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ORIGINAL ARTICLE

Low technology, mild controlled hypothermia for necrotizing enterocolitis treatment: an initiative to improve healthcare to preterm neonates.

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Abstract

Necrotizing enterocolitis (NEC) treatment remains unchanged for years. Data suggest that mild controlled hypothermia could potentially improve NEC outcomes. Our units presented unfavourable outcomes on NEC. The aim was to assess our experience with low technology, mild controlled hypothermia on NEC outcomes, and improve preterm infants' healthcare. This was a single-center quality improvement study with retrospective cohort design at the neonatal intensive care unit in the university hospital. Forty-three preterm infants with NEC (Modified Bell's Stage II/III) were included: 19 in the control group (2015–2018) and 24 in the hypothermia group (2018–2020). The control group received standard treatment (fasting, abdominal decompression, and broad-spectrum antibiotics). The hypothermia group underwent cooling to 35.5 °C for 48 h after NEC diagnosis, along with conventional treatment. The primary outcomes are intestinal perforation, need for surgery, duration of parenteral nutrition, death, and extensive resection of the small intestine. There was no statistical difference in the NEC score. The hypothermia group required less surgery (aRR 0.40; 95% CI 0.19–0.85), presented less bowel perforation (aRR 0.39; 95% CI 0.18; 0.83), had a shorter duration of parenteral nutrition (aHR 5.28; 95% CI 1.88–14.89), did not need extensive intestinal resection, (0 vs 15.7%), and did not experience any deaths (0 vs 31.6%).

Conclusions: In our experience, low technology, mild controlled hypothermia was feasible, not related to adverse effects, and effective treatment for NEC Modified Bell's Stage II/III. It avoided surgery, bowel perforation, and extensive intestinal resection; reduced mortality; and shortened parenteral nutrition duration.

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What is Known:
- *New approaches have been proposed to avoid enterocolitis incidence; however, the treatment of enterocolitis stage 2 has been the same for decades, and unfavourable outcomes remain despite conventional management.*
- *Studies suggest that hypothermia can be an alternative to enterocolitis treatment.*

What is New:
- *Mild controlled hypothermia can be an additional practice to treat enterocolitis stage 2, is feasible, and is not related to adverse effects to preterm infants.*
- *It can decrease surgery needs, duration of parenteral nutrition, and death and avoids extensive intestinal resection in preterm infants.*

Keywords Necrotizing enterocolitis · Hypothermia · Preterm infant · Small intestine

Introduction

Necrotizing enterocolitis (NEC) is one of the main causes of mortality in very low birth weight (VLBW) preterm infants, resulting in a great burden to families and the health system [1–3]. Its incidence varies from 5 to 10%, and its mortality is high (15 to 30%) [1–4]. Moreover, 30–50% of NEC patients require surgery and many develop intestinal failure, prolonged dependence on parenteral nutrition, and worse neuropsychomotor development [1–10].

Despite being extensively investigated, NEC incidence has not decreased in recent years [1–10]. Moreover, while most current research addresses NEC prevention, its treatment has not changed in the last decades [11–13].

In 2010, Hall and colleagues reported the feasibility and safety of mild controlled hypothermia in the treatment of moderate NEC in preterm infants [14] based on previous studies [15–19].

In our centre, despite the adoption of the best practices and evidence-based protocols for NEC treatment, NEC incidence in VLBW preterm infants was high and outcomes were poor. We investigated the risk factors associated with the worst outcome; however, no predictor was identified. The NEC surgery rate (2015–2018) was 78.9%, lethality rate was 31.5%, and short bowel syndrome was 15.7% [20].

Therefore, since 2018, we have been proposing to families low technology, mild controlled hypothermia as a compassionate treatment, which is an adjunctive therapy for NEC-Modified Bell's stage II/III cases [21].

This study aimed to report our experience on the feasibility and efficacy of this intervention on NEC outcomes.

Methods

In this quality improvement study with retrospective cohort design, we included preterm infants from a university, tertiary level NICU (neonatal intensive care unit) admitted from 2015 to 2020. This study was approved by the Institutional Review Board of Ribeirão Preto Medical School, University of São Paulo (FMRP-USP, CAAE number 29879620.5.0000.5440, approval number 3.920.223). The study followed the SQUIRE guidelines [22].

