Bovine Lactoferrin Prevents Invasive Fungal Infections in Very Low Birth Weight Infants: A Randomized Controlled Trial

WHAT'S KNOWN ON THIS SUBJECT: Lactoferrin is a glycoprotein with anti-infective activities being part of the innate defensive network. Bovine and human lactoferrin share high homology. Bovine lactoferrin can prevent late-onset sepsis in preterm very low birth weight neonates.

WHAT THIS STUDY ADDS: In preterm very low birth weight infants, bovine lactoferrin is able to prevent not only late-onset sepsis but also systemic fungal infections. This protection is achieved independently from their colonization status.

AUTHORS: Paolo Manzoni, MD,[a] Ilaria Stolfi, MD,[b] Hubert Messner, MD,[c] Silvia Cattani, MD,[d] Nicola Laforgia, MD,[e] Mario G. Romeo, MD,[f] Lina Bollani, MD,[g] Matteo Rinaldi, MD,[h] Elena Gallo, MD,[a] Michele Quercia, MD,[e] Milena Maule, MD,[i] Michael Mostert, MD, PhD,[j] Lidia Decembrino, MD,[g] Rosario Magaldi, MD,[h] Fabio Mosca, MD, PhD,[k] Federica Vagnarelli, MD,[l] Luigi Memo, MD,[m] Pasqua M. Betta, MD,[f] Mauro Stronati, MD,[h] and Daniele Farina, MD,[a] on behalf of the Italian Task Force for the Study and Prevention of Neonatal Fungal Infections–the Italian Society of Neonatology

[a]Department of Neonatology and NICU, S. Anna Hospital, Torino, Italy; [b]Department of Neonatology, Policlinico Umberto I, Rome, Italy; [c]Department of Neonatology, Ospedale Regionale, Bolzano/Bozen, Italy; [d]Department of Neonatology and NICU, University of Modena and Reggio Emilia, Modena, Italy; [e]Department of Neonatology and NICU, Policlinico University Hospital, Bari, Italy; [f]Department of Neonatology, Azienda Ospedaliera Universitaria Policlinico di Catania, Italy; [g]Department of Neonatology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [h]Department of Neonatology, Ospedali Riuniti, Foggia, Italy; Departments of [i]Biomedical Sciences and Human Oncology, Cancer Epidemiology Unit, and [j]Pediatrics, University of Torino, Torino, Italy; [k]NICU, Istituto di Ricerca e Cura a Carattere Scientifico, Mangiagalli Hospital, Milano, Italy; [l]NICU, Arcispedale, Reggio Emilia, Italy; and [m]Department of Neonatology, Ca' Foncello Hospital, Treviso, Italy

KEY WORDS

lactoferrin, VLBW neonates, *Candida*, fungal sepsis, prophylaxis

ABBREVIATIONS

bLF—bovine lactoferrin
CI—confidence interval
DOL—day of life
IFI—invasive fungal infection
LF—lactoferrin
LGG—*Lactobacillus rhamnosus* GG
RCT—randomized controlled trial
RR—risk ratio
VLBW—very low birth weight

Some data from this study were presented in abstract form as preliminary data at the Pediatric Academic Societies Meetings; May 2–4, 2009; Baltimore, MD.

Dicofarm SpA had no role in the original study design and enrollment of patients; the collection, analysis, and interpretation of the data; or the preparation and submission of any article related to the data generated by the original study.

This trial has been registered with the ISRCTN Register (http://isrctn.org) (identifier ISRCTN53107700).

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abstract

BACKGROUND: Lactoferrin is a mammalian milk glycoprotein involved in innate immunity. Recent data show that bovine lactoferrin (bLF) prevents late-onset sepsis in preterm very low birth weight (VLBW) neonates.

METHODS: This is a secondary analysis of data from a multicenter randomized controlled trial where preterm VLