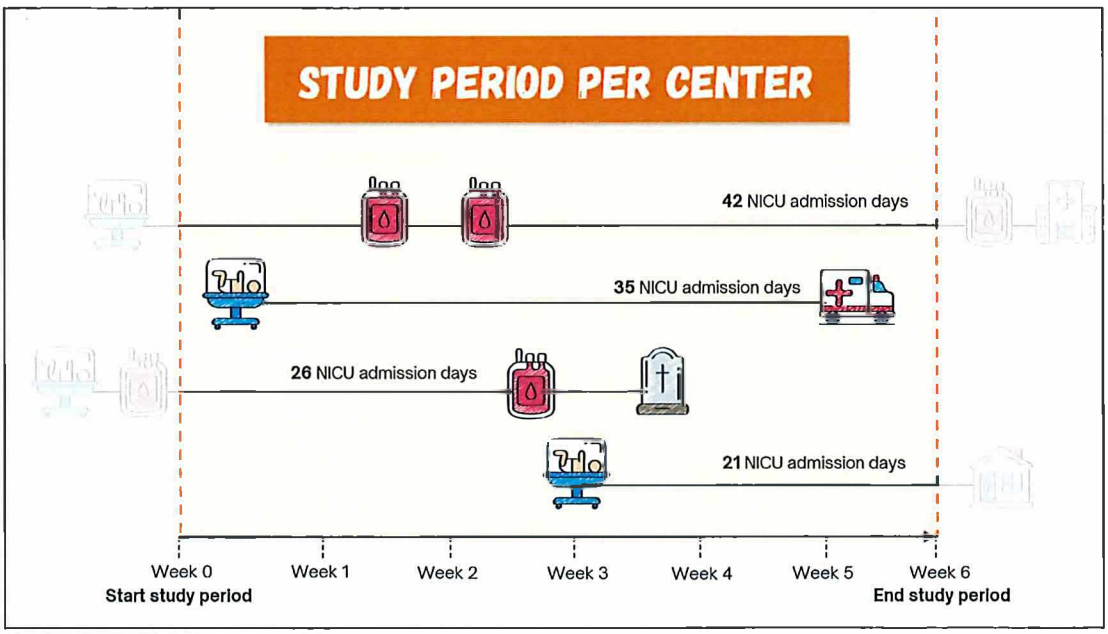
1. STUDY SUMMARY

Study rationale: Premature infants are a highly transfused group, though robust evidence supporting neonatal transfusion practice is scarce. The PlaNeT-2/MATISSE platelet transfusion trial found that a 25 x10^9/L platelet count threshold was superior to a 50 x10^9/L threshold in preterm infants, with higher mortality and major bleeding rates in the latter liberal threshold group. (1) There are no 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 practices in Europe, including the use of platelet transfusions, are lacking.

Study population: preterm infants born below 32 weeks gestation admitted to a tertiary level Neonatal Intensive Care Unit (NICU)

Study design: prospective, international, multicenter, observational study

Study data collection: data collection took place from September 2022 to August 2023. All participating centers collected data during a fixed six-week study period. Local sites documented transfusion use in all infants in their NICU during these weeks, including infants already admitted at the start of the study period or newly admitted during the study period. Consequently, not all infants were followed from birth, and the duration of study follow-up varied, with a maximum follow-up of 42 days per included patient.



Infographic was made using images of Juicy_Fish from FlatIcon.

2. STUDY OUCOME MEASURES

We aim to publish separate papers describing neonatal RBC, platelet and plasma transfusions. ***Therefore, this SAP only focuses on the use of platelet transfusions.***

2.1 Main study outcome measures

1. Platelet transfusion day prevalence rate
2. Case-mix adjusted platelet transfusion day rate
3. Cumulative incidence of receiving at least one platelet transfusion during first 28 days of life

2.2 Other study outcome measures

4. Primary indications for platelet transfusion
5. Volume, duration and infusion rate of platelet transfusion
6. Platelet count prior to platelet transfusion
7. Platelet transfusion increment, stratified for transfusion volume
8. Transfusion related adverse effects

3. DATA CLEANING

3.1 Data cleaning during study follow-up

The site specific patient data was checked and cleaned after the completion of the study period in that participating center. This data quality check was used to detect outliers and impossible combinations of data entry (e.g. non-chronological dates, transfusions after end of study follow-up , etc.). Additionally, we checked for missing data in the baseline characteristics and outcome data. In case of missing data or ambiguities, the local investigator was contacted and incorrect values were directly corrected in the data management system Castor.

