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Study protocol and SAP

Study title: Regular intestinal lavage to promote enteral feeding and prevent necrotizing enterocolitis in extremely preterm infants. A randomized controlled trial.

Protocol summary

Optimizing enteral nutrition (EN) is challenging in extremely preterm infants due to feeding intolerance that relates to the functional gastrointestinal immaturity. Early feeding is a safe way to promote postnatal gastrointestinal maturation and, when compared with delayed enteral feeding, provide benefit, such as reduced time to full enteral feedings (TFF) and number of parenteral nutrition (PN) days. Failure to develop oral feeding competence often leads to growth failure, longer hospital stays, dependence on PN and its complications, and influences long-term growth and developmental outcomes. Feeding with human breast milk has a protective effect against necrotizing enterocolitis (NEC) compared with formula, whereas feeding intolerance is one of the early signs of NEC. Delayed passage of meconium is a risk factor for feeding intolerance in preterm VLBW neonates and specific meconium microbiota characteristics have been linked to increased risk of NEC. This randomized controlled trial (RCT) aims at evaluating the effect of regular intestinal lavage using normal saline on the TFF and severe complications such as NEC and sepsis, in extremely preterm infants. We aim also to follow children´s neurological development until 5,5 years of age. The study will include one intervention group of 100 subjects that will receive regular rectal washout with normal saline and equal number of control subjects, treated according to current routine. The trial is preliminarily estimated to last between year 2018 and 2022. Investigators will monitor closely for possible adverse events. The results are going to be published in reviewed medical journal.

Background

Optimizing EN is challenging in preterm very low birth weight (VLBW) neonates, due to feeding intolerance that relates to the functional immaturity of their gastrointestinal (1,2), neurologic, cardio-respiratory, and oral-motor systems (1). The introduction of enteral feeds for extremely preterm or VLBW infants is often delayed for several days or longer after birth due to concern that early introduction may not be tolerated and may increase the risk of NEC, a devastating intestinal disease. However, a recent Cochrane meta-analysis indicated that delayed introduction of progressive enteral feeds do not reduce the risk of developing NEC in very preterm or VLBW infants (3). Moreover, strong evidence suggests that early feedings are a safe way to promote postnatal gastrointestinal maturation, and when compared with delayed enteral feeding, provide benefit to this vulnerable population, such as reduced TFF and number of parenteral nutrition (PN) days (4).

Failure to develop oral feeding competence often leads to poor nutritional status, growth failure, longer hospital stays, increased costs of care, and influences long-term growth and neurodevelopmental outcomes (2). Feed intolerance is associated with delayed maturation of gut function as well as prolonged TFF and dependence on PN (1,5,6). PN provides life-saving artificial nutrition and adequate growth in infants with insufficient intestinal function due to prematurity and/or major abdominal gastrointestinal surgical procedures but is also linked to severe complications. More specifically, PN is associated with increased rates of bacterial and fungal sepsis due to the requirement for central line for infusion, and intestinal bacterial overgrowth caused by enteral starvation and immature immune function (1,7,8). Moreover, the use of PN increases the risk of mechanical complications related to venous line placement as well as miscalculations and errors in PN manufacture, supply and administration (7). Intestinal failure-associated liver disease (IFALD) is a potentially life-threatening problem in patients receiving long-term PN, and preventive strategies include early enteral feeding, weaning of PN, reduced dose lipid emulsions and early recognition and treatment of sepsis (5–8). Inadequate breastfeeding and severe weight loss are known risk factors for

unconjugated hyperbilirubinemia in term and late preterm babies; however, there is paucity of data about the effect of enteral feeding on the risk of jaundice in extremely preterm infants (9).

