Treatment Outcomes of Infants With Cyanotic Congenital Heart Disease Treated With Synbiotics

WHAT’S KNOWN ON THIS SUBJECT: Several studies have suggested that probiotics may prevent necrotizing enterocolitis and death in preterm infants. However, there are no data on the preventive effect of probiotics in infants with cyanotic congenital heart disease.

WHAT THIS STUDY ADDS: Although duration of hospitalization was not significantly decreased, *Bifidobacterium lactis* plus inulin appears to decrease the rate of nosocomial infection, necrotizing enterocolitis, and death in infants with cyanotic congenital heart disease.

abstract

OBJECTIVES: The goal was to investigate the effect of orally administered synbiotics on outcome of infants with cyanotic congenital heart disease (CCHD).

METHODS: A prospective, blinded, randomized controlled trial was conducted to evaluate the effect of synbiotics on outcome of infants with CCHD. The infants with CCHD were assigned randomly to 2 groups. Infants in the study group were given synbiotic (*Bifidobacterium lactis* plus inulin) added to breast milk or mixed feeding until discharge or death. Infants in the placebo group were fed with breast milk or mixed feeding. The outcome measurements were nosocomial sepsis, necrotizing enterocolitis (NEC; Bell stage ≥2), length of NICU stay, and death.

RESULTS: A total of 100 infants were enrolled in the trial: 50 in each arm. There were 9 cases of culture-proven sepsis (18%) in the placebo group and 2 cases (4%) in the synbiotic group (*P* = .03). Length of NICU stay did not differ between the groups (26 [14–36] vs 32 days [20–44], *P* = .07). There were 5 cases of NEC (10%) in the placebo group and none in the synbiotic group (*P* = .03). The incidence of death was lower in synbiotic group (5 [10%] of 50 vs 14 [28.0%] of 50, respectively, *P* = .04).

CONCLUSIONS: Synbiotics administered enterally to infants with CCHD might reduce the incidence of nosocomial sepsis, NEC, and death. *Pediatrics* 2013;132:e932–e938

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KEY WORDS synbiotics, cyanotic congenital heart disease, sepsis, necrotizing enterocolitis

ABBREVIATIONS
CCHD—cyanotic congenital heart disease
CHD—congenital heart disease
CI—confidence interval
IQR—interquartile range
NEC—necrotizing enterocolitis
NNT—number needed to treat
OR—odds ratio
RACHS-1—Risk Adjustment in Congenital Heart Surgery

Dr Dilli conceptualized and designed the study, and drafted the initial manuscript; Drs Aydin, Özyazıcı, Beken, and Okumuş carried out the initial analyses and reviewed and revised the manuscript; Dr Zenciroğlu designed the data collection instruments, coordinated and supervised data collection at 2 of the 4 sites, and critically reviewed the manuscript; and all authors approved the final manuscript as submitted. This trial has been registered at www.clinicaltrials.gov (identifier NCT01810978).

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Congenital heart disease (CHD) is associated with substantial morbidity and accounts for 4% of all neonatal deaths. Infants with CHD have more frequent infections and exposures to antibiotics than healthy infants. These infants are frequently exposed to major surgical procedures, multiple transfusions, and prolonged hospital stays that may increase their risk of sepsis.

Among term infants, necrotizing enterocolitis (NEC) is rare and frequently associated with congenital diseases. The infants with cyanotic CHD (CCHD) have a strikingly high incidence of NEC. Prolonged hospital stays and exposure to nosocomial microbes, delay in feeding, decreased rates of human milk feeding, poor nutritional status, courses of broad-spectrum antibiotics, and the inflammation associated with cardiac surgery may have role in the pathogenesis of NEC in these infants.