Inclusion criteria were preterm infants admitted to the NICU of Ribeirão Preto Medical School, University of São Paulo, with a confirmed diagnosis of NEC-Modified Bell's Stage II/III (mild to moderate systemic illness, absent bowel sounds, pneumatosis intestinalis, or portal venous gas). Major congenital malformations, genetic abnormalities, missing data in medical records for the correct diagnosis, no parental consent, NEC modified Bell's Stage 1, NEC fulminant, and spontaneous perforation were not included. The NEC fulminant is characterized by rapid clinical progression and severe hemodynamic instability (need of two or more vasoactive drugs), with 24 h or less from the onset of symptoms to death. Diagnosis and stage classification were performed by the staff at the moment of onset and checked retrospectively by two researchers. Parents were informed about the compassionate treatment and agreed to its conduction.

The participants were divided into two groups, before (control) and after (hypothermia) we introduced low technology, mild therapeutic hypothermia in our service. The control group received standard treatment (normothermia, fasting, abdominal decompression, and broad-spectrum antibiotic therapy) from February 2015 to May 2018. The hypothermia group underwent cooling to 35.5 °C for 48 h after NEC diagnosis, along with standard treatment, from June 2018 to February 2020. All patients received human milk at birth (increased daily by 20 mL/kg, full feed 180 mL/kg). According to our nutrition guidelines, premature infants receive human milk until reaching the full feed (180 ml/kg). Premature infants (<1500 g) receive fortified breast milk when the feed reaches 100 ml/kg. After the good tolerance of the full feed, if the mother chooses not to breastfeed or does not have enough milk production, it is transitioned to preterm formula.

Histamine-2 receptor blockers were not used; antibiotic use was supervised by the hospital infection control committee; the indication of blood products follows the hospital guidelines and is supervised by the institution's blood bank. Diagnosis and staging of NEC, followed by the indication of hypothermia, were performed by attending physicians.

The institutional standard surgical treatment for NEC is surgery if pneumoperitoneum radiological diagnosis, progressive clinical deterioration despite maximal medical therapy, or positive paracentesis.

Low technology, mild controlled hypothermia protocol

The low technology, mild hypothermia protocol consisted of cooling patients with NEC-modified stage Bell II/III to 35.5 °C (± 0.5 °C) as the target temperature for 48 h. The mild hypothermia protocol was elaborated based on Hall et al. (2010) [14]. Patients in the hypothermia group were cooled for 1 h by turning off the incubators' heating source, exposing them to a room temperature of 24 °C (± 1 °C) on automated room conditioning. Temperature was continuously monitored with a transoesophageal probe connected to a multi-parameter monitor (Dixtal Dx 2022). During the cooling phase, temperature and vital signs were monitored by the team every 15 min. If temperature variations were lower than expected, the incubator's heating mechanism was activated, and a target temperature of 35.5 °C was established. If there was difficulty in lowering the temperature, ice packs were placed under the mattress.

After the target temperature was reached, it was maintained for 48 h, with cardiac rhythm and vital signs monitored every 30 min. After this period, rewarming was performed at 0.5 °C per h, up to 36.5 °C, using the servo-control of the incubator. Neonatal Infant Pain Scale (NIPS) was performed for all patients at the moment at the time of NEC diagnosis; when NIPS is >3, the patient received analgesia with fentanyl (0.5 mcg/kg/h). During hypothermia, the CONFORT scale was performed, and the dose of fentanyl was titrated according to the result (17–26 score was considered adequate analgesia). Laboratory tests were collected before hypothermia and at each 12 h until rewarming. Fulminant NEC did not undergo hypothermia treatment.

The main efficacy outcomes were (a) intestinal perforation confirmed by surgical findings; (b) need for surgery due to signs of intestinal perforation or significant clinical worsening of the patient's general condition despite maximal clinical therapy or positive paracentesis; (c) duration of parenteral nutrition, considering the time required for full enteral feeding after NEC; (d) death within 30 days after NEC diagnosis or anytime if related to extensive bowel resection; and (e) extensive resection of the small intestine ≥40 cm, with ileocecal valve removal, or ≥ 60 cm, without valve resection.