NEC is a multifactorial disease primarily affecting extremely preterm and VLBW infants, leading to profound inflammation and intestinal injury with severe morbidity and mortality if not identified and treated early (10). Clinical signs and symptoms are nonspecific and include temperature instability, bradycardia and apnoea, hypotension, abdominal wall erythema, increased pregavage residuals, abdominal distention, emesis, blood in the stool, absent bowel sounds, abdominal tenderness, and occasionally a right lower quadrant mass. Feeding intolerance is one of the early signs of NEC and with timely identification the disease can be treated conservatively with bowel rest and antibiotics. If untreated, NEC progresses rapidly to more advanced stages that require surgical intervention (11). As mentioned above, early feeding seems to be beneficial for extremely preterm and VLBW infants and is not associated with increased risk for NEC (4). Besides, there is good evidence that feeding with human breast milk has a protective effect against NEC, whereas formula increases the risk of this gastrointestinal condition (10). Meconium is not sterile and higher detection rates of potentially pathogenic bacteria in faeces of NEC infants suggest that a range of potentially pathogenic bacteria may collectively contribute to NEC pathogenesis (12). Considering also that serial rectal irrigation performed before surgery in children with Hirschsprung disease prevents enterocolitis (13), one could hypothesize that early meconium evacuation in extremely preterm born infants, which have immature intestinal motility, could prevent NEC, by counteracting both mechanical obstruction and overgrowth of potentially harmful pathogens, and by facilitating the flow of gastrointestinal content.

Delayed passage of meconium is identified as one of the risk factors for feeding intolerance in preterm VLBW neonates (1,14,15). The use of medications such as prokinetics (e.g. erythromycin, cisapride) to improve gut motility has failed to show significant benefits in VLBW neonates and is associated with adverse events (1). Nevertheless, several studies have evaluated the effect of induced meconium evacuation on TFF and feeding intolerance in neonates, with controversial results (1,16–25). In an observational study in 2007, Shim et al. reported that routine use of glycerine enema in infants resulted in full enteral feeds earlier than in the control group (median 16.0 vs 22.9 days; P

value <0.001) with a hazard ratio of 2.9 (95% confidence interval 1.8 to 4.8). This difference was greater for infants with birth weight (BW) < 1000 g (median 17.3 vs 28.1 days; P value < 0.001). Investigators also reported that the rate of sepsis was lower for VLBW infants in the glycerine enema group than for those in the control group (7.7% vs 27.8%; P value = 0.02). Thereafter, several RCTs have been conducted to further examine these findings (16,18–23), and have been subsequently summarized in systematic reviews and meta-analyses (1,24–27).

Ibrahim et al. conducted an open-label, pilot, randomized controlled trial (RCT) including infants with BW ≤1,500 g comparing early aggressive meconium evacuation with twice-daily normal saline rectal washout to conventional management with glycerine suppositories, until full enteral feeds were reached. The study resulted in shorter TFF in children with birth weight 750-999 g receiving rectal washout as compared to those receiving glycerine suppositories, but no difference was noted in children with birth weight 1000-1500 g (16). However, an open-label RCT study with regular administration of glycerine suppositories to babies born at gestational age (GA) between 24 and 32 weeks for 10 days did not significantly reduce the TFF. There was no impact on secondary outcomes including sepsis, NEC, feed tolerance, duration of oxygen requirement, growth or age at discharge. Passage of first meconium was earlier in intervention group babies than controls, but the clinical significance was unclear (19). Similarly, a controlled multicentre RCT by Mena et al. that evaluated the use of normal saline enemas with glycerol versus simulation in infants with BW 500 - 1250 g did not find significant differences regarding the TFF and secondary outcomes including the number of episodes of late sepsis, hyperbilirubinemia, NEC, and intraventricular haemorrhage (IVH) (21). Two other RCTs, one that evaluated small volume saline enemas versus no intervention in infants with BW ≤1500 g and GA ≤32 weeks to accelerate meconium evacuation in VLBW infants (20), and another that compared the efficacy of glycerine suppository versus no intervention in preterm VLBW (BW 1000 - 1500 g) and preterm (GA 28 - 32 weeks) neonates to improve feeding tolerance (18), found no evidence of efficacy. Moreover, rectal stimulation and/or small volume saline enemas did not accelerate the normalization of stooling pattern in infants with a gestational age ≤28 weeks (22). Finally, another RCT found that the administration of both glycerine enema and oral probiotics

(Golden diplococci) can significantly improve feeding tolerance and shorten the TFF (23). Nevertheless, the last two studies were subject to the limitation of combining two different interventions in the same group, thus making it difficult to distinguish their effect (22).

