Effect of a Dual-Strain Probiotic on Necrotizing Enterocolitis in Neonates with Ductal-Dependent Congenital Heart Disease: A Retrospective Cohort Study

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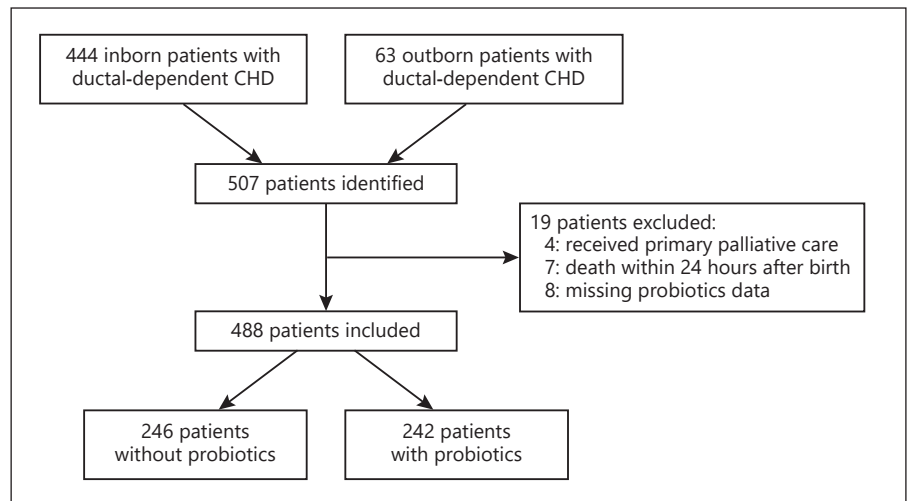
Abstract

Background: Newborns with ductal-dependent congenital heart disease (CHD) are at increased risk for developing necrotizing enterocolitis (NEC). ***Objectives:*** To investigate whether the use of dual-strain probiotics is beneficial for prevention of NEC in CHD patients, as demonstrated for premature infants. ***Study Design:*** Single-center retrospective cohort study of newborns with ductal-dependent CHD before and after implementation of oral dual-strain probiotics containing *Bifidobacterium infantis* and *Lactobacillus acidophilus*, on each day of exposure to prostaglandin E1 (PGE1). ***Results:*** Birth weight, gestational age, and distribution of heart defects were similar in both cohorts. NEC occurred in 6 of 247 (2.4%) patients without probiotics, and in 3 of 242 (1.2%) patients who received probiotics ($p = 0.504$). NEC-related mortality (0.4 vs. 0.4%, $p = 1.000$) and overall mortality (11.0 vs. 8.7%, $p = 0.448$) were likewise not different. PGE1 exposure was 1,788 and 2,455 days, respectively. In subgroup analysis of 152 infants with aortic arch malformations, such as coarctation of the aorta and interrupted aortic arch, we observed a significant reduction of NEC frequency (5.6 vs. 0.0%, $p = 0.048$). ***Conclusions:*** This is the first study to investigate the effect of a dual-strain probiotic on NEC in CHD patients. Infants with aortic arch malformations appear to benefit from dual-strain probiotics. Due to the scarcity of concurrence of ductal-dependent CHD and NEC, a clinical trial on probiotics to decrease risk of NEC in infants with ductal-dependent CHD would require several thousand infants.

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Introduction

Necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease and predominantly affecting premature infants [1]. Presence of a congenital heart disease (CHD), particularly a complex or ductal-dependent CHD, is a major risk factor for the development of NEC in near-term and full-term neonates [2–4]. The incidence of NEC in term infants with CHD has been reported to be 3–11%, which is substantially higher than rates reported for the entire population of late preterm and term newborns [2, 5, 6].

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Fig. 1. Flow diagram of patient enrollment. CHD, congenital heart disease.

The pathogenesis of NEC is multifactorial and incompletely understood, but genetic predisposition, alterations in gut flora, inadequate intestinal barrier function, regulation of the gastrointestinal and systemic inflammatory response, and hypoxia/ischemia might contribute to intestinal necrosis [7, 8]. Enteral supplementation of probiotics has the potential to change the intestinal microbiota, leads to a reduction in the frequency of NEC in very low birth weight (VLBW) premature infants, and has become one of the most studied interventions in neonatal medicine in recent years [9, 10].