Although the exact pathogenesis of NEC in infants with CCHD is not clear, it is likely that both the acute mesenteric blood-flow reduction and chronic cyanosis may alter the development of the gut microbiota and the integrity of the gut barrier, increasing risk for NEC. It has been proposed that the changes in the microbial community structure and function within the gut among these term infants with CCHD can be modified with probiotic bacteria. Animal studies and in vitro research suggested several mechanisms of probiotics, such as competitive inhibition of pathogen-adhesion receptor-binding sites, decreased expression of proinflammatory cytokines, increased expression of anti-inflammatory cytokines, secretion of enzymes and beneficial metabolites, modulation of cellular redox potential, functional enhancement of epithelial tight junctions, induction of intestinal mucin production, signaling interactions with cell surface molecules involving cytoprotective and immune responses (with local and systemic effects), increased expression of innate immune antimicrobial peptides, and evoking neuroendocrine and hormonal responses involved in growth and development.

It was hypothesized that prophylactic administration of probiotics may alter the intestinal microbiota in this high-risk population, decreasing the NEC risk. In recent research, Ellis et al stated that probiotic therapy might help reduce the incidence of NEC in infants with CCHD. However, there is no study evaluating the association between probiotic use and morbidity in infants with CCHD in either the National Institutes of Health National Library of Medicine (PubMed) database or the ClinicalTrials.gov registry. Therefore, this study aimed to investigate the effect of orally administered symbiotics on outcomes of infants with CCHD.

**METHODS**

**Design and Setting**

From June 2011 to April 2013, a prospective, blinded, single-center, randomized controlled trial was conducted in the NICU of Dr Sami Ulus Maternity and Children Research and Training Hospital, a level III neonatal center in the central part of Turkey.

**Patients and Methods**

During the study period, the infants with CCHD who were admitted to the NICU were included in the study.

**Inclusion Criteria:**

- Infants with CCHD
- Infants ≥35 weeks’ of gestational age
- Infants born at or transferred to Dr Sami Ulus Maternity and Children Research and Training Hospital
- Infants who fed enterally and survived beyond the seventh day after admission

**Exclusion Criteria:**

- Congenital anomalies of the intestinal tract
- Infants who were not fed enterally or died before the seventh day after admission

**Randomization**

Infants were randomized into the symbiotic or placebo groups by balanced blocks using sealed envelopes when they fed enterally and survived beyond the seventh day after admission. Informed and written parental consents were obtained before randomization. The study group received symbiotic (Bifidobacterium lactis, 5 × 10^9 colony forming units, 30 mg plus inulin, 900 mg, Maflor, Mamsel, Istanbul, Turkey), 1 sachet per day with breast milk or formula until discharge or death, whichever came first. The placebo (control) group was fed with breast milk or formula without addition of probiotic or prebiotic and received maltodextrin.

Group assignment was made by the investigators (A.Z., N.O.) who were not involved in the care of the infant. A member of the feeding team who was blinded and not involved in the care of the infant followed orders from the sealed envelope prepared by the investigators. Synbiotic or maltodextrin was mixed with breast milk or formula in the kitchen by this person. The sachets containing the synbiotic were similar in appearance to the placebo. The nurses who fed the infants were blinded to the group assignments.

Feeding was given when the infant had stable vital signs, active bowel sounds without abdominal distension, and no bile or blood from the nasogastric tube. In both groups, feeding was stopped if there was any sign of feeding intolerance (defined as the presence of gastric aspirate in the amount that was more than half of the previous feeding, twice, with abdominal distension.)
Feeding was stopped or restarted by the physician who was involved in the care of the infant, but unaware of the patient’s group. The same physicians (D.D., B.A., E.U., and S.B.) were in charge of the care of the infants during their hospital stay. The residents who rotated through the NICU provided care following the established protocols of the unit.