A Cochrane meta-analysis by Anabrees et al. showed that prophylactic administration of glycerine laxatives did not reduce the TFF and did not influence secondary outcomes, including duration of hospital stay, mortality, patent ductus arteriosus (PDA), IVH, NEC, retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD) and weight at hospital discharge. Prophylactic administration of glycerine laxatives resulted in improved stool passage over the first 48 hours of life (24). Similarly, another meta-analysis including the same RCTs concluded that the evidence for the use glycerine enemas or suppositories in premature infants is inconclusive (25). Less strict inclusion criteria were applied in a meta-analysis conducted by Deshmukh et al, suggesting that the use of glycerine suppositories, small-volume enema with glycerine or normal saline, or the administration of an oral osmotic agent, such as gastrografin, to evacuate the meconium did not reduce the TFF in preterm VLBW neonates. The authors proposed that the reason for this might be that these interventions do not influence the right colon or the small bowel. Additional limitations included the small sample size, inherent bias due to a lack of blinding of the intervention in some studies, and lack of a standard definition for full enteral feeding (1). Of note, other investigators conducted a systematic review of the available studies, choosing not to proceed to meta-analysis, pointing out that only two studies examined the same primary outcome. Furthermore, the authors underlined that all the interventions were carried out in a different way. The studies that examined the effect of enemas displayed slight differences regarding the use of enema volumes, the administration of enemas and the time between these administrations. Besides, the included studies were carried out all over the world, in different hospitals and all the hospitals had different policies on enteral feeding, use of TPN and infusion, which might have influenced the outcome of the studies and made it hard to draw an unambiguous conclusion (26).

The study by Haiden et al. is the one with the lowest GA and BW studied so far, namely 24 weeks and 491g, respectively (20). In none of the available studies the infants were fed exclusively with

breast milk. Furthermore, no study started enteral feeding soon after birth (within the first two hours of life) (1). To our knowledge, there are two ongoing RCTs evaluating TFF with induction of meconium evacuation: one including infants with GA 24 – 32 weeks and BW 500 – 1,500 g, comparing glycerine suppository vs sham suppository (registry number NCT02153606) and another including infants with BW <1,250 g, comparing glycerine suppository vs no intervention (registry number NCT01799629). One more study intending to compare three groups (glycerine suppository, rectal stimulation, and sham placebo) among preterm infants with BW ≤ 1500 g was also identified (registry number NCT02149407), but its recruitment status is unknown (28).

The Neonatal Intensive Care Unit (NICU) of University Children's Hospital in Uppsala is a tertiary unit caring for extremely preterm infants born as early as GA 22+0. The incidence of NEC, IVH, and PDA in infants born in GA 22+0 and 26+6 is approximately 15%, 20%, and 60%, respectively, and almost all infants are diagnosed with various degrees of BPD and ROP. All extremely preterm infants are fed exclusively with breast milk (mother's and/or donated), the first feeding given within one to two hours from birth. We strive to reach full enteral feeding within about 7 and 10 days in infants born in GA ≤ 26 weeks and 5 days in infants born GA 26-28 weeks, respectively.

Study hypothesis

Regular intestinal lavage in preterm infants of GA between 22+0 and 26+6 weeks facilitates faeces evacuation, shortens the TFF and time to regaining of birth weight, thereby decreasing the risk of sepsis (through shorter intravascular access) and NEC.

Study design

Interventional study design

The study will be designed and conducted as a two-arm parallel, open-label, randomized controlled trial (RCT) at a tertiary-care hospital.

Masking

No masking will be applied, in order not to expose the control group to sham interventions of no benefit or potential harm.

Allocation

A blinded research coordinator will randomly assign eligible infants using a computer software-based randomization system (default) or sealed, numbered, and opaque envelopes (back-up), with a 1:1 allocation ratio, as soon as possible and not later than 24 hours from birth.

