**Project Title:** The Impact of Neurobehavior on Feeding Outcomes in Neonates with Congenital Heart Disease

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**Introduction**

This single-center retrospective cohort study aims to understand the association between neonatal attention and feeding outcomes in infants aged 0 to 4 weeks with congenital heart disease (CHD) who underwent surgery. The study focuses on infants who received at least one perioperative (pre-operative or post-operative) Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) score between August 2015 and October 2017. The main objective is to investigate the relationship between lower pre- and post-op attention scores and the percentage of oral feeds at the time of discharge. Furthermore, it seeks to examine whether lower pre- and post-op attention scores are associated with extended time to achieve full oral feeds following surgery.

**Investigator’s Description**

The prolonged time required to attain full oral feeds following neonatal cardiac surgery has been associated with lengthened hospital stays, poor weight gain, and increased stress experienced by parents. One factor that contributes to the challenges of achieving full oral feeds among infants with congenital heart disease (CHD) is neurodevelopment delays. Neonatal neurobehavior abnormalities are associated with poor long-term feeding outcomes among premature infants. Similarly, infants with CHD who have undergone cardiac surgery have also exhibited abnormal neurobehaviors, with poor attention emerging as a common feature of their neurobehavioral state.

Despite the variation in neurobehaviors among infants, previous research has yet to investigate the relationship between neonatal attention and feeding outcomes among infants undergoing CHD surgery. The Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) is a standardized assessment that evaluates infant neurobehaviors across 12 domains, including neonatal attention. In this context, the NNNS defines neonatal attention as the ability of an infant to direct and sustain attention toward a specific stimulus or object.

The overarching aim of this study is to evaluate neonatal attention's impact on feeding outcomes among infants undergoing congenital heart defect surgery during the first four weeks of life. More specifically, we aim to examine the association between lower pre- and post-op attention scores and decreased percentage of oral feeds at discharge. Additionally, we seek to investigate whether lower pre- and post-op attention scores are associated with an extended time to achieve full oral feeds following cardiac surgery.

**Project Endpoints**

Publication, poster presentation, and oral presentation

**Research Objectives**

1. Investigate the relationship between lower pre- and post-op attention scores and the percentage of oral feeds at the time of discharge.
2. Investigate whether lower pre- and post-op attention scores are associated with extended time to achieve full oral feeds following surgery.

**Data**

The NNNS score dataset provided consists of a single-center retrospective cohort of infants aged 0 to 4 weeks with congenital heart disease (CHD) who underwent surgery and received at least one perioperative (pre-operative or post-operative) Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) score between August 2015 to October 2017. There were 132 infants admitted to the cardiac intensive care unit during the study period who were enrolled in the study. Infants with a congenital upper airway or neurological abnormality were excluded (n = 4). The dataset with 128 observations provided included the variables listed in Table 1 below.