Outcomes
Primary outcomes were nosocomial sepsis and NEC. Nosocomial sepsis, also known as hospital-acquired sepsis, is defined as infections that develop at least 48 hours after birth. Sepsis evaluation was made according to previously suggested diagnostic criteria.9 Infants whose culture results were positive were diagnosed as having proven sepsis. NEC is categorized by modified Bell classification.10 The diagnosis and classification of NEC was made by 2 independent senior neonatologists who did not know the group assignment of the infant. Only children with confirmed NEC (Bell stage IIA–IIB: typical symptoms plus pathognomonic radiologic signs) were included. Illness severity was evaluated by the Score for Neonatal Acute Physiology Perinatal Extension, which is a scoring system developed and validated by Richardson et al in 2001.11 Risk Adjustment in Congenital Heart Surgery (RACHS-1) classification, in which category 6 has the highest risk for death, was used to define intervention risk.12 Secondary outcomes were length of NICU stay and death.

Ethics
The study protocol was approved by the local ethics committee. All parents were fully informed about the investigational nature of this study as well as its aim. The parents were informed of potential side effects of probiotics. A written consent was obtained from all parents. This trial is registered at www.clinicaltrials.gov (identifier NCT01810978).

Sample-Size Calculation and Statistics
According to our previous NICU experience, the incidence of NEC among infants with CCHD is 12%. Power analysis showed that setting the error 0.05 and β error 0.55 (2-tailed) and an absolute reduction of the incidence of NEC by 50%, the total number needed to verify our hypothesis was 100 (50 in each group). According to intention-to-treat analysis, every subject who was randomized was included.

All data analyses were performed by using the Statistical Package for the Social Sciences, version 15.0 (IBM SPSS Statistics, IBM Corporation, Chicago, IL). Differences for continuous variables between the 2 groups were analyzed by the Student’s t test or Mann-Whitney U test according to spread of data. The χ² test was performed for categorical variables. Variables are given as mean ± SE of means or median (interquartile range [IQR] or range), as appropriate. A P value of <0.05 was accepted as significant. Multivariate analysis was conducted by entering possible confounding variables (gestational week, birth weight, duration of mechanical ventilation and umbilical catheterization, surgery, probiotic use, feeding type, red blood cell transfusions and antibiotic days, and RACHS-1 score) to analyze the synbiotic effects on the outcome variables (nosocomial sepsis and death). Odds ratios (ORs) are presented with 95% confidence intervals (CIs). A model could not be performed for NEC, as there was no case in synbiotic group. Number needed to treat (NNT) was calculated for proven sepsis, NEC, and death.

RESULTS
There were 178 infants with CCHD admitted to our NICU during the 22-month study period. Of these infants, 78 were not included in the study; 22 were <35 weeks of gestational age, 46 died before 7 days after admission, 2 had diaphragmatic hernia, and 8 were without parental consent. Finally, 100 infants (50 in each arm) enrolled the study (Fig 1).

Fig 2 describes the different types of CCHD found in all infants. Obstruction of the right ventricular outflow tract was dominant (51 of 100). Although hypoplastic left heart cases with the highest RACHS score of 6 were included only in the placebo group, there was no significant difference between the groups. Average age at cardiac surgery was 8 days (range 2–54 days). Prostaglandin E1 (0.01–0.1 μg/kg/min) was administered to 80 cases (80%) at the preinterventional period. The median postoperative feeding time was 3 days (range 2–5 days).

The maternal and infants’ demographic characteristics did not differ between the 2 groups (Table 1).

Table 2 shows the infants’ clinical characteristics. The age at enrollment did not differ between the 2 groups (4 vs 5 days, P = .08). The median duration of oral probiotic or placebo administration was 19 days (range 5–96 days). The durations of total parenteral nutrition (7.0 vs 12.5 days, P = .001) and mechanical ventilation (3 vs 5 days, P = .004) were longer in the placebo group.

The incidence of nosocomial clinical sepsis was significantly lower in the synbiotic group (4 [8%] of 50 vs 14 [28%] of 50, respectively; P = .01). There were 9 cases (18%) of culture-proven sepsis with 11 episodes in the placebo group, and 2 cases (4%) with 3 episodes in the synbiotic group, corresponding to an NNT of 7 (95% CI 4–68).