Sample size calculation and estimated duration of the study

Sample size calculation is based on the incidence of NEC, as the rarest of the events that will be studied, which is currently about 15% among extremely preterm infants at our unit, as mentioned above. For study power of at least 70%, confidence level of 5%, and an expected decrease of NEC incidence from 15% to 8%, each study arm should include about 100 participants (29). Each year, our unit treats about 70 infants born in GA 22+0 – 26+6. Supposing a participation rate of 70%, it is estimated that the recruitment period should last between 3 and 4 years, preliminarily starting during 2018. However, a recruitment period of maximum 5 years will be allowed.

Eligibility criteria

All infants born extremely preterm in GA between 22+0 and 26+6 weeks at the NICU of University Children's Hospital in Uppsala will be consecutively recruited in the study during the years 2018 – 2022. Written informed consent will be obtained from both parents before enrollment in the study.

Exclusion criteria

Infants with major dysmorphic features and major congenital anomalies, such as gastrointestinal disorders, as well as infants with circulatory instability during the first hours of life will be excluded from the study.

Feeding regimen and feeding intolerance guidelines (common for both groups)

Feeding regimen:

All infants born between GA 22+0 and 26+6 are fed exclusively with breast milk (mother's and/or donated), the first feeding given as soon as possible after birth (usually within one to two hours) and then 2-hourly. At day 1, the following scheme is applied:

- GA 26 – 28 v: total volume 80-90 ml/kg/day; enteral nutrition 2ml/kg every two hours, and the remaining is administered as PN through a central venous access.
- GA <26 v: total volume 90-100 ml/kg/day; enteral nutrition 1ml/kg every two hours, and the remaining is administered as PN through a central venous access.

The total feeding volume is advanced progressively on an individual basis, usually by 10-20ml/kg/day, to a final volume between 150 and 200ml/kg/day, striving not to exceed a weight loss of 10%, and depending on feeding tolerance. Full enteral nutrition is pursued within 5 days for GA 26 – 28 weeks and within 7-10 days for GA ≤26 weeks. PN is discontinued when enteral feeding reaches about 150 - 200 ml/kg/day, considering the child´s growth development. Protein fortification is usually initiated when enteral nutrition constitutes more than 75-100 ml/kg/d (~50%) of total fluid volume.

Feeding intolerance:

In case of feeding intolerance, the feeding strategy is modified accordingly, depending on the nature and severity of symptoms.

Specific symptoms that should lead to continued treatment:

- In case of occasional vomiting and / or biliary retention without any other symptoms, the position of the nasogastric catheter is checked, a clinical assessment is performed and EN is eventually withheld or reduced. One must specifically consider the potential onset of infection or gastrointestinal disease such as NEC, intestinal obstruction etc. Depending on the course of symptoms, the same or reduced food volume is continued.
- In case of more severe symptoms, such as cardio-respiratory instability, impaired abdominal status (e.g. inflated abdomen, abdominal pain) in combination with repeated vomiting, bilious

residuals or lack of defecation, EN is withheld, paediatric surgeon is consulted and radiological and laboratory investigation including early treatment with antibiotics (tazobactam / piperacililin + gentamicin) is considered. If NEC and / or sepsis diagnosis is not established / suspected and the child continues to show signs of food intolerance, fasting should continue for at least 24 hours and the clinical status carefully monitored with repeated laboratory / radiological assessment. EN is resumed when symptoms have passed / are ameliorated and most importantly infection / NEC has been ruled out.

Intervention

The following intervention will be applied to the intervention group: specially trained pediatric surgeon will administer 10ml/kg pre-warmed (37^{o}C) normal saline via a single-use rectal tube of size 6FR twice per day, aiming at a depth of maximum 10 cm/kg, starting after randomization and not later than 24 hours of age, and continued until full enteral nutrition of 170ml/kg/day is achieved or NEC diagnosis (Bell stage II or more) is established, which one comes first. However, the intervention will be applied at a maximum of 2 weeks from birth. Besides, the intervention will be withheld in case of suspicion of infection or NEC but will be resumed if antibiotics are not introduced and discontinuation of feeds does not exceed 48 hours. Early stopping criteria include infection / sepsis or NEC suspicion necessitating prolonged discontinuation of feeds > 48 hours and / or antibiotics, circulatory instability or adverse events. The intervention will only be applied at the NICU of University Children's Hospital in Uppsala and will be discontinued if the infant is transferred to another hospital. Standard departmental guidelines will be followed regarding ventilation, choice of PN, invasive monitoring, and management of sepsis and/or necrotizing enterocolitis (NEC) for both the intervention and the control groups.