To assess the repercussions of low technology, mild hypothermia, we studied the following outcomes, only in the hypothermia group, during cooling and after rewarming: arterial blood gas analysis, blood cell counts, and coagulation panel (only in case of bleeding) performed before, during, and after hypothermia for arterial blood sampling; and cranial ultrasound, obtained before and 7 days after hypothermia. The radiology team performed ultrasound reports according to the Volpe criteria [23].

To monitor signs of intestinal involvement (pneumatosis, oedema, bowel loop perfusion), radiological and/or ultrasound images of the abdomen were performed and revised by the institution's neonatology and radiology teams immediately, before and up to 12 h after hypothermia, according to literature recommendations on POCUS [24], using the Philips HD11 XE Ultrasound System (Philips Medical Instruments Bothell, WA, USA) with a 12–8 MHz microconvex transducer.

Patient characterization

To characterize and compare the groups of patients, we used a mortality risk >4 at the time of diagnosis according to the neonatal Sequential Organ Failure Assessment (nSOFA) [25]. To evaluate the risk of developing NEC, we used a GutCheck^{NEC} score > 32 (high and very high risk) [26].

We also recorded the following data: antenatal corticosteroid use, gestational age according to a first-trimester ultrasound, presence of maternal chorioamnionitis (assessed by the obstetrics staff), adequacy of birth weight for gestational age according to Intergrowth 21st standards [27], and postconceptional age and gestational age at NEC onset.

At the time of NEC diagnosis, feeding was categorized as human milk (when the newborn received pasteurized, raw, or fortified breast milk), fasting, or preterm formula. Ventilation support was defined as the use of mechanical ventilation or continuous positive airway pressure (CPAP).

Body temperature was closely monitored for 48 h after cooling started (hypothermia group) or after NEC diagnosis (control group).

Statistical analysis

To compare the groups regarding baseline variables, Wilcoxon's non-parametric or Fisher's exact tests were used, where appropriate. Regarding the time course of clinical and laboratory variables in the hypothermia group, linear regression models were adjusted under the Bayesian approach, along with a random effect to address intra-individual variability, estimating differences between means and 95% credible intervals (95% CrI). The RJAGS package of R 4.0.2 software was used.

To estimate the relative risks for the hypothermia group (control group as a reference) regarding intestinal outcomes, simple and multiple log-binomial regression models were adjusted considering nSOFA and gestational age as covariates in the estimation of adjusted relative risks through PROC GENMOD from the SAS 9.4 software. To compare the duration of parenteral nutrition between groups, we fitted a Cox proportional hazards model, estimating the hazard ratio with a

95% confidence interval. A significance level of 5% was adopted.

Results

A total of 677 preterm babies were born weighing less than 1500 g during the study period, with an overall incidence of NEC of 9.6% (65 cases/677 births). The incidence of NEC was 9.0% (35 cases/390 births) in the control group (2015–2018) and 10.7% (30 cases/287 births) in the hypothermia group (2018–2020). After the diagnosis review, 22 patients were not included [NEC Bell's Stage I ($n = 7$/ control group) due to NEC fulminant diagnoses ($n = 3$/ 2 control group; 1 hypothermia group), absence of data for proper characterization ($n = 3$/ control group), genetic syndromes or major malformations ($n = 7$/ 4 control group; 3 hypothermia group), and spontaneous intestinal perforation diagnosed by laparotomy ($n = 2$/hypothermia group)]. Thus, 43 patients with confirmed NEC (modified Bell's Stage II/III) were included in the study: 19 in the control group and 24 in the hypothermia group. Table 1 shows the patients' baseline characteristics.

The proportion of patients with an nSOFA score > 4 (8.3% vs. 47.3%; $p < 0.01$) was significantly lower in the hypothermia group than in the control group, while postconceptional age at onset (32.6 vs. 30.3 weeks, $p < 0.02$) was significantly higher in the hypothermia group than in the control group; therefore nSOFA and postconceptional age at onset were considered covariables to statistical adjustment.

Figure 1 shows that infants in the hypothermia group maintained the target low temperature during the protocol, while infants in the control group had temperatures within the normal range. The mean of the time to reach the targeted temperature (35.5 C) was 154.5 min (SD 128.87 min).