3.2 Data cleaning after completion study follow-up

A final data quality check will be performed once all the centers completed the study period using the same data quality check code. Possible ambiguities that were overlooked during the center specific check will be corrected, where any modifications made will be recorded in the data cleaning code.

4. DATA ANALYSES

4.1 General remarks

All statistical analyses will be performed using R statistical software (Version 4.1.17, R Core Team, 2021) or STATA statistical Software (Version 16.1, Texas, USA). Continuous data will be presented as mean (standard deviation (SD)) or as median (interquartile range (IQR)), where appropriate.

4.2 Missing data

Missing values were limited in the eCRF since the majority of the collected variables were marked as mandatory to fill in. Therefore, we expect very few missing values in the dataset.

We decided in advance how to handle missing data for the following variables:

Variables	Commentary
Platelet count prior to transfusion	Only recorded if platelet count was available in the 24 hours before transfusion. If left blank, the value is recorded as 'not available'.
Platelet count after transfusion	Only recorded if platelet count was available in the 24 hours after transfusion. If left blank, the value is recorded as 'not available'.
Congenital anomalies	If not recorded, we assume that congenital anomalies were unlikely and the value is recorded as '0'.
Bleeding disorders	If not recorded, we assume bleeding disorders were not present and the value is recorded as '0'.
Major bleeding	If not recorded we assume no major bleeding occurred and the value is recorded as '0'.
NEC	If not recorded, we assume no NEC occurred and the value is recorded as '0'.
Sepsis	If recorded as unknown, we assume no sepsis occurred and the value is recorded as '0'.
Mechanical ventilation	If recorded as unknown, we assume no mechanical ventilation occurred and the value is recorded as '0'.
Surgical procedures	If recorded as unknown, we assume no surgical procedures occurred and the value is recorded as '0'.

For all other variables, the total number of missing values will be reported, with the number of infants who had one or more missing values for the variable.

4.3 Data transformations

For the clinical events, investigators only collected the start date of clinical events (except mechanical ventilation) so no information is available on the duration of the event or the

time to clinical remission. Therefore, we made assumptions about the duration of clinical events, which allows us to use these variables for the case-mix adjusted prevalence rates.

The variables for clinical events were defined as followed:

Clinical events	Definitions
Major bleeding	The duration of a major bleeding episode is defined as 7 days from the day of detection of the major bleeding. For each individual admission day, one of the values will be assigned: – **0:** No major bleeding – **1:** Current bleeding episode – **2.** Status post bleeding
NEC	The duration of a NEC episode is defined as 7 days from the day of the first symptoms. For each individual admission day, one of the values will be assigned: – **0:** No NEC – **1:** Current NEC episode – **2.** Status post NEC
Sepsis	The duration of a sepsis episode is defined as 7 days from the day of the positive blood culture. For each individual admission day, one of the values will be assigned: – **0:** No sepsis – **1:** Current sepsis episode – **2:** Status post sepsis
Mechanical ventilation	The duration of a mechanical ventilation episode is defined as the period from the postnatal day of start mechanical ventilation until the postnatal day of stop mechanical ventilation. For each individual admission day, one of the values will be assigned: – **0:** No mechanical ventilation – **1:** Current mechanical ventilation episode – **2:** Status post mechanical ventilation
Surgical procedures	The duration of a surgical procedure episode is defined as 7 days from the day of the surgical procedure. For each individual admission day, one of the values will be assigned: – **0:** No surgical procedure – **1:** Current surgy episode – **2:** Status post surgery

For these assigned values, it is not possible for infants to go back to a status with a lower value, example given from "1" to "0".

Example. *Patient A was followed in the study from postnatal day 1 (equal to day of birth) to postnatal day 28, during which the patient developed a culture proven sepsis episode on day 4. In the study database, the patient was represented by 28 rows each representing one admission day in study follow-up. For this sepsis episode, day 4 and the following six days until day 10 were recorded as "1". Prior to sepsis, day 1 to 3 were recorded as "0" (no sepsis), day 11 to 28 as "2" (status post sepsis).*

4.4 Patient and center characteristics

The following descriptive data on patient and center characteristics will be presented.