The types of microorganisms were Pseudomonas spp (n = 3), Streptococcus viridans (n = 2), Enterococcus spp (n = 2), Burkholderia gladioli (n = 2), Candida spp (n = 2), Brevundimonas vesicularis (n = 1), and Escherichia coli (n = 1). Our microbiology laboratory was not able to grow and identify bifidobacteria in the blood. There were 5...
cases (10%) of NEC (Bell stage 2 \(n = 3\), Bell stage 3 \(n = 2\)) in the placebo group and none in the synbiotic group. This corresponded to an NNT of 10 (95% CI 5–111). NEC developed at the preoperative period (range 6–21 days) in 3 cases, and postoperative period (range 3–14 days) in 2 cases. Two patients already presented with intestinal perforation at the time of diagnosis of NEC. The types of CCHD were right sided in 3 patients and left sided in 2. Four infants with NEC died.

Length of NICU stay did not differ between the groups (26 [14–36] vs 32 [20–44] days, respectively; \(P = .07\)). The incidence of death was lower in the synbiotic group (5 [10%] of 50 vs 14 [28%] of 50, respectively), giving an NNT of 6 (95% CI 3–40). In multivariate analysis, with adjustment for various confounding variables (birth weight, 5-minute Apgar score, need of surgical interventions, need of angiographic intervention, synbiotic use, feeding type, blood transfusion, antibiotic day, and RACHS-1 score), synbiotic use (OR 0.4, 95% CI 0.18–0.90) and breast milk feeding were associated with decreased risk of proven nosocomial sepsis (OR 0.4, 95% CI 0.17–0.80). By adding proven nosocomial sepsis to the previous model, the decreased risk of mortality was associated only with synbiotic use (OR 0.5, 95% CI 0.25–0.93).

**DISCUSSION**

In premature infants, probiotics decrease the incidence of NEC with a relative risk of 0.33,13 and prebiotics increase stool colony counts of bifidobacteria and lactobacilli, but a decrease in NEC or sepsis has not been demonstrated.14 Reports of synbiotic use in preterm infants are limited. In term infants with CCHD, NEC and sepsis are common. A small pilot study of *Bifidobacterium infantis* demonstrated feasibility of probiotics administration in this population, but no significant differences between probiotics and placebo in the fecal microbiota or several cytokines associated with NEC.15 In this study, we present the first randomized controlled trial of a synbiotic product in term infants with CCHD.
Nosocomial infection in the NICU is associated with prolonged hospitalization, morbidity, and mortality. Of all neonates admitted to the NICU, ~6.2% to 33.0% developed nosocomial infection. Infants with CHD also have more frequent infections and exposures to antibiotics than healthy infants. It was suggested that the infants with CHD were frequently exposed to major surgical procedures, multiple transfusions, and prolonged hospital stays that might increase their risk of sepsis. The higher incidence of sepsis in our study may be explained by different risk factors, and population differences in cardiac complexity, gestational age, birth weight, and the proportion of infants undergoing cardiac surgery.

The commensal bacteria of the gastrointestinal tract have a key role in the development of healthy immune responses. Healthy term infants acquire these commensal organisms rapidly after birth. However, colonization in hospitalized infants is adversely affected by the intensive care environment. Altered microbiota composition may lead to increased colonization with pathogenic bacteria, poor immune development, and susceptibility to sepsis in these infants. Probiotics may also favorably modulate host immune responses in local and remote tissues. Although the underlying mechanisms of probiotics are still unclear, they may include strengthening of the non-immunologic gut barrier, interference with pathogen adhesion and growth inhibition, and the enhancement of the local mucosal immune system in the gut, as well as of the systemic immune response.

