Chapter 3

Research Design and Methodology Overview

Hypothesis :

The failure rates and the number of events of side effect were the same between using normal saline and fresh frozen plasma for partial exchange transfusion in polycythemic neonates.

Research Questions :

Primary question : was there any difference in the failure rates, defined as post partial exchange hematocrit $\geq$ 65%, between the polycythemic neonates who had hematocrit $\geq$ 70% treated with standard doses of normal saline and fresh frozen plasma for partial exchange transfusion within 6 hours of life ?

Secondary questions : 1. was there any difference in number of events of side effect between the two groups ?

: 2. which alternative cost more between fresh frozen plasma and normal saline for partial exchange transfusion?

Objectives :

1. To compare the failure rates in decreasing hematocrit from $\geq$ 70% to < 65% between using normal saline and fresh frozen plasma for partial exchange transfusion.

2. To identify the side effects that might occur between using normal saline and fresh frozen plasma for partial exchange transfusion.

3. To compare the cost of fresh frozen plasma and normal saline for partial exchange transfusion, to determine the least expensive way of

achieving the outcome which is a decrease in the hematocrit to <65% and to do a sensitivity analysis to determine the threshold point of re-transfusion rate between the two treatment strategies.

Research Design :

Stratified randomized clinical trial with single blind. In order to get equally distributed population for the two different treatment groups, the stratifications were justified for 5 predisposing factors that had the influences upon the prognosis of infants in this study, both immediate and longterm. The study population was first divided into 5 relevant strata, and a random sample was then selected from each stratum for treatment with normal saline or fresh frozen plasma using blocks of 2.

Five groups of infants at high risk for developing polycythemia and hyperviscosity were :

1. intrauterine hypoxic infants from all causes, from history and physical examination, the infants could be categorized into this group from small for gestational age appearance. The causes of small for gestational age were intrauterine infections, varieties of syndrome, high altitude, malnutrition, chromosomal abnormalities, infants who were exposed to intrauterine toxic substances, etc.

2. infants with decreased placental functions such as posterm infants, placental infarction, diseases of the placenta and maternal pre-eclampsia.

3. infants with abnormal hormonal stimulation such as infants of diabetic mother, maternal hyperthyroidism, maternal ovarian tumors, etc.

4. infants with increased placental transfusion such as the infants who were born before arrival at the hospital, delayed cord clamping, feto-fetal transfusion and materno-fetal transfusion.

5. infants who did not have any risk factors or no apparent predisposing factor but hematocrit was high of unknown cause.This group consisted of normal fullterm infants who appeared absolutely normal and were appropriately for gestational age but hematocrit was at abnormally high level.

All 5 groups of infants had no proven influence on neither prognosis of immediate outcome nor decreasing hematocrit post partial exchange transfusion,but certainly had major influence on prognosis of longterm outcome of neurodevelopment of the infants.

For economic study, since the outcome of both alternatives is axpecled to be equal and to successfully decrease the hematocrit in all cases to <65%, a cost-identification (cost - minimization) analysis is appropriate for this study[24,25]. Decision tree is shown (Appendix 3).

Research Methodology :

Population and sample :

Umbilical cord blood hematocrit screening was done in all the neonates born at term at Pramongkutklao Hospital. In any infants with cord blood hematocrit > 55% peripheral venous hematocrit was determined. Approximately 1% of the cord blood samples were discarded because of such factors as clotting and inadequate sample. Peripheral venous hematocrit was measured in these infants.

Inclusion criteria :

Any infants 37 weeks gestation and above who had peripheral venous hematocrit ≥ 70% who were born in Pramongkutklao hospital and parents signed the informed consents.

Exclusion criteria :

Any infants whose parents refused to sign the informed consents, preterm and any critically ill infants whom the procedure would make the condition worse such as shock, severe perinatal asphyxia with seizure and disseminated intravascular coagulopathy, infants with blood dyscrasia, intracranial hemorrhage and infants who were born outside Pramongkutklao hospital.

Sample size :

The total number of infants were 38 in each group on the basis of a risk of alpha error of 5% and power of 80% to detect difference.

From pilot study, the proportion of infants that had post partial exchange Hct of >65% in plasma group of 13 infants = 0, there was no failure rate, It could have been from small sample size. From clinical practice the failure rate was also extremely low, therefore, for the purpose of sample size calculation the failure rate was estimated to be 0.005. In saline group of pilot study the failure rate in 13 infants = 0.15. In clinical practice the acceptable re-exchange rate was up to 20%

$$n = \frac{2\,(Z\alpha + Z\beta)^2 \times \pi\,(1-\pi)}{(P_1-P_2)^2}$$

$Z\alpha$ = type I error = 5% = 1.96 two tailed

$Z\beta$ = type II error = 20% = 0.84

P_1 = Expected event rate in plasma group = 0.005

P_2 = Expected event rate in saline group = 0.20

$$\pi = \frac{P_1 + P_2}{2} = \frac{0.005 + 0.20}{2} = 0.1025$$

$$n = \frac{2(1.96 + 0.84)^2 \times 0.1025\,(1-0.1025)}{(0.005-0.20)^2}$$

n = 38/ group

Intervention :

Step 1. The parents of any infants with Hct $\geq$ 70% were verbally informed and have signed the written informed consents.

Step 2. The infants who met the eligibility criteria were stratified by the predisposing factors into 5 strata.

Step 3. With each stratum, infants were randomly allocated to treatment with normal saline or fresh frozen plasma using blocks of 2. The normal saline and fresh frozen plasma group AB (universal donor) were made available at hand in the nursery.

Step 4. The baseline multiple variables were recorded just before starting the procedure according to observations and measurements.

Step 5. Umbilical venous line was used for partial exchange transfusion. The volume of blood taken out was pre-calculated by using the following formula :

Volume of blood taken out

$$= \frac{\text{Observed Hct-desired Hct x blood volume x body weight}}{\text{Observed Hct}}$$

Where :

Observed Hct = hematocrit at immediate pre exchange

Desired Hct = mean hematocrit of the normal fullterm infants
= 55%

Blood volume = volume in ml. of blood according to Rawlings et al nomogram[19]

Body weight = birthweight in kilogram

The amount of fluid replacement was equal to the volume of blood taken out. It was done via umbilical venous line by taking the blood out 20 ml. at a time and alternately pushing fluid in 20 ml. at a time simultaneously, in order not to interfere with normal body physiology.

The first syringe of the drawn blood was sent for all required biochemical measurement which was determined by micromethod. The second syringe of blood was drawn immediately post exchange. Only the observer or pediatric resident knew the type of fluid given.The assessor or the investigator did not have any access to know which infants belong to which group.

Step 6. Body weight at 24 hours was recorded.

Observations and measurements :

Administrative variables :

Maternal name, ID, address.

Baseline variables :

Infant's sex, gestational age, type of delivery, Apgar Score at 1 and 5 minutes, birthweight, date and time at diagnosis, when treatment started, when treatment ended, volume of blood taken out and volume of fluid used.

Outcome variables :

Main outcomes were failure rate of post exchange hematocrit, define as hematocrit $\geq$65%, at immediate post exchange, 4 hours and 24 hours post exchange. Biochemical derangements at immediate post exchange were secondary outcomes.

At diagnosis : hematocrit and blood sugar were measured in % and mg/dl.

At the start of treatment : hematocrit, serum sodium, chloride, potassium, bicarbonate, calcium, blood urea nitrogen, creatinine, total protein and albumin were measured.

At the end of treatment : hematocrit and all previous biochemical substances were measured again. Hematocrit at 4 hours and 24 hours after the treatment ended were also measured.

Note : hematocrit at immediate post partial exchange transfusion was used to compare the real efficacy of normal saline to fresh frozen plasma.

: Hct at 4 hours was tested after the equilibration of blood. This value tells whether the normal saline can be maintained continuously inside blood vessel.

: Blood sugar was done at diagnosis to find out which infants was hypoglycemic (which is the complication of polycythemia) and when treatment for hypoglycemia started immediately. There was comparison between blood sugar of pre and post exchange to see the effect of each fluid used in the procedure. Once the treatment was efficacious, the blood sugar should be within the normal limit in both groups.