Patient characteristics (for total and per country)

- Number of patient included
- Total number of admission days in study follow-up
- Duration of study follow-up per patient, in days
- Postnatal age at start follow-up in the study, in days
- Sex, % female
- Gestational age at birth, in days
- Birth weight, in grams
- Multifetal pregnancy, % singleton
- Patients with congenital anomalies, %
- Patients with intrauterine growth restriction, %
- Patients with at least one major bleeding during study follow-up, %
- Patients with at least one NEC episode during study follow-up, %
- Patients with at least one sepsis episode during study follow-up, %
- Patients with at least one day of mechanical ventilation during study follow-up, %
- Patients with at least one surgical procedure during study follow-up, %

Participating centers (for total and per country)

- Number of academic centers, %
- Number of centers that perform NEC surgery, %
- Number of larger centers (caring >100 preterm infants with a GA below 32 weeks yearly on average), %

5. ANALYSIS OF STUDY OUTCOME MEASURES DESCRIBING PLATELET USE

5.1 Platelet transfusion day prevalence rates

The prevalence rate is the rate of events (new and recurring events) over a specified time period. The platelet transfusion day prevalence rate in a certain time period is defined as the number of platelet transfusion days per 100 NICU admission days in this period. A platelet transfusion day is defined as any day after birth on which the infant received at least one platelet transfusion.

$$\textbf{\textit{Prevalence rate}} = \frac{Number\ of\ RBC\ transfusions\ days\ during\ study\ period}{Number\ of\ admission\ days\ in\ follow\ up\ during\ study\ period} \times 100\ days$$

The platelet transfusion day prevalence rate is calculated in each center and pooled per country. We expect a large between country variability, therefore all prevalence rates are pooled using random effects Poisson models described by Stijnen et al. (2010). This model uses the exact within-study likelihood instead of the approximate normal within-study likelihood which is known to perform better with low event rates and small sample sizes. Additionally, this method does not require the use of an continuity correction in case centers have a prevalence rate of 0 platelet transfusion days. (2) Pooling is done using the function "metarate" of the R-package Meta.

Subgroup rates and overall rate will be presented with 95% confidence intervals in a forest plot. Prevalence rates will only presented on country level.

5.2 Case-mix adjusted platelet transfusion day prevalence rates

As variation between countries in platelet prevalence rates could be explained by differences in population characteristics and disease severity, we will calculate case-mix adjusted platelet transfusion day prevalence rates adjusted for several patient demographics and clinical variables to allow fair comparison between prevalence rates.

Case-mix adjusted platelet transfusion day prevalence rates will be calculated as followed:

1. A logistic regression model is fitted on all data, with case-mix variables and clinical variables as (possible time varying) predictors and platelet transfusion as response variable.
2. The model is used to estimate the expected platelet transfusion day prevalence rate per country
3. Observed / expected prevalence rate ratio per country are calculated
4. Rate ratio are multiplied with overall unadjusted platelet prevalence rate

5.2.1. Average expected platelet transfusion day prevalence rate per country

A logistic regression model is used to estimate the probability that a NICU admission day is a platelet transfusion day based on several covariables, for each individual admission day in study follow-up. To account for the repeated measures within an infant, the model is fitted using generalized estimation equations (GEE).
Probabilities are calculated individually for each patient for each NICU admission day in study follow-up and summarized per country to calculate the average predicted platelet transfusion day prevalence rate.

$$\textbf{\textit{Average expected RBC transfusion day prevalence rate per country}} = \frac{\textit{Sum of predicted probability per admission day over admission days over infants per country}}{\textit{Number of admission days per country}}$$

The following variables are included in the regression analyses. The clinical variables could vary over time and are included under the assumption that experiencing these events leads to an increased risk of transfusion and not vice versa.

- **Baseline variables**: sex, gestational age at birth, birth weight, congenital anomalies, intrauterine growth restriction (birth weight below 10th percentile) (3)
- **Clinical variables:** major bleeding, NEC, sepsis, mechanical ventilation, surgical procedure, postnatal day

5.2.2. Observed / expected prevalence rate ratio per country

The prevalence rate ratio per country is defined as the country's observed platelet transfusion day prevalence (as calculated in section 5.1) compared to the country's average expected platelet transfusion day prevalence rate.

$$\boldsymbol{Prevalence\ rate\ ratio\ per\ country} = \frac{Observed\ platelet\ day\ prevalence\ rate\ per\ country}{Average\ expected\ platelet\ day\ prevalence\ rate\ per\ country}$$

Observed / expected prevalence rate ratios will be presented in an observed/expected funnel plot.