Online resource present estimates for the differences in the laboratory and clinical variables from the time of NEC diagnosis up to 24 or 48 h for infants in the hypothermia group (Table 1 online resource).

Arterial pH significantly increased in the first 24 h. Haemoglobin, arterial lactate, and platelet counts increased over the initial values of the 48 h protocol, while bicarbonate decreased. Other variables did not change significantly between these time points.

Only one patient (4.6%) in the hypothermia group experienced bleeding and had a confirmed coagulation disorder (prothrombin time, 14 s; activated partial thromboplastin time, 37.3 s), requiring fresh frozen plasma transfusion (10 mL/kg).

None of the patients needed to receive or increase the use of vasoactive drugs or saline expansion during or after hypothermia. During hypothermia, two infants (8.3%) required ventilation support because of apnoea. All patients were receiving analgesia (fentanyl 0.5 mcg/kg/h) when the cooling started, prescribed at NEC diagnosis due NIPS >3.

Due to technical problems, no patients in the control group, and only ten neonates in the hypothermia group (41.6%) underwent bowel ultrasound monitoring between 2 and 6 days after NEC diagnosis. In four of them (40%), ultrasonographic findings remained unchanged, and in six (60%), imaging signs of improvement were detected after cooling, as shown in Fig. 1 (online resource).

On cranial ultrasound, performed up to 7 days after hypothermia, 23 (95.8%) patients exhibited the same radiological pattern, and only one (4.1%) patient, who had previously had unilateral intraventricular haemorrhage grade I, presented with bilateral intraventricular haemorrhage grade I at the end of hypothermia.

Efficacy outcomes

The efficacy outcomes are summarized in Table 2. Patients in the hypothermia group needed less surgery (aRR 0.40; 95% CI 0.19–0.85) and had less intestinal perforation (aRR 0.39;

Table 1 Baseline clinical and demographic characteristics of participants

Variables	Hypothermia (n=24)	Control (n=19)	*p value*
Birth weight, g, median (IQR)	1197 [840–1870]	1000 [780–1140]	0.07
Gestational age, weeks, median (IQR)	30.3[28.6–31.9]	28.1[26.4–29.3]	<0.01
Antenatal steroids, n (%)	20 (83.33%)	17 (89.47%)	0.68
Chorioamnionitis, n (%)	0 (0.00)	3 (15.79%)	0.08
Small for gestational age*, n (%)	9 (37.50%)	2 (10.53%)	0.08
Apgar 5th min, median (IQR)	8.5 [8–9]	8 [7–9]	0.07
Chronologic age at NEC** onset, days, median (IQR)	12 [7.5–22.5]	16 [6–29]	0.63
Postconceptional age at NEC onset, days, median (IQR)	231 [215–244]	214 [193–233]	0.02
nSOFA >4, n (%)	2 (8.3%)	9 (47.3%)	< 0.01
Human milk, n (%)	17 (70.8%)	13 (68.4%)	0.42
GutChec^{NEC} score>32, n (%)	18(70.8%)	18(94.7%)	0.06
Platelet count at NEC onset, $\times 10^3/mm^3$, median (IQR)	234 [134–503]	217 [104–324]	0.39

