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ORIGINAL ARTICLE

Remote ischemic conditioning in necrotizing enterocolitis: study protocol of a multi-center phase II feasibility randomized controlled trial

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Abstract

Purpose Remote ischemic conditioning (RIC) is a maneuver involving brief cycles of ischemia reperfusion in an individual's limb. In the early stage of experimental NEC, RIC decreased intestinal injury and prolonged survival by counteracting the derangements in intestinal microcirculation. A single-center phase I study demonstrated that the performance of RIC was safe in neonates with NEC. The aim of this phase II RCT was to evaluate the safety and feasibility of RIC, to identify challenges in recruitment, retainment, and to inform a phase III RCT to evaluate efficacy.

Methods RIC will be performed by trained research personnel and will consist of four cycles of limb ischemia (4-min via cuff inflation) followed by reperfusion (4-min via cuff deflation), repeated on two consecutive days post randomization. The primary endpoint of this RCT is feasibility and acceptability of recruiting and randomizing neonates within 24 h from NEC diagnosis as well as masking and completing the RIC intervention.

Results We created a novel international consortium for this trial and created a consensus on the diagnostic criteria for NEC and protocol for the trial. The phase II multicenter-masked feasibility RCT will be conducted at 12 centers in Canada, USA, Sweden, The Netherlands, UK, and Spain. The inclusion criteria are: gestational age < 33 weeks, weight ≥ 750 g, NEC receiving medical treatment, and diagnosis established within previous 24 h. Neonates will be randomized to RIC (intervention) or no-RIC (control) and will continue to receive standard management of NEC. We expect to recruit and randomize 40% of eligible patients in the collaborating centers (78 patients; 39/arm) in 30 months. Bayesian methods will be used to combine uninformative prior distributions with the corresponding observed proportions from this trial to determine posterior distributions for parameters of feasibility.

Conclusions The newly established NEC consortium has generated novel data on NEC diagnosis and defined the feasibility parameters for the introduction of a novel treatment in NEC. This phase II RCT will inform a future phase III RCT to evaluate the efficacy and safety of RIC in early-stage NEC.

Keywords Necrotizing enterocolitis · Remote ischemic conditioning · Feasibility · Randomized clinical trial · Phase II · RCT

Introduction

Background

Necrotizing enterocolitis (NEC) is a devastating intestinal disease that affects 4–9% of preterm infants and remains a major unsolved clinical challenge in neonatology [1]. Gut immaturity in neonates with NEC can lead to intestinal

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inflammation, progressing in severe cases to necrosis and perforation. NEC results in high mortality [2], neurodevelopmental impairment [3, 4], intestinal failure [5], reduced quality of life, and high treatment costs estimated at $500 M-$1B per year in the USA [2]. Although mortality of premature infants continues to decrease, mortality due to NEC remains high [6]. A systematic review of recent large cohort studies (January 2010–January 2018) reported that contemporary overall mortality was 24% in all neonates with confirmed NEC (Bell stage II and above), 35% in neonates that underwent surgery for NEC, 41% in preterm infants with medical NEC, and 51% in those with surgical NEC [7].

Some progress has been made on the prevention of NEC with exclusive use of human milk and probiotics. However, despite ongoing research and advancements in neonatal care, innovations in the treatment of NEC are lacking. Present treatments are primarily supportive; they include antibiotics, bowel rest, and surgery to remove necrotic bowel if necessary. There is an urgent need for devising a novel treatment strategy to avoid the progression from initial inflammatory changes to more advanced intestinal injury in NEC.

The etiology of NEC remains imperfectly understood. However, NEC is considered multifactorial with prematurity and formula feeding being two of the most important risk factors [8–12]. Prematurity is strongly associated with NEC, with its highest incidence in infants with extremely low birth weight (ELBW) [11]. After birth, premature infants develop NEC following 2–4 weeks of being parenterally fed [13, 14]; more than 90% of infants with NEC have been enterally fed [11, 12]. Formula feeding is strongly associated with intestinal ischemia and hypoxia in human NEC [15]. After feeding, intestinal blood flow increases to above baseline to fulfill the increased intestinal oxygen demand, a process known as postprandial hyperaemia [14]. In adult humans, the mean mesentery blood velocity increases by > 150% after enteral feeding [14]. However, in preterm infants, postprandial hyperemia is remarkably compromised such that the mean mesenteric blood velocity increases by only 30% [16–18]. Emerging evidence suggests that derangements in intestinal microcirculation are associated with experimental NEC and play a significant role in disease development [19–22]. Therefore, modulating the immature intestinal microvasculature could prove to be a viable strategy to counteract the feeding-induced hypoxia and prevent the progression of NEC.

Remote ischemic conditioning (RIC) is a therapeutic strategy for the protection of distant organs against the detrimental effects of ischemia and hypoxia. RIC involves the application of brief cycles of ischemia and reperfusion to a limb to protect distant organs from sustained ischemic damage. To investigate the effectiveness of RIC against NEC, we used a well-established experimental model of this disease in mouse pups [23] and determined the efficacy and mechanism of action of RIC [15]. RIC, when administered to neonatal mouse pups at postnatal day 5 (P5), counteracted the feeding-induced hypoxia observed in these pups due to immaturity of the intestinal microcirculation [15]. When administered in the early stages of experimental NEC, RIC decreased intestinal injury and inflammation and prolonged survival [15]. The mechanism of action of RIC involved increasing intestinal perfusion through vasodilation mediated by nitric oxide and hydrogen sulfide [15]. RIC was also safe and did not alter the hindlimb motor function [15]. These experimental findings indicate that RIC may be a novel viable and non-invasive treatment strategy for the *treatment* of NEC.

Clinical trials have been performed in adults [24–26] and in children [27–31] which suggest benefits from RIC protecting various organs including the heart, lung, and kidney. In addition, a systematic review and meta-analysis evaluating randomized trials found that compared with controls, RIC significantly reduced the recurrence of stroke or cerebral transient ischemic attacks [32]. However, the advantage of RIC on the heart remains controversial as randomized controlled trials showed no improvement after myocardial infarction [33] or cardiac surgery [34, 35]. Only 3 trials in adults have focused on the effects of RIC on the intestine [36–38]. One trial demonstrated benefit after abdominal aortic aneurism repair, after which intestinal ischemia/reperfusion-induced injury is expected [36]. In contrast, the other two trials found no intestinal changes after cardiopulmonary bypass which can cause moderate and transient intestinal injury [37] or in the course of chronic intestinal inflammation such as ulcerative colitis [38]. To the best of our knowledge, RIC has never been given to small preterm neonates with an *acute* intestinal injury such as NEC.