In contrast to prematures, the underlying cardiac disease can lead to abnormal vascular flow in mesenteric vessels and intestinal hypoxia-ischemia in CHD patients, with secondary inflammation [11]. The role of microbial bowel colonization in the pathogenesis of NEC in CHD patients is unknown, but intestinal hypoperfusion and hypoxia, low cardiac output, antibiotic therapies, and delayed enteral feeding due to respiratory or cardiovascular instability might, alone or in combination, alter the intestinal microbiota in these patients [12, 13].

No study has yet investigated whether the supplementation of probiotics influences the risk for NEC in neonates with CHD [12, 14]. Following the emergence of strong evidence that probiotics reduce rates of NEC and mortality in premature infants, the most widely studied probiotics consisting of *Bifidobacterium infantis* and *Lactobacillus acidophilus* were routinely administered to all newborns considered at increased risk of NEC [15]. VLBW premature infants and infants with CHD requiring continuous infusion of prostaglandin E1 (PGE1) were equally considered to be at risk of NEC, and the shift in institutional policy was indeed associated with a NEC reduction in VLBW infants [2, 16]. In this retrospective cohort study, we investigate the potential influence of a dual-strain probiotic containing *Bifidobacterium infantis* and *Lactobacillus acidophilus* on newborns with ductal-dependent CHD.

Materials and Methods

Inclusion Criteria

A retrospective analysis was performed on all neonates born between January 2005 and December 2014 with ductal-dependent CHD, who received PGE1 to maintain ductal patency. Exclusion criteria were death within 24 h after birth, primary palliative care, and missing data on the administration of probiotics.

Intervention

The routine use of probiotics during administration of PGE1 in children with ductal-dependent CHD was gradually implemented during 2009. Patients received ½ capsule of Infloran® (Laboratorio Farmaceutico, Mede, Italy) containing *Bifidobacterium infantis* and *Lactobacillus acidophilus* twice daily (total daily dose 1×10^9 colony-forming units of each organism) on each day of PGE1 exposure. Two subgroups were formed for comparison: the control group of patients without any supplementation of probiotics and the group of infants receiving enteral probiotics during PGE1 administration. Enteral feeding was routinely started within 24 hours after birth with maternal breast milk, if available, or formula.

Study Measures

All data were retrieved from medical files. Extracted data for each patient include demographic details, type of ductal-dependent CHD, duration of PGE1 administration, data on the perinatal and perioperative clinical course and management, NEC, and mortalities, until hospital discharge.

Table 1. Demographic data and clinical course

Variable	No probiotics ($n = 246$)	Probiotics ($n = 242$)	p value
Demographic data[a]			
Sex, male	154 (62.6)	133 (55.0)	0.086
Gestational age, weeks	38.7 (25.4–42.0)	38.6 (28.1–41.4)	0.945
Prematurity (<37 weeks' gestational age)	46 (18.8)	36 (14.9)	0.250
Birth weight, g	3,205 (640–4,800)	3,110 (706–4,650)	0.437
Very low birth weight (<1,500 g)	8 (3.3)	7 (2.9)	0.812
Multiple gestation	17 (6.9)	16 (6.6)	0.895
Data on delivery[a]			
Inborn delivery	207 (83.7)	221 (91.3)	0.011
Caesarean section	86 (35.0)	110 (45.5)	0.021
Apgar score at 5 min[b]	9 (2–10)	9 (1–10)	0.010
Postnatal clinical course[a, c]			
Early-onset infection, suspected[d]	42 (17.8)	32 (13.2)	0.167
Late-onset infection, suspected[d]	13 (5.5)	17 (7.0)	0.487
Postnatal antibiotic therapy	131 (53.3)	107 (44.4)	0.051
Duration of antibiotic therapy, days	4 (1–91)	3 (1–37)	0.078
Hemoglobin at birth, g/L	172 (88–247)	172 (56–236)	0.977
Red blood cell transfusion	34 (14.0)	31 (12.8)	0.702
Platelet transfusion	6 (2.4)	7 (2.9)	0.761
Mechanical ventilation	110 (45.1)	77 (32.0)	0.003
Administration of surfactant	14 (5.7)	4 (1.7)	0.028
Cardiac management[a]			
Cardiac catheterization including BAS, pre	120 (49.2)	123 (50.8)	0.717
PGE1 infusion	246 (100.0)	242 (100.0)	N/A
PGE1, duration of administration, days	6 (1–99)	8 (1–60)	<0.001
PGE1, total duration of exposure, infant days	1,788	2,455	