Legend: *, based on Intergrowth 21st standards; ** necrotizing enterocolitis

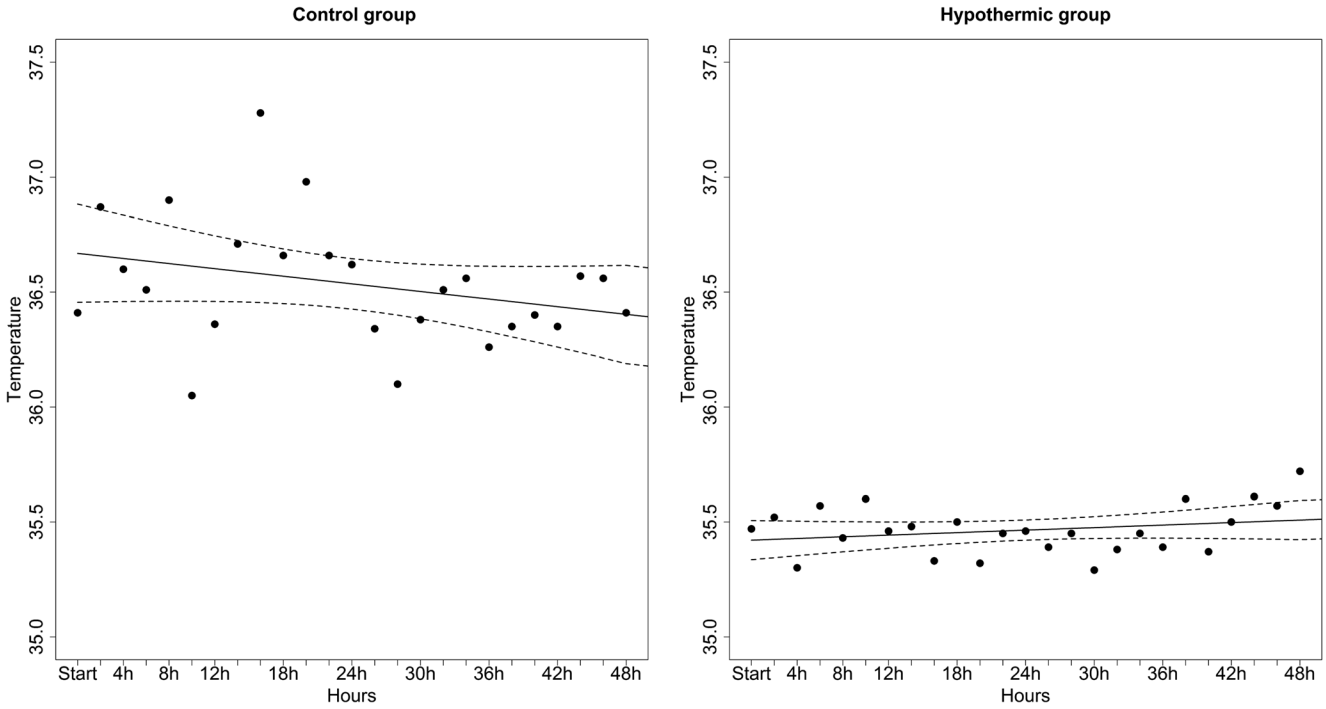

Fig. 1 Distribution of average temperature in the groups during 48 h after NEC diagnosis. Intervention: **(a)** Hypothermia group: cooling. **(b)** Control group: conventional treatment

95%CI 0.18–0.83) and days of parenteral nutrition (aHR 5.28; 95%CI 1.88–14.89). No deaths occurred in the hypothermia group compared with 6 (31.6%) in the control group ($p < 0.01$). Extensive intestinal resection did not occur in the hypothermia group, compared to 3 (15.7%) in the control group ($p = 0.08$). All pneumoperitoneum cases were diagnosed by radiography and confirmed via laparotomy. The process control chart (Fig. 2) presented the intervention effects (hypothermia) on efficacy outcomes during the study period.

Discussion

We herein report a single-center successful experience with low technology, mild controlled hypothermia as adjuvant therapy for moderate NEC management. This intervention induced only minor changes in laboratory and clinical parameters, while significantly reducing the need for surgery, intestinal perforation frequency, length of parenteral nutrition, and mortality. The initiative improved NICU outcomes and presented a positive impact on neonatal care (Fig. 2).

In general, baseline characteristics were similar between the two groups. Regarding the GutCheckNEC score, no differences between groups were found in the. Moreover, the platelet count was not different between the groups at the time of diagnosis, suggesting that the NEC severity was similar between them, and also the groups presented similar chronological age at NEC onset. Gestational age and the proportion of patients with nSOFA >4 were different between the groups; therefore, our analyses were adjusted for gestational age and nSOFA levels.