RIC can be implemented in human neonates with NEC to potentially prevent disease progression and minimize the need for aggressive surgical intervention. Although RIC is a simple and attractive maneuver, there is a need to evaluate feasibility and safety in this patient population via a randomized controlled trial (RCT). We recently completed a non-randomized single-center phase I pilot safety study at the Hospital for Sick Children (Toronto, CA), proving that RIC is safe in premature infants with NEC [39]. The pathway to clinical implementation of RIC requires a phase II RCT to prove the feasibility and acceptability of this maneuver in the multicenter setting. This is necessary before embarking on designing and conducting a randomized multicenter phase III RCT to investigate the efficacy of RIC in NEC. This manuscript reports the protocol of our phase II randomized trial.

Rationale

The rationale for the proposed trial is based on the following:

a. *Therapeutic potential of RIC*: Our clinical observations indicate that in neonates with NEC, there is intestinal hypoxia in the most affected segment of the bowel [8, 10, 15]. Our group has been the first to demonstrate the beneficial effects of RIC in experimental NEC. RIC counteracted early-stage NEC and prolonged survival by preserving intestinal microcirculation [15]. Importantly, RIC was effective when administered in the early stage of NEC, but not once intestinal necrosis has already occurred [16].
b. *Window of opportunity for RIC intervention*: Based on a multicenter study, one-third of infants with NEC progress to severe advanced disease which is associated with poorer prognosis [40]. Based on a retrospective review of all neonates treated in the neonatal unit of Mount Sinai Hospital (Toronto, CA) in the last 9 years, 176 inborn neonates developed NEC and 147 (83%) presented with medical NEC. In 59 (40%) of them, NEC progressed, requiring an abdominal operation and 42% of these neonates eventually died despite surgical intervention. The progression from medical to surgical NEC occurred after a mean of 3 days (range 1–7 days) despite conventional medical treatment for NEC as well as respiratory and hemodynamic support. Thus, 24 h is an ideal "window of opportunity" for the RIC intervention.
c. *Safety of RIC in infants:* RIC has never been evaluated in preterm infants with NEC. Therefore, we recently completed a phase I pilot safety study in human preterm neonates with NEC (ClinicalTrials.gov: NCT03860701) and demonstrated that RIC, given as 4 cycles per day of 4 min of limb ischemia followed by 4 min of reperfusion and on two consecutive days, was safe in 5 preterm neonates as it did not produce deleterious effects such as limb ischemia, peripheral nerve damage, new onset of cutaneous injury in the limb where RIC was performed, or severe pain [39].
d. *Need for a phase II feasibility trial:* There are three main knowledge gaps to the successful design and completion of a future phase III efficacy RCT for RIC in preterm infants with NEC: (i) a large proportion of phase III pediatric trials fail due to flawed study design, insufficient recruitment and randomization, inappropriate statistical endpoints, or underpower [41]. This is particularly relevant in trials focused on NEC treatment as to our knowledge, the only two trials on NEC treatment had suboptimal recruitment [42, 43]; (ii) the outcomes for NEC are poorly defined and partly influenced by subjectivity; and (iii) we do not know whether it is acceptable to parents/caregivers and doctors and nurses to perform an RCT on RIC in neonates with NEC. Thus, although we are excited about our novel experimental data and the safety of RIC in preterm neonates as demonstrated in our phase I pilot safety study [39], a *phase II RCT* is necessary before embarking on a precisely powered multicenter international *phase III RCT* to prove, on a large scale, the efficacy of RIC in the treatment of early-stage NEC.

Objectives

The *objectives* of this phase II Feasibility RCT are to determine:

i. Feasibility of identifying, recruiting, randomizing, and applying masked intervention to neonates within 24 h from confirmed diagnosis of medical NEC;
ii. Feasibility of recording NEC outcome measures to calculate sample size for the future phase III RCT;
iii. Satisfaction of key trial stakeholders (parents and healthcare workers) with the recruitment process and the intervention.

Proposed hypothesis

We *hypothesize* that it is feasible to conduct a multicentre randomized controlled trial to evaluate RIC during the management of neonates with NEC.

Study methods

Trial registration

This trial will be registered on www.clinicaltrials.gov/ under the following name: "RIC-NEC multicenter Phase II Randomized Controlled Trial: Remote ischemic conditioning in necrotizing enterocolitis".

Trial design

The RIC-NEC trial is an interventional, prospective, randomized, controlled, masked, multicenter, phase II trial (Fig. 1). This study protocol is reported in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 guidelines [44].

Ethical considerations

Approval of the Research Ethics Committee relevant to all collaborating centers will be obtained before starting the trial. The parents/caregivers of all study participants