|  |  |  |
| --- | --- | --- |
| **Table 1.** Variables provided in the dataset (n = 128 participants) | | |
| **Codename** | **Variable** | **Values** |
| **record\_id** | **Participant ID** | **text** |
| **sex..1.M..2.F.** | **Sex of infant** | **1=Male**  **2=female** |
| **Age.at.Surgery..days.** | **Age at surgery** | **Continuous** |
| **Premature** | **Premature** | **1=Yes**  **2=No** |
| **Genetic.Syndrome.or.Chromosomal.Abnormality** | **Genetic Syndrome or Chromosomal Abnormality** | **1=Yes**  **0=No** |
| **Cardiac.Anatomy** | **Cardiac Anatomy** | **1=Single ventricle w/o arch obstruction**  **2=Single ventricle w/ arch obstruction**  **3=Two ventricle w/o arch obstruction**  **4=Two ventricle w/ arch obstruction** |
| **GI.Complication** | **Gastrointestinal Complications** | **1=Yes**  **0=No** |
| **Length.of.Stay..days.** | **Length of stay** | **Days (continuous)** |
| **Length.of.intubation..days.** | **Length of intubation** | **Days (continuous)** |
| **Extubation.failure** | **Failure of extubation** | **Y=Yes**  **N=No** |
| **bypass.used.** | **Bypass used** | **Y=Yes**  **N=No** |
| **bypass.time..min.** | **Time on bypass** | **Minutes (continuous)** |
| **Neurologic.Complication** | **Neurological complications** | **1=Yes**  **0=No** |
| Pre.Op.NNNS.habituation.score | Pre-operation NNNS habituation score | Continuous |
| **Pre.Op.NNNS.attention.score** | **Pre-operation NNNS attention score** | **Continuous** |
| Pre.Op.NNNS.handling.score | Pre-operation NNNS handling score | Continuous |
| Pre.Op.NNNS.Quality.of.Movement.Score | Pre-operation NNNS quality of movement score | Continuous |
| Pre.Op.NNNS.Regulation.Score | Pre-operation NNNS regulation score | Continuous |
| Pre.Op.NNNS.Non.Optimal.Reflexes.Score | Pre-operation NNNS optimal reflexes score | Continuous |
| Pre.Op.NNNS.Stress.Score | Pre-operation NNNS stress score | Continuous |
| Pre.Op.NNNS.Arousal.Score | Pre-operation NNNS arousal score | Continuous |
| Pre.Op.NNNS.Hypertonic.Score | Pre-operation NNNS hypertonic score | Continuous |
| Pre.Op.NNNS.Hypotonic.Score | Pre-operation NNNS hypertonic score | Continuous |
| Pre.Op.NNNS.Asymmetry.Score | Pre-operation NNNS asymmetry score | Continuous |
| Pre.Op.NNNS.Excitability.Score | Pre-operation NNNS excitability score | Continuous |
| Pre.Op.NNNS.Lethargy.Score | Pre-operation NNNS lethargy score | Continuous |
| **Percent.of.feeds.taken.by.mouth.at.discharge** | **Feeds taken by mouth at discharge** | **% (continuous)** |
| **Date.PO.feeds.started** | **The date that PO feeds started** | **date** |
| **Date.Reaching.Full.PO** | **Date reaching full PO** | **date** |
| **Date.Identified.as.not.yet.full.PO** | **Date identified as not yet full PO** | **date** |
| Post.Op.NNNS.habituation.score | Post-operation NNNS habituation score | Continuous |
| **Post.Op.NNNS.attention.score** | **Post-operation NNNS attention score** | **Continuous** |
| Post.Op.NNNS.handling.score | Post-operation NNNS handling score | Continuous |
| Post.Op.NNNS.Quality.of.Movement.Score | Post-operation NNNS quality of movement score | Continuous |
| Post.Op.NNNS.Regulation.Score | Post-operation NNNS regulation score | Continuous |
| Post.Op.NNNS.Non.Optimal.Reflexes.Score | Post-operation NNNS optimal reflexes score | Continuous |
| Post.Op.NNNS.Stress.Score | Post-operation NNNS stress score | Continuous |
| Post.Op.NNNS.Arousal.Score | Post-operation NNNS arousal score | Continuous |
| Post.Op.NNNS.Hypertonic.Score | Post-operation NNNS hypertonic score | Continuous |
| Post.Op.NNNS.Hypotonic.Score | Post-operation NNNS hypotonic score | Continuous |
| Post.Op.NNNS.Asymmetry.Score | Post-operation NNNS asymmetry score | Continuous |
| Post.Op.NNNS.Excitability.Score | Post-operation NNNS excitability score | Continuous |
| Post.Op.NNNS.Lethargy.Score | Post-operation NNNS lethargy score | Continuous |

The primary focus of this statistical analysis is investigating the relationship between lower pre- and post-op attention scores and the percentage of oral feeds at the time of discharge. Therefore, we will examine the outcome variables, pre- and post-operation NNNS attention scores, in relation to the predictor variable, the percent of feeds taken by mouth at discharge.

**Analyses**

**Data Cleaning and Exploration**

1. Data Cleaning
2. Missingness and Imputation

The data has high rates of both structural and non-structural missingness. Structural missingness includes dates of censoring reached for observations where censoring does not occur. Missingness in these variables will be re-coded with flags for intended missingness and non-intended missingness, and non-intended missingness will be considered non-structural in further analysis.