The Cochrane meta-analysis showed that enteral probiotics supplementation did not affect the rate of nosocomial sepsis (typical relative risk 0.90, 95% CI 0.76–1.07). Srinivasjois et al in 2009 published a systematic review/meta-analysis of 4 trials on the efficacy and safety of prebiotic oligosaccharide supplementation of formula in reducing the incidence of NEC and sepsis in preterm infants. The authors stated that only 1 trial reported that NEC did not occur in any of the enrolled neonates and others did not report on NEC or sepsis. Rojas et al reported that *Lactobacillus reuteri* did not appear to decrease the rate of sepsis, but...
the current study, we showed that synbiotic use reduced the rates of nosocomial clinical or proven sepsis. CCHD has been suggested to be a predisposing factor for the development of NEC in neonates born at or close to term.4,25 McElhinney et al1 reported the incidence of NEC as 3.3% among all infants with CCHD, and 7.6% in patients with hypoplastic left heart syndrome. The authors observed that the patients with obstruction of the left ventricular outflow tract and especially with univentricular heart disease have a higher risk to develop NEC than those without. In other research, Kargl et al26 found the overall NEC incidence in infants with CCHD as 2.5%. Sweet et al27 also showed that the infants with CCHD appeared to be at a greater risk of gastrointestinal complications, including NEC, in the days following cardiac catheterization. In our study, there were 5 cases (10.0%) of NEC in the placebo group and none in the synbiotic group, with an overall NEC incidence of 5.1% among infants with CCHD. NEC developed during the preoperative period in 3 cases, and during the postoperative period in 2 cases. Two patients already presented with intestinal perforation at the time of diagnosis of NEC. The types of CCHD were right sided in 3 patients, and left sided in 2.

In McElhinney et al’s study,4 although most cases of NEC were managed successfully with medical measures and hospital mortality did not differ significantly from that in control patients, all of the deaths in patients who developed NEC were directly attributable to NEC, not to their heart disease. A possible explanation might be the major contribution of intestinal hypoxia-ischemia and the lesser role of intestinal immaturity and microbial invasion in pathogenesis of NEC in these children. In the current study, 4 of 7 infants with NEC died because of the disease severity.

The incidence of NEC in our experience is higher than that in the study by McElhinney et al.4 It is not clear why this is the case. However, the composition of their study population differed substantially from ours with respect to types of heart disease. On the other hand, most of our patients were newborn, so preoperative care was not optimal in these cases. Regardless of the differences in findings between our investigation and that of McElhinney et al,4 both studies support the belief that NEC is an important concern in the neonate with CHD.

Altered mesenteric blood flow may be an important factor in the pathophysiology of NEC in neonates with CCHD.6 In addition, many of these children are monitored with umbilical arterial catheters, exposed to prostaglandin, and some undergo cardiac catheterization, which have been reported as risk factors for NEC. It may be fact that underlying CHD and its management rather than the prostaglandins or umbilical lines cause to decreased reserve in circulatory physiology in these patients.4 In our patients, the duration of prostaglandin E1 infusion and umbilical catheterization did not differ between the groups.

In patients with severe CHD requiring cardiopulmonary bypass surgery, NEC occurring in the postoperative period can be devastating.28 It has been suggested that early surgical correction of CCHD defects prevents postoperative development of NEC.29 This hypothesis might be suitable for patients presenting with episodes of low cardiac output or poor systemic perfusion before the correction of the heart defect. In our patients, there was no correlation between age at cardiac surgery and development of NEC.

There are several limitations to our study. First, the number of study patients is too small to definitely report that synbiotics reduce morbidity and mortality in infants with CCHD. The sample size required in future studies should be higher than 280 to reach 80% power. Second, there are different types of CCHD in our study population. Most infants with severe CCHD die within the first week of life. On the other hand, our microbiology laboratory was not able to grow and identify bifidobacteria in the blood. Nevertheless, to our knowledge, this study is the first randomized controlled trial evaluating the effects of synbiotics on nosocomial sepsis, NEC, and mortality in infants with CCHD.

CONCLUSIONS

In a population of 100 neonates with complex CCHD, synbiotics, in the form of _Bifidobacterium lactis_ plus inulin, administered enterally to infants with CCHD, might reduce the incidence of nosocomial sepsis, NEC, and death. Further multicentered randomized controlled studies conducted on a selective group of patients with CCHD are needed to support the effects of synbiotics on neonatal morbidity and mortality in this population.

**REFERENCES**


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