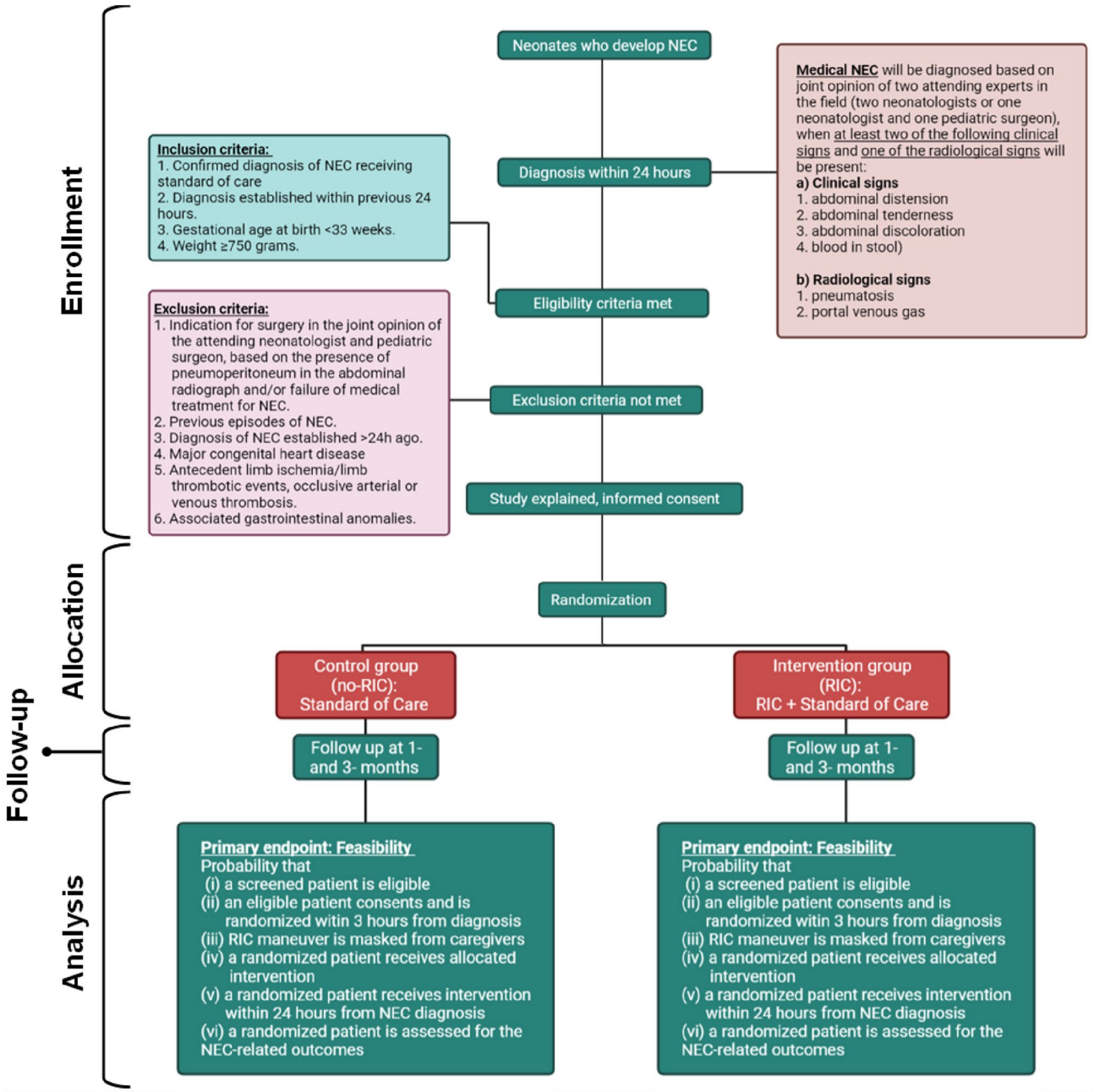

Fig. 1 Trial flowchart according to CONSORT guidelines

will be asked to provide a written informed consent. The person obtaining informed consent from parents/caregivers will not be part of the circle of care. Families will be fully informed that the clinical outcomes of RIC in NEC remain unproven and are therefore being investigated. We will seek permission from families to hold their child's details in a secured registry and to contact them in the future to determine in a longer follow-up study the effect of NEC and RIC on outcome.

Study setting

This trial will be conducted in 12 sites in six countries (Table 1). All collaborating centers are level III neonatal intensive care units (NICUs) with considerable expertise in establishing NEC diagnosis and implementing medical and surgical treatment. All collaborators have been involved in the development of this protocol. The selection of sites for this trial is based on previous successful

Table 1 Twelve collaborating centers

	Collaborating Centers
CANADA	1. The Hospital for Sick Children, Toronto, ON, Canada (Coordinating Center)
	2. Mount Sinai Hospital, Toronto, ON, Canada
	3. Sunnybrook Health Sciences Center, Toronto, ON, Canada
	4.McMaster Children's Hospital—Hamilton Health Sciences, Hamilton, ON, Canada
USA	5. Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
	6. Children's Hospital of Orange County, Orange County, California, USA
	7. Texas Children's Hospital, Houston, Texas
EUROPE	8. Karolinska University Hospital, Stockholm, Sweden
	9. Erasmus MC Sophia Children's Hospital, Rotterdam, The Netherlands
	10. The UCL Great Ormond Street Institute of Child Health, London, UK
	11. Southampton Children's Hospital, Southampton, UK
	12. La Paz University Hospital, Madrid, Spain

collaboration in multicenter observational and randomized trials [43, 45–63] involving the centers from Canada (Toronto), UK (London, Southampton), Sweden (Stockholm), the Netherlands (Rotterdam), Spain (Madrid), and the US (Cincinnati and Orange County).

Study participants

The neonates can be inborn or transferred from lower-level NICUs following NEC diagnosis.

Inclusion and exclusion criteria

The inclusion criteria for this study are as follows:

1. Preterm neonates with gestational age < 33 weeks [64].
2. Current weight ≥ 750 g.
3. Confirmed diagnosis of "medical" NEC based on the joint opinion of two attending physicians (two neonatologists or one neonatologist and one pediatric surgeon). For the trial, NEC will be defined according to pragmatic modification of prospectively evaluated criteria suggested by Battersby et al [65]. We selected the criteria associated with an odds ratio > 5 (NEC vs no NEC) [65] except for the presence of mucus in stool as we are concerned about the sensitivity of this feature. The criteria that will be used to diagnose "medical" NEC will be that at least two of the following clinical signs and one of the radiological signs need to be present:
    a. *Clinical signs* (1. abdominal distension; 2. abdominal tenderness; 3. abdominal discoloration; 4. blood in stool);
    b. *Radiological signs* (1. pneumatosis; 2. portal venous gas).
4. NEC diagnosis established 24 h before receiving study intervention.

The exclusion criteria for this study are:

1. Patients who have indication for surgical intervention in the joint opinion of the attending neonatologist and pediatric surgeon (i.e. surgical NEC). This diagnosis is based on the presence of pneumoperitoneum in the abdominal radiograph and/or failure of medical treatment for NEC;
2. Previous episodes of confirmed NEC;
3. Diagnosis of NEC established > 24 h ago;
4. Major congenital heart disease which needs surgical repair;
5. Antecedent limb ischemia/limb thrombotic events, or occlusive arterial or venous thrombosis;
6. Associated gastrointestinal anomalies including gastroschisis or congenital diaphragmatic hernia.

Recruitment

A neonatologist and a pediatric surgeon will provide local coordination in each center and will be supported by a local research fellow and/or nurse with medical expertise. NEC will be diagnosed in the joint opinion of two attending experts in the field (two neonatologists or one neonatologist and one pediatric surgeon). The attending neonatologist will be alerted to the presence of "suspected" NEC and will discuss the diagnosis of "confirmed" NEC with another attending neonatologist or pediatric surgeon. To expedite the decision-making process, the attending experts will communicate in person, by phone or, if needed, virtually through a platform dedicated to the trial. We believe that this can be done within our 24-h time frame. The 24-h time frame will begin as soon as the diagnosis of NEC is established. When the neonate is diagnosed with NEC, the local coordinators, research fellow and/or nurse, not involved in the circle of care, will be notified and will (i) screen for eligibility; (ii) seek permission from the circle of care to approach the parents/caregivers of patients who meet the inclusion criteria;

(iii) approach parents/caregivers of patients who meet the inclusion criteria to explain the study by providing in-person or virtually a leaflet written in lay terms; (iv) seek written in-person informed consent within 2 h of explaining the study and maintaining randomization to not exceed the 24 h from NEC diagnosis. Patients will be recruited as early as possible in their phase of illness. Information regarding the precise time of RIC administration will be collected to assess the time-dependent effect.

The leaflet and consent forms will be translated to various languages including Swedish and Dutch for our collaborating centers in Stockholm and Rotterdam. If consent to randomization is not given, the parents/caregivers will be asked if they consent to the collection of anonymized demographics and outcomes of their child.

At present, there is no ongoing trial competing for patient recruitment with the proposed feasibility phase II RCT. The total number of preterm neonates (gestational age < 33 weeks) at risk of developing NEC admitted during the last year in the 12 collaborating centers is 2675. Of these, we expect 244 neonates to be eligible during the 30 months of trial recruitment. Considering the retrospective evaluation of our eligibility criteria and the definition of "medical" NEC, we expect this number to be further reduced by 20%, therefore having 195 neonates eligible for the trial. On the basis of our safety trial [39], we expect to approach 80% of these patients ($n = 156$) and to recruit and randomize 40% of them (78 patients, 39 per arm). Our phase I pilot safety study did not show any dropouts during the trial [39]. However, an RCT may encounter some dropouts as well as other limitations. One of the aims of the proposed multicenter phase II trial is to determine the precise dropout rate as well as to identify other limitations to patient enrollment and retention in the trial.

Randomization

Patients will be randomized in a 1:1 ratio to RIC (intervention) or no RIC (control) by a trained research fellow and/or nurse not involved in the circle of care and using a 24-h internet-based randomization service (www.Randomize.net). Randomization will be stratified by clinical site employing random block sizes of 4 and 6. This service will be accessed via the trial website which will also be a source of information for the collaborating centers, parents, and clinicians. Study documentation will be available in a secured area.

Intervention

Intervention description

Neonates randomized to the intervention arm will receive RIC as well as the standard medical management (i.e. standard of care) for NEC. The RIC procedure will consist of 4 cycles of limb ischemia (4 min) followed by reperfusion (4 minutes) and a 5-minute gap before the next cycle of ischemia, repeated on two consecutive days. An appropriately sized blood pressure cuff (covering 2/3 of the distance between the shoulder and the elbow) will be applied by a trained research fellow and/or nurse to an arm (or leg if the arm is not available because of medical reasons such as central line insertion). The systolic blood pressure will be measured before the first RIC cycle using a different cuff of the same size connected to a monitor. During ischemia time, the cuff will be inflated to a pressure of 15 mmHg above the child's systolic pressure.

Control arm description

Neonates randomized to the control arm (no RIC) will continue to receive standard medical management (i.e. standard of care) for NEC without variation from current practice.

Masking

RIC will be masked from the local team of healthcare workers and parents. A trained research fellow and/or nurse not involved in the circle of care will perform, behind a portable sliding medical privacy screen, inflation/deflation of the cuff (RIC intervention) or sham inflation/deflation of the cuff connected to a dummy neonatal arm to mimic the noise of cuff inflation (control). Both limbs (receiving and not receiving RIC) will be blinded from the circle of care via the use of the privacy screen. Following the RIC procedure, the nurses and doctors in the patient's circle of care (masked to treatment allocation) will monitor the return of perfusion or occurrence of adverse events (see "Safety monitoring" and "Adverse events definition and reporting" sections).

In the case of the occurrence of serious adverse events (see "Adverse events definition and reporting" section), the study blind or participant code will be broken—the blood pressure cuff will be removed from the neonate's limb immediately and the team taking care of the patient will be notified. A member of the research team will notify verbally the circle of care including the nurse, staff neonatologist and surgeon of breaking the code. This event will be also (i) recorded in the data collection form and (ii) communicated to the Data Safety and Monitoring Committee (DSMC).

Anticipated risks

RIC implies inflating a standard blood pressure cuff around a limb, aiming to interrupt distal arterial blood supply to produce transient skeletal muscle ischemia. This may potentially lead to limb ischemia. In our phase I pilot safety study, limb perfusion was continuously monitored

during the RIC procedure and cautiously assessed after RIC, both visually and via pulse oximetry [39]. During RIC, the limb became dusky (venous congestion), and the pulse wave was lost due to total arterial compression (as expected and desired. Once the blood pressure cuff was deflated, quick reperfusion followed, usually with evident hyperemia in visual inspection [39]. Capillary refill time (CRT) was assessed before and after every ischemic cycle to quantify limb perfusion, which was always recovered within four minutes and the normal range (≤ 3 seconds) [39]. To monitor any effects on limb perfusion after the RIC procedure in this proposed trial, limb perfusion will be assessed in the safety monitoring of the RIC intervention (please see "Safety monitoring" section).

RIC could potentially lead to microhemorrhages (petechiae, ecchymosis, bruising). Also, the presence of an inflated blood pressure cuff over a more extended than usual period—blood pressure measurement—could lead to skin breakdown especially in this vulnerable population (neonates). In our phase I pilot study, we assessed the limb skin before and after RIC and found that no neonates had any issues regarding skin integrity or cutaneous bleeding [39]. Please see "Safety monitoring" section for a description of our assessment of limb skin in the safety monitoring for RIC.