Other elements of our management bundle have been described in the literature: wide use of human milk, protocolised feeding advancements and blood transfusion, judicious use of

Table 2 Comparison of the efficacy outcomes in the groups

Variables	Hypothermia (n=24)	Control (n=19)	Risk Ratio (95% CI)	Adjusted Risk Ratio (95% CI) * or p value***
Need of surgery, n (%)	6 (25.0)	15 (78.9)	0.32 (0.15; 0.65)	0,40 (0.19; 0.85)
Bowel perforation, n (%)	3 (12.5)	14 (73.7.)	0.17 (0.05; 0.50)	0.39 (0.18; 0.83)
Death related to NEC, n (%)	0 (0.0)	6 (31.5)	–	<0.01
Extensive intestinal resection, n (%)	0 (0.0)	3 (15.7)	–	0.08
Days of parenteral nutrition**	16.5 (8.1)	65.9 (86.1)	–	5.28 (1.88; 14.89)

*AdjRR: Gestational age and N-SOFA. ** Hazard ratio *** Fisher's exact test

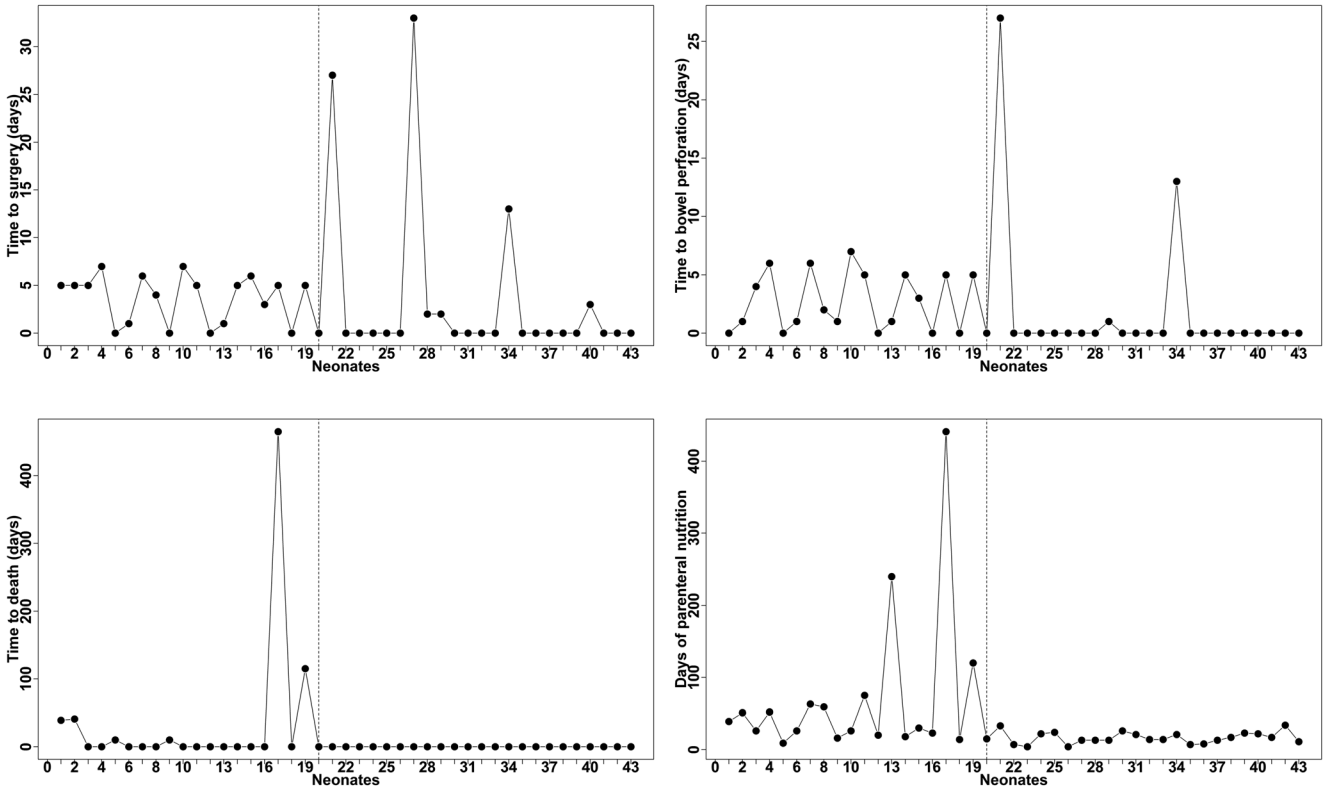

Fig. 2 Process control chart. Time from diagnosis of NEC to death (time to death), time from diagnosis of NEC to bowel perforation (time to bowel perforation), time from diagnosis of NEC to surgery (time to surgery), and days of parenteral nutrition is depicted for neonates; Control group: 1 to 19 and Hypothermia group: 20–43. The hatched line represents the start (first patient) of intervention (hypothermia). The optimal outcomes were considered the zero line (the no outcome occurrence)

antibiotics, and avoidance of antacids. Most of these interventions have an impact on the incidence of NEC in different NICUs [11–13]. In both periods of our study, our patients were treated according to the same institutional protocols, and the NEC incidence was the same [11–13]. This enhances the comparability of the two study groups.

