Title: Can Mixed Lipid Emulsion Prevent Parenteral Nutrition Associated Cholestasis?

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**Type of Investigation:**
Prevention

**Question:**
In extremely low birth weight infants (<1000g) does a mixed lipid emulsion compared to a soybean oil-based lipid emulsion reduce the incidence of parenteral nutrition associated cholestasis (PNAC)?

**Methods:**

*Design:* Single center, double blind randomized control trial

*Allocation:* Concealed (randomized using permuted blocks, ratio 1:1, block size of 4)

*Blinding:* Double blinded

*Follow-up period:* 44 weeks postmenstrual age, discharge, or transfer to another hospital

*Setting:* A single level 4 NICU

*Patients:* Inclusion criteria: infants who were <1000g at birth and admitted to the University Children’s Hospital Vienna before 24 hours of life between June 2012 and October 2015.
Exclusion criteria: infants with conjugated bilirubin levels >1.5mg/dL before intervention, conditions associated with cholestasis independent of parenteral nutrition and higher order multiples.
**Intervention:** Double-blind randomized controlled trial of infants who were <1000g at birth and randomized to either a mixed lipid emulsion (intervention) or a soybean oil-based lipid emulsion (standard).

**Outcomes:** Primary Outcome: reduction in PNAC in infants receiving a mixed lipid emulsion (intervention) compared to those who received the standard lipid emulsion. Secondary outcomes: evaluate the impact of the study lipid on other morbidities (death, retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), liver function tests (LFTs), sepsis, intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA), and pulmonary hypertension).

**Analysis and Sample size:** 100 infants in each group would provide an 80% power to detect a 60% reduction in PNAC (from 25% to 10%). They assumed an 18% drop out rate and, therefore, aimed to enroll 122 patients per group. There were 274 infants eligible for the study and 230 were ultimately randomized, 113 in the intervention group and 117 in the standard group. The intention to treat analysis was performed on 223 infants, 110 in the intervention group and 113 in the standard group. The randomization was stratified according to sex and birthweight (<750g vs ≥750g). If twins were included, the first twin was randomized and the second twin was assigned to the opposite treatment. For the primary outcome, the $X^2$ test was used to evaluate the effect of the intervention on PNAC. A univariate logistic regression model for significant influence was conducted on possible confounders that the authors had identified from the literature (male sex, sepsis, compound outcome of NEC, focal intestinal perforation and gastrointestinal surgery, birthweight, total days on parenteral nutrition, $z$ score of birthweight, and enteral in the first week of life per kg birth weight). The secondary outcomes were analyzed using the $X^2$ test for categorical data and the Mann-Whitney $U$ test for continuous data.
Patient Follow Up: 3 infants initially randomized to the intervention group were excluded due to not receiving the intervention (2) or having a genetic syndrome predisposing them to cholestasis (1). In the analysis per protocol an additional 5 were excluded due to death <28 days. In the standard group there were 4 infants initially randomized that were excluded in the intention to treat analysis due 1 death prior to receiving the intervention, 2 infants who had cholestasis associated with a disease, and 1 infant who developed cholestasis prior to the intervention. The analysis per protocol was performed on 105 infants in the intervention group (93%) and 101 in the standard group (86%).

Main Results:
11 (10%) infants in the intervention group and 18 (16%) infants in the standard group were diagnosed with PNAC. Although the incidence of PNAC in the intervention group was lower, it was not found to be statistically significant (p=0.2) In addition, the timing of development of PNAC was also not different between the groups (median day of life was 23 in the intervention group and 20 in the standard group). Morbidities and mortality were also not statistically significant between the groups. Therapy adherence between the 2 groups were equal and both groups received parenteral nutrition and lipids for similar amounts of time. Triglyceride levels and incidence of hypertriglyceridemia did not differ significantly between groups. Feeding volumes in the first week of life, the use of mother’s own milk, and the growth parameters were not found to be different between the 2 groups.

Study Conclusion:
The study found that a mixed lipid emulsion composed of soybean oil, medium chain triglycerides (MCT), olive oil, and fish oil did not significantly reduce the incidence of PNAC in extremely low birth weight infants.

**Commentary:**

It is well known that long term parenteral nutrition is associated with the development of cholestasis in preterm infants. However, despite recent advancements in postnatal nutrition practices including early enteral feeding, increase protein intake, and increased calorie intake, ELBW infants are still dependent on parenteral nutrition including lipids for an extended period. As a result, these infants are at high risk for developing parenteral nutrition associated cholestasis (PNAC) with reported prevalence rates anywhere from 10-50% (1). The development of PNAC is multifactorial with gestational age having a major influence (2, 3). The proinflammatory nature of the omega-6 fatty acids as well as the phytosterols found in the soybean based lipid emulsion preparations these infants receive are also thought to play a critical role (4). Recent studies have suggested that using a lipid formulation composed primarily of omega-3 fatty acids, which are less proinflammatory and do not contain phytosterols, reverses PNAC in extremely low birth weight (ELBW) infants (3, 5, 6).