Non-structural missingness will be assessed for appropriateness of multiple imputation strategies by checking for the likelihood of MNAR patters in the following ways:

* Patterns of missingness will be explored visually using heat maps to look for large chunks of unit missingness.
* Pre and post-op attention scores will be plotted as box plots next to each other and Wilcoxon signed rank tests will be run. This will be done for the whole group and then separately for participants with missing percent of oral feeds at discharge observations. Change in trend direction, large deviations in test statistics, or large deviations from significance to non-significance at an inflated alpha level (0.1) between the wilcox model results for the groups with missing or non-missing values (of percent of oral feed at discharge) against each other or the large group model will indicate MNAR data. In this case multiple imputation will not be used.

In the case that MNAR data is suspected, observations without percent oral feed at discharge or a missing pre or post-op attention score will be deleted. Other missing values will be imputed using univariate imputation regression models constructed from the covariates in the reduced model described in the analysis of the primary objective section.

In the case that MNAR data is not-suspected, multiple imputation will be used for both the primary and secondary objectives. Multiple imputation will be carried out using the MICE package in R. Structural missingness will be artificially preserved or induced during the imputation process. Standard diagnostic plots from the mice package will be used to assess the success of the multiple imputations. Primarily, kernel density estimates for the marginal distributions of the observed and imputed data will be plotted on top of each other to check for major differences in any of the variables of interest.

The same set of data will be used for analyses of both primary and secondary objectives. Analyses will be carried out on all imputed datasets. The number of imputations will start at 20. Before pooling of the estimates, distribution of the estimates will be examined using a qq plot to determine whether normality is reasonably approximated. If significant deviation from normality is observed in the estimates of parameters of interest in any of the models, the number of imputations will be increased to see if that fixes the issue. If it does, all models will be re-run using the new imputed data sets. Estimates for coefficients in the regression will be pooled to give a final estimate for the primary objective. Estimates for the risk ratios in the survival analyses will be pooled to give a final estimate for the secondary objective.

1. Data Visualization

Our data visualization methods will be discussed in the analysis section of this SAP.

1. Summary Statistics

Descriptive summaries of the variable will include mean (SD), medians with interquartile ranges (IQR), and counts (%).

**Analysis of the Primary Objective**

Due to the occurrence of zero proportions of oral feeds at discharge, including instances where patients did not consume any oral feeds, we will employ zero-inflated beta-regression models in our analysis and will use gamlss package in R.

Zero-inflated Beta regression:

The zero-inflated beta distribution is given as

if

if . The parameters satisfy and .  
Here and .

Rare instances with a value of one (indicating 100% oral feed) were also present. Given our constrained sample size, we will transform non-zero values for analysis using the formula (y[n-1] + 0.5)/n, where y represents the proportion of oral feeds at discharge, and n is the count of non-zero values.

The initial segment of our model as we can see in the formula of zero-inflated beta regression model above is focused exclusively on patients who were orally feeding at discharge. Their outcomes will be modeled using a beta distribution with a logit link function.

In the subsequent part, we will model oral feeds at discharge being 0%, in contrast to >0%, for all patients in our cohort. This will be achieved using logistic regression with a logit link function.

We want to include as much as variables that we can. So, we will use Pre and post attention scores and all other covariates except other NNNS scores.

We will implement three models. One model includes both post and pre attention scores after adjusting for sex, genetic syndrome, age at surgery, prematurity, cardiac anatomy, length of intubation, extubation failure (Y/N), and gastrointestinal complications. The second model includes just pre-attention scores and the same covariates. The third model includes post-attention scores and the same covariates.

**Model 1:**

**% oral feeds** at discharge  
 Age.at.Surgery..days Post-attention scores

**Model 2:**

**% oral feeds** at discharge  
 Age.at.Surgery..days Pre-attention scores

**Model 3:**

**% oral feeds** at discharge  
 Age.at.Surgery..days Post-attention scores

Table (2): odds ratio of oral feeds when oral feed is >0

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| variables | Estimated values | Lower CI | Upper CI | p-values |
| Sex |  |  |  |  |
| Age |  |  |  |  |
| Genetic |  |  |  |  |
| Cardiac |  |  |  |  |
| …… |  |  |  |  |
| Pre\_attention score |  |  |  |  |
| Post\_attention score |  |  |  |  |

Table (3): odds ratio of oral feeds when oral feed is 0

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| variables | Estimated values | Lower CI | Upper CI | p-values |
| Sex |  |  |  |  |
| Age |  |  |  |  |
| Genetic |  |  |  |  |
| Cardiac |  |  |  |  |
| …… |  |  |  |  |
| Pre\_attention score |  |  |  |  |
| Post\_attention score |  |  |  |  |

