Pediatr Gastroenterol Hepatol Nutr. 2023 May;26(3):146-155
https://doi.org/10.5223/pghn.2023.26.3.146
pISSN 2234-8646·eISSN 2234-8840

Pediatric Gastroenterology, Hepatology & Nutrition
pghn

Original Article

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The Optimal Time for Initiating Probiotics for Preterm and Very-Low-Birth-Weight Infants: A 10-Year Experience in a Single Neonatal Intensive Care Unit

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OPEN ACCESS

Received: Nov 16, 2022
Revised: Mar 24, 2023
Accepted: Apr 12, 2023
Published online: May 11, 2023

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ABSTRACT

Purpose: The starting time for probiotic supplementation in preterm infants after birth varies widely. This study aimed to investigate the optimal time for initiating probiotics to reduce adverse outcomes in preterm or very low birth weight (VLBW) infants.
Methods: Medical records of preterm infants born at a gestational age (GA) of <32 weeks or VLBW infants in 2011–2020 were reviewed respectively. The infants who received *Saccharomyces boulardii* probiotics within 7 days of birth were grouped into an early introduction (EI) group, and those who received supplemented probiotics after 7 days of birth were part of the late introduction (LI) group. Clinical characteristics were compared between the two groups and analyzed statistically.
Results: A total of 370 infants were included. The mean GA (29.1 weeks vs. 31.2 weeks, $p<0.001$) and birth weight (1,235.9 g vs. 1491.4 g, $p<0.001$) were lower in the LI group (n=223) than in the EI group. The multivariate analysis indicated that factors affecting the LI of probiotics were GA at birth (odds ratio [OR], 1.52; $p<0.001$) and the enteral nutrition start day (OR, 1.47; $p<0.001$). The late probiotic introduction was associated with a risk of late-onset sepsis (OR, 2.85; $p=0.020$), delayed full enteral nutrition (OR, 5.44; $p<0.001$), and extrauterine growth restriction (OR, 1.67; $p=0.033$) on multivariate analyses after adjusting for GA.
Conclusion: Early supplementation of probiotics within a week after birth may reduce adverse outcomes among preterm or VLBW infants.

Keywords: Probiotics; Preterm infant; Very low birth weight infant

INTRODUCTION

Intestinal microbiota has been proven as a key modifier of morbidities among preterm infants [1,2]. To maintain or promote the intestinal microbial environment and prevent necrotizing enterocolitis (NEC) or sepsis, probiotics have been widely used in preterm infants

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Funding
None.

Conflict of Interest
The authors have no financial conflicts of interest.

[3-6]. Intestinal microbiota can interact with the intestinal epithelium. The intestinal mucosa acts as a natural barrier to block pathogenic bacteria and toxins from the environment [7]. It regulates responses to endotoxins, prevents colonization of pathogens, and enhances immune responses [8]. In newborns, the intestinal mucosal barrier is immature and intestinal microbiota is affected by gestational age (GA) and birth weight (BW), therefore, intestinal dysbiosis commonly occurs in preterm infants [9]. Intestinal dysbiosis is an important risk factor for NEC and sepsis in preterm infants [10]. Probiotic supplementation during the neonatal period can regulate the intestinal microbiota composition and provide resistance to pathogens to promote the intestinal mucosal barrier in infants [1-4,7]. Recent studies and meta-analyses have also reported that probiotics can reduce morbidity and mortality of NEC and sepsis in preterm infants [5,6]. Therefore, probiotics are applied to various medical fields. In our neonatal intensive care unit (NICU), preterm infants have been given probiotics to reduce morbidity and mortality for more than 10 years. Although probiotics are widely used in medical fields, there are still concerns about the side effects, such as systemic infections, detrimental metabolic activities, excessive immune responses, transfer of antibiotic-resistant genes to pathogenic bacteria, and intestinal gas formation, especially in immunocompromised individuals or preterm infants [5,11,12]. Our NICU has two isolated beds to prevent the spread of infection, but there is always a concern about the spread of nosocomial infections because the NICU is a single large unit. Due to this concern, antibiotics are used liberally when sepsis is suspected in a sick baby. Discontinuation of antibiotics is decided based on as soon as possible based on the negative results of subsequent culture tests. With concerns for the survival of probiotics and the delivery of antibiotic-resistant genes by probiotics under our sepsis management protocol, *Saccharomyces boulardii* was instigated for preterm infants [12].

Despite concerns about potential side effects, extensive studies on probiotics in preterm infants have consistently identified the beneficial strains and optimal amounts for improving neonatal outcomes [5,6,11,12]. However, the optimal starting time for probiotic supplementation remains unclear.

This 10-year retrospective study aimed to determine the optimal timing for initiating probiotics in preterm infants to reduce poor outcomes, by investigating the clinical differences between two groups of preterm infants who received *S. boulardii* at different timing after birth.

MATERIALS AND METHODS

Patients

The clinical characteristics of preterm infants born at a GA of <32 weeks or VLBW infants who weighed <1,500 g at birth and admitted to the NICU of our hospital between January 2011 and December 2020, were reviewed. Infants who did not receive probiotics were excluded. The infants were divided into an early introduction (EI) group (within one week after birth) and a late introduction (LI) group (after one week of life) according to the timing of probiotic initiation. Encoded clinical data were obtained from Gyeongsang National University Hospital Biobank, a member of the Korea Biobank Network, reviewed, and analyzed.

Nutrition and probiotic supplementations in our NICU

Enteral nutrition (EN) was initiated in preterm or VLBW infants without gastrointestinal obstruction after stabilization along with parenteral nutrition (PN). EN was started at a dose of 10–20 mL/kg/day of breast milk (BM) or preterm formula. Minimal EN was maintained for several days after birth. The feeding volume was then increased at a rate of 20–30 mL/kg/day depending on the individual's medical condition. Under the EN strategy for preterm or VLBW infants, full EN (FEN ≥120 mL/kg/day) was usually achieved 2–3 weeks after birth in our NICU. Probiotics supplementation is a routine practice in our NICU. This has been performed for more than 10 years although an ideal initiation time was not determined. A dose of 5×10^9 CFU of *S. bouardii* CNCM I-745 (Bioflor 250 powder®, Kuhnil) was administered with breast or formula milk twice a day. The supplementation starting time was determined by the pediatricians or neonatologists.

Clinical definitions

Clinical data were reviewed as follows. GA was determined based on the last menstrual period. BW was obtained. Small for gestational age (SGA) was defined as <10th percentile of BW for GA at birth and sex according to the Fenton growth charts [13]. Prolonged rupture of the membrane (PROM) was defined as the rupture of the amniotic membrane for 18 or more hours before delivery. Apgar score (AS) was obtained. A poor AS was defined as 0–3 at 5 minute after birth. Respiratory distress syndrome (RDS) of the newborn was defined as the detection of ground glass opacity on chest X-ray. Patent ductus arteriosus (PDA) was defined as left to right shunt via PDA on echocardiography. Since PDA in preterm infants born at <28 weeks of gestation with RDS was treated with ibuprofen or indomethacin prophylactically in our NICU, surgical ligation was determined as hemodynamically significant PDA even after medical treatment. Early hypotension was defined as a mean arterial pressure (MAP) below that for GA, MAP <30 mmHg within a week after birth, or medications for hypotension including inotropes, such as dopamine, epinephrine, hydrocortisone, and others, such as vasopressin. Late onset circulatory collapse (LCC) was defined as late-onset hypotension and oliguria resistant to intravascular volume expanders and inotropes that occurred abruptly without an underlying cause (including hemodynamically significant PDA, sepsis, bleeding, or NEC) after a transitional period [14]. Intraventricular hemorrhage (IVH) was diagnosed via cranial ultrasonography or magnetic resonance imaging. IVH grade 2 and more (IVH2) was obtained. NEC was defined as ≥stage 2 according to the modified Bell's criteria. Late-onset sepsis (LOS) was defined as a positive blood culture and antibiotic treatment for ≥5 days after one week of age. PN associated liver disease (PNALD) was defined as cholestasis with a direct bilirubin level of ≥2 mg/dL or 20% of total bilirubin without underlying hepatobiliary disorders in the PN setting. Bronchopulmonary dysplasia (BPD) was defined as the need for oxygen or respiratory support at 36 weeks of gestation or postnatal 28th day based on the severity-based definition for BPD of the National Institute of Health consensus [15]. When an infant achieved EN of ≥120 mL/kg/day, FEN was determined, and the date was collected. The first day of EN and/or probiotic supplementation was obtained. Hospital stays, postmenstrual age (PMA), and weight at discharge, were obtained. Extra-uterine growth retardation (EUGR) was defined as weight <10th percentile for PMA and sex at discharge. It was assessed using the Fenton growth charts [13].

Statistical analyses

Continuous variables are reported as means and standard deviations. They were compared using an independent *t*-test or Mann-Whitney U-test depending on the normality test outcomes. Categorical variables are reported as numbers and percentages. They were compared

https://doi.org/10.5223/pghn.2023.26.3.146

using the Chi-square or Fisher's exact test. To evaluate the risk factors associated with the LI of probiotics, univariate linear or non-linear regression analysis was performed with each significantly different factor identified by comparing the EI and LI groups. Multivariate regression analyses were performed with the statistically significant factors from the univariate analyses to evaluate the outcomes associated with the timing of probiotics initiation after adjusting for GA. Data were analyzed using R software version 4.1.3 (R Development Core Team, 2022; http://www.r-project.org). Two-sided $p<0.05$ was considered significant.

Ethics statement

This study was approved by the Institutional Review Board (IRB) of Gyeongsang National University Hospital (GNUH 2022-04-017), and the need to obtain informed patient consent was waived.

RESULTS

Clinical characteristics of the infants

A total of 416 preterm or VLBW infants were admitted to the NICU of our hospital between 2011 and 2020. Among them, 370 infants received probiotics and were included in this study. Forty-six infants were not supplemented with probiotics and therefore, excluded. These included 15 infants who required laparotomy due to intestinal obstruction or spontaneous intestinal perforation, 4 infants who were transferred to another hospital, and 27 infants with mortality within 2 weeks after birth because of severe hemodynamic instability. The mean GA was 29.9 weeks and the BW was 1,337 g. There were 118 (50.8%) boys 251 (67.8%) infants born by cesarean section, and 44 (11.9%) infants who were SGA. RDS occurred in 313 (84.6%) infants, PDA ligation in 29 (7.8%), early hypotension in 91 (24.6%), IVH2 in 21 (5.7%), LCC in 30 (8.1%), NEC in 26 (7.0%), LOS in 46 (12.4%), and moderate to severe BPD in 64 (17.3%) (**Table 1**). EN was started at a mean of 2.1 days after birth. Probiotics were initiated at a mean of 12.0 days after birth. The rate of breastfeeding was 68.0%. FEN was achieved in 370 infants at a mean of 18.9 days after birth. The mean duration of PN was 21.6 days. Infants were hospitalized on average for 59.3 days and discharged at a PMA of 38.5 weeks, weighing 2,617.3 g. Eleven (3.0%) infants died due to multiple organ failure (n=3), sepsis (n=2), NEC (n=3), or pulmonary arterial hypertension (n=3).

Comparisons of the clinical characteristics between the EI and LI groups

Clinical characteristics were investigated and compared between the EI and the LI groups (**Table 1**). The mean GA was 31.2±2.0 weeks in the EI group and 29.1±2.1 weeks in the LI group ($p<0.001$). The mean BW was 1,491.4±298.3 g in the EI group and 1,235.9±318.2 g in the LI group ($p<0.001$). SGA was more prevalent in the EI group (n=30, 20.4%) than in the LI group (n=14, 6.3%, $p<0.001$). The rates of PROM (35.5% vs. 21.9%, $p=0.007$) and poor AS (5.8% vs. 0.7%, $p=0.010$) were higher in the LI group than in the EI group. There were no significant differences between the two groups for sex, maternal diabetes, or maternal hypertension.

The incidence of morbidities associated with premature birth was significantly higher in the LI group than in the EI group, such as RDS (90.6% vs. 75.5%, $p<0.001$), PDA ligation (12.1% vs. 1.4%, $p<0.001$), early hypotension (31.8% vs. 13.6%, $p<0.001$), LCC (12.2% vs. 2.0%, $p<0.001$), IVH2 (8.6% vs. 1.4%, $p=0.003$), PNALD (23.8% vs. 9.8%, $p=0.002$), LOS (17.5% vs. 4.8%, $p<0.001$), and moderate to severe BPD (25.1% vs. 6.1%, $p=0.037$). NEC occurred more frequently in the LI group (9.0%) than in the EI group (4.1%), although the difference was

https://doi.org/10.5223/pghn.2023.26.3.146

Table 1. Clinical characteristics of preterm or very low birth weight infants and comparisons between the early and late probiotics introduction groups

Variable	Total (n=370)	EI (n=147)	LI (n=223)	*p*-value*	OR	95% CI		*p*-value**
						Lower	Upper	
Perinatal variables								
GA (wk)	29.9±2.3	31.2±2.0	29.1±2.1	<0.001	1.68	1.475	1.946	<0.001
BW (g)	1,337.0±334.4	1,491.4±298.3	1,235.9±318.2	<0.001	1.00	1.002	1.004	<0.001
SGA	44 (11.9)	30 (20.4)	14 (6.3)	<0.001	0.26	0.130	0.504	<0.001
Sex (male)	188 (50.8)	78 (53.1)	110 (49.3)	0.524	1.16	0.766	1.764	0.482
CS	251 (67.8)	97 (66.0)	154 (69.1)	0.570	1.15	0.736	1.792	0.536
Poor AS	14 (3.8)	1 (0.7)	13 (5.8)	0.010	9.06	1.776	165.5	0.035
Multigestation	112 (30.3)	35 (23.8)	77 (34.5)	0.029	1.69	1.062	2.720	0.029
Maternal DM	29 (7.9)	9 (6.1)	20 (9.0)	0.430	1.52	0.690	3.598	0.316
Maternal HTN	45 (12.2)	19 (12.9)	26 (11.7)	0.747	0.89	0.477	1.701	0.727
PROM	110 (30.1)	32 (21.9)	78 (35.5)	0.007	1.96	1.220	3.192	0.006
Feeding start (d)	2.1±3.2	1.0±1.1	2.9±3.9	<0.001	1.75	1.457	2.167	<0.001
BM	247 (68.0)	92 (62.6)	155 (69.5)	0.108	0.68	0.436	1.066	0.093
Initiation of probiotics (d)	12.0±23.7	3.1±2.0	17.9±29.0	<0.001				
Neonatal morbidities and associated variables								
RDS	313 (84.6)	111 (75.5)	202 (90.6)	<0.001	3.12	1.751	5.683	<0.001
PDA ligation	29 (7.8)	2 (1.4)	27 (12.1)	<0.001	9.99	2.930	62.54	0.002
Early hypotension	91 (24.6)	20 (13.6)	71 (31.8)	<0.001	2.97	1.741	5.247	<0.001
IVH2	21 (5.7)	2 (1.4)	19 (8.6)	0.003	6.74	1.917	42.72	0.011
LCC	30 (8.1)	3 (2.0)	27 (12.2)	<0.001	6.65	2.291	28.21	0.002
PNALD	55 (18.2)	12 (9.8)	43 (23.8)	0.002	2.86	1.478	5.905	0.003
NEC2	26 (7.0)	6 (4.1)	20 (9.0)	0.095	2.33	0.963	6.495	0.077
LOS	46 (12.4)	7 (4.8)	39 (17.5)	<0.001	4.24	1.953	10.61	<0.001
BPD	64 (17.3)	9 (6.1)	55 (25.1)	0.037	2.78	1.111	7.296	0.032
Outcomes and associated variables								
Fullfeeding (d)	18.9±NA	12.7±7.9	23.1±14.9	<0.001	1.11	1.074	1.142	<0.001
PN duration (d)	21.6±17.9	13.8±10.0	26.7±20.0	<0.001	1.09	1.062	1.117	<0.001
Hospital stays (d)	59.3±31.0	45.8±20.0	68.2±33.7	<0.001	1.04	1.027	1.052	<0.001
PMA discharge (wk)	38.5±3.5	37.7±2.3	39.0±4.0	0.001	1.14	1.058	1.232	0.001
Wt. discharge (g)	2,617.3±671.0	2,523.5±538.0	2,679.4±740.7	0.013	1.00	1.000	1.001	0.032
EUGR	174 (47.0)	62 (42.2)	112 (50.2)	0.137	1.38	0.910	2.109	0.130
Mortality	11 (3.0)	1 (0.7)	10 (4.5)	0.056	6.85	1.292	126.5	0.068

Values are presented as mean±standard deviation or number (%).
EI: early probiotics introduction (<1 week after birth), LI: late probiotics introduction (≥1 week after birth), OR: odds ratio, CI: confidential interval, GA: gestational age, BW: birth weight, SGA: small for gestational age, CS: cesarian section, Poor AS: 5-minute apgar score less than 3, maternal DM: maternal diabetes, maternal HTN: maternal hypertension, PROM: prolonged rupture of amniotic membrane, BM: breast milk, RDS: respiratory distress syndrome, PDA ligation: ligation of patent ductus arteriosus, IVH2: intraventricular hemorrhage ≥grade 2, LCC: late onset circulatory collapse, PNALD: parenteral nutrition-associated liver disease, NEC2: necrotizing enterocolitis stage ≥2, LOS: late onset sepsis, BPD: moderate to severe bronchopulmonary dysplasia, NA: not available, PN: parenteral nutrition, PMA discharge: postmenstrual age at discharge, wt. discharge: weight at discharge, EUGR: extrauterine growth restriction.
*p-value was obtained by Mann-Whitney U-test or Fisher's exact test. **p-value was obtained by univariate linear or non-linear regression analysis.

not statistically significant (p=0.095). Infants in the LI group needed longer PN durations (26.7±20.0 vs. 13.8±10.0 days, p<0.001) and lengthier hospitalization (68.2±33.7 vs. 45.8±20.0 days, p<0.001) than those in the EI group. Infants in the LI group were discharged at more advanced PMA (39.0±4.0 vs. 37.7±2.3 weeks, p=0.001) and higher weights (2,679.4±740.7 vs. 2,523.5±538.0 g, p=0.013) than infants in the EI group. The rate of EUGR at discharge was higher in the LI group than in the EI group, although the difference was not statistically significant (50.2% vs. 42.2%, p=0.137).

Risk factors associated with the LI of probiotics

To assess factors associated with the timing of probiotics initiation, we performed univariate regression analyses to identify the statistically significant variables (**Table 1**). Among perinatal variables, factors associated with the initiation of *S. boulardii* were GA (odds ratio [OR], 1.68; p<0.001, per week decrease), SGA (OR, 0.26; p<0.001), multigestation (OR, 1.69; p=0.029), PROM (OR, 1.96; p=0.006), poor AS (OR, 9.06; p=0.035), and the day of EN

https://doi.org/10.5223/pghn.2023.26.3.146

initiation (OR, 1.75; p<0.001). Among the neonatal morbidities, RDS (OR, 3.12; p<0.001), PDA ligation (OR, 9.99; p=0.002), early hypotension (OR, 2.97; p<0.001), IVH2 (OR, 6.74; p=0.011), LCC (OR, 6.65; p=0.002), PNALD (OR, 2.86; p=0.003), LOS (OR, 4.24; p<0.001), and moderate to severe BPD (OR, 2.78; p=0.032) were significantly associated with the late initiation of *S. boulardii*. Among the short-term outcome variables, FEN and PN durations, hospital stays, PMA at discharge, and weight at discharge were statistically significant factors associated with the late initiation of *S. boulardii*, although the ORs of these variables were not significantly high (**Table 1**). To assess the factors affecting the initiation of *S. boulardii*, multivariate logistic regression analysis was performed with statistically significant perinatal variables or neonatal morbidities on the univariate regression analyses that usually occur in the transitional period (**Table 2**). The results demonstrated that GA at birth (OR, 1.52; p<0.001) and the day of EN initiation (OR, 1.47; p<0.001) were factors that influenced the timing of probiotic initiation in our NICU.