Data are presented as number of patients (%) or median (range). [a] The following information (No. of infants) was missing: gestational age = 1, prematurity = 1, birth weight = 1, very low birth weight = 1, Caesarean delivery = 2, Apgar score = 3, early-onset infection = 10, late-onset infection = 9, duration of antibiotic therapy = 16, hemoglobin at birth = 43, red blood cell transfusion = 3, platelet transfusion = 1, mechanical ventilation = 2, administration of surfactant = 1, cardiac catheterization = 2, PGE1 duration = 4 in the no probiotics group, and Apgar score = 1, postnatal antibiotic therapy = 1, duration of antibiotic therapy = 2, hemoglobin at birth = 17, mechanical ventilation = 1, PGE 1 duration = 1 in the probiotics group. [b] 25th and 75th centiles are 8 and 9 for the control group, and 8 and 10 for the probiotics group, respectively. [c] Before cardiac surgery or discharge. [d] Based on clinical signs and laboratory tests (interleukin-6, C-reactive protein, and hematological parameters).

Outcomes

Primary outcome of our study was diagnosis of Modified Bell Stage IIa or higher NEC, either before or following cardiac surgery [17]. Secondary outcomes were NEC-related mortality, defined as mortality attributed to worsening of NEC, and overall mortality before discharge from hospital. Results of blood cultures were obtained for safety analysis.

Statistics

The data were analyzed using IBM SPSS Statistics, version 24.0 (IBM Inc., Armonk, NY, USA). Descriptive data for continuous variables are presented as median and range, categorical variables as relative frequencies. Differences for continuous variables between the two groups were analyzed using Mann-Whitney U test. Chi-square and two-tailed Fisher's exact test were used for com-

parison of dichotomous variables. Sample size calculation was performed by the Kelsey method using OpenEpi, version 3.01 (Open Source Epidemiologic Statistics for Public Health, Atlanta, GA, USA). A p value <0.05 was considered statistically significant.

Results

Inclusion criteria were met by 507 patients. Four patients with primary palliative care, 7 patients who died within 24 hours after birth, and 8 patients with missing data on administration of probiotics were excluded. The study cohort of 488 patients was divided into two sub-

Table 2. Types of congenital heart malformations in the study cohort

Cardiac lesion	No probiotics (n = 246)	Probiotics (n = 242)	p value
Aortic atresia + coarctation of the aorta	0 (0.0)	1 (0.4)	0.496
Aortic atresia + interrupted aortic arch	0 (0.0)	1 (0.4)	0.496
Aortic stenosis	7 (2.8)	3 (1.2)	0.339
Aortic stenosis + coarctation of the aorta	4 (1.6)	3 (1.2)	1.000
Coarctation of the aorta	60 (24.4)	57 (23.6)	0.829
Dextro-transposition of the great arteries	76 (30.9)	74 (30.6)	0.940
Dextro-transposition of the great arteries + coarctation of the aorta	4 (1.6)	11 (4.5)	0.074
Double outlet right ventricle	3 (1.2)	2 (0.8)	1.000
Ebstein's anomaly	4 (1.6)	2 (0.8)	0.687
Hypoplastic left heart syndrome	30 (12.2)	29 (12.0)	0.943
Interrupted aortic arch	3 (1.2)	7 (2.9)	0.219
Pulmonary atresia	19 (7.7)	26 (10.7)	0.249
Pulmonary stenosis	7 (2.8)	6 (2.5)	0.802
Shone's complex	2 (0.8)	1 (0.4)	1.000
Tricuspid atresia	3 (1.2)	3 (1.2)	1.000
Total anomalous pulmonary venous return	5 (2.0)	1 (0.4)	0.216
Tetralogy of Fallot	7 (2.8)	7 (2.9)	0.975
Unbalanced atrioventricular septal defect	2 (0.8)	2 (0.8)	1.000
Others[a]	10 (4.1)	6 (2.5)	0.325

Data are presented as number of patients (%). [a] Multiple cardiac anomalies including other ductal-dependent single ventricle anomalies.

groups: 246 patients (50.4%) who did not receive any enteral probiotics formed the control group, and 242 patients (49.6%) with administration of dual-strain probiotics formed the intervention group (Fig. 1).

