PROTOCOL

Effect of Medium Chain Triglyceride Intake on Colonization of Preterm Infants with *Candida*

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I. Purpose and Background

Systemic candidiasis has become the fourth leading cause of hospital acquired sepsis in the United States, making these infections as common as those caused by enteric Gram-negative bacilli.[1] Premature neonates are an immunocompromised population that has seen a concurrent increase in the incidence of these serious fungal infections. A report of neonatal late-onset sepsis in the NICHD neonatal network identified fungi as the cause of 12% of these infections, with *Candida* species being the 2nd most common etiologic agent overall (after coagulase-negative *Staphylococcus*).[2] Further, infections with *C. albicans* carried a mortality rate of 44%.[2] Thus, despite the dramatic advances in neonatal intensive care and the availability of antifungal drug therapies, these infections still carry an unacceptably high cost in terms of morbidity and mortality.

Antifungal prophylaxis has been shown to reduce invasive fungal infections in neonatal intensive care units; indeed, it has been suggested that all NICUs caring for extremely preterm infants should use antifungal prophylaxis.[3] However, this strategy is associated with risks of drug toxicity and emergence of resistance to antifungal drugs. Alternative strategies to reduce colonization and invasive disease are therefore warranted. Colonization with *C. albicans* generally precedes the development of invasive infection, and the gastrointestinal tract is a common site of colonization.[4] In an adult mouse model of colonization, dietary fatty acids impact the level of *C. albicans* colonization. Crucially, dietary medium chain triglyceride (MCT) oil decreases *C. albicans* colonization.[5] MCT oil has been an ingredient in infant formula since the 1950's. In a pilot study, we found that dietary supplementation of preterm infants with MCT oil for 7 days reduced colonization in infants who received it but not in control infants (Arsenault, et al., submitted). This is the first evidence that MCT oil can decrease colonization in preterm infants who are at risk for invasive disease with this organism.

This protocol describes an observational, follow-up study in which we will examine whether the suppression of *Candida* colonization with dietary MCT supplementation will be maintained over a longer time period of supplementation. Data collected in this study will be used to further inform a future interventional trial in which MCT oil can be formally tested for its ability to reduce *Candida* colonization in premature infants at risk for disseminated candidiasis.

Specific Aims: 1. To determine the frequency, timing, level, and dietary risk factors of *Candida* species colonization in the gastrointestinal tracts of preterm infants.
2. To examine whether dietary supplementation with MCT oil reduces gastrointestinal colonization by *Candida* species.

II. Characteristics of the Research Population

Any premature infant admitted to the Neonatal Intensive Care Unit (NICU) at Women and Infants Hospital will be eligible for this study. There will be no distinction based on gender or ethnicity. We anticipate that 800 different individuals will be adequate to provide the number of samples required for these experiments. Because we are interested in supplementing infants colonized with *Candida* with MCT oil, we will restrict the targeted group to those infants who are already receiving dietary supplements through either preterm or transitional formula or fortified breast milk. These infants are generally under 2000 grams birth weight.

Inclusion criteria: Any premature infant admitted to the NICU at Women & Infants Hospital who is on full enteral feeds of either preterm or transitional formula or fortified breast milk and who is anticipated to have a minimum stay of two weeks.

Exclusion criteria: Infants not on full enteral feeds with preterm formula or fortified breast milk or whose NICU stay is anticipated to be less than two weeks.

III. Methods and Procedures

1. Obtaining Specimens: Preterm infants admitted to the NICU will be screened for inclusion. Each medical record will be reviewed to confirm that the standard hospital consent form was signed on admission and that there were no exclusions indicated for the collection of stool for research purposes. A subject ID number will be assigned. Stool samples identified only by subject ID number will be collected and processed to determine whether they contain *Candida* species by standard laboratory methods in the Bliss research laboratory. Because colonization with *Candida* may develop over time, we will collect stool samples from eligible patients weekly throughout their hospital stay. Parent(s) of colonized infants will be approached for informed consent. Once consent is obtained, infants will be randomized into control and supplementation groups. After baseline sample collection, infants in the supplementation group will receive 0.5 ml/oz additional MCT oil (4 kcal/oz) as a nutritional supplement for 21 days or until hospital discharge. Sample collection from enrolled infants will include a stool sample and 0.5 ml of maternal breast milk obtained from a bottle provided to the patient's room. Samples will be collected three times during the study period: before supplementation is started, 1 week after supplementation is started, and at the conclusion of the supplementation period.. The following information will be extracted from the medical record of consented infants: gestational age, birth weight, day of life, gender, admitting diagnosis, prenatal infection screening labs, method of delivery, antibiotic use (duration and drug class), corticosteroid use, presence of central lines, amount and types of feeds (breast milk only, breast milk and formula, formula only; formula brand; type and amount of fortifiers and other nutritional supplements) and feeding modality (breast, bottle, NG, G-tube) will be recorded. If any infant subsequently develops an invasive fungal infection or oral thrush, that will also be noted. All information extraction will conform to HIPAA and institutional standards for protected health information.

2. Data Analysis: Stool samples from preterm infants admitted to the ICU will be processed by standard laboratory methods in the Bliss research laboratory to determine whether they contain *Candida* species. The presence and concentration of organisms will be recorded. Once infants are enrolled, stool samples and breast milk will be collected and stored for later testing at Tufts University. The presence and concentration of microbes in stool samples will be quantified, and the fatty acid content of breast milk samples will be analyzed. All identifiers will be removed from all samples collected for this study; they will be identified only by subject study ID number. No PHI will be provided to Tufts. Laboratory data generated from these studies will be maintained independent from any patient identifying information. Patient identifiers will be maintained in coded fashion secured in Dr. Bliss' office at Women & Infants Hospital. This office is in a building distinct from that of the laboratory.

Microbiological Methods: Stool samples will be weighed and suspended in 10 ml of sterile saline. Serial dilutions will be cultured on YPD agar media (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) containing 100 μg/mL streptomycin and 50 μg/mL ampicillin to inhibit bacterial growth and to allow quantification of the yeast content per gram of stool. Colonies will be evaluated by light microscopy to confirm that they are comprised of yeast. Yeast will be speciated using the FDA-approved Vitek-2 system.

3. Safety Assessments: Because MCT oil is already a component of infant formulas and breast milk, and because recommended dietary intakes will not be exceeded, there are no specific procedures, laboratory evaluations, or measures required to monitor infants receiving the supplement. Routine clinical care and assessments will be undertaken, with the option to discontinue the supplement in the unlikely event that there is any clinical suspicion of intolerance. In particular, enrolled patients receiving supplemental MCT oil will be monitored for the following signs/symptoms:

1. Feeding intolerance – defined as any clinical event related to feeding (gastric aspirates, emesis, abdominal distension) that results in a reduction or withholding of enteral feedings for a period of 24 hours or more.
2. Diarrhea
3. Necrotizing enterocolitis
4. Poor weight gain
5. Excessive weight gain

Adverse event

An adverse event is any untoward medical occurrence in a patient or trial subject administered a pharmaceutical product; the event does not necessarily have a causal relationship with the treatment or usage. Adverse events include the following:

- Abnormal test findings, as specified below.
- Clinically significant signs and symptoms.
- Changes in physical examination findings.
- Progression/worsening of underlying disease.

Abnormal test findings

An abnormal test finding, e.g., abnormal laboratory analysis results or vital signs, should be recorded as an adverse event in any of the following situations:

- The investigator considers the test result to be clinically significant.
- The test is associated with accompanying symptoms. Note, that the symptom, not the test result, should be recorded as an adverse event.
- The test result leads to a medical/surgical intervention including withdrawal of investigational product or discontinuation from the study. Repeat/confirmatory testing is not considered a medical intervention.

Serious adverse event (SAE)

An adverse event that meets one or more of the following criteria/outcomes is classified as serious:

- Results in death.
- Is life-threatening (i.e., at immediate risk of death).

• Requires prolongation of hospitalization.
• Results in persistent or significant disability/incapacity.
• Other medically important adverse event that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or may require medical or surgical intervention to prevent one of the outcomes listed above.

Adverse Event Grading Scale

The adjectives MILD, MODERATE, or SEVERE will be used to describe the maximum severity of the adverse event. For the purpose of consistency, these intensity grades are defined as follows:

- MILD: Does not interfere with patient's usual function.
- MODERATE: Interferes to some extent with patient's usual function.
- SEVERE: Interferes significantly with patient's usual function.

Note the distinction between the gravity (seriousness) and the intensity (severity) of an adverse event. **Severe** is a measure of intensity; thus, a **severe** reaction is not necessarily a **serious** reaction. For example, a headache may be severe in intensity, but would not be classified as serious unless it met one of the criteria for serious events listed above.

Causality assessment

For each adverse event, a causality assessment will be made to determine if there is a reasonable possibility that the investigational product caused the adverse event. The adverse event is assessed using the following classification and criteria:

Not related: This relationship suggests that there is no association between the investigational product and the reported event.

Unlikely: This relationship suggests that the temporal sequence of the clinical event, including a laboratory test abnormality, with investigational product administration makes a causal relationship improbable, and that other drugs or underlying diseases provide plausible explanations.

Possible: This relationship suggests that treatment with the investigational product caused or contributed to the adverse event, i.e., the event follows a reasonable temporal sequence from the time of drug administration or follows a known response pattern to the investigational product, but could also have been produced by other factors.

Probable: This relationship suggests that a reasonable temporal sequence of the event with investigational product administration exists and, based upon the known pharmacological action of the drug, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the investigator's clinical experience, the association of the event with the investigational product seems likely.

Definite: This relationship suggests that a definite causal relationship exists between investigational product administration and the adverse event, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event.

"**Not related**" and "**unlikely**" will be included in the category "**unrelated**" (i.e., not having a reasonable suspected causal relationship to the investigational product).

"Possible", "probable" and **"definite"** will be included in the category **"related"** (i.e., having a reasonable causal relationship to the investigational product).

IV. Risk/Benefit Assessment

This is a minimal risk study. Collection of stool samples presents no risk to the patient. The very small volume of breast milk that is required for research purposes will not impact that which is available to the baby, but in the event that there is any concern about breast milk supply, the samples will not be collected. Some babies will receive a supplement of MCT oil to their feedings. MCT oil is already a component of preterm formulas and is present to varying degrees in breast milk as well. It is commercially available and in routine use as a caloric supplement in premature infants. The amount of MCT oil that will be supplemented (4 kcal/oz) is within the range recommended and maintains the appropriate fat:total calorie ratio for infant feeding. Because colonization with *Candida* is a recognized risk factor for invasive candidiasis in premature infants, if MCT oil supplementation reduces colonization with this potential pathogen, the subjects could potentially benefit from participation.

Colonization of the gastrointestinal tract with *Candida* species is a very common occurrence in premature infants, occurring in 30-60% of infants in recent series. Determination of colonization status for *Candida* is not a part of routine clinical care. Although colonization has been defined as a risk factor in research settings, the vast majority of infants who are colonized with *Candida* species will not develop invasive disease. The infants who are found to have *Candida* in their stool therefore do not exceed a risk threshold that would warrant any specific treatment for this condition in current clinical practice.

V. Subject Identification, Recruitment, and Consent/Assent

Subjects will be identified by the investigators. The subject's hospital admission consent form will be reviewed to confirm that it has been signed and that the patient has not indicated any restrictions on the use of stool samples for research purposes.

There are no costs to the patient or financial incentives for the patient for participation in this study.

VI. References

1. Calderone, R.A., *Candida and Candidiasis*, 2002, ASM Press: Washington D.C.
2. Stoll, B.J., et al., *Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network.* Pediatrics, 2002. **110**(2 Pt 1): p. 285-91.
3. Kaufman, D.A., *"Getting to Zero": preventing invasive Candida infections and eliminating infection-related mortality and morbidity in extremely preterm infants.* Early Human Development, 2012. **88 Suppl 2**: p. S45-9.
4. Huang, Y.C., et al., *Association of fungal colonization and invasive disease in very low birth weight infants.* Pediatr Infect Dis J, 1998. **17**(9): p. 819-22.
5. Gunsalus, K.T., et al., *Manipulation of host diet to reduce gastrointestinal colonization by the opportunistic pathogen Candida albicans.* mSphere, 2016. **1**(1).