Previous research has focused on strategies to prevent NEC, but only a few studies propose new treatments [11–13, 28, 29]. Stem cell therapy is under investigation, but this strategy is still in the early stages of development [30, 31].

Mild controlled hypothermia as a treatment for NEC was initially studied a few years ago [14–19]. In 2010, Hall et al. published their results on the safety of hypothermia (three temperature ranges: 35.5, 34.5, and 33.5 °C) for advanced NEC and failure of at least three organs (mean nSOFA of 10). They did not observe changes in mortality or in the occurrence of side effects in the 15 preterm infants with a mean gestational age between 26 and 30 weeks that underwent hypothermia for 48 h. The Great Ormond Street Hospital for Children group proposed a clinical trial (CoolNEC) to test the effectiveness of hypothermia on NEC, but the results were not released to the best of our knowledge [32].

We used a different protocol from the aforementioned study. We started low technology, mild hypothermia (35.5 °C) with passive cooling NEC-modified Bell's Stage II/III, with no end-organ failure, to minimize inflammation before intestinal damage and organ's injury was irreversible, as demonstrated in some studies [16, 19].

We chose the temperature range of 35.5 C because it is a temperature tested by Hall et al. [14]. We considered a range that would be safer and more feasible for the intervention proposed to preterm infants in our neonatal units.

In the last decade, hypothermia safety in preterm infants has been discussed, especially after reports associating hypothermia at NICU admission with increased mortality rates [33]. However, this is a different situation than that presented in our study. Hypothermia associated with mortality occurred at birth, the temperature was not controlled, and temperature ranges were widely variable. Even at birth, studies have shown that a target temperature of approximately 35.5 °C, even at birth, is possibly safe [34, 35].

In our study, achieving hypothermia through passive cooling was feasible and no related to adverse effects, as well as inexpensive. This method demands greater attention by the staff to track and adjust the temperature because of concerns about temperature being out of range [36], which was not observed in our study possibly due to the constant supervision and monitoring of the temperature.

Although we observed statistical differences in pH, haemoglobin, lactate, and bicarbonate were of no clinical

significance, except for the platelet count, which moderately increased within 48 h of hypothermia [37, 38]. None of the patients in the hypothermia group exhibited hemodynamic or ventilation instability, and only one patient had a coagulation disorder, possibly related to disease progression.

Concerning the repercussions of cooling on the brain, we performed a cranial ultrasound in the group that underwent hypothermia and did not find any worsening of pre-existing brain conditions, suggesting the non-maleficence of the method. Neurological involvement and severe brain inflammation associated with NEC may occur [39, 40], while studies have demonstrated the protective effect of hypothermia in brain injury [41]. Therefore, the neuroprotective effect of hypothermia in patients with NEC deserves further investigation.

Regarding the impact of hypothermia on the progression of intestinal injury, we have shown stabilization or amelioration of ultrasonographic patterns of the bowel, sometimes with reversion of pneumatosis. Since the literature has already demonstrated the association between pneumatosis and the need for surgery, our results suggest a favourable disease course [37, 38, 42].

In 2018, Rolnitsky et al. reported the implementation of quality measurements in a Canadian NICU. Although they observed a reduction of moderate NEC in patients with gestational age < 33 weeks (4.4–1.7%) and a reduction in the need for surgery (53.3– 50.0%), they did not observe a decrease in mortality [12].

In a survey on NEC management, applied in 153 units from eight countries, it was observed that, despite the implementation of practices to avoid unfavourable outcomes related to NEC, mortality remained between 15 and 30% and the need for surgery between 30 and 50% [10].