Patients' Characteristics

Demographic characteristics did not differ between the two groups. The rate of inborn births was increased, and there were more Caesarean deliveries in the intervention group (Table 1). The rate of suspected early- and late-onset infections did not differ between the two groups, but we observed an insignificant trend towards less frequent and shorter postnatal antibiotic courses in the intervention group. Two infants who developed postoperative NEC in the control group had received transfusions of red blood cells after cardiac surgery, 1 or 2 days, respectively, before onset of NEC. No other infant was diagnosed with NEC within 48 h of a blood transfusion. Patients who received probiotics were significantly longer exposed to PGE1 (median 6 vs. 8 days, $p < 0.001$), and the amount of total days of PGE1 exposure was 37% higher in the probiotic group compared to the control group, with 2,455 vs. 1,788 days (Table 1). The frequencies of various types of ductal-dependent CHD were similarly distributed between the two groups in our study cohort (Table 2). Most common lesions were dextro-transposition of the great arteries and coarctation of the aorta, both also with additional variations.

Outcomes

There was no significant difference in the frequency of NEC (2.4 vs. 1.2%, $p = 0.504$), NEC-related mortality (0.4 vs. 0.4%, $p = 1.000$), and overall mortality (11.0 vs. 8.7%, $p = 0.448$) between the two study groups. NEC was diagnosed on median day 10 of life (5–35 days). One patient of the control group was treated conservatively, whereas 8 out of 9 infants with NEC required abdominal surgery. The diagnosis of NEC was additionally confirmed by characteristic findings, as seen during laparotomy or in histological examinations in 7 of these. In one infant who received probiotics, histological examination revealed cecal perforation with local inflammation, and spontaneous intestinal perforation could also be considered for differential diagnosis in this patient. We found no significant difference in outcomes in the subgroups of VLBW and non-VLBW infants (Table 3). Subgroup analysis of infants stratified by types of CHD revealed a reduction of NEC incidence in patients with aortic arch malformations leading to decreased perfusion of the aorta (5.6 vs.

Table 3. Effect of probiotics on NEC and mortality stratified by birth weight

Variable	No probiotics	Probiotics	p value
All infants	246 (100.0)	242 (100.0)	
NEC, overall	6 (2.4)	3 (1.2)	0.504
NEC, after cardiac surgery	3 (1.2)	0 (0.0)	0.249
NEC-related mortality[a]	1 (0.4)	1 (0.4)	1.000
Mortality, overall	27 (11.0)	21 (8.7)	0.448
Birth weight <1,500 g[b]	8 (100.0)	7 (100.0)	
NEC, overall[c]	2 (25.0)	1 (14.2)	1.000
NEC, after cardiac surgery	0 (0.0)	0 (0.0)	1.000
NEC-related mortality	1 (12.5)	1 (14.2)	1.000
Mortality, overall	6 (75.0)	2 (28.6)	0.132
Birth weight ≥1,500 g[b]	237 (100.0)	235 (100.0)	
NEC, overall[c]	4 (1.7)	2 (0.9)	0.686
NEC, after cardiac surgery	3 (1.3)	0 (0.0)	0.248
NEC-related mortality	0 (0.0)	0 (0.0)	N/A
Mortality, overall	21 (8.9)	19 (8.1)	0.869

Data are presented as number of patients (%). [a] Patient with preoperative onset of NEC. [b] Information on birth weight was missing for one patient in the no probiotics group, who was $35^{1}/_{7}$ weeks of gestational age at birth and did not develop NEC. [c] Difference between frequency of NEC in VLBW infants and infants with birth weight ≥1,500 g: $p = 0.013$ (no probiotics group) and $p = 0.085$ (probiotics group), respectively.

0.0%, $p = 0.048$), such as coarctation of the aorta and interrupted aortic arch (Table 4).

Probiotic Bacteremia

A total of 86 patients (35.5%) of the intervention group had blood cultures, all being negative for *Bifidobacterium* or *Lactobacillus* bacteremia. Organisms identified were *Acinetobacter* or *Staphylococcus* in 2 blood cultures in the control group, and *Escherichia coli*, *Staphylococcus*, or *Streptococcus* in 6 blood cultures in the intervention group.

Sample Size Calculation

A randomized controlled trial with the aim to demonstrate a significant reduction of NEC incidence from 2.4 to 1.2% by a single intervention, such as administration of probiotics, would need to include at least 3,786 patients (1,893 in each group, allocation ratio 1:1), for a two-sided type I error of 5% and a power of 80%.