These results make using a fish oil based lipid emulsion an attractive potential solution to prevent the development of PNAC in ELBW infants. The current study looked at whether a fish oil based lipid emulsion would decrease the incidence of PNAC in ELBW compared to a soybean based lipid preparation when started shortly after birth. They found no statistical difference in PNAC between the two groups. They also found that infants in each group were on
parenteral nutrition for similar amounts of time, had similar incidences of hypertriglyceridemia, had no difference in liver enzymes values, and the day of life that infants developed cholestasis was also not different. Infants were randomized and stratified based on sex and birth weight but there was no analysis or mention of the effect of the intervention lipids on infants in different weight groups. Since lower birth weight infants are more at risk for developing PNAC it would be interesting to see in the ongoing trial whether the infants who were <750g at birth and who received the study lipids had a decreased incidence of PNAC as compared to the infants who were >750g at birth. In addition, no statistical differences between the groups based on demographic characteristics was noted, but there were 34% SGA infants in the standard group and 20% SGA infants in the study group. How this difference may have impacted the results is not described. One might assume that a more mature infant, even SGA, would have a more mature liver and be able to tolerate lipids better than an infant of similar weight but lower gestational age. Infants were started on the study protocol within the first 120 hours of life which could potentially mean that infants randomized to the study lipid emulsion could have received 5 days of the soybean based lipid emulsion prior to starting the intervention. The exposure to soybean based lipids may have predisposed infants to cholestasis but this was not discussed in the paper.

Dr. Repa and colleagues published a study in 2016 evaluating an aggressive versus conservative feeding practice of ELBW infants in their NICU and the impact on growth and the development of cholestasis (7). They found that adopting a more aggressive feeding protocol decreased their rate of cholestasis in ELBW infants from 45% to 27% but did not change the length of time that infants were on parenteral nutrition. The authors of the current study mention a significant limitation to their study was that although they assumed the incidence of PNAC to be 25%, the
actual incidence of PNAC at the start of the study was 15.9%. Despite this drop in incidence, how this affected the results was not mentioned. They attributed this drop to their unit’s adoption of an accelerated parenteral nutrition weaning protocol, presumably the one studied in the 2016 article, but did not mention the specifics of their unit’s feeding protocol during this study. Based on an initial chi-square analysis a need for 100 infants per group to achieve a power of 80% was estimated. However, when the incidence of 15.9% of PNAC was determined, the group size was not recalculated. If one were to use a starting incidence of 16%, you would need 492 infants per group to detect a decrease in incidence of PNAC from 16% to 10%. Therefore, the current study lacks the power to detect a true difference between soybean based lipids and fish oil based lipids.

A recent Cochrane Review in 2015 evaluated studies that compared the administration of fish oil based lipid preparations versus the traditional soybean based lipid emulsion (8). This review concluded that a fish oil based lipid emulsion appeared to be well tolerated and safe for use in preterm infants but that there was no statistical difference in the incidence of PNAC between those infants that received the fish oil based lipid emulsion and those that received the traditional soybean oil based lipid emulsion. They concluded that there is insufficient evidence to recommend changing the current standard practice of using a soybean based lipid emulsion. Additionally, the FDA has not approved fish oil lipid emulsion in the United States due to potential contamination risks. The majority of the studies reviewed are from outside the US and it’s unclear how pure the formulations are and what the exact preparations and proportions are in order to truly compare their effectiveness in preventing PNAC in ELBW infants.

The conflicting outcomes of the recent studies comparing fish oil based formulations to soybean oil based preparations illustrate the complex and multifactorial pathogenesis of the development
and reversal of PNAC. Currently, although fish oil preparations appear safe, they are not standard of care in most institutions as the clear benefits, as seen in this study, have not been elucidated. Future studies should attempt to standardize the formulation of the fish oil based preparation and compare outcomes when altering only one component of the emulsion in order to better define the impact each component or each amount of fish oil has on PNAC and other morbidities.

**EBM Lesson:**

**Chi-square test**

A Chi-square test (also known as the Pearson Chi-square test) is a non-parametric statistical test commonly used in clinical research to determine whether categorical variables are independent from each other; that is that the observed values are significantly different from those values that would be expected by chance. The results obtained with this test are most reliable when the data is collected from randomly selected subjects and when sample sizes are sufficiently large that they have adequate statistical power. There are several requirements, or assumptions, that must be met in order to use this statistic:

1. The data in the cells should be frequencies or number of cases.
2. Each category is mutually exclusive such that each data point or subject only fits into one category.
3. Each data point or subject only contributes to one cell (i.e. you can’t use multiple data points from one subject).
4. The study groups being compared are independent of each other.
5. There are 2 variables that are both measured as categories.

6. The expected number of data points or subjects within each cell should have a minimum of 5 in at least 80% of the cells and no cell should have a value less than 1. This test is extremely sensitive when the numbers are too small (9). For sample sizes less than 20 it is recommended to use a more exact test such as a Fisher’s exact test.

In this case, chi-square test was used to determine whether using a fish oil based lipid emulsion reduced the incidence of PNAC in ELBW infants. The incidence of PNAC in the group receiving the intervention lipid was 10% as compared to 16% in the standard soybean oil lipid preparation group. This difference was not found to be statistically significant.

References:


