Protocol

Research Design. A randomized clinical trial will be conducted to determine the benefits of omitting aspiration of residual gastric contents (RGC) prior to feedings on nutritional outcomes and gastrointestinal integrity and functioning of premature, very low birth weight (VLBW) infants. VLBW infants will be randomly assigned to one of two groups. Group 1 will receive standard care; that is, physical parameters of feeding intolerance and NEC will be assessed and RGC will be aspirated prior to each feeding. In Group 2, physical parameters for feeding intolerance and NEC will be assessed but RGC will not be aspirated. To determine nutritional outcomes, the following factors will be evaluated: (1) weekly enteral intake for the first 6 weeks of life, (2) time to reach full enteral feedings, (3) hours of parenteral nutrition (PN), (4) diagnosis of parenteral nutrition associated liver disease (PNALD), (5) growth indices, (6) hours of central venous line (CVL) access, (7) episodes of late onset sepsis (LOS), (8) length of hospital stay. The presence of gastrointestinal bleeding and inflammation will be measured by (1) presence of blood in the stool, and (2) fecal calprotectin levels. Furthermore, plasma levels of gastrointestinal peptides including serum gastrin and motilin will be measured as indicators of gastrointestinal function. See Table 1.

Sample and Setting. The proposed study will follow a prospective cohort (N = 120) of racially and economically diverse VLBW infants for 6 weeks following birth. The infants will be born to mothers who are English or Spanish speaking and who are 18 years of age or older. VLBW infants will be sampled by convenience from the neonatal intensive care unit (NICU) at Shands Children's Hospital, a Level III tertiary care center. The hospital is part of the University of Florida and includes a 52 bed NICU. The hospital catchment area encompasses north central Florida and southern Georgia, a predominantly rural and semi-rural population. Approximately 100-110 VLBW infants are delivered there per year. In the preliminary study, 70% of mothers consented for the study. Inclusion criteria for the infants are as follows: (1) born at 32 weeks or less of gestational age, (2) birth weight equal to or less than 1,250 grams, and (3) infant receiving some enteral feedings by 72 hours of age and parenteral feedings by 24 hours of age. Exclusion criteria for the infants are as follows: (1) congenital or chromosomal abnormalities, (2) complex congenital heart diseases and congenital anatomic gastrointestinal abnormalities, (3) Infants will be withdrawn from the study if any of the following occur: grossly bloody stools, radiologic evidence of NEC, or other intestinal complications such as perforation.

Sample Size Determination / Power Analysis. In the preliminary study, 40 premature, VLBW infants had a mean enteral intake at 14 days of 72 mL/kg/d (SD=65). To detect a 50% improvement to 108, we would require 104 evaluable subjects in a non-sequential design for Primary 1.1 to achieve 80% power at P=0.05 (two-sided). To accommodate one interim analysis, we employed the minimax method of Shuster et al (2002), with first look after 66 evaluable subjects have been accrued. If $|Z|>2.28$ we shall stop for significance. If $|Z|<1.12$, the study will be reviewed for possible futility. There will be no stopping for futility. Otherwise, the study will accrue 120 evaluable subjects (54 more). No competing two stage design can achieve a lower worst case scenario average sample size than this one's 89 evaluable. Intent-to-treat will be followed as closely as possible. However, to accommodate dropouts including deaths (15%) and infants who develop NEC (7%) we expect to accrue 160 subjects.

Randomization. Within 72 hours of life and within 24 hour of initiating feeds of less than 20 mL/kg/d, informed consent will be obtained from the mothers, and infants will be randomly assigned to one of two groups by random length permuted blocks of sizes 4, 6, or 8 to maintain approximate balance assigned to each treatment group.

Standard Infant Feeding Protocol. As per standard NICU protocol, all infants will begin receiving PN within 24 hours of life. In addition, all infants will have an orogastric (OG) or nasogastric (NG) tube placed upon admission to the NICU and begin receiving enteral feeding of less than 20 ml/kg/d within 72 hours. Length of insertion of the OG/NG tube is determined by measuring from the tip of the nose to the tip of the ear lobe and then halfway between the xyphoid process and umbilicus (Ellett et al., 2011). Minimum insertion length based on the weight of the infant (Cordero et al., 2011). If the infant receives an X-ray upon admission, placement of the OG/NG tube is verified. Placement is also verified if additional X-rays are taken during the study as per usual OG/NG placement protocol in the NICU. Depending on the infant's gestational age, weight, and clinical status, feedings are initiated at up to 20mL/kg/d and advanced daily by no more than 20mL/kg/d toward a goal of 120-150mL/kg/d divided into 8 equal feedings per day using the established University of Florida Children's Hospital NICU feeding guidelines. Mom's own breast milk is encouraged, and donor breast milk provided if mothers own breast milk is unavailable. Prior to each feeding, the OG/NG tube is checked for proper placement by the bedside nurse verifying the insertion length with the initial measured insertion length. In addition, the nurse assesses the infant for any signs or symptoms of feeding intolerance or NEC (i.e., abdominal distension and/or tenderness, increased abdominal girth, visible bowel loops, presence of emesis, and visible blood in the stool). It is standard protocol to aspirate RGC prior to each feeding. However, for this study, this will only occur in infants randomized to Group 1. Kangaroo Care (holding of an infant dressed only in a diaper upon someone's bare chest) can positively affect growth and improve feeding tolerance (Conde-Aqudelo et al., 2011)) Kangaroo care is encouraged in this NICU and nursing protocols have been established to provide support to all mothers regarding provision of kangaroo care.

Research Procedure. The procedures used in this study will be the same as those successfully used in the preliminary study. Following consent by the mothers, infants will be randomized, and baseline demographic data will be collected from their medical charts. Over the first 6 weeks of life the following additional information will be collected at specified time periods from their medical charts: weekly enteral intake, days to full enteral feedings (120 mL/kg/d), hours of PN, evidence of PNALD (level of direct bilirubin, alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), growth indices (weekly weight, head circumference and length), medications, hours with a CVL, episodes of presumed or culture proven LOS (occurring > 7 days of life), days to discharge, evidence of stage 2 or greater NEC, evidence of aspiration pneumonia on chest radiograph, episodes of ventilator associated pneumonia and guaic status of stools. Additionally, one stool sample will be collected at 3 and 6 weeks to test for fecal calprotectin levels. 1mL of blood will be collected during a routine blood draw between 7 and 14 days of life to test for serum gastrin and motilin levels. If ventilated, tracheal aspirate samples will be collected on days 1, 3, 5,7,14, 21, 28 and 35 from routine tracheal aspirate suctioning. As kangaroo care is a potentially confounding factor, episodes and duration of kangaroo care between mother and infant will recorded on the Kangaroo Care log sheet located at the infant's bedside by either the mother or the nurse. See Table 1.

Aim 1. The primary aim of this study is to determine the risks and benefits of aspirating RGC and the clinical benefits of omitting aspiration of RGC prior to enteral feedings on the nutritional outcomes of premature VLBW infants. Both groups will have a sign placed on their isolettes stating which group they are assigned to and whether the nurse should or should not aspirate RGC. Although already documented on the infant's flow sheet, the sign will also include an area for the nurse to document the OG/NG insertion depth. Those personnel who are not involved with the study infants will be unable to be blinded since information regarding gastric residual volume and color is placed in the chart by the nursing staff and monitored by clinicians making decisions regarding enteral feeding plans. The PI is a neonatal nurse practitioner in the NICU

and will not be involved in the feeding plans for any subject in the study. The nurses at this research institution were willing to comply with the protocol in the past and have agreed to do so again. During the pilot study there were no known protocol deviations involving nursing staff. The only difference in care is that the infants in Group 1 will continue to have RGC aspirated prior to each feeding, and Group 2 will not have RGC aspirated. RGC are obtained by gentle aspiration of gastric contents from the indwelling OG/NG tube into a syringe. Decisions regarding advancement of feedings will be made according to the University of Florida Children's Hospital NICU feeding guidelines, including consideration of the physical assessment for signs and symptoms of feeding intolerance and NEC. Data to be collected for Aim 1 are found in Tables 1 and 2.

Aim 2. Aim 2 will determine the effect of routine RGC aspiration on gastrointestinal bleeding and inflammation. All stools will be tested for blood by the bedside nurse for the first 6 weeks using point of care guiac test kits, with the results recorded on the infant's flow sheet. Subsequently, this information will be collected and recorded by the project coordinator. Stool will be collected by the bedside nurse at 3 and 6 weeks of age and placed in a vial which is enclosed in a Ziploc® plastic bag and attached to the infant's isolette. Initial inflammatory changes have been seen in the intestinal tract of infants 4 days after an insult and the decision to test at 3 and 6 weeks was made to allow sufficient time for intestinal inflammation to occur from GRC aspiration (Saarinen et al., 2002). . The vial will be collected by the project coordinator who will take the sample to the co-investigator's (J. Neu, MD) existing research laboratory where the calprotectin level will be analyzed. This lab is currently successfully involved with and is proficient in fecal analysis of calprotectin levels. The results will be collected and recorded by the project coordinator.

Aim 3. Aim 3 will determine the effect of routine RGC aspiration on gastrointestinal function as evidenced by gastrointestinal peptide activity. Serum levels of gastrin and motilin will be measured on all infants between 14 and 21 days depending upon the timing of a routine blood draw. Since all GRCs are not discarded, waiting until between 14-21 days will allow adequate time for potentially one or more GRC to be discarded. 1 mL of blood will be taken during a routine blood test. All samples will be sent to the laboratory at Shands Children's Hospital at the University of Florida for testing, and lab values will be extracted and recorded by the project coordinator.

Statistical Analysis. Data will be entered into the RedCap system by the research assistant. For the primary hypothesis (P1.1), the total weekly enteral intake will be compared between the two randomized treatment groups via a two-sided Welch-corrected T-test. For sample sizes of this magnitude, the central limit theorem assures us that this is an assumption-free analysis, even if the underlying population variances differ. An interim analysis of P1.1 will also be done as described in the Power Analysis section below. Secondary hypotheses S1.1-S1.7 will be tested in the same manner as the primary hypothesis P1.1. For secondary Hypothesis S2.1 (S3.1), [S3.2], we shall compare the personal fractions of positive stools (gastrin levels) [motilin levels] respectively via two-sided Welch-corrected T-tests. For secondary hypothesis S2.2, we shall use a repeated measures analysis of variance to compare the groups with respect to week 3 and week 6 calprotectin levels. . Actual P-values will be reported, and each hypothesis will be accompanied by a point estimate and 95% confidence interval estimate for effect size. For descriptive purposes, a P-value of under 5% will be declared significant. The study is powered strictly around the primary hypothesis P1.1. Withdrawal rates will be compared between the treatments in a tertiary manner using survival analysis (time to withdrawal), via a logrank test.

Expected outcomes. Based on the preliminary study, it is reasonable to expect that infants who do not receive routine aspiration of RGC will have better nutritional outcomes, evidence of less gastrointestinal bleeding and inflammation, and improved gastrointestinal function.

Data Entry and Management. All data will be entered into a REDCAP database having integrated data quality and consistency checks (e.g., data-range) as part of the data procedure. Data quality will be monitored and assured: 1) as reported; and 2) as entered into the database. For the former, all hardcopy forms will be visually inspected before data entry. Furthermore, a manual comparison of randomly selected data hardcopy forms with data output listing generated from the study database will be performed, and consistency checks will be generated by SQL or SAS programs as part of routine data cleaning procedures. All subjects eligible for enrollment will be registered and entered into the study database designed by the project coordinator.

Table 1. Instruments

Variable	Measurement
Demographic data	Gestational age, birth weight, maternal history, prenatal and perinatal complications and medication, mode of delivery, Apgar scores, resuscitation at birth and neonatal acuity (SNAP II) score, medications and disease processes associated with decreased intestinal motility, episodes and duration of KC
Nutritional factors (1-8) below	
1. Enteral intake	Weekly 24-hour enteral feeding intake in mL/kg for first 6 weeks; Type of feeding received
2. Time to full feeds	First day infant received $\geq$120 mL/kg/d of enteral feedings
3. Hours of TPN	Number of hours infant received some PN for days 1-42
4. PNALD	Weekly or biweekly liver function tests (level of direct bilirubin, alkaline phosphatase AST and ALT) for first 6 weeks
5. Hours of central venous line access	Number of hours infant has a central venous line for days 1-42
6. Episodes of late onset sepsis	Episodes of culture proven or presumed sepsis (treated with 7-10 days of antibiotics but with negative cultures) during week 1-6 weeks of life
7. Growth indices	Weekly weight. length, and head circumference
8. Length of hospital stay	Days infant remains in hospital from birth until discharge
Episodes of NEC	Episodes of radiologic evidence of NEC during the first 6 weeks
Gastrointestinal bleeding and inflammation (1-2) below	
1. Presence of blood in stools	Positive or negative guiac of all stools for first 6 weeks
2. Fecal calprotectin levels	Level of fecal calprotectin at 3 and 6 weeks
Gastrointestinal function	Serum levels of gastrin and motilin at 14-21 days
Respiratory aspiration of gastric contents (1-3) below	
1. Pepsin level in tracheal aspirates	Pepsin level in the tracheal aspirates of ventilated infants on day ,7,14, 21, 28 and 35
2. Presence of aspiration pneumonia	Presence of aspiration pneumonia on all radiographs
3. Presence of ventilator associated pneumonia	Presence of positive tracheal aspirate on ventilated infants

Table 2: Timetable for Collection of Data

Data	Time Period Collected
Demographic data	Upon entry into study and during 6 week study period
24-hour enteral feeding intake in mL/kg	Weekly for 6 weeks
Time to full feedings	Recorded daily until 120mL/kg/d reached
Hours of PN	Daily for 42 days
Evidence of PNALD	Weekly or biweekly liver function tests (level of direct bilirubin, alkaline phosphatase AST and ALT)for first 6 weeks
Hours of central venous line access	Daily for 42 days
Episodes of late onset sepsis	All incidences of culture proven or presumed (treated for 7-10 days but with negative cultures) for weeks 1-6.
Growth indices	Weekly weights, head circumferences and lengths
Days to discharge	Days infant is hospitalized
Episodes of radiologic evidence of NEC	All incidences for 6 weeks
Results of stool guaic	Every stool for 6 weeks
Fecal calprotectin levels	3 and 6 weeks
Serum levels of gastrin and motilin	14-21 days
Pepsin level of respiratory aspirate	Day 1,3,5,7,14 21, 28 and 35 in ventilated infants
Evidence of aspiration pneumonia	All radiographs for 6 weeks
Respiratory cultures	All respiratory cultures done on ventilated infants for 6 weeks