Discussion

Our results indicate that oral administration of a dual-strain probiotic might be beneficial to reduce the rate of NEC in infants with aortic arch malformations. The low frequencies of overall and postoperative NEC in our center are rather in the lower range of the various NEC frequencies reported in previous studies, making analyses whether the administration of probiotics has an influence on the frequency of NEC in CHD patients challenging [2, 5, 6].

Differences between both groups, notably more Caesarean deliveries and a trend towards less frequent antibiotic use in the intervention group, might impact our results. Both factors have been shown to influence the composition of the intestinal microbiota by reducing the amount of *Bifidobacteria* and *Bacteroides fragilis* group in fecal samples in infants at 1 month of age [18]. However, the impact of mode of delivery and antibiotic therapy on NEC incidence is still critically discussed [19]. Furthermore, we could not investigate whether the infants were fed with breast milk or formula. Breast milk has been shown to have a beneficial effect in reducing the incidence of NEC in premature infants; however, in contrast, no difference in NEC incidence was found between infants with ductal-dependent CHD fed with breast milk or formula [19, 20].

Growing evidence leads to the assumption that NEC in CHD patients is a clinically distinct entity from NEC in premature infants, distinguished by different demographics, pathogenic mechanisms, and outcomes [6, 11, 12]. Previous studies show contradictory results regarding the age at onset of NEC in CHD patients, especially in

Table 4. Effect of probiotics on NEC and mortality stratified by type of CHD

Variable	No probiotics	Probiotics	p value
CoA and IAA, all (n = 152)[a]	72 (100.0)	80 (100.0)	
NEC, overall	4 (5.6)	0 (0.0)	0.048
NEC, after cardiac surgery	2 (2.8)	0 (0.0)	0.223
NEC-related mortality	1 (1.4)	0 (0.0)	0.474
Mortality, overall	6 (8.3)	5 (6.3)	0.757
d-TGA, all (n = 165)[b]	80 (100.0)	85 (100.0)	
NEC, overall	1 (1.3)	1 (1.2)	1.000
NEC, after cardiac surgery	0 (0.0)	0 (0.0)	1.000
NEC-related mortality	0 (0.0)	1 (1.2)	1.000
Mortality, overall	6 (7.5)	4 (4.7)	0.526
HLHS (n = 59)	30 (100.0)	29 (100.0)	
NEC, overall	1 (3.3)	1 (3.4)	1.000
NEC, after cardiac surgery	1 (3.3)	0 (0.0)	1.000
NEC-related mortality	0 (0.0)	0 (0.0)	1.000
Mortality, overall	8 (26.7)	6 (20.7)	0.761

Data are presented as number of patients (%). [a] Includes: aortic stenosis + CoA = 4, d-TGA with CoA = 4, interrupted aortic arch = 3, multiple cardiac anomalies = 1 (unbalanced atrioventricular septal defect, single right-sided ventricle + CoA) in the no probiotics group, and aortic atresia + CoA = 1, aortic atresia + IAA = 1, aortic stenosis + CoA = 3, d-TGA with CoA = 11, interrupted aortic arch = 7 in the probiotics group. [b] Including infants with d-TGA and CoA (4 and 11, respectively).

comparison to NEC in premature infants [2, 4, 6, 11, 19]. In premature infants, NEC is seen as primarily inflammatory disease with secondary necrosis, whereas impaired splanchnic blood flow and oxygen delivery leading to splanchnic ischemia is discussed as major factor in the pathophysiology of NEC in neonates with CHD [11]. Infants with aortic arch obstruction, lesions with significant systemic-to-pulmonary runoff, including aortopulmonary window, hypoplastic left-heart syndrome, and truncus arteriosus, and episodes of low cardiac output are predisposed to mesenteric circulatory insufficiency and have been shown to be at high risk for NEC [2]. Apart from obstruction of systemic circulation or "pulmonary steal" phenomenon, complex CHD can lead to low saturated arterial blood directed towards the lower parts of the body, forming an additional risk factor for (chronic) intestinal hypoxia [11]. Altered mesenteric blood flow in Doppler and lower splanchnic regional oxygenation, as measured by means of near-infrared spectroscopy, have been shown to be associated with development of NEC in infants with critical CHD [21–23].