RIC may also cause pain in the limb receiving the RIC stimulus. To assess the pain due to RIC in our phase I pilot study, pain was assessed before and after the RIC procedure [39]. Persistent pain was considered at 6 hours, as NEC is also a cause of pain, and it would be challenging to separate pain changes due to RIC or to the NEC itself. The pain was assessed using the validated Premature Infant Pain Profile (PIPP) score [66, 67]. In our phase I pilot study, there was minimal change in PIPP score comparing its value before and six hours after RIC [39]. Compared to the assessment before RIC, the PIPP score did not change from 'no pain' or 'mild-moderate to 'severe' pain six hours after the last ischemic cycle [39]. The PIPP score in 5 neonates (33%) increased from 'no pain' to 'mild-moderate pain, and in 2 neonates (13%) decreased from 'mild-moderate pain to 'no pain'. Please see "Safety monitoring" section for the description of our assessment of pain in the safety monitoring of RIC in this proposed study.

Muscle ischemia could lead to muscle necrosis and rhabdomyolysis. The most severe potential complication of rhabdomyolysis is oliguric acute kidney injury secondary to renal tubules obstruction with myoglobin affecting >50% of the kidneys. In our phase I pilot study, urine output 24 hours before and after RIC was assessed, and no significant differences were found, with no neonates becoming oligo-anuric (defined as urine output < 1 ml/kg/h) after RIC [39]. To monitor any effects on urine output after the RIC procedure in this proposed study, we will assess 24-hour urine output (ml/kg/h) before and after RIC to exclude potential secondary effects on renal function (please see "Safety monitoring" section).

Safety monitoring

Our phase I pilot safety study indicated no adverse events or complications in neonates with NEC undergoing RIC [39]. However, this study was based on 15 neonates in total, with 5 of them receiving RIC on two consecutive days. Therefore, we will extend our evaluation of the safety of RIC in the proposed phase II RCT studying a larger population of neonates in the intervention arm. This will be accomplished by assessing the proportion of neonates experiencing any adverse events following RIC (evaluation by the circle of care following RIC to maintain masking). Please see "Adverse events definition and reporting" section for the definition of adverse events.

Before starting RIC, skin integrity and perfusion (colour and CRT) will be assessed and a baseline measurement of blood pressure, heart rate, and oxygen saturation (via pulse oximetry) will be established. After each RIC cycle, the limb undergoing RIC will be assessed by the circle of care 6 h after RIC and re-evaluated at 24 and 48 h both clinically (skin integrity, visual inspection of colour and CRT), and by pulse oximetry (pulse waveform display and arterial oxygen saturation returning to baseline). Heart rate and blood pressure will also be assessed after the last cycle. In addition, we will record the 24-h urine output (ml/kg/h) before and after RIC to exclude potential secondary effects on renal function, and the premature infant pain profile (PIPP) score [66, 67] assessed 15 min before RIC (baseline), 30 s after each ischemic cycle, and 6 hours after the last cycle.

Adverse events definition and reporting

An *adverse event (AE)* is any untoward medical occurrence associated with the use of an intervention in a study participant, which does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign, symptom or disease temporally associated with the use of the intervention, whether or not considered related to the investigational intervention.

Stable chronic conditions which are present before entry in the study or worsen due to the progression of NEC are not considered AE. Anything that is a condition of prematurity or is related to the regular clinical course of the patient will not be an AE. These pre-existing conditions will be documented in the neonate's medical history.

A qualified neonatologist and a surgeon who is part of the study team will be responsible for determining whether an AE is expected or unexpected. An AE will be considered

unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

AE are classified as *serious* or *non-serious*:
Non-serious AE due to RIC include:

- *Skin breakdown* and/or skin microhemorrhages, petechiae, ecchymosis, and bruising in the limb receiving RIC persisting after 48 h post-RIC.

Serious adverse events (SAE) due to RIC include:

- *Limb ischemia* or lack of limb reperfusion 4 min after each ischemic cycle (assessed visually, and via pulse oximetry and CRT not returning to baseline values ± 3%). AE is considered if perfusion does not return to normal after 30 min of implementing strategies described in Sect. "Criteria for discontinuing or modifying intervention" (rescue intervention).

The occurrence of an AE or SAE may be detected during the safety monitoring of the RIC intervention (Please see "Safety monitoring" section), reported by a neonatologist, surgeon, or nurse in the circle of care, or identified by research staff. AE will be reported to The Hospital for Sick Children Research Ethics Board according to the hospital's AE Reporting requirements and as per local institutional and regulatory requirements at each participating site.

Criteria for discontinuing or modifying intervention

Interruption of RIC and rescue intervention: RIC will be stopped if there is no return of perfusion (clinical inspection) and no return of saturation (pulse oximetry) to baseline values ± 3% within 4 minutes in the arm/leg receiving RIC. If perfusion does not return during the expected time, a stepwise rescue intervention will be performed consisting of warming and elevation of the limb (10 minutes), followed by application of a nitroglycerine patch and Doppler ultrasound.

Discontinuation of the RIC intervention: On the second day of the RIC intervention, patients in the control (standard of care for NEC) and intervention group (RIC + standard of care for NEC) will be reassessed for (i) meeting the inclusion criteria, and (ii) not meeting the exclusion criteria. In the case that there is a change in the clinical status of the neonate, or the neonate has become clinically unwell or meets the exclusion criteria, the RIC intervention will not continue on Day 2, and the patient will be discontinued from the second day of the intervention. Discontinuation from the RIC intervention does not mean discontinuation from the study, and remaining study procedures should be completed as per the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the principal investigator will determine if any change in participant management is needed.

Trial stopping rules

Stopping rules: These are defined as one or more of the following: lack of limb reperfusion, or loss of limb cutaneous integrity after RIC in ≥5% of randomized patients at half-enrollment. The Data Safety and Monitoring Committee (DSMC) will assess all major adverse events within 24 hours and evaluate whether reperfusion, the urine output, and pain profile were related to the disease process (i.e. progression of NEC) or to the RIC maneuver.