5.2.3 Case-mix adjusted prevalence rates

The standardized platelet transfusion day prevalence rates are defined as the prevalence rate ratio for a country multiplied with the overall unadjusted platelet prevalence rate.

$$\boldsymbol{Case-mix\ adjusted\ prevalence\ rate\ per\ country} = Prevalence\ rate\ ratio\ per\ country * overall\ unadjusted\ platelet\ prevalence\ rate$$

5.3 Cumulative incidence of receiving at least one platelet transfusion

The cumulative incidence (with 95% confidence interval) of receiving at least one platelet transfusion during the first 28 days of life will be calculated where death and discharge are considered as competing events. Infants that were already present at the NICU at the start of the study period are excluded, because the first platelet transfusion for these infants could have happened before the study period. Event time for infants who were transferred or discharged during the first 28 days of life were censored. The cumulative incidence was calculated in R with the package "cmprsk" using function "cuminc".

6. ANALYSIS FOR OTHER STUDY OUTCOME MEASURES

6.1 Primary indications for platelet transfusion

We will present the primary indications for platelet transfusion. The indications reported under 'other indications' will be categorized where possible at the discretion of the primary investigators and will be reported.

6.2 Transfusion volume, duration and infusion rate

Transfusion infusion rates will be calculated by dividing the volume (mL/kg) by the transfusion duration (in hours). Transfusion infusion rates will be presented in two bubble plots, separately for platelet transfusions given based on platelet count threshold and platelets transfusion given for active bleeding, prevention of major bleeding, lumbar puncture, surgical procedure and any other primary indications.

6.3 Platelet count prior to transfusion

We collected the last platelet count measured prior to platelet transfusion, if available within maximum 24 hours before transfusion.

The available platelet count values prior to transfusion will be presented in two box plots, separately for the platelet transfusions given based on platelet count threshold and platelets transfusion given for active bleeding, prevention of major bleeding, lumbar puncture, surgical procedure and any other primary indications.

We will present the proportion of platelet counts prior to transfusions given based on threshold that is below the restrictive threshold ($<25 \times 10^9$/L), between the restrictive and below the liberal threshold (25×10^9/L to $<50 \times 10^9$/L), and above the liberal threshold ($\geq 50 \times 10^9$/L) as compared in the PlaNeT2-Matisse trial. (1)

6.4 Platelet transfusion increment

We also collected the post-transfusion platelet count at the most recent measurement after the platelet transfusion, if available within maximum 24 hours after transfusion. All platelet transfusions given based on platelet count threshold in which both pre-transfusion and post-transfusion platelet count are available will be used for the following plots. Transfusion increment will be calculated by subtracting post-transfusion platelet count from pre-transfusion platelet count.

Transfusion increments will be plotted separately for the different transfusion volume categories as well as for all transfusions. Transfusion increment of a transfusion volume group will not be plotted if 5 or less platelet transfusions given at this volume.

6.5 Transfusion related adverse effects following platelet transfusion

In the absence of clear definitions of transfusion-associated side effects in preterm infants, local investigators were asked to register any perceived transfusion-associated adverse effects if they consider the adverse event to be potentially associated with the preceding transfusion.

We will report the percentage of platelet transfusions for which an adverse events was registered and the mean/median time between occurrence of the adverse effect and the platelet transfusion, in days. The observed adverse effects will be categorized where possible at the discretion of the primary investigators and will be reported in text.

7. GENERAL REMARKS

Reporting guidelines

The study results will be presented according to the recommendations of the STROBE guidelines.

Publication

The statistical analysis plan will be published in the ISRCTN registry.

8. STATEMENT

We hereby declare that this statistical analysis plan has been drafted prior to analysis of the INSPIRE platelet data for the manuscript, and that this plan will be followed during the analysis phase. If any deviations from the plan are made, they will be incorporated and explained in an amendment.

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Version:

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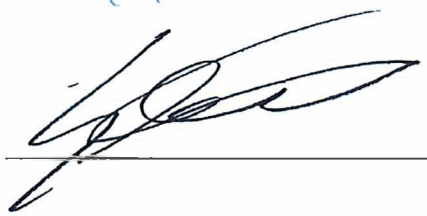

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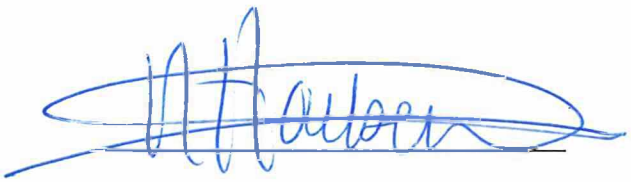

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