Clinical outcomes associated with the LI of probiotics

To assess the outcomes associated with the timing of probiotic initiation, multivariate linear or nonlinear regression analyses were performed with neonatal morbidities and short-term outcome variables adjusted with GA (**Table 3**). Late probiotic initiation was associated with delayed FEN (OR, 5.44; p<0.001), LOS (OR, 2.85; p=0.020), and EUGR (OR, 1.67; p=0.033).

Table 2. Risk factors associated with late probiotics introduction in preterm or very low birth weight infants

Variable	OR	95% CI		*p*-value
		Lower	Upper	
GA (per week decrease)	1.52	1.264	1.842	<0.001
Feeding_start (per day increase)	1.47	1.225	1.828	<0.001
SGA	1.09	0.413	2.804	0.865
Poor AS	1.54	0.233	30.73	0.701
Multigestation	1.74	0.999	3.076	0.053
PROM	1.63	0.936	2.852	0.087
RDS	1.05	0.513	2.173	0.888
PDA ligation	3.13	0.780	21.13	0.154
Early hypotension	1.15	0.591	2.258	0.684
IVH2	0.70	0.149	5.066	0.678

OR: odds ratio, CI: confidential interval, GA: gestational age, SGA: small for gestational age, Poor AS: 5-minute apgar score less than 3, PROM: prolonged rupture of amniotic membrane, RDS: respiratory distress syndrome, PDA ligation: ligation of patent ductus arteriosus, IVH2: intraventricular hemorrhage ≥grade 2.
p-value was obtained by multivariate logistic regression analysis.

Table 3. Neonatal morbidities and outcomes associated with late probiotics introduction in preterm or very low birth weight infants

Variable	OR	95% CI		*p*-value
		Lower	Upper	
NEC2	1.51	0.473	4.844	0.424
LOS	2.85	1.236	7.419	0.020
Fullfeeding (d)	5.44	2.718	8.152	<0.001
Hospital stays (d)	5.25	0.128	10.62	0.056
EUGR	1.67	1.046	2.684	0.033
Mortality	2.79	0.455	53.79	0.352

OR: odds ratio, CI: confidential interval, NEC2: necrotizing enterocolitis stage ≥2, LOS: late onset sepsis, EUGR: extrauterine growth restriction.
p-values were obtained by multivariate linear or non-linear regression analyses after adjusting for gestational age at birth.

https://doi.org/10.5223/pghn.2023.26.3.146

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DISCUSSION

Deshpande et al. [16] suggested that probiotics could be beneficial for preterm infants as early as possible after birth. To date, reports on the optimal or appropriate timing for probiotic initiation in premature infants are limited [5]. As the optimal protocol for probiotic administration in preterm infants has not been undetermined, most investigators have decided on the timing of probiotic supplementation based on clinical stability. The timing of probiotics initiation is varied in the literature, from the first day of birth to unknown [17,18]. To determine the optimal timing for probiotic initiation for reducing poor outcomes, we analyzed the clinical characteristics of preterm or VLBW infants based on their starting time of probiotic supplementation. In this study, the factors affecting the time of *S. boulardii* supplementation initiation were GA at birth and the EN starting day (**Table 2**). Since the initiation of probiotics was affected by GA and the EN starting day, individual medical conditions during the perinatal period associated with GA may also affect the timing of probiotics initiation. However, probiotic initiation more than one week after birth could increase the risk of poor outcomes, especially LOS, delayed FEN, and EUGR after adjusting for GA (**Table 3**).

The overall frequency of NEC was 7.0%. NEC was more frequent in the LI group (9.0%) than in the EI group (4.1%). However, there was no statistically significant difference between the frequency of NEC (p=0.095, **Table 1**) or the risk of NEC and LI of *S. boulardii* (OR, 1.51; 95% confidential interval, 0.473-4.844; p=0.424, **Table 3**). In this study, the lack of statistical significance for the frequency and the risk of NEC may be due to both groups of infants being given probiotics although at different timing. In recent studies, *S. boulardii* supplementation has been shown to reduce the risks of NEC and LOS, frequency of feeding intolerance, as well as length of FEN and hospital stays, in preterm and VLBW infants [5,6].