Data collection and management

Data being collected

1. Patient confidential demographics (gestational age, birth weight (g), postnatal age (weeks + days) at RIC, and weight at RIC (g))
2. Clinical monitoring parameters once at inclusion (baseline), then 12-hourly for 3 days, and then daily until discharge from the NICU. These include physiological parameters (body core temperature, respiratory rate, heart rate, oxygen saturation, and blood pressure), bright red blood from rectum, and abdominal examination for presence or absence of bowel sounds, abdominal tenderness, cellulitis, or right lower quadrant mass. The severity of illness will be scored according to the Neonatal Sequential Organ Failure Assessment Score (nSOFA) [68, 69];
3. Abdominal X-ray (pneumatosis intestinalis, portal venous gas, and pneumoperitoneum) when clinically indicated for deterioration;
4. Adverse events occurring within 24 h after RIC (please see "Safety monitoring" and "Adverse events definition and reporting" sections);
5. Primary and secondary outcomes as described in Sect. "Outcomes";
6. Proportion of patients for which the above clinical outcomes are not recorded as well as reasons for incomplete recording of outcomes;
7. Frequency of participants who completed the study;
8. Frequency of protocol deviations.

Data collection

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Study data will be entered into REDCap (Research Electronic Data Capture), a secure, web-based application designed exclusively to support data capture for research studies. REDCap is developed and maintained by a team at Vanderbilt University and licensed free of charge by the Research Institute at The Hospital for Sick Children. The application and data are housed on servers provided by The Hospital for Sick Children. These servers are located within the Hospital for Sick Children secure data centre. Local support for REDcap is provided by the Hospital for Sick Children Research IT.

Data Management and Record-Keeping

Data will be collected and stored in accordance with local Data Protection and Privacy regulations and transmitted electronically to the coordinating center (The Hospital for Sick Children). Data will be recorded electronically and de-identified on a password-protected custom-made database with a secure backup copy. A customized REDCap database will be developed for this project to facilitate and coordinate multi-site data entry and to streamline data transfer and management. This will be carried out at the lead center by the Data Manager in coordination with the Institution's Clinical Data Management Group. The database will be hosted on the Hospital's web and database servers with appropriate security measures in place to ensure data confidentiality. Credentialing to access REDCap will be managed centrally. Individual access will be limited to data from individual sites.

Outcomes

Primary outcome

The primary outcome of this study is to determine the feasibility of recruitment, randomization, and delivery of intervention to eligible neonates within 24 h from the diagnosis of NEC. We have shown that the beneficial effects of RIC in a preclinical experimental model of NEC are present in the early stages of the disease and not when the intestine has suffered irreversible damage such as necrosis [16]. Therefore, we aim to ascertain whether it is feasible to recruit neonates with NEC within the optimal time window of treatment opportunity (24 h from diagnosis). To assess this, we will examine the probability that:

1. a screened patient is eligible;
2. an eligible patient consents and is randomized within 24 h from diagnosis;
3. the RIC maneuver is masked from caregivers;
4. a randomized patient receives allocated intervention;
5. a randomized patient receive intervention within 24 h from NEC diagnosis;
6. a randomized patient is assessed for the NEC-related outcomes which we have selected as potential elements of "primary composite outcome" of the future phase III trial.

Feasibility of masking the RIC intervention is to determine the feasibility of masking of healthcare workers (responsible nurse, neonatologist, surgeon) and parents to treatment allocation (RIC or no RIC). As the application of RIC requires approximately 45 minutes and can be masked, we aim to determine whether it is feasible to mask this procedure from health care workers and parents. Please see "Masking" section for information regarding masking of the RIC intervention.

In a series of meetings held among all trial investigators including our trial statistician, we reached consensus and defined the "primary endpoint" of the future p*hase III* efficacy trial. We deemed the most objective and valid outcome to assess RIC effectiveness in NEC to be a composite of "survival at 1 month and 3 months without NEC-related intestinal complications including perforation, necrosis or stricture". Development of NEC-related intestinal complications is defined by the presence of one or more of the following: (i) *Intestinal perforation*: diagnosed by the presence of free intraperitoneal air on an abdominal radiograph or visualized at laparotomy. Intestinal perforation can be due to spontaneous idiopathic intestinal perforation or NEC. However, as these conditions may represent different endpoints of the same underlying pathway, similarly to previous studies, the outcome definition has deliberately been designed to encompass both entities [42, 43, 70]. (ii) *Intestinal necrosis*: diagnosed by visual inspection at laparotomy. (iii) *Intestinal stricture*: diagnosed at laparotomy and confirmed by histopathology of the resected specimen. It is expected that neonates who develop intestinal necrosis or stricture would undergo a laparotomy or die from the disease.

Collecting the following NEC-associated outcomes is important but we recognize that they can be influenced by subjectivity and/or have unclear dependency from NEC, hence the reason for considering them secondary outcomes. During the 90 days post-randomization, the clinical status of the patient including the length of hospital stay will be monitored. During the same period, we will record the timing and cause of death considering whether it was possible to determine if death was related to complications

of NEC or to a disease process in other systems including cardiac, neurological, respiratory, renal, metabolic. NEC can result in severe illness, requirement of surgery, affect growth, and can increase the risk of developing myriad of clinical sequelae not only in the intestine but also in other organs such as brain, lung, eye, as well as prolonged hospitalization. The following outcomes related to severity of NEC and effect on enteral feeding will be collected during the 90 days post-randomization: (i) Administration of inotropes (number of days of administration) [71]. (ii) Abdominal operation performed. These include insertion of peritoneal drainage or laparotomy [72]. We will record whether one or more operation was performed and when the operation was performed. (iii) Intestinal function will be assessed by measuring at 90 days post-randomization the number of patients receiving parenteral nutrition. The injury to other organs will be assessed by recording: (i) Development of severe neurological injury: This is based on head ultrasound and defined as the presence of IVH, ventricular enlargement or parenchymal echogenicity or PVL. According to Canadian Neonatal Network [64], PVL is defined as grade 3 IVH (intraventricular hemorrhage with ventricular enlargement) or grade 4 IVH (intraventricular hemorrhage and persistent parenchymal echogenicity) or persistent parenchymal echogenicity. Head ultrasound examination will be performed according to published guidelines [73]. (ii) Development of CLD: Defined as respiratory support given at 36 weeks' postmenstrual age or at discharge (if earlier than 36 weeks' postmenstrual age) to level 2 NICU and classified in different degrees of severity from mild to moderate to severe chronic lung disease (CLD) according to the criteria published in the Canadian Neonatal Network (CNN) Annual Report (2019) [64]. (iii) Development of severe ROP: Stage 3, 4 or 5 retinopathy of prematurity (ROP) as defined by the International Classification of ROP and/or those infants requiring treatment (laser or intraocular injection) [74]. ROP will be scored as the highest stage in either eye identified at any time.