The mechanisms by which hypothermia is beneficial to NEC outcomes are not fully understood to date. Recent studies have proposed that both fever and hypothermia can be physiological responses to aggressions caused by pathogenic microorganisms [43]. In addition, hypothermia may modulate inflammation, which is a major component in NEC pathophysiology [44]. Hall et al. observed a decrease in interleukin (IL)-10 levels during hypothermia, and, even after rewarming [14], these levels were maintained. Other possible influences of hypothermia on the intestinal biome and sepsis have been described [45, 46], but further research is needed to elucidate the mechanisms by which hypothermia is beneficial in preterm infants with NEC.

This report has several limitations. Most importantly, this was not a non-randomized open-label clinical trial. Therefore, no prior sample size calculation was performed, and no randomisation was made. This was actually a retrospective report of a single-center experience. Consequently, we have some missing data; intestinal ultrasound recordings from all patients were not available, and historical controls were used. For all these reasons, it is necessary to clarify hypothermia effects in future studies.

Also, we used passive cooling in our study, a low-technology method with a greater risk of changes in the ideal temperature range than active cooling, requiring more work by the health team. Active cooling could offer benefits to therapy concerning safety in thermal control, though the passive cooling method's viability and effectiveness have been described in the literature for use in neonatal asphyxia [47–49].

Furthermore, our data suggest that passive cooling is possible, with temperature within the proposed range though it requires constant vigilance from the nursing team. Our study data also presented the alternative of offering therapeutic hypothermia passively, with low technology, which would enable the use in low-resource hospitals, which are very common in developing countries, where NEC incidence is often elevated [50].

In contrast, although hypothermia has been previously studied and reported [14, 32], our approach is distinct and unprecedented; it is the first paper looking into the efficacy of hypothermia on NEC, especially regarding passive cooling and a moderate target temperature (35.5 °C). Moreover, a report on low technology, mild controlled hypothermia in 24 preterm infants with an average gestational age of 30 weeks, without any adverse effects, adds novel knowledge to the field. The next step in our research group is a prospective study of mild controlled hypothermia on NEC.

In our experience, low technology, mild controlled hypothermia is feasible and no related to adverse effects in VLBW newborns with modified Bell's stage II/III, and improved main outcomes by avoiding surgery, and extensive intestinal resection, and lowering mortality, bowel perforation, and duration of parenteral nutrition.

List of abbreviations CPAP, Continuous positive airway pressure; NEC, Necrotizing enterocolitis; NICU, Neonatal intensive care unit; nSOFA, Sequential organ failure assessment; VLBW, very low birth weight

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Authors' contributions Walusa Assad Gonçalves–Ferri: Conception and design of the study. Statistical analysis. Contribution to analysis. Interpretation of data. Drafting of the study.

Cristina Helena Faleiros Ferreira. Acquisition of the data. Contribution to analysis Interpretation of data. Drafting of the study. Revising it critically for important intellectual content.

Laryssa de Carli: Acquisition of the data. Contribution to analysis Interpretation of data. Revising it critically for important intellectual content.

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Fabio Carmona. Conception and design. Acquisition of the data. Contribution to analysis Interpretation of data. Revising it critically for important intellectual content.

Davi Casale Aragon. Conception and design. Statistical analysis. Contribution to analysis Interpretation of data. Drafting of the study. Revising it critically for important intellectual content.

Gerson Crott. Acquisition of the data. Contribution to analysis Interpretation of data. Drafting of the study. Revising it critically for important intellectual content.

Marisa Mussi. Conception and design. Contribution to analysis Interpretation of data. Revising it critically for important intellectual content.

Jose Simon Camelo Junior. Contribution to analysis Interpretation of data. Drafting of the study. Revising it critically for important intellectual content.

Anelise Roosch. Contribution to analysis interpretation of data. Drafting of the study. Revising it critically for important intellectual content.

Lourenço Sbragia Neto: Contribution to analysis. Interpretation of data. Drafting of the study. Revising it critically for important intellectual content.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the study.

Data availability all the data are available for inquiry or analysis.

Code availability N/A

Declarations

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethics approval This study was approved by the Institutional Review Board of Ribeirão Preto Medical School, University of São Paulo (FMRP-USP, CAAE number 29879620.5.0000.5440, approval number 3.920.223). The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent to participate N/A

Consent for publication N/A

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