Table (4): odds ratio of oral feeds when oral feed is> 0

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| variables | Estimated values | Lower CI | Upper CI | p-values |
| Sex |  |  |  |  |
| Age |  |  |  |  |
| Genetic |  |  |  |  |
| Cardiac |  |  |  |  |
| …….. |  |  |  |  |
| Pre\_attention scores |  |  |  |  |

Table (5): odds ratio of oral feeds when oral feed is 0

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| variables | Estimated values | Lower CI | Upper CI | p-values |
| Sex |  |  |  |  |
| Age |  |  |  |  |
| Genetic |  |  |  |  |
| Cardiac |  |  |  |  |
| …….. |  |  |  |  |
| Pre\_attention scores |  |  |  |  |

Table (6): odds ratio of oral feeds when oral feed is> 0

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| variables | Estimated values | Lower CI | Upper CI | p-values |
| Sex |  |  |  |  |
| Age |  |  |  |  |
| Genetic |  |  |  |  |
| Cardiac |  |  |  |  |
| …….. |  |  |  |  |
| Post\_attention scores |  |  |  |  |

Table (7): odds ratio of oral feeds when oral feed is> 0

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| variables | Estimated values | Lower CI | Upper CI | p-values |
| Sex |  |  |  |  |
| Age |  |  |  |  |
| Genetic |  |  |  |  |
| Cardiac |  |  |  |  |
| …….. |  |  |  |  |
| Post\_attention scores |  |  |  |  |

**Analysis of the Secondary Objective**

To investigate whether lower pre- and post-operative attention scores are associated with extended time to achieve full oral feeds following surgery, we will employ Cox proportional hazard regression models. For the analysis, our time will be measured in days from surgery to the date the infant achieved full oral feed (event) or until the data of discharge (censored). In order to implement the survival analysis, two variables will be created using the “Length.of.Stay..days.” and “Date.Reaching.Full.PO” variables to capture the time elapsed until an occurrence and a binary variable to distinguish between the event being achieve or the subjected being censored.

Statistical modeling will employ Cox Proportional Hazard Regression models, generating Hazard Ratios (HRs) and 95% Confidence Intervals (CIs) for achieving full oral feeds. HR interpretation will focus on an increase (HR < 1) or decrease (HR > 1) in time to achieve full oral feeds. Univariable analyses will be conducted separately for preoperative and postoperative attention scores, reporting HRs, 95% CIs, and p-values to assess the association with feeding outcomes.

In the multivariate analysis, Cox proportional hazard regression models will be constructed, incorporating preoperative and postoperative attention scores alongside relevant covariates. The combined effect of attention scores and covariates on time to achieve full oral feeds will be assessed, with results reported in terms of HRs, 95% CIs, and p-values. Model evaluation will involve assessing goodness-of-fit, checking the proportional hazards assumption, and evaluating performance metrics such as concordance, Likelihood Ratio Test, Wald Test, and Log Rank Score Test.

We will construct various models and compare them using ANOVA methods; these will include both univariate models looking at the simple relationship between our “survival” and our pre- and post-surgery attention scores to multivariate models with variables previously selected in the above process.

The results will be presented in a table similar to the one presented below:

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Pre - Op | | | | Post - op | | | |
|  | Univariate model | | Multivariate | | Univariate model | | Multivariate | |
|  | HR (95% CI) | P-value | HR (95% CI) | P-value | HR (95% CI) | P-value | HR (95% CI) | P-value |
| Attention scores |  |  |  |  |  |  |  |  |
| Additional Predictors |  |  |  |  |  |  |  |  |

**Quality Control (QC) Plan**

In our quest to maintain the quality of this analysis, we plan to engage in double coding for all aspects within the given time frame. In addition, group members will systematically review and validate the code at crucial points during its creation, ensuring its alignment with the intended analysis and the production of accurate results. This spot-checking process will also focus on code validation, logical flow, and adherence to coding standards. Documentation will be maintained throughout the analysis, including explanations and comments for future reference. Rigorous quality assurance testing will validate the correctness and functionality of the code, addressing any potential issues or errors. A final review will take place after all analyses and code has been consolidated for the final report.