Although hypoperfusion and hypoxia are postulated to be inciting events in the development of NEC in infants with CHD, the process is nevertheless likely to be multifactorial, and the role of gut microbial colonization in the pathogenesis of NEC in newborns with CHD is still unknown [14]. Acute mesenteric hypoperfusion and chronic hypoxia may alter the development of gut microbiota and the gut barrier integrity, or lead to a chronic state of inflammation [3, 12]. In older children with cyanotic CHD, chronic hypoxia results in an altered diversity of gut microbial communities with a marked decrease in *Lactobacilli* [24]. Changes in the gut microbial community structure and function and potential modifications with probiotic bacteria might influence the risk for NEC [12]. Ellis et al. [25] described significant intestinal dysbiosis in CHD patients of at least 34 weeks of gestational age, characterized by decreased total bacteria and decreased *Actinobacteria* (predominantly *Bifidobacteria*). However, oral administration of *Bifidobacterium infantis* did not result in an increase of fecal *Bifidobacteria* in this pilot study. In contrast, perioperative administration of the probiotic *Bifidobacterium breve* to neonates undergoing surgery for CHD significantly changed their intestinal environment [13]. These diverging results raise the question whether or not other influence factors such as enteral feeding, antibiotic treatment, and hypoxia play a more dominant role in shaping the intestinal microbiota, compared to probiotics [25].

Despite different pathophysiological mechanisms of NEC in premature and CHD patients, the use of probiotics for prevention of NEC has been repeatedly discussed, but current evidence is extremely limited [11, 12, 14]. The only published study evaluating the association between probiotic bacteria and NEC, a small prospective, single-center, randomized controlled trial on 100 infants from Turkey, showed a significant reduction of NEC rate (10 vs. 0%) and mortality (28 vs. 10%) in infants with cyanotic CHD who received a synbiotic preparation containing *Bifidobacterium lactis* and inulin [26]. No study has yet investigated the effect of probiotic mixtures on NEC in CHD patients.

Our retrospective observational study has notable limitations. The study is not inclusive of all known and suspected risk factors associated with NEC. Due to the long study period of 10 years, clinical management of CHD patients might have changed over time, as indicated by less frequent mechanical ventilation in the intervention group, and whether these changes influence the frequency of NEC and make the comparison of the two study cohorts less conclusive is unclear. Subgroup analyses might not be adequately powered and only be useful to generate hypotheses. Lack of analysis for fecal microbiota impedes us to investigate po-

tential changes in the composition of the microbiota related to the administration of probiotics. For safety analysis, blood cultures were not available for all infants for investigation of systemic infection from probiotic species. Furthermore, the incidence of probiotic bacteremia might be underestimated due to the practice of requesting only aerobic blood cultures for neonates with suspected infection.

The investigation of a potential impact of probiotics on an already low frequency of NEC in infants with ductal-dependent CHD is challenging. However, our results warrant further research. A well-designed multicenter trial to further investigate whether the simple approach of administering probiotics is effective to prevent NEC in the high-risk population of newborns with ductal-dependent CHD would be of great interest. Assuming a 50% reduction of NEC based on a low NEC incidence of 2.4% as found in our cohort, a clinical trial would need to include almost 4,000 patients with ductal-dependent CHD, an impossible task for a single center. Despite the limitations, this is the first study to investigate the potential impact of probiotic mixtures on NEC in newborns with ductal-dependent cardiac malformations.

Conclusion

Enteral administration of a dual-strain probiotic containing *Bifidobacterium infantis* and *Lactobacillus acidophilus* might have the potential to reduce NEC in infants with ductal-dependent CHD, particularly in those with reduced intestinal perfusion.

Statement of Ethics

Written informed consent of the study participants (or their parents or guardians) was not obtained, as all data were collected as part of routine clinical care and have been anonymized for the purpose of analysis and presentation. Publication of the results was approved by the Institutional Review Board (Charité Berlin, EA2/069/17).

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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Author Contributions

L.K. collected, analyzed, and interpreted the data and drafted the initial manuscript. C.B. contributed to the study design, interpretation of data, and critically reviewed the manuscript for intellectual content. F.B. contributed to the interpretation of data, and critically reviewed the manuscript for intellectual content. V.B. conceptualized and designed the study, contributed in data collection and analysis, supervised data collection and interpretation, and critically reviewed and revised the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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