Secondary outcome

The secondary outcome of this study relates to stakeholder's satisfaction to determine the facilitators and barriers to participation in a future *phase III* trial, and the acceptability and accurate recordability of outcome measures. We will use qualitative methods to evaluate the view of healthcare workers and parents/caregivers on the recruitment process and RIC intervention. This will allow us to identify factors affecting enrollment and adherence to the trial such as healthcare workers' and parents/caregivers' attitudes, beliefs and behaviour. Ultimately, we will benefit from the input of all these stakeholders. A questionnaire will be distributed to parents/caregivers and healthcare workers not involved in the trial at enrollment and 1 month and 3 months from randomization to evaluate their view on the trial and to assess their comments. The same questionnaire will be given to the research personnel in the trial. A simpler questionnaire will be distributed to parents/caregivers who do not consent on randomization to evaluate reasons for declining and/or withdrawing consent. Anonymity will be maintained in collecting this information. Questionnaires are available on request.

Exploratory outcomes

Intestinal oxygenation and perfusion: In 30 patients (15 per group) from 2 centers (Hospital for Sick Children in Toronto and Karolinska University Hospital in Stockholm), we will determine whether the hemodynamic response to RIC can be quantified by assessing:

1. *Intestinal oxygenation by near-infrared spectroscopy (NIRS)* at baseline (before RIC) and continuously for 48 h after RIC. NIRS monitors real-time tissue oxygenation and detects changes in splanchnic tissue oxygenation. Readings from NIRS will be blinded from patients' circle of care and will only be collected by our research team for later analysis. Of note, the use of NIRS has not been validated in monitoring the progression of NEC but only for diagnosis Therefore, we do not currently have a standardized procedure for NIRS and this is something we aim to establish in this trial; hence, NIRS will be assessed as an exploratory outcome only in the named centers which use NIRS as part of the standard of care for neonates with NEC.
2. *Intestinal perfusion by abdominal colour doppler ultrasonography* at baseline (before RIC) and 48 h after RIC. As it is very challenging to standardize ultrasound imaging across the various centers and in 6 countries, we will instead perform ultrasound only in the centers which routinely perform this investigation as part of standard of care such as the coordinating center, The Hospital for Sick Children, where the use of bowel ultrasound in the diagnostic evaluation of NEC was described and first established. This will enable us to first explore the feasibility and sensitivity of using ultrasound in our study and to later translate our knowledge to the other centers and provide standardized training to specialists at other facilities for the future phase III trial on the efficacy of RIC for neonates with NEC. The findings from abdominal ultrasound alone will not be considered diagnostic enough for eligibility. We will collect information on the abdominal ultrasound in the sites which conduct this

investigation as part of standard of care for neonates with NEC but will not use it as diagnostic or mandatory criteria.

Determination of sample size

In the 12 international collaborating centers, we expect to randomize, in 30 months, 78 patients with NEC receiving standard of care (39 per arm) which represents 40% of approached eligible neonates. If the phase III trial is, in truth, feasible, then the evidence from the phase II trial, based on approaching 244 eligible patients, will be sufficient to conclude that the phase III trial is feasible and will have adequate power for clinically important differences in the primary outcome.

To determine the adequacy of the proposed sample size, consider the null hypothesis that the phase III trial is not feasible. The specific alternative hypothesis has three components, namely that in the phase III trial (i) the probability that an eligible patient consents and is randomized is at least 0.4, (ii) the probability that a randomized patient is treated in the arm to which they were assigned is at least 0.95, and (iii) the probability that a randomized patient is evaluated for the primary outcome is at least 0.95. We postulate that under this hypothesis, the phase III trial is feasible. Under the assumption of the alternative hypothesis, we used simulations to determine, given the information (and associated uncertainty) from approaching eligible 200 patients in the phase II trial, the maximum number of eligible patients that need to be approached (Y) and the maximum number of patients that need to be randomized (X) to achieve the required power for the phase III trial [75]. The number of patients in the phase III trial to achieve the required power will depend on the smallest clinically important difference (SCID) for the primary outcome. From the simulations, if the SCID is 20 percentage points, then $Y = 500$ and $X = 175$. That is, a strategy of approaching eligible patients until either the number approached is 500 or the number randomized is 175 has an expected power of 80%. In summary, if the alternative hypothesis is true, i.e. the phase III trial is feasible, then the evidence from the phase II trial, based on approaching 200 eligible patients, will be sufficient to conclude that conducting the phase III trial is feasible and will have adequate power for clinically important differences in the primary outcome.

Statistical methods

The following feasibility parameters are of interest:

1. The probability that a screened patient is eligible;
2. The probability that an eligible patient consents and is randomized within 24 h from diagnosis;
3. The probability that the RIC maneuver is masked from caregivers;
4. The probability that a randomized patient receives allocated intervention;
5. The probability that randomized patients receive intervention within 24 h from NEC diagnosis;
6. The probability that a randomized patient is assessed for the NEC-related outcomes which we have selected as potential elements of the "primary composite outcome" of the future Phase III trial.

Bayesian methods will be used to combine uninformative prior distributions with the corresponding observed proportions from the phase II trial to determine posterior distributions for the parameters. The corresponding predictive distributions of these posteriors will be used to determine the feasibility of successfully conducting the phase III trial [75]. That is, the predictive distributions from the Phase II trial can be used to determine Y, the maximum number of eligible patients that need to be approached and X the maximum number of patients that need to be randomized to achieve the required power for the phase III trial. If enough clinical sites can be recruited for the phase III trial so that it is reasonable to expect that Y eligible patients can be approached during the proposed recruitment period, the phase III trial will be considered feasible. Beta distributions will be used for the priors and posteriors, with the corresponding predictive distributions being Beta-binomial. All screened patients will be included in item 1. All eligible patients will be included in item 2. All randomized patients will be included in items 3–6. Similar Bayesian methods will be used to provide posterior distributions for the probability of recording each outcome measures.

