Fortification of Expressed Breast Milk with Preterm Formula Powder vs. Human Milk Fortifier in Preterm (28-34 weeks' Gestation) Very Low Birth Weight Neonates: a Randomized non inferiority trial

Statistical Analysis Plan

1. Introduction

Very low birth weight (VLBW) neonates require fortification of human milk to meet the recommended dietary allowances of nutritional intake. Commercial human milk fortifiers are costly and therefore, sparingly used in low and middle income (LMIC) countries, leading to postnatal growth restriction. This has significant public health implications in resource restricted countries where burden of prematurity, neonatal mortality and morbidity is extremely high. The study intervention will evaluate a low-cost alternative to the commercial fortifiers. If the study demonstrates benefit of the new intervention, it can lead to significant cost savings to an already overburdened healthcare system in LMIC. Our study will test the hypothesis that preterm formula fortification will be non-inferior to fortification by a human milk fortifier in very low birth weight neonates.

2. Descriptive statistics

Patient characteristics will be summarized by randomization group, which will be labelled as preterm formula (PTF) and human milk fortifier (HMF). For continuous variables, mean with standard deviation or median with interquartile range will be reported depending on the skewness of the data. Student t test will be used to compare means of the two groups. Where appropriate, Wilcoxon rank sum test will be used for continuous data. Categorical outcomes will be represented as counts and percentages and compared by using the χ_2 test. Any group characteristics that are identified as either statistically significantly different ($p<0.05$) or clinically disparate will be considered as covariates.

3. Primary Analysis

3.1 Primary outcome: n-hospital weight gain (g/kg/d), i.e., from enrolment until discharge or 40 weeks PMA, whichever is earlier

3.2 Secondary outcome: head circumference and length (in cm) at discharge, extrauterine growth restriction, feed intolerance, late onset sepsis, intraventricular hemorrhage, anemia of prematurity, late metabolic acidosis, metabolic bone disease, retinopathy of prematurity, bronchopulmonary dysplasia, mortality.

3.3 Analysis plan: The primary hypothesis to be tested is whether in-hospital weight gain after fortification with PTF is non-inferior to HMF group. The absolute non-inferiority margin is set at 2g/kg/d. The null hypothesis for the formal statistical test is that the PTF is inferior to HMF group with a 2g/kg/day non-inferiority margin ($\mu_{PTF} - \mu_{HMF} < -2$g/kg/d). The

alterative hypothesis is that PTF is non-inferior ($\mu_{PTF} - \mu_{HMF} > -2$g/kg/d). If the lower bound of confidence interval is more than -2g/kg/d, the non-inferiority of the intervention will be established. This will be tested based on a 2-sided confidence interval for the true difference in means ($\mu_{PTF} - \mu_{HMF}$).

Potential covariates that shall be considered for adjusting primary analysis include gestation, gender, birth weight, and small for gestational age. SGA status will be stratified at the time of randomization. Covariate adjustment will be done only if significant imbalances are found in between the two groups for the individual covariates.

Both intention-to-treat and per-protocol analysis will be performed.

The feed intolerance related outcomes will be represented as incidence density (number of episodes per 1000 patient-days). Poisson or negative binomial regression model will be used for calculating the incidence rate ratio (depending on the dispersion of the count data for feed intolerance). The other secondary outcome data will be handled as mentioned for descriptive statistics above.

3.4 Sample size and power: The unit data for weight gain in VLBW neonates with human milk fortifier is available for HIJAM, which is 13.5 g/kg/day (SD 3.8). The fortifier currently being used is PreNAN (Nestle and Co., India), the weight gain data for which is not available. Assuming that fortification with preterm formula powder (Dexolac Special Care, DANONE India) will result in weight gain not lower than 2 g/kg/day i.e., a minimum weight gain of 11.5 g/kg/day with the same SD, and an alpha error of 5% and power of 90%, we have to enrol 62 neonates in each group.