In this study, probiotics initiation more than one week after birth was associated with delayed FEN and an increased risk of LOS and EUGR (**Table 3**). Although the neonatal outcomes associated with probiotic supplementation initiation have rarely been reported, many species of probiotics including *S. boulardii* have been studied and found to improve poor neonatal outcomes [3-5,17,19-22]. Recent studies have also suggested the use of multiple strains for probiotic supplementation to further reduce the risks of poor neonatal outcomes in preterm infants as opposed to using a single strain [5,6]. A combination of *Lactobacillus* spp., *Bifidobacterium* spp. and *S. boulardii* for probiotic supplementation may be more effective in shortening the FEN length and reducing the risk of sepsis than other combinations [6].

The optimal clinical probiotics dosage for preterm infants has not yet been determined. Previous studies have reported that doses of 10^5–10^{10} CFU did not provide statistically significant differences in neonatal outcomes among preterm infants [19,20]. Recently, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Committee suggested that *L. rhamnosus* GG ATCC53103 at a dose of 1×10^9–6×10^9 CFU or the combination of *B. infantis* Bb-02, *B. lactis* Bb-12, and *Str. thermophiles* TH-4 each at a dose of 3–3.5×10^8 CFU may reduce the risk of stage 2 or 3 NEC [5]. In the present study, *S. boulardii* CNCM I-745 at a dose of 5×10^9 CFU was used twice a day for all the infants, regardless of body weight. Thus, outcome differences according to various dosages could not be analyzed.

The ESPGHAN committee does not recommend routine use of *S. boulardii* for patients with a central venous catheter, critically ill patients, or immunocompromised patients due to safety

https://doi.org/10.5223/pghn.2023.26.3.146

reasons [5,23,24]. In contrast to the ESPGHAN recommendation, at our NICU, we have been using *S. boulardii* supplementation for all preterm or VLBW infants for more than 10 years. Our NICU is a single large unit, and thus, the potential spread of nosocomial infections is always a concern. As a result, antibiotics treatment has been relatively liberal when sepsis is suspected in a sick baby. Considering the characteristics of *S. boulardii* with its resilience against broad-spectrum antibiotics and low risk of antibiotic resistance transmission, it is a good choice for probiotic supplementation [21,22]. Over the last 10 years in our NICU, two cases of fungemia caused by *S. cerevisiae* have occurred. In both cases, complete recovery was possible after using antifungal agents. These cases of fungemia may have been caused by contamination during the mixing of *S. boulardii* into the formula in the incubator. However, fungemia has not occurred again after cautioning about environmental contamination.

This study has several limitations. First, this was a retrospective study in a single hospital. Second, there was a possibility of selection bias because the initiation of probiotics supplementation was determined based on the initial clinical condition of the preterm or VLBW infants as affected by GA.

Despite these limitations, this study has strength in that it investigated the optimal timing of probiotic initiation in a large number of preterm or VLBW infants and reported the long experience of *S. boulardii* use in the NICU setting.

In conclusion, although GA at birth and neonatal medical conditions in the transitional period may affect the initial timing of *S.boulardii* supplementation in preterm and VLBW infants, probiotic initiation later than one week after birth may contribute to a higher risk of LOS, delayed FEN, and EUGR even after adjusting for GA. Therefore, the EI of *S. boulardii* to infants within one week after birth may reduce the risk of adverse outcomes in preterm or VLBW infants. To avoid environmental contamination, the mixing of probiotics into BM or formula must be performed with caution. In the future, large-scale prospective studies to verify our findings are warranted.

ACKNOWLEDGEMENTS

Statistical analyses in this study were supported by the Medical Research Statistics Department of the Gyeongsang National University Hospital. Encoded clinical data used in this study were obtained from Gyeongsang National University Hospital, a member of the Korea Biobank Network.

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