STUDY PROTOCOL

A randomized multicenter open-label controlled trial to show that mucous fistula refeeding reduces the time from enterostomy closure to full enteral feeds
(**MUC**ous **FI**stula **RE**feeding ("**MUC-FIRE**") trial)

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LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine transaminase
AST	aspartate transaminase
CVL	central venous line
DMC	Data Monitoring Committee
eCRF	electronic case report form
FIP	focal intestinal perforation
FTT	failure to thrive
FU	follow-up
GGT	gamma-Glutamyltransferase
ZKS	Zentrum für klinische Studien der MHH (Center for clinical trials at MHH)
ICD	International Statistical Classification of Diseases and related Health Problems
ICH-GCP	International Conference on Harmonisation – Good Clinical Practice
IMC	intermediate care ward
IVH	intraventricular hemorrhage
ITT	intention to treat
IVH	intraventricular hemorrhage
MFR	mucous fistula refeeding
MHH	Medizinische Hochschule Hannover (Hannover Medical School)
NEC	necrotizing enterocolitis
NEK	Nekrotisierende Enterokolitis
NG	nasogastric
NICU	neonatal intensive-care units
OR	operation room
PC	phosphatidylcholine
PNALD	parenteral nutrition-associated liver disease
POD	postoperative day
SAE	serious adverse event
SBBO	small bowel bacterial overgrowth
SOP	standard operating procedure
TPN	total parenteral nutrition
WHO	World Health Organisation

STUDY SYNOPSIS

Title of Study	A randomized multicenter open-label controlled trial to show that mucous fistula refeeding reduces the time from enterostomy closure to full enteral feeds (**MUC**ous **FI**stula **RE**feeding ("**MUC-FIRE**") trial)
Short Term	MUC-FIRE
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Study Design	Randomized, multicenter, open-label, controlled, parallel group research study
Patient Population	Infants who underwent creation of an enterostomy receiving postoperative care and awaiting enterostomy closure
Participating Study Sites	Total: n = 17 Germany: n = 13 Austria: n = 2 Netherlands: n = 2
Sample Size	To be assessed for eligibility: n = 230 To be assigned to the study: n = 120 To be analysed: n = 120
Objectives	The primary objective of this study is to demonstrate that mucous fistula refeeding between enterostomy creation and enterostomy closure reduces the time to full enteral feeds after enterostomy closure compared to standard of care.

Endpoints	<u>Primary efficacy endpoint:</u> Time to full feeds (hours), defined as time to actual enteral intake of the age-dependent caloric requirements per day (defined as 90 or 120 kcal/kg/24h) for at least 24 hours and a concomitant reduction of parenteral fluids to < 20 ml/kg/24h [32, 33]. The decision about the full feed kcal goal is made before randomization by the treating physician, depending on the birth weight of the infants and mother's gestation week at birth: 1) The nutrition aim is 120 kcal/kg/24h for premature infants with a birth weight < 1000 g or premature infants with a birth weight ≥ 1000 g and mother's gestation week at birth before 37+0. 2) The nutrition aim is 90 kcal/kg/24h for born mature infants, e.g. mother's gestation week at birth at least 37+0. <u>Key secondary endpoints:</u> 1) Reoperation 2) Time to first bowel movement after enterostomy closure (mucous stool is considered a bowel movement) Cleaning and changing of infants diapers will be performed according to a fixed schedule in order to uniformly document the time to first bowel movement following enterostomy closure. 3) Postoperative weight gain (g/d) (daily documentations recommended, minimum 2x per week), regular Z-Score (standard deviation score) documentation [WHO - weight-for-age] (daily documentations recommended, minimum 2x per week). This will be carried out according to a fixed schedule during morning rounds prior to feeding in an unclothed status. 4) Days of postoperative total parenteral nutrition (> 20 ml/kg/24h) before and after the 2nd operation (=ostomy takedown). Days of total parenteral nutrition (TPN) are counted, starting on the day of enterostomy closure and ending on the day of full enteral nutrition. The parenteral nutrition is manufactured by the hospital pharmacy on a daily basis, while considering the simultaneous enteral caloric intake. 5) Laboratory parameters indicating cholestasis (conjugated bilirubin, GGT, ALT, AST, hemoglobin) and sodium resorption (sodium in urine). Time points for harvesting of blood samples: Baseline at the time of randomization, then every 2 weeks until enterostomy takedown and at the 3-months follow-up in cases of pathological clinical signs (jaundice, acholic stools) 6) Weight gain during the subsequent 5 days after reaching the primary endpoint

	7) Central venous line (CVL) duration (days) and number of CVL infections (definition of infection: Neo-Kiss Guidelines) 8) Length of hospital stay (days) 9) Estimated ratio of the diameter of the two bowel loops which are anastomosed. 10) Time to full age-dependent volume intake per day (defined as 150 ml/kg/24h for premature infants and 120 ml/kg/24h for mature born infants as well as corrected mature infants) (in hours) [32,33] a) The volume aim is 150 ml/kg/24h for premature infants with a birth weight < 1000g or premature infants with a birth weight ≥ 1000g and mother's gestation week at birth before 37+0. b) The volume aim is 120 ml/kg/24h for born mature infants, e.g. mother's gestation week at birth at least 37+0. 11) <u>Assessment of safety:</u> Assessment of possible (serious) adverse events (AEs/SAEs) after randomization (e.g. death, sepsis, bowel perforation, stoma prolapse, abscess)
Inclusion and Exclusion Criteria	<u>Key inclusion criteria:</u> • Infants < 366 days • Ileostomy / Jejunostomy, • double loop enterostomies and split enterostomies (with mucous fistula) • Signed written informed consent obtained by parents/legal guardians and willingness of parents/legal guardians to comply with treatment and follow-up procedures of their child Notice: All patients with meconium ileus are included. If later diagnostics verify cystic fibrosis, diagnostics and diagnoses need to be documented in the eCRF and in further analysis subgroups will be established. <u>Key exclusion criteria:</u> • resection of ileocecal valve, • colostomy, • small bowel atresia, • multiple ostomies (more than just an enterostomy and a mucous fistula), • chromosomal abnormalities (if known at the time of randomization),

	• Hirschsprung's disease, • participation in another drug-intervention study • Intestinal perforation due to congenital heart defects with impairment of hemodynamics Reoperation (e.g. relaparotomy) prior to randomization is not an exclusion criterion, these patients may still be included in the study. Short bowel syndrome is not a criterion for exclusion.
Intervention	All patients will receive standard care with standardized enterostomy creation and closure and will be treated according to a standardized feeding protocol. Experimental intervention: Perioperative mucous fistula refeeding between enterostomy creation and enterostomy closure Control intervention: No perioperative mucous fistula refeeding between enterostomy creation and enterostomy closure Duration of intervention per patient of the intervention group: minimum 21 days/3 weeks until patient's weight > 2000 g (averaged 6 weeks between enterostomy creation and enterostomy closure). Follow-up per patient: 3 months and 6 months postoperatively, following enterostomy closure (12-month follow-up only applicable for patients that are recruited early enough to complete this follow-up within the 48 month of overall study duration).
Study Duration	Recruitment: approx. 60 months (258 weeks) Study duration per patient: Maximum 58 weeks to minimum 32 weeks Duration of the entire study (first patient in to last patient out): 72 months (310 weeks)
Statistical Analysis	Efficacy: The type-one error rate is set to 5% (two-sided). Description of the primary efficacy: The primary analysis is performed on the intention to treat population (ITT). The aim of this study is to demonstrate superiority of perioperative mucous fistula refeeding compared to no mucous fistula refeeding in reducing the time to full enteral feeds after enterostomy closure. The treatment effect will be estimated with a Cox-regression adjusted for treatment, weight and mother's

	gestation week at birth (< 1000 g and before 37+0 / ≥ 1000 g and before 37+0 / ≥ 1000 g and at least 37+0), study center as well as height of the stoma (jejunostomy/proximal Ileostomy or terminal ileostomy) and will be assessed by the estimated hazard ratio (refeeding vs no refeeding) for reaching full enteral feeds. Superiority of the refeeding procedure will be concluded if the lower bound of the corresponding two-sided 95%-confidence interval for the treatment effect hazard ratio is greater than 1. Safety: (Serious) adverse events (AEs/SAEs) will be compared between treatment groups with a chi-square test and other appropriate tests. P-values will be assessed descriptively. Secondary endpoints: All secondary analyses will be explorative.

RESPONSIBILITIES

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1 SCIENTIFIC BACKGROUND AND STUDY RATIONALE

Enterostomies in infants may be created for different reasons. During the presence of an enterostomy, the regular stool transfer is interrupted since the distal part of the bowel (the part following the enterostomy) does not participate in the processing of stool. Therefore, it does not contribute to the resorption of enteral nutrients. As a consequence, these infants need additional parenteral nutrition. Due to the negative side-effects of parenteral nutrition all patients should return to enteral nutrition as soon as possible. Consequently, many pediatric surgical centers worldwide routinely perform mucous fistula refeeding (MFR) into the former unused bowel after enterostomy creation because case reports and retrospective analyses show low complication rates and faster postoperative weight gain. Several providers, however, shy away from this approach because to date there is still no high-quality evidence for the benefit of this treatment. The aim of this study is to assess the effects of mucous fistula refeeding in a prospective randomized trial. We hypothesize that MFR between enterostomy creation and enterostomy closure reduces the time to full enteral feeds after enterostomy closure compared to standard care. Moreover, the side effects of parenteral nutrition may be reduced and the postoperative hospital care of infants undergoing ostomy closure shortened.

1.1 The Medical Problem

After creation of any enterostomy the bowel distal to the enterostomy is not in use. Therefore, the physiologic passage of stool, nutrient uptake and growth of the bowel distal of the enterostomy are interrupted. At the time of enterostomy takedown, the surgeon often sees an enormous discrepancy in diameters of the proximal and the distal loops of the bowel. In these cases, the postoperative increase of enteral feeds and the dependence of infants on parenteral nutrition may be prolonged. Furthermore, it is well known that continuous parenteral nutrition is associated with several side effects including cholestasis and central line infections [1]. The physiological passage of stool through the bowel is important for enterohepatic circulation, resorption of fluids, electrolytes, vitamins, and enteral growth. Moreover, the passage of stool per rectum is important for developing a regular defecation reflex.

1.2 Evidence

Gause et al. presented their results on MFR in neonatal patients [2, 3]. In their retrospective analysis of 28 patients (13 in the MFR group and 15 in the control group) a shorter duration of parenteral nutrition and a faster time to full enteral feeds in the MFR group were reported. Recently, Yabe et al. reported on the beneficial effect on MFR in low-birth-weight infants, showing better weight gain and again a shorter duration of parenteral nutrition compared to a historical control group. [31] In 2006, Richardson et al. performed a systematic review on case reports and small case series of MFR after enterostomy creation [4]. The authors concluded that MFR was safe, as no complications were identified in any of the cited publications. In conclusion, studies published so far showed a faster weight gain in the group of MFR compared to controls [2, 4, 5, 6, 7]. These promising results need to be confirmed by a randomized, controlled study, which is the intention of this proposal.

1.3 The need for a study

As suggested by Gause et al. [3] a multicenter study of MFR is warranted in order to address the limitations of retrospective studies carried out so far. The results of this randomized controlled study may strongly influence the perioperative care of neonates within the pediatric surgical community. If our hypothesis is confirmed, the postoperative hospital stays of infants undergoing ostomy closure will be shortened. The benefits of MFR include a shorter duration and therefore less side effects of parenteral nutrition. Moreover, an economic benefit through lower costs for TPN and a shorter hospital stay may be reached.

1.4 Risk-Benefit-Assessment

Many pediatric surgical centers worldwide routinely perform MFR after enterostomy creation. However, due to a lack of prospective studies the level of evidence showing a benefit of this treatment strategy is low. Although the systematic review by Richardson et al. [4] showed no complications using this technique, MFR into the distal bowel loop may potentially cause complications such as bowel perforation. The risk for possible complications can be minimized by careful and standardized manipulation of the enterostomies. The local condition of the ostomy will be investigated twice daily.
If our hypothesis is confirmed, the postoperative hospital care of infants undergoing ostomy closure will be shortened. The benefits of MFR may include a shorter duration and therefore less side effects of parenteral nutrition, especially PN-associated liver disease (PNALD). Moreover, an economic benefit through lower costs for TPN and a shorter hospital stay may be reached.
The results of the current study may influence the standard of neonatal intensive care. Therefore the potential benefits of MFR outweigh the possible risks of this study.

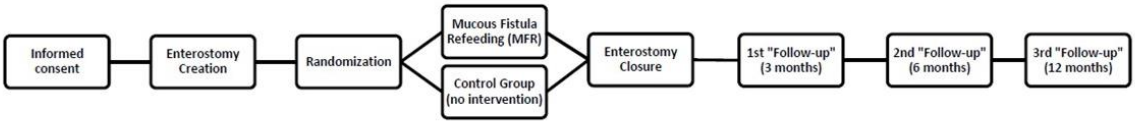
Results of data analyses including all data how to perform MFR will be published. If the results of this study will show significant differences between the intervention group and controls, MFR will become the new standard of care for neonates with enterostomies.
In Germany, the current national guideline for neonatal and surgical treatment of necrotizing enterocolitis (NEC) is currently in revision [Leitlinie 024-009: Nekrotisierende Enterokolitis (NEK)]. One of the principal investigators of the study (Prof. Dr. Martin Lacher) is coauthor of this guideline. If the current study proves the hypothesis that MFR is beneficial for these infants it may not only change the national guideline for the best treatment after enterostomy creation in Germany but in other countries too.

2 STUDY DESIGN, OBJECTIVES AND ENDPOINTS

2.1 Study Design

This is a randomized, multicenter, open-label, parallel group, controlled research study to demonstrate that mucous fistula refeeding between enterostomy creation and enterostomy closure reduces the time to full enteral feeds after enterostomy closure compared to standard of care.

Intervention scheme/Study Flow



2.2 Study Objectives

The primary objective of this study is to demonstrate that mucous fistula refeeding between enterostomy creation and enterostomy closure reduces the time to full enteral feeds after enterostomy closure compared to standard of care.

2.3 Study Endpoints

2.3.1 Outcome measures

Time to full enteral feeds (age-dependent caloric requirements per day) after enterostomy closure (hours) was chosen as the primary outcome parameter because of its clinical relevance representing the influence of MFR on the intestinal autonomy in the course of the disease. The endpoint is highly objective due to the strict and well-defined feeding protocol (see 3.1). In most of the referenced publications, postoperative weight gain early after surgery was chosen as the primary outcome parameter.

However, body weight is always affected by the shift of body fluids into the third space. Therefore, postoperative weight does not always correlate with enteral/parenteral caloric supplementation as a sign of enteral resorption. For this reason, it was not selected as the primary outcome parameter but will be assessed as secondary outcome measure.

Secondary outcome measures further include the time to full age-dependent volume intake, number of days of postoperative total parenteral nutrition (TPN) and the cholestasis parameters (conjugated bilirubin, GGT, ALT, AST) as indicators for hepatotoxicity of parenteral nutrition. The “time to first bowel movement” (hours) which correlates to the postoperative transanastomotic passage of stool, will be another secondary outcome parameter. A bowel movement consisting of only mucous rather than stool is also considered a bowel movement. Finally, all outcome parameters including possible complications will be assessed during the follow-up 3, 6 and 12 months (12-month follow-up only applicable for patients that are recruited early enough to complete this follow-up within the 48 month of overall study duration) after enterostomy closure.