Comparison group

The following guidelines are currently applied at our unit for extremely preterm infants that do not defecate adequately:

If the child has not passed meconium or has already passed meconium / feces but has not defecated for 3-4 days:

- If there are no symptoms: continued EN, follow-up of clinical status and defecation pattern. If the child has not defecated for another 1-2 days (and remains asymptomatic), consider the following treatment strategy to facilitate defecation:
    1. Tactile perineal stimulation is performed by a nurse, using a room temperature damp compress
    2. Cautious rectal stimulation is performed by a nurse, using a rectal catheter size 6FR; depth 1 - 2 cm
    3. Step 1 & 2 is repeated depending on effect and defecation pattern
    4. If Step 1 & 2 does not result in defecation and the clinical status is unchanged, enema with 4mL / kg sodium chloride 9mg / mL is given
    5. In case of no effect, a pediatric surgeon is consulted, and consideration is given to continued treatment; (1) watchful waiting alternatively repeated enema (2) rectal washout with 10 mL / kg sodium chloride 9mg / mL (3) further radiological and laboratory investigations
- In case of symptoms (cardiorespiratory instability, impaired abdominal status, vomiting, bilious residuals, etc.): EN is withheld, pediatric surgeon is consulted and radiological / laboratory investigations as well as early treatment with antibiotics (tazobactam / piperacililine + gentamicin) is considered.

Glycerin suppositories / enemas are not used at our unit.

Primary outcomes

- TFF, defined as the period between birth and achievement of enteral nutrition of 170ml/kg/d.
- NEC, Bell stage II or more

Secondary outcomes

Time to regaining of birth weight, growth, BPD, IVH, PDA, ROP, small intestinal perforation, sepsis, hyperbilirubinemia, mortality, adverse events, age at the time of discharge from hospital, number of days on PN, IFALD, and neurological development.

Follow-up

All extremely preterm infants hospitalized in our unit are discharged home at about GA 35+0. Infants regionally belonging to another hospital are usually transferred to the "home hospital unit" not earlier than GA 28+0. After hospital discharge, all extremely preterm infants are routinely followed-up according to a nationwide schedule, until the age of 5,5 years. Data mostly concerning children's growth as well as neurological and psychomotor development are collected, and interventions are made as needed.

Study participants will be followed-up until full term corrected age (GA 40 +0), and medical information including possible treatments as well as diagnosis of NEC or other gastrointestinal complication, sepsis, PDA, BPD, ROP, IVH, hyperbilirubinemia, growth parameters (weight, length, head circumference) and mortality will be collected by the investigators from the treating physicians and electronic medical records. All the clinical, laboratory and radiological findings of infants treated at our unit are routinely and continuously registered in detail, with the use of medical computer software, during the whole hospitalization period, and these data will be readily accessible to the investigators. Besides, data on children's growth and neurological development will be collected from the electronic medical records, until the age of 5,5 years. The above-mentioned data of study participants that have been transferred to the home or other hospital unit will be collected by the investigators though communication with the treating physician at the respective hospital.

Documentation

All data relevant to the study collected by the investigators will be registered and de-identified in electronic database with password-protected access allowed only to the investigators.

Refuse to enrollment and subject dropouts

Refusals to enroll the study and dropouts of the study and the respective reasons will be documented and reported in the results. Effort will be made to replace each of these subjects with another infant of the same gestational age.

Completion of the study

The study will be completed when the number of 100 subjects per study arm is reached (a maximum of 130 subjects per study arm will be allowed), estimated to be achieved by the year 2022 (and not later than year 2023).

Statistical analysis

Numerical data with a gaussian distribution will be expressed as means (standard deviation), otherwise as medians (ranges). We will use the Anderson-Darling test to assess the normality of the data. Numerical values will be compared between intervention and control groups using t-tests or the Mann-Whitney U test. Categorical variables will be compared using χ^2 or Fisher's exact tests. Times to achieve full enteral feeding will be compared by the log rank test. Multiple Cox regression analysis will be used to adjust for covariates, such as gestational age and birth weight. IBM SPPS software (current version at the time of publication) will be used for all calculations and a p-value of <0,05 will be considered significant.

Interim analysis will be conducted when 35 infants have been recruited in each study arm (estimated about 1,5 years from study start). Another interim analysis will be conducted when 65 infants have been recruited in each study arm (estimated about 3 years from study start). Unplanned interim analysis will also be conducted in case of subjectively estimated too obvious difference between the groups regarding the primary outcomes, at any time during the study. The study will be discontinued as soon as an interim analysis shows statistically significant results regarding the primary outcomes or severe adverse events.

Ethical considerations

The study protocol will be submitted for approval to the Regional Ethical Review Board in Uppsala and subsequently registered at the international registry of clinical trials, ClinicalTrials.gov. The parents of the infants will be informed about the study by specially trained personnel, both verbally and in writing, before the child is born or, whenever that is not possible, as soon as possible after birth. Upon agreement to participate, written informed consent will be obtained from the parents, before enrollment in the study. Additionally, the parents will be clearly informed about the possibility to leave the study any time they wish.

None of the studies published so far reported any adverse events by induced meconium evacuation, with the exception of a higher proportion of NEC (although not statistically significant), vomiting, nausea and bradycardia with the use of oral gastrografin (1,22), and a non-significant trend towards increased risk of NEC with the use of glycerine enemas or suppositories compared with no treatment (25). However, in the RCT study described herein, both health personnel involved in the participating infants' daily care and the investigators are going to closely monitor for possible adverse events. Possible adverse events could be: rectal bleeding (including occult rectal bleeding-induced anemia), fluid loss due to overstimulation of immature intestines of preterm infants or the opposite effect, namely intestinal absorption of given saline, leading to hypervolemia and electrolyte disturbances, mainly hyponatremia. A safety monitoring committee will monitor adverse events with predefined stopping rules. Early stopping criteria will be applied in case of medical instability, as mentioned above. Furthermore, the whole study will be discontinued in case of too obvious positive effect, making it unethical to deprive the control group of the intervention, or in case of severe adverse events.

The inclusion of study participants in other interventional studies could intervene with the outcomes intended to be investigated in the study herein, rendering it difficult to retrieve definitive conclusions. Thus, study participants will not be included in other interventional studies at the same time.

The intervention will be discontinued if the infant is transferred to another hospital, as mentioned before. The decision to transfer a study subject to home or other hospital unit will only be based on current criteria and will not be affected by the participation in the study. Study participation is not expected to delay the transfer to home hospital and prolong the stay at our NICU in Uppsala, unless a severe adverse event should occur.

Reporting and dissemination

Following the study, data will be analysed by the research group and the results will be published in reviewed medical journals and possibly presented in a neonatology conference, hopefully during year 2023. It is difficult to provide a precise time schedule. Patient data collection is estimated to last about 4 years. Data analysis thereafter is time consuming and may last one more year until the whole project is completed and ready for publication. All the principal investigators will have free access to raw data and the right to publication.

Further specifications:

Sepsis definition: positive blood, urine and/or CSF culture, clinical deterioration and laboratory inflammatory response*.

A blood culture indicating growth of coagulase-negative staphylococci (CoNS) will be considered as false positive if following criteria are not fulfilled:

1. clinical symptoms
2. two separate blood cultures with the same antibiotic resistance pattern and/or central venous/arterial line prior to onset.
3. laboratory results indicating infection*

Culture-proven sepsis will be further classified as early (<72 hours postpartum) or late (≥72 hours postpartum) onset and if caused by CoNS or not.

* Laboratory inflammatory response (one of the following is needed):

1. LPK < 5 or > 20 (x10^9 cells/L)
2. TPK < 100 (x10^9 cells/L)
3. CRP > 15 mg/L

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Appendix

- Parental informed consent (in Swedish)

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