Data monitoring

To ensure that the trial progresses in accordance with guidelines for good clinical practice in multicenter trials, a Data Safety and Monitoring Committee (DSMC) will monitor trial data for ethical or safety considerations. The DSMC will be independent of both the trial management group and those providing therapy; this will be chaired by independent clinician or surgeon not involved in trial management or design and will include: (i) a pediatric surgeon and a neonatologist independent of the trial; (ii) a parent representative; (iii) a statistician. DSMC Terms of reference and Charter will be developed, based on the DAMOCLES (Data Monitoring Committees: Lessons, Ethics, Statistics) Study Group [76] and StaR Child health Standard for Research with Children [76, 77] and agreed at an initial meeting at the beginning of the trial prior to the onset of recruitment.

The DSMC will meet on a planned basis every six months. Additional meetings will be convened as required and as directed by the committee's chairperson and/or the TSC. The DSMC Statistician will be independent from the Trial and will receive data from the Trial Statistician. The DSMC will review initial and final analyses and other safety data have the power to recommend modification or closure of the trial to the steering committee. Efficacy will not be assessed during the trial and thus, the closure of trial will not occur for "excessive benefirt" or "futility". Final decision will be made by the steering committee of the trial. Further details about the DSMC Charter can be found along with the trial registration on www.clinicaltrials.gov.

Key roles and study governance

The *coordinating center*, based at The Hospital for Sick Children in Toronto, will comprise of (i) the principal investigator who will assume overall responsibility for successful study completion; (ii) part-time research coordinator and (iii) part-time clinical research fellow. They will coordinate the activities of all collaborating centers, monitor progress, prepare reports and facilitate links among the trial committees.

Each *collaborating center* will be composed of two link persons (neonatologist and pediatric surgeon) in addition to the local research personnel supported by the trial.

The day-to-day management of the trial will be coordinated by a *Trial Management Group (TMG)* based at the coordinating institution and comprising the principal applicant and the personnel appointed for this RCT. This resource has been successfully implemented by some of the applicants in previous multicenter RCTs [7, 63, 78]. The TMG will prepare and distribute study documentation and case report forms, check data quality, monitor recruitment and provide day-to-day support to participating centers by e-mail. The TMG will work together and formally meet on a weekly basis for the duration of the trial.

A *Trial Steering Committee (TSC)* will be convened to provide overall supervision of the trial and ensure that the trial is conducted to rigorous scientific, clinical, and ethical standards. The committee will be chaired by Dr. Prakesh Shah and there will be one co-investigator from each recruitment site as well as two independent members (Pediatric Surgeon and Neonatologist) from centers not involved in the trial. The TSC will be convened at the beginning of the trial to agree on the terms of reference. Responsibilities will include the operation of the trial and decisions on major changes that need to be made to the study protocol. The committee will communicate via e-mail and have monthly conference calls to discuss study issues. Three-monthly meetings are planned to discuss study progress and any relevant issues.

Please see "Data monitoring" section for information regarding the composition and responsibilities of the DSMC.

Participant discontinuation/withdrawal from the study

Parents/caregivers are free to withdraw their neonate from participation in the study at any time upon request. The PI may discontinue or withdraw a participant from the study for the following reasons:

- Withdrawal of informed consent (participant or parent/guardian withdraw for any reason)
- If any clinical AE, laboratory abnormality or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Significant study intervention non-compliance
- Disease progression (e.g. need for surgical intervention) which requires discontinuation of the study intervention
- Requirement of prohibited concomitant medication(s) that requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant is unable to receive the RIC intervention for two consecutive days.

The reason for participant discontinuation or withdrawal from the study will be recorded in the data collection log. Participants for whom parents/caregivers sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will be replaced. The data from participants who are withdrawn or discontinued from the study will be used in the analysis unless the parents/caregivers of the patient request otherwise.

Confidentiality

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the Sponsor (The Hospital for Sick Children, Agostino Pierro). This confidentiality is extended to cover clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. All research activities will be conducted in as private a setting as possible.

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Discussion

The main contribution of the proposed trial will be to determine whether a masked multicenter phase II RCT for RIC in neonates with early-stage medical NEC is feasible.

To the best of our knowledge, the safety and feasibility of RIC have not been investigated in preterm neonates with NEC to date. The findings of our phase I pilot safety study conducted at The Hospital for Sick Children, including 15 neonates, demonstrated no adverse events or complications due to the RIC intervention. However, the safety of RIC needs to be evaluated in a multicenter setting and a larger patient population. In our proposed phase II RCT, we will be using the same parameters as in our phase I study to further validate the safety of RIC in neonates with NEC.

A phase II Feasibility RCT is also necessary to address existing knowledge gaps related to the design of a future phase III RCT to evaluate the efficacy of RIC in the *treatment* of neonates with NEC. These knowledge gaps include: (i) uncertainties related to the recruitment, randomization and retention of patients, masking and acceptability of the RIC intervention by healthcare workers and parents/caregivers; and (ii) the outcomes of NEC are poorly defined and partly influenced by subjectivity. To our knowledge, 40% of RCTs in children were discontinued prematurely [79] and the only two trials published on NEC treatment have not reached the predetermined power due to difficulties in multicenter recruitment, randomization, and acceptability of the RCT [42, 43]. Therefore, a stepwise approach of conducting a phase II Feasibility RCT before embarking on a large efficacy phase III RCT, is necessary to anticipate and correct issues related to enrollment, randomization, retention, masking, acceptability of the RCT, and measurement of clinical outcomes.

The protocol illustrated in this article has been finalized before the recruitment into the trial started and reports important aspects of the design, conduct, reporting as well ethical considerations. The protocol has been prepared following the SPIRIT 2013 guidelines [44].

Conclusion

This phase II trial could determine the feasibility of identifying, recruiting, randomizing, and treating neonates with RIC within the optimal window of treatment opportunity (24 h from confirmed diagnosis of medical NEC), the feasibility of masking the RIC intervention, the feasibility of recording NEC outcome measures and calculating the sample size needed to establish efficacy in the future *phase III* RCT, and the satisfaction of parents/caregivers and healthcare workers with the recruitment process and RIC intervention.

Data availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Conflict of interest No conflict of interest has been declared by the authors in relation to the study itself.

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