: The side effects of normal saline were shown from :

1. Dilutional effect of blood chemistries out of the following normal limits : serum potassium = 6.4$\pm$0.9 mMol/L, bicarbonate = 16$\pm$3 mMol/L, calcium = 2.38$\pm$0.25mMol/L, blood urea nitrogen = 25.7$\pm$9.3mMol/L, creatinine = 93$\pm$31mMol/L, total protein = 72.5$\pm$12.5mMol/L, albumin = 39$\pm$13 mMol/L.

2. Hypernatremia : serum sodium >152.4 mMol/L, hyperchloremia : serum chloride >108.07 mMol/L.

3. Inability to stay long in vascular compartment as shown from significant increase in body weight at 24 hours after the treatment ended.

: The side effects of plasma were mostly long term and unable to be shown here, but the chance of blood chemistry derangement was also as possible as normal saline, though expected to be fewer.

Due to technical difficulties and co-operational problems the 24 hours urine output and sodium excretion were unable to be collected.

The variable in economic study is cost. It usually means the service offered to the patients as well as the hospital expenses incurred in providing such service. In a broad sense, these costs include the capital cost and the benefits obtained both by the personnel and the patients. It is very difficult and complicated to calculate the exact value of the cost for each patient and oftentimes charge is used to represent the cost although these two are not equal most of the time.

In this study, the patients are neonates and stay in the hospital for only a short time for the treatment of polycythemia. The outcome used here is the objective value of hematocrit. Therefore it is appropriate to calculate only the direct medical cost, not including the direct non-medical cost, indirect cost, intangible cost and the effect of time[25].

According to the treatment procedure, following are the 9 items of cost calculated :

1. Cost of screening and diagnoses of polycythemia.
2. Cost of partial exchange transfusion.
3. Physician's fee.
4. Nurse's fee.
5. Cost of retest of hematocrit four times.
6. Cost of serum lab tests.
7. Cost of re-transfusion.
8. Cost of managing complications.
9. Other cost of hospitalization

Point of view : the health care provider perspective is taken into account in this analysis.

Data Collection :

All data were recorded in the data collection form (Appendix1) and software program Epi Info Version 6. by two persons, and rechecked for accuracy of data entry.

For economic study, consultation of corresponding sections or departments of the hospital for financial informations were done.

Statistical Analysis :

The main purpose of this study was to compare between the normal saline and fresh frozen plasma to detect the differences in the baseline and outcome variables.

For continuous data (such as birthweight, age at diagnosis,Hct, etc.) the means, standard deviations and student - t test were calculated when the data were normally distributed.

For dichotomous data (such as sex, Apgar score, symtomatology) proportion or percentage and Chi-square or Fisher exact test were calculated.

The comparison between baseline variables among control and treatment groups to see whether randomizations were successful and the comparsion of outcome variables, were analysed by programs Epi Info version 6 and SPSS/Pc+.

Ethical Consideration :

Fresh frozen plasma and normal saline had been used for partial exchange transfusion in our hospital. The partial exchange transfusion also had been accepted as a standard treatment for the polycythemic infant, however, the procedure, normal saline and fresh frozen plasma carried their own complications, the parents were verbally informed all of the following :

1. What polycythemia means and its complications.

2. The treatment procedure and the equal chance of fluid type that the infant would get.

3. The complications of the procedure, side effects of fluids and the outcome after treatment (failure and re-exchange).

4. The study comparison between normal saline and fresh frozen plasma was discussed in detail. The written informed consent (appendix 2) was obtained from one of the parents who agreed to participate in this study.

Pramongkutklao Hospital Ethical Committee had given approval of this study.

In the case of treatment failure, the infant would receive second partial exchange transfusion using fresh frozen plasma.

Expected Benefits and Applications :

In Thailand there have been several times in 3-4 decades when the polycythemic infants were transferred from far away places to regional hospitals just for partial exchange transfusions because of lacking of fresh frozen plasma. At present, with the use of microcentrifugal machine to do hematocrit for diagnosis of polycythemia and the normal saline, the treatment could be carried out with saving both time and money.

In most regional centers and medical school hospitals such as Pramongkutklao Hospital, the blood bank process of doing typing and cross matching to get plasma took over 3 hours compared to less than 1 hour for getting the procedure started with the available normal saline.

Also by avoiding the use of plasma, the risk of newborn infants contracting viral infections would be decreased. At present the incidence of HIV infection is 1 : 20,000 transfusion.