2.3.2 Determination of primary and secondary measures

Primary efficacy endpoint:

Time to full feeds (hours), defined as time from enterostomy closure to actual enteral intake of the age-dependent caloric requirements per day for at least 24h and a concomitant reduction of parenteral fluids to < 20 ml/kg/24h.

For determining the time to full enteral feeds, the feeding advancement will be carried out according to the predefined nutritional protocol after 6-8 tolerated feedings in 3-4 hour intervals (24 hours). “Full feeds” is therefore defined age-dependent as 90 or 120 kcal/kg/24h (full feed kcal goal) actual enteral intake [8, 9, 32, 33]. The nurses will document any increase and decrease of nutrition precisely and daily controls will be carried out by the responsible neonatologist and pediatric surgeon.

The decision about the full feed kcal goal is made before randomization by the treating physician, depending on the birth weight of the infants and mother’s gestation week at birth:

- The nutrition aim is 120 kcal/kg/24h for premature infants with a birth weight < 1000 g or premature infants with a birth weight ≥ 1000 g and mother’s gestation week at birth before 37+0.
- The nutrition aim is 90 kcal/kg/24h for born mature infants, e.g. mother’s gestation week at birth at least 37+0.

In the case unforeseen circumstances lead to an unexpected maturation of the infant, at the time of enterostomy closure an infant formerly classified as “premature” can be re-classified as “mature”, following justified reasoning concerning laboratory parameters and consultation with the principal investigators. To rule out a biased decision by the investigators, an independent reviewer will review these decisions at the end of the study.

As the full feed kcal goal is implemented firstly in the study protocol version 3.0 the independent reviewer will also be provided with the data of patients that were randomized before study protocol version 3.0 and that did not achieve time to full feeds with the initial kcal goal of 120 kcal/kg/24h. On the basis of the individual patient information the independent reviewer will assess the primary endpoint with respect to the specific kcal goal defined above.

Secondary endpoints:

1) Reoperation
2) Time to first bowel movement after enterostomy closure (mucous stool is considered a bowel movement)
   Cleaning and changing of infants diapers will be performed according to a fixed schedule in order to uniformly document the time to first bowel movement following enterostomy closure.
3) Postoperative weight gain (g/d) (daily documentations recommended, minimum 2x per week), regular Z-Score (standard deviation score) documentation [WHO - weight-for-age] (daily documentations recommended, minimum 2x per week), This will be carried out according to a fixed schedule during morning rounds prior to feeding in an unclothed status.
4) Days of postoperative total parenteral nutrition (> 20 ml/kg/24h) before and after the 2nd operation (= ostomy takedown) (TPN)
   Days of postoperative total parenteral nutrition (TPN) are counted, starting on the day of enterostomy closure and ending on the day of full enteral nutrition. The parenteral nutrition is manufactured by the hospital pharmacy on a daily basis, while considering the simultaneous enteral caloric intake.
5) Laboratory parameters indicating cholestasis (conjugated bilirubin, GGT, ALT, AST, hemoglobin) and sodium resorption (sodium in urine).
   Time points for harvesting of blood samples during clinical routine blood withdrawal: Baseline at the time of randomization, then every 2 weeks until enterostomy takedown, at the 3-months follow up and in cases of pathologic clinical signs (jaundice, acholic stools)
6) Weight gain during the subsequent 5 days after reaching the primary endpoint
7) Central venous line (CVL) duration (days) and number of CVL infections (definition of infection: Neo-Kiss Guidelines)
8) Length of hospital stay (days)
9) Estimated ratio of the diameter of the two bowel loops which are anastomosed
10) Time to full oral volume intake (based on age-dependent daily fluid requirements), for at least 24h. The feeding advancement will be carried out according to the predefined nutritional protocol. “Full oral volume intake” is therefore defined as 150 ml/kg/24h (premature infants) and 120 ml/kg/24h (born mature as well as corrected mature infants) actual enteral volume intake (as in synopsis) [32,33]
11) Assessment of safety: Assessment of possible (serious) adverse events (AEs/SAEs) after randomization (e.g. death, sepsis, bowel perforation, stoma prolapse, abscess)

2.4 Study Duration

Recruitment:
Approximately 60 months (258 weeks)

Study duration per patient:
Maximum 58 weeks to minimum 32 weeks

Duration of the entire study (first patient in to last patient out):
72 months (310 weeks)

3 STUDY POPULATION

3.1 Study Population

Infants who underwent creation of an enterostomy receiving postoperative care and awaiting enterostomy closure:

to be assessed for eligibility: n = 230
to be assigned to the study: n = 120
to be analysed: n = 120

Duration of intervention per patient of the intervention group: minimum 21 days/3 weeks until patient's weight >2000 g, averaged 6 weeks between enterostomy creation and enterostomy closure

Follow-up per patient: 3 months, 6 months and 12 months following enterostomy closure (12-month follow-up only applicable for patients that are recruited early enough to complete this follow-up within the 48 months of overall study duration).

3.2 Inclusion Criteria

1. Only infants younger than 366 days of age with status post ileostomy or jejunostomy creation (double loop enterostomies and split enterostomies (with mucous fistula)) will be included in the study to create a homogenous cohort of patients with similar diseases (e.g. necrotizing enterocolitis [NEC], focal intestinal perforation [FIP]). Also, infants of this age group are unique in several respects such as the response to parenteral nutrition and its hepatic toxicity resulting into neonatal cholestasis.
   The ostomy localization is restricted to the jejunum and ileum. Therefore, the cohort of patients shows a similar bowel length for fluid-, vitamin- and electrolyte resorption.
2. All patients with meconium ileus are included into the study. If later (required) diagnostics verify cystic fibrosis, the diagnostics as well as the diagnosis need to be documented in the eCRF and in further analysis subgroups will be established.
3. Signed written informed consent obtained by parents/legal guardians and willingness of parents/legal guardians to comply with treatment and follow-up procedures of their child.

3.3 Exclusion Criteria

1. The resection of the ileocecal valve is an exclusion criterion because of its association with extensive bowel resection and therefore prolonged parenteral nutrition [10]
2. Colostomy
3. Patients with small bowel atresia are excluded because of prenatally underdeveloped bowel distal to the atresia
4. Multiple ostomies (more than just an enterostomy and a mucous fistula)
5. Patients with chromosomal abnormalities (if known at the time of randomization) are excluded because of potential malabsorption and malnutrition due to an underlying syndrome.
6. Hirschsprung disease secondary exclusion
7. Participation in another drug-intervention study
8. Intestinal perforation due to congenital heart defects with impaired hemodynamics

Specifications:
The application of prokinetic drugs is not allowed throughout the study especially after enterostomy closure.

Reoperation (e.g. relaparotomy) prior to randomization is not an exclusion criterion, these patients may still be included in the study.

3.4 Feasibility of recruitment

The participating sites represent institutions treating a large patient volume and are located in different regions of Germany, Austria and the Netherlands. All of them are academic (university) hospitals with large neonatal intensive-care units (NICU).

3.5 Achievability of recruitment rate

In order to achieve a total sample size of 120 patients during 72 months, 17 sites participate to the study.

The number of participating sites was increased by new partners during the course of the study. All sites have experience in adhering to scientific protocols and have participated in prospective studies. The adapted required patient number (n = 120) calculated by the power analysis will be achieved in a five-year period according to the patient numbers of the individual sites.

The Hannover Medical School and the University of Leipzig have already participated in several (multicenter) prospective studies without any problems in patient recruitment after being properly counseled and informed about study objectives, protocols and the potential complications in relation to the estimated benefits [11-25].

Patients in this study are recruited after enterostomy creation. As patients should be clinically stable at this time, parents/legal guardians (will) have sufficient time to decide whether they want their infant to participate in the trial.

3.6 Discontinuation Criteria

The following reasons may lead to discontinuation of the study in a patient 3:

1. Death
2. Iatrogenic bowel perforation of the distal bowel loop during catheterization for refeeding
3. Withdrawal of written consent

Patients who discontinue treatment for any reason will remain in the study to be evaluated for efficacy and safety endpoints, and will be expected to continue study visits.

4 STUDY PROCEDURES

No study procedures are allowed to be conducted until the parents'/legal guardians' written informed consent has been obtained (please also refer to chapter 9.1). The investigator is responsible for obtaining the parents'/legal guardians' written informed consent after adequate explanation of the aim, study assessments, potential risks and benefits and consequences of the study as well as alternative treatment options.

4.1 Study Calendar

	Enterostomy Creation	Screening	Pre-Treatment Phase	Treatment Phase (Refeeding or Control)	End of Treatment**** (**Enterostomy Closure**)	Post-Treatment Phase	FU 1 3 months 3 months after Entero-stomy Closure)	FU 2 6 months 6 months after Entero-stomy Closure)	(FU 3 12 months)* 12 months after Entero-stomy Closure)
			at least 1 week up to 6 weeks	at least 3 weeks up to 8 weeks	approx. 6 weeks after Enterostomy Creation	at least 2 weeks up to 8 weeks			
Data Assessment			daily	daily		daily	Outpatient clinic	Outpatient clinic	Outpatient clinic
Randomization			x						
Demographic data		x							
Informed consent		x							
In-/ Exclusion criteria		x							
Operation protocol	x				x				
Body weight		x	x	x		x **	x	x	x
Laboratory			x ***	x ***		x ***			
Refeeding protocol				x					
Nutrition protocol				x		x			
Medical history		x							
Adverse events			x	x	x	x	x	x	x
Time to first bowel movement after enterostomy closure [hours]						x			

*only applicable for patients that are recruited early enough to complete the 12-month follow-up within the 48 months of overall study duration

**weight is measured during the subsequent 5 days after reaching the primary endpoint

***every 2 weeks starting at randomization and in cases of pathologic clinical signs (jaundice, acholic stools); Laboratory analysis: During routine blood withdrawal, laboratory analysis for the blood parameters of GGT, ALT, AST, hemoglobin and conjugated bilirubin will be performed every 2 weeks starting from randomization until enterostomy closure. Additionally, in urine, sodium concentration is determined in the same time interval. No additional sample volume is necessary for this study.

****the day of enterostomy closure (day of operation) is the last day within the treatment phase, the day after operation is set as the 1st day of the post-treatment phase

4.2 Standardized protocol for creation of a small bowel enterostomy (*all patients*):

- Exploratory laparotomy (transverse preferred)
- Possible resection of necrotic bowel
- Identification of bowel for the enterostomy
- Proximal and distal limbs of the bowel loop are pulled through the abdominal wall muscles and skin (Loop enterostomy) via the abdominal incision or separate incision (preferred).
- Measurement of the length of small bowel between
  a) the ligament of Treitz (or if malrotation the first mobile part of the duodenum) and the enterostomy and
  b) the enterostomy and the ileocecal valve [cm].
  The measurement should be undertaken at the antimesenteric wall of the bowel.
- Closure of laparotomy:
  - Fascia with continuous suture Polyglactin 2-3/0
  - Subcutaneous interrupted sutures Polyglactin 4/0
  - Intracutaneous interrupted sutures Poliglecaprone 5/0
- Documentation of operative time (OR-Time in minutes).
- Daily documentation of the patient´s weight recommended (minimum 2x per week).

4.3 Standardized protocol on perioperative mucous fistula refeeding (*MFR*):

Definition: Infants are considered capable for MFR after 2 weeks following enterostomy creation if no contraindications for MFR, like sepsis, are present.

- Start 14-42 days after enterostomy creation (modified according to Wong et al. [6])
- Content to be transferred: the infant's own stool.
- Intervals of stool transfer: 6-8 hours as a bolus or continuously via a catheter introduced into the distal bowel loop (blocked with 0.5 ml of water).
- Amount of stool transfer: Initiation with 0.5 ml/kg/h per day. Increase of 5 ml/kg/24h or as tolerated.
- If the stool is too thick to be transferred, it may be diluted with normal saline 0,9%. (or glucose 5% in case of hypernatremia), no dilution with formula.
- Maximum amount of stool transfer (goal): whole amount of own stool.
- Documentation of time point and amount
  a) when the maximum amount of feeds are tolerated
  b) if and when the entire amount of stool is transferred
  c) if the entire amount of stool cannot be transferred, the maximum amount transferred is quantified and documented in this "partial-MFR subgroup".
- Duration of refeeding: at least 3 weeks and until the infant's weight exceeds 2000 g.
- Probiotics may be given as per protocol of the local institution.
- Prokinetic agents are not allowed during the entire trial.
- MFR should at least be performed for 21 days.
- Documentation whether the full amount of stool has been transferred (yes/no).

4.4 Standardized protocol for enterostomy closure (*all patients*):

- Timing of surgery: at least three weeks of MFR or standard treatment and an infant's body weight of > 2000 g
- Preoperative contrast study of the distal loop of the enterostomy to rule out stenosis is only necessary if the infants have not reached MFR of the total stool amounts of the preceding 24h. For all other infants preoperative contrast studies can be performed voluntarily. This study may be performed on the NICU by plain abdominal X-ray with enteral contrast (water-soluble isoosmolar).
- Central line placement if an adequate amount of calories cannot be provided via a peripheral line.
- No preoperative bowel preparation
- Placement of nasogastric (NG) tube in the operation room (OR)
- Size NG tube:
    - Premature infants up to 3 months of age: 6F catheter
    - 3 to 12 months of age: 8F catheter
- Small bowel anastomosis: Interrupted sutures with
    - 5/0 Polyglactin in infants below 6 months of age
    - 4/0 Polyglactin in infants above 6 months of age

- Perioperative antibiotic therapy: type and length based on bacteria profile have to be documented. Suggestion: Perioperative single shot antibiotic treatment. Different antibiotic regimes, adjusted to microbe profiling is possible, but should be documented precisely.

4.5 Standardized protocol on parenteral nutrition during treatment phase (*all patients*):

It is referred to the recommendations in "Neugeborenenintensivmedizin" by Rolf Maier and Michael Obladen (9th edition, 2017) on nutrition:

- fluid (ml/kg body weight/24h) 110 – 180
- energy (kcal/kg body weight/24h) 80 – 160
- amino acid (g/kg body weight/24h) 2 – 4
- lipid (g/kg body weight/24h) 2 – 3

4.6 Standardized protocol for management of nutrition after enterostomy closure (*all patients*):

Parenteral nutrition:

- Calories of the parenteral nutrition [8, 9] if there is no hyperglycemia (> 200 mg/dl), sepsis, hemodynamic instability that require a different caloric intake.
    - Day of surgery, starting 6h post operation: 50 – 90 kcal/kg/24h
    - POD (postoperative day) #1: 80-120 kcal/kg/24h
    - POD #2: 80-120 kcal/kg/24h
    - POD #3: 80-120 kcal/kg/24h
    - POD #4: 80-120 kcal/kg/24h
- Composition of lipid, amino acid and energy may vary according to the need of the patient and depending on the options (CVL or peripheral catheter)
- Trophic oral feeding of <3 ml x 8 (max 24 ml/24h) is allowed on day of surgery

Enteral nutrition:

- Initiation: POD #1

- Standardized feeding source
    - In all infant´s age-specific feeding sources will be used
    - Breast milk (if available) as there is a general consensus that breast milk (70 kcal/100ml) is the most effective protection against the development of necrotizing enterocolitis (Good et al.[26]) (document amount used each day)
    - Alternative 1: donor breast milk (document amount used each day)
    - Alternative 2: Formula for preterm infants (name, manufacturer, the kcal/ml and the amount should be documented in the eCRF)
    - Condition of the milk needs to be documented (raw or pasteurized)
    - Caloric enhancement of the milk: pure human milk or preterm formula is given until a feeding amount of 100ml/kg/d is tolerated, then the energy content of human milk can be enhanced – type and extent of caloric enhancement should be documented precisely

      Notice: As a large variety of institutional protocols on the fortification of the milk exist, the type of fortification is left to the discretion of the institution but should be documented.
    - Documentation of the selected fortifiers, their amount and the caloric content of the milk
- parallel to the increase of oral food concomitant reduction of parenteral fluids to < 20 ml/kg/24h

- Prerequisite: continuous measurement of the gastric residual via the nasogastric tube prior to the next feeding

Protocol 1: Gastric residual is below 3 ml/kg/nursing-shift or 10 ml/kg/24h

Feeding protocol (modified protocol of Bohnhorst et. al [27])

- Initial amount of enteral nutrition: 20 ml/kg/24h (in intervals of 3 or 4 hours)
- Increase by 30 ml/kg/24h, when 8 (or 6, depending on feeding intervals) consecutive feedings were accepted

Example (infant's weight 2000 g):

POD # 1: 8 x 5 ml	= 40 ml	(20 ml/kg/24h)
POD # 2: 8 x 12,5 ml	= 100 ml	(50 ml/kg/24h)
POD # 3: 8 x 20 ml	= 160 ml	(80 ml/kg/24h)
POD # 4: 8 x 27,5 ml	= 220 ml	(110 ml/kg/24h)
POD # 5: 8 x 35 ml	= 280 ml	(140 ml/kg/24h)

Protocol 2: Gastric residuals prior to the feeding is 20-50% of the previous feeding

For the consecutive feeding, 20% of the preceding feeding volume (= accepted gastric residual) is added to the current volume while the previous gastric residual (> 20%) is subtracted of the total volume:

Adapted amount of feeding volume =

current feeding-volume + 20% of the preceding volume – whole amount of previous gastric residuals

1. Example:

Enteral intake 6 x 60 ml; gastric residual 21 ml (= gastric residual 35%)

Calculation:

60 mL (feeding) + 12 ml (20% of the previous feeding)

– 21 ml (gastric residual prior to the feeding) → **51 ml**

2. Example:

Enteral intake 6 x 72 ml; gastric residual 30 ml (= gastric residual 42%)

Calculation:

72 ml (feeding) + 14 ml (20% of the previous feeding)

– 30 ml (gastric residual prior to the feeding) → **56 ml**

The next feeding is continued regularly and the feeding volume is then again increased after six consecutive accepted feeds.

Protocol 3: Gastric residuals prior to the feeding exceeds 50% of the previous feeding

If gastric residuals exceed 50% of the previous feeding volume or infant´s vomiting, one feeding is skipped

Protocol 4: Gastric residuals prior to the feeding reaches 100% of the previous feeding

If gastric residue reaches 100% of the previous feeding volume or infant´s vomiting, two feedings are skipped.

4.7 Further documentations after enterostomy closure (*all patients*):

1. Duration (minutes) of surgery (enterostomy closure)
2. Postoperative duration of assisted respiration (hours) prior versus post extubation
3. Daily documentation of the administration of opioids (influencing bowel movement and therefore our primary outcome)
4. Documentation of analgesia type (especially peridural anesthesia catheters, influencing postoperative bowel motility)

4.8 Additional treatments

The additional treatment of the patient (intervention) group involves the MFR (see 4.3 „standardized protocol on perioperative MFR“) with daily introduction of a catheter into the distal bowel loop followed by stool transfer.
Despite the standardized MFR no additional surgical or drug therapy is planned.

4.9 Control(s)/Comparator(s)

Infants of the control group will receive the current perioperative care.

4.10 Frequency and scope of study visits

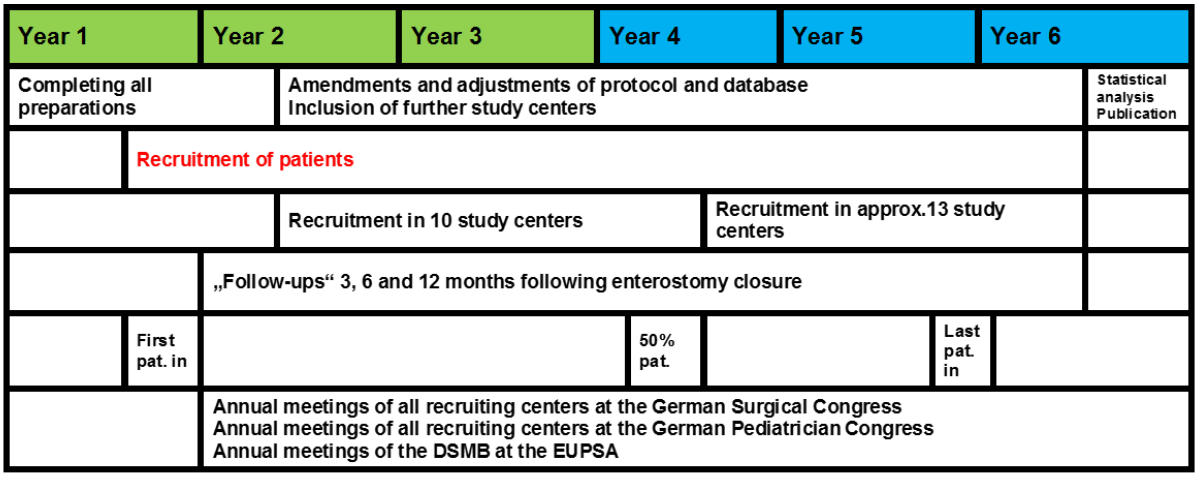
All patients will be continuously monitored on the intensive care unit (NICU) or intermediate care ward (IMC) by neonatologists, pediatric surgeons, and nursery staff. Medical records will be analyzed including vital signs, weight, oral intake, and medications.

All participating sites will be visited by the coordinating investigators before the start of the study. During the the complete study period, investigators from all sites will meet once a year, either digitally (during pandemic periods) or in person, to share information about the feasibility of the protocols and to discuss complications and serious adverse events related to the study. Study coordinators are in close contact with the study investigators via phone and email. Medical information will be exchanged electronically via encrypted email, phone, and fax on a regular basis during the course of the study.

4.11 Assessment of safety

Assessment of possible (serious) adverse events (AEs/SAEs) after surgery (e.g. intestinal bleeding, bowel perforation, sepsis, abscess) from randomization at least until reaching the primary endpoint and 12 months following enterostomy closure
AEs and SAEs will be classified at the end of the trial to achieve standardized grouping and minimize intergroup differences. The Clavien-Dindo system will be used to classify postoperative complications (see chapter 5).

4.12 Timeframe complete study



5 ADVERSE EVENTS

The documentation of all adverse events and complications (AEs/SAEs) occurring in patients within the study period is obligatory.

Current data suggest a low complication rate of the mucous fistula refeeding. Lau et al. [28], with the largest study population to date (n=77), documented no major complications. However, in a retrospective analysis by Haddock et al. [25] with an inhomogeneous population, the risk of bowel perforation, bleeding, and death associated with mucous fistula refeeding was reported.

Despite the obligation to document all events and complications in our study population, we selected adverse events of special interest (AESIs) based on the current literature.

Those AESIs include:

- events related to the underlying disease and the management in the NICU, e.g. ursodeoxycholic acid-therapy due to cholestasis and cholestasis (without ursodeoxycholic acid therapy), sepsis, and central line complications (except sepsis)
- events related to the surgery, e.g. small bowel obstruction (ileus), stoma stenosis, parastomal hernia and stoma prolapse
- events related to the mucous fistula refeeding, e.g. bowel perforation

All AEs/SAEs have to be documented and reported in the eCRF and on paper forms (source data) collected in the TIF.

All documented AEs/SAEs are classified according to the Clavien-Dindo classification and complications are assessed daily.

If an AE or SAE causally related to the intervention occurs, it will be immediately reported by the study site to the study coordination in Leipzig and from there to the ethics committees. All other AEs/SAEs not related to the intervention will be summarized and sent once a year by the study coordination in Leipzig to the ethics committees via the Safety Report.

6 STATISTICAL ANALYSIS

The primary analysis will be performed on the ITT population, i. e. all randomized patients will be analyzed in the treatment group to which they have been initially allocated. The treatment effect will be assessed by the Hazard Ratio for reaching full enteral feeds estimated with a Cox regression adjusted for center, weight and mother's gestation week at birth (< 1000 g and before 37+0 /< 1000 g and before 37+0 / ≥ 1000 g /and at least 37+0), height of stomata and treatment (jejunostomy/proximal Ileostomy or terminal ileostomy), and the respective 95% confidence interval. Superiority of the refeeding procedure will be concluded if the lower bound of the two-sided 95%-confidence interval for the Hazard Ratio (refeeding vs no refeeding) is greater than 1. In case of missing information on the time to full feeds, patients will be censored at the last known status before full feeds.

Extensive sensitivity analyses will be performed to assess the impact of the specification of the primary endpoint definition with study protocol version 3.0, specifically consistency of patient population before and after the specification will be analyzed and subgroup analyses for the defined strata will be performed.

Furthermore, time to full feeds after randomization (in days) will be analyzed in line with the cox regression model for the primary endpoint.

All secondary analyses will be tested exploratory using a two-sided significance level of 5%. For all analyses estimates, their associated two-sided 95% confidence intervals and p-values from regression models will be provided and conducted on the ITT population. The analysis strategy for the key secondary endpoints will be as followed:

- The secondary endpoints "Time to first bowel movement after enterostomy closure" and "Time to full oral volume intake" will be analyzed using a cox-regression model in line with the primary analysis strategy.
- The secondary endpoint "Reoperation" will be analyzed using a logistic regression model to compare both treatment arms stratified by center, weight and mothers gestation week at birth.
- The secondary endpoints "Postoperative weight gain (g/d)", "Days of postoperative total parenteral nutrition (TPN)", "Laboratory parameters indicating cholestasis (conjugated bilirubin, GGT, ALT, AST, hemoglobin) and sodium resorption (sodium in urine)", "Weight gain during the subsequent 5 days after reaching the primary endpoint", "Central venous line (CVL) duration (days) and number of CVL infections", "Length of hospital stay (days)" and "Estimated ratio of the diameter of the two bowel loops which are anastomosed" will be analyzed using a linear regression model to compare both treatment arms stratified by center, weight and mothers gestation week at birth.

To evaluate the feasibility of the intervention and the refeeding protocol 2 additional subgroup analyses will be performed, each comparing 3 groups in the MFR treatment arm. A period of

7 days prior to the last day before the surgery for enterostomy closure is considered resulting in classifications for these groups:

First additional subgroup analysis:

1. No refeeding: 0 days of refeeding protocol adherence (0%)
2. Partially refeeding: 1-3 days of refeeding protocol adherence (1% - 50%)
3. Full refeeding: 4-7 days of refeeding protocol adherence (51% - 100%)

Second additional subgroup analysis:

1. No refeeding: 0 days of total volume refed (0%)
2. Partially refeeding: 1-3 days of total volume refed (1% - 50%)
3. Full refeeding: 4-7 days of total volume refed (51% - 100%)

These subgroup analyses will each be performed descriptively using a two-sided Kruskal-Wallis-Test comparing the respective three groups.

Safety analyses

Occurrence of AEs and SAEs will be analyzed descriptively using absolute and relative frequencies for the whole population and separately for the intervention and control groups and will be compared with chi-squared tests. The grading of each event according to the Clavien-Dindo classification, the outcome of the event and ICD-10 terms will be compared descriptively. Furthermore, events classified as AESIs will be analysed descriptively additionally in sub-group investigations.

6.1 Methods against bias

This is an open-label study. Blinding is not possible because active refeeding of stool in the intervention group is obvious to any person participating in the medical care of the patient. Randomization will be performed centrally via fax (with variable block length) and stratified by study center, height of stomata (jejunostomy/proximal Ileostomy or terminal ileostomy) [29, 30], weight (< 1000g / ≥ 1000g) and mother's gestation week at birth (before 37+0 / at least 37+0), as this are important prognostic factors for the primary endpoint. Randomization will take place after enterostomy creation in order to reduce the amount of missing values due to patient exclusion after surgery (e.g. due to unforeseen need for resection of ileocecal valve) and after the treating physician has determined the full feed kcal goal on the basis of the stratification. The primary analysis will be performed on the ITT population as this is an open study and parents/legal guardians may have preferences not outspoken before randomization. A per protocol analysis will be conducted as a sensitivity analysis in all patients that have no substantial protocol deviations. Consistency between the findings in the ITT population and the per-protocol population will be examined, as it is an important pre-requisite for a successful interpretation of the study.

In general, dropouts are not expected because all patients will constantly undergo neonatal intensive care and will therefore not be lost to follow-up. If parents/legal guardians withdraw their infant from study participation, they will be asked to allow data collection at a final analysis to avoid wasting that information. Nevertheless, up to now, we recognized that for some individual patients it is not feasible to abide by the protocol, especially the nutrition protocol after enterostomy closure. Therefore, if missing values occur (e.g. due to death, or parents'/legal guardians' refusal of data collection), observations will be censored at the last time point with known enteral feeding status. Since this censoring may be informative, missing values for time to full feeds will be replaced by the worst observation in each group in a sensitivity analysis in order to check how censoring may have influenced the results. If any death occurs before the respective patient reaches full enteral feeds, a sensitivity analysis will be performed for all surviving patients.

To avoid direct influence on the change of kcal goal of the treating physician during the study, an independent reviewer will assess at least all changes of kcal goals at the end of the study.

6.2 Proposed sample size/Power calculations

The literature of MFR is scarce and information on the primary endpoint "time to full enteral feeds" is limited [4]. A recently published retrospective analysis of 24 patients [3] of whom 13 received refeeding of stool to the mucus fistula and 11 of whom did not receive refeeding of stool showed a median time from enterostomy takedown to enteral feeds of 7 days in the control group and 4 days in the refeeding group. The data presented for the control group is in line with retrospective data of 42 patients collected at Hannover Medical School. These 42 patients are all patients fulfilling the inclusion criteria who were treated at Hannover Medical School between 2005 and 2015. They did not receive refeeding of stool and had a median time to full enteral feeds of 7 days. According to Gause et al. [3], a survival analysis is appropriate. In their respective publication, median times are reported corresponding to a hazard ratio of 1.751 for time to enteral feeds (4 days vs 7 days), 2.331 for parenteral nutrition discontinuation (6 days vs 14 days) and 2.667 for goal feeds (7.5 days vs 20 days). Because time to enteral feeds in this publication is in line with our retrospective data of time to full feeds, a hazard ratio of 1.751 is assumed for the treatment effect. In order to show a treatment effect with a power of 80% and a two-sided type I error probability of 5% with a logrank test, a total of 100 events (full enteral feeds) is required, if the hazard ratio for the treatment effect is 1.751. Since patients will be in neonatal intensive care, every patient is expected to reach full enteral feeds. Nonetheless, to account for possible deaths and patients that are not able to reach full enteral feeds or abide the study protocol, the sample size was increased by 20 patients, resulting in a total of 120 patients. Sample size was estimated in nQuery Advisor 7.

6.3 Compliance/Rate of loss to follow up

Several retrospective data analyses show low complication rates related to MFR. During 14 years of MFR, a group from the University of Hong Kong observed no major complications associated with refeeding in 77 patients with necrotizing enterocolitis [28].
All centers participating in the current study have experience with MFR and did not record major complications in any of the centers. This observation is well in line with data from 13 patients undergoing MFR at the Department of Pediatric Surgery at Johns Hopkins University

School of Medicine in Baltimore. The authors did not document major complications associated with refeeding but observed benefits of the intervention [3].

We are very confident that the loss of follow-up in this study at the end will be quite small. Due to the severe course of the diseases, parents/legal guardians of patients with neonatal surgical conditions have an intense emotional relationship with the treating surgeons and neonatologists. Almost all parents/legal guardians prefer follow-up appointments at the treating hospital after their infants have been discharged from the hospital. We therefore do not expect any loss of follow-up. However, as a precaution, the patient recruitment was increased to 13 centers with 230 expected patients.

7 DATA MANAGEMENT

All study data will be collected by the investigator and/or other study personnel. A validated clinical trial data base (electronic case report form) is provided in which the data are entered. AEs and SAEs and further relevant diagnoses will be coded using ICD 10 coding system (International Statistical Classification of Diseases and Related Health Problems).

Authorized and trained staff of the study sites will enter the data in the eCRF in a timely manner.

Verification of the data in the eCRF occurs by risk-based monitoring as well as via range, validity and consistency checks programmed in the system. Additionally, manual queries can be raised in the system if discrepancies are detected. Based on the queries, the investigator can review the data and resolve the discrepancy or justify the entered data directly in the system. All changes of data entered in the eCRF are documented in an audit trail. A quality control will be performed before the database is closed. This procedure is documented. Finally, data transfer takes place for statistical evaluation.

The data management plan contains further details about data management processes.

8 QUALITY ASSURANCE AND MONITORING

All initiation visits, onsite monitoring visits, close-out visits and in-house monitoring will be conducted by monitors of ZKS (ZKS, MHH) or an external qualified CRO. ZKS SOPs will be utilized. Prior to the start of the study, pre-study visits by the primary investigators will be conducted to be able to instruct the local investigators in how to follow the study protocol and documentation of data. Initiation visits will be done in each study site prior to patient recruitment to ensure adherence with all study procedures by the monitor of ZKS or an external CRO and the study coordinators. To assure high data quality and patients safety, regular on-site monitoring visits will be performed by monitors. Checking of signed informed consents and source data verification will be carried out according to a risk adapted approach. At the end of the study, close out visits will be performed at all study sites. Project managers, monitors, study coordinators and PIs will be in close and regular contact throughout the study and with all study sites.
Monitoring details will be summarized in a monitoring plan which will be prepared by the lead monitor (ZKS). The monitoring plan will be reconciled with the coordinating investigator and members of the clinical project management. It will serve as guiding document for all monitors and will contain details on monitoring activities, responsibilities and interfaces between study team, data management, source data and adverse events. In-house monitoring will assure high data quality. Data capture will be achieved by electronic data capture (electronic CRF). On-site source data verification will be done according to a risk adapted monitoring afterwards. In total, 2 monitoring and 1 close-out visits are planned per study site.

8.1 Data Monitoring Committee

An independent Data Monitoring Committee (DMC) is implemented to detect possible harms and to assure continuous risk/benefit assessment. A DMC is a group of independent experts external to the study assessing the progress, safety data and, if needed, critical efficacy endpoints. Details of the definition of DMC, its composition and its roles and responsibilities can be found in the separate DMC charter.

9 ETHICAL AND LEGAL CONSIDERATIONS, ADMINISTRATION

The study will be conducted in accordance with the principles of ICH-GCP (as far as possible for this kind of study) and the Declaration of Helsinki.
Study protocol and patient consent form will be submitted to the Ethics Committees for review prior to the start of the study. No amendment to the protocol may be made without consideration by the Ethics Committee.

9.1 Patient Information and Informed consent

The investigator is responsible for obtaining the parents'/legal guardians written informed consent after adequate explanation of the aim, study assessments, potential risks and benefits and consequences of the study as well as alternative treatment options. Parents/legal guardians will have sufficient time to ask questions before deciding on whether or not to participate in the study. The patient information/informed consent form has to be signed in duplicate by the patient's parents/legal guardians and the investigator. One document will be given to the parents/legal guardians, the other one will be kept at the participating study sites. No study procedures are allowed to be conducted until parents'/legal guardians' written informed consent has been obtained.
The patient information/informed consent form has to be revised whenever important new information becomes available that may be relevant to the parents'/legal guardians' consent.
In case of the infants transfer into another clinic, the investigator obtained the informed consent from the parents/legal guardians to release the physicians in the external clinic from their medical confidentiality to retrieve the data for the study.
Participation in this clinical trial is voluntary. Withdrawal from the study at any time and for any reason is without any disadvantages to the patient's further treatment.

9.2 Patient Insurance

The trial will be covered by a participant insurance in case the trial site (clinic) does not cover the study by its liability insurance (Haftpflichtversicherung). All subjects (parents/legal guardians) will be informed about their rights and obligations in regard to insurance policies before participating in the study. A copy of the insurance policies will be handed out to each patient (parents/legal guardians).

9.3 Data Protection

Data collection, handling, storage and analysis will be in accordance with national regulations. All study staff are obliged to duly observe data protection and medical confidentiality.
If the participant withdraws the previously given informed consent, the participant has the right to demand the deletion of all data collected so far. If the participant withdraws and does not demand the deletion of data, the data collected up to that point will be anonymised and used for the statistical analysis.

9.4 Registration

The study is registered at ClinicalTrials.gov: NCT03469609

9.5 Record Retention

The original study documents wil be stored in an archive of the participating study site for at least 10 years after the final study report.

9.6 Financing

The clinical trial is funded by public funds through the German Research Foundation.

9.7 Patient Involvement

The steering committee of the patient organization “Das frühgeborene Kind e. V.” is aware of the study. This organization consists of parents of former preterm infants and also actively involved in other scientific studies in Germany. Regular telephone conferences with this organization will be held to exchange information, and a representative is invited to the investigator meetings. The PIs are available for consultations by the patient organization concerning mucous fistula refeeding and will update the steering committee on recent publications and evidence-based recommendations.

10 HANDLING OF BIOMATERIAL

Biomaterials in the main study include the analyses of sera, plasma and urine, rectal stool and the use of enterostomy stool for MFR. Sera, plasma and urine will be collected and analyzed using the current concepts of each department. Therefore, no additional trauma will be present. Enterostomy losses will be collected in strict intervals [1x (continuous refeeding) or 3x (separated refeeding every 8 hours) daily] for the refeeding. Stool will not be stored for the MFR. The necessary amount will be transferred and the surplus will be thrown away.

11 PUBLICATION

After completion of the trial, data analyses will be performed by the Institute of Biostatistics (MHH). The results and the study protocol including all data used to perform MFR will be published. If the results of this study show significant differences between the intervention group and controls, MFR will become the new standard of care for neonates with enterostomies. In Germany, the current national guideline for neonatal and surgical treatment of necrotizing enterocolitis (NEC) is currently in revision [Leitlinie 024-009: Nekrotisierende Enterokolitis (NEK)]. One of the principal investigators of the trial (Prof. Dr. Martin Lacher) is co-author of this guideline (Delphi method). If the current trial proves the hypothesis that MFR is beneficial for these infants, it may not only change the national guideline for the best treatment after enterostomy creation in Germany, but also in other countries.

13 REFERENCES

1. Jolin-Dahel et al “Parenteral nutrition-induced cholestasis in neonates: where does the problem lie?” Gastroenterol Res Pract. 2013;2013:163632
2. Gause et al “Mucous fistula refeeding decreases parenteral nutrition exposure in post-surgical premature neonates” AAP experience, National Conference & Exhibition, Washington DC
3. Gause et al “Mucous fistula refeeding decreases parenteral nutrition exposure in postsurgical premature neonates” J Pediatr Surg. 2016 Nov;51(11):1759-1765
4. Richardson et al “What is the evidence on the practice of mucous fistula refeeding in neonates with short bowel syndrome?” J Pediatr Gastroenterol Nutr. 2006 Aug;43(2):267-70
5. Al-Harbi et al “Mucous fistula refeeding in neonates with short bowel syndrome” J Pediatr Surg. 1999 Jul;34(7):1100-3
6. Wong KK „Mucous fistula refeeding in premature neonates with enterostomies.” J Pediatr Gastroenterol Nutr. 2004 Jul;39(1):43-5
7. Gardner et al “A case study utilizing an enteral refeeding technique in a premature infant with short bowel syndrome.” Adv Neonatal Care. 2003 Dec;3(6):258-68; quiz 269-71
8. Klein CJ et al “Nutrient requirements for preterm infant formulas” J. Nutr. 132:1395S-1577S, 2002
9. Agostoni C, Buonocore G, Carnielli VP et al for the ESPGHAN Committee on Nutrition. Enteral nutrient supply for preterm infants: Commentary from the European Society for Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. Journal of Pediatric Gastroenterology and Nutrition 2010;50:1-9
10. Caniano et al “Newborns with massive intestinal loss: Difficult choices” N Engl J Med 318:703-707, 1988
11. Ahlenstiel-Grunow T, Koch A, Grosshennig A et al. “A multicenter, randomized, open-labeled study to steer immunosuppressive and antiviral therapy by measurement of virus (CMV, ADV, HSV)-specific T cells in addition to determination of trough levels of immunosuppressants in pediatric kidney allograft recipients (IVIST01-trial): study protocol for a randomized controlled trial.” Trials 2014;15:324-6215-15- 324- IF 2,117
12. Reismann, Ure BM et al “Fast-track concepts in routine pediatric surgery: a prospective study in 436 infants and children” Langenbecks Arch Surg. 2009 May;394(3)
13. Engelmann C, Ure BM et al “Laparoscopic anterior hemifundoplication improves key symptoms without impact on GE in children with and children without neurodevelopmental delays” J Pediatr Gastroenterol Nutr. 2010 Oct;51(4):437-42
14. Dingemann J, Ure BM et al “Perioperative analgesia strategies in fast-track pediatric surgery of the kidney and renal pelvis: lessons learned” World J Urol. 2010 Apr;28(2)215-9
15. Petersen C, Ure BM et al “Postoperative high-dose steroids do not improve mid-term survival with native liver in biliary atresia” Am J Gastroenterol. 2008 Mar;103(3):712-9
16. Bagci S, Lacher M, Ure BM, et al “More than fetale urine: enteral uptake of amniotic fluid as a major predictor for fetal growth during late gestation” Eur J Pediatr. 2016 Jun;175(6):825-31
17. Van der Steeg HJ, Lacher M, et al “European consensus meeting of ARM-Net members concerning diagnosis and early management of newborns with anorectal malformation” Tech Coloproctol. 2015 Mar;19(3)181-5

18. von der Leyen HE et al. “A prospective, single-blind, multicenter, dose escalation study of intracoronary iNOS lipoplex (CAR-MP583) gene therapy for the prevention of restenosis in patients with de novo or restenotic coronary artery lesion (REGENT I extension)”. Hum Gene Ther. 2011 Aug; 22(8):951-8
19. Samuels S, von der Leyen H, Wittkop U, et al. “Precision medicine in cancer: challenges and recommendations from an EU-funded cervical cancer biobanking study”. Br J Cancer. 2016 Nov 22
20. Scheper V, von der Leyen H, Koch A, et al. “ACEMg-mediated hearing preservation in cochlear implant patients receiving different electrode lengths (PROHEARING): study protocol for a randomized controlled trial”. Trials. 2016 Aug 8;17:394
21. Lindhardt M, von der Leyen H, et al. „Proteomic prediction and Renin angiotensin aldosterone system Inhibition prevention Of early diabetic nephRopathy in TYpe 2 diabetic patients with normoalbuminuria (PRIORITY): essential study design and rationale of a randomised clinical multicentre trial”. BMJ Open. 2016 Mar 2;6(3):e010310
22. Rohde GG, Koch A, Welte T; ABACOPD study group. “Randomized double blind placebo-controlled study to demonstrate that antibiotics are not needed in moderate acute exacerbations of COPD--the ABACOPD study”. BMC Pulm Med. 2015 Jan 27;15:5
23. Dindo D, Demartines N, Clavien PA. “Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey.” Ann Surg 240:205-13 (2004)
24. Sethi MV, Zimmer J, Ure B, Lacher M. “Prospective assessment of complications on a daily basis is essential to determine morbidity and mortality in routine pediatric surgery.” J Pediatr Surg. 51(4):630-3 (2016)
25. Haddock CA, Stanger JD, Albersheim SG et al. “Mucous fistula refeeding in neonates with enterostomies.” J Pediatr Surg. 2015 May;50(5):779-82
26. Good et al “Evidence based feeding strategies before and after development of necrotizing enterocolitis” Expert Rev Clin Immunol. 2014 July ; 10(7): 875-884
27. Bohnhorst et al “Early feeding after necrotizing enterocolitis in preterm infants”, J Pediatr 2003;143:484-7
28. Lau et al “Beneficial effects of mucous fistula refeeding in necrotizing enterocolitis neonates with enterostomies” J Pediatr Surg. 2016 Sep15
29. Kargl et al “Ileostomy Complications in Infants less than 1500 grams –Frequent but Manageable” J Neonat Surg. 2017 Jan 1
30. Richardson et al “What is the evidence on the practice of mucouse fistula refeeding in neonates with short bowel syndrome” J Pediatr Gastroenterol Nut. 2006:43:267
31. Yabe et al „Safety and efficacy of mucous fistula refeeding in low-birth-weight infants with enterostomies“ Pediatr Surg Int. 2019 Oct;35(10):1101-1107
32. Nutrition Committee, Canadian Paediatnc Society; CAN MED ASSOC J o JUNE 1, 1995, 152 (1 1)
33. Gregory et al ”Update on Nutrition for Preterm and Full-Term Infants “JOGNN, 34, 98-108; 2005

14 SIGNATURES

This document has been approved by the following primary investigators:

2022-12-12

Prof. Dr. med. Martin Lacher — Date
Coordinating Investigator

2022 – 12 – 06

PD Dr. med. Omid Madadi-Sanjani — Date
Coordinating Investigator

Prof. Dr. Armin Koch — Date
Trial Statistician

Sören Wiesner — Date
Trial Statistician

14 SIGNATURES

This document has been approved by the following primary investigators:

Prof. Dr. med. Martin Lacher — Date
Coordinating Investigator

PD Dr. med. Omid Madadi-Sanjani — Date
Coordinating Investigator

13.12.22

Prof. Dr. Armin Koch — Date
Trial Statistician

14.12.22

Sören Wiesner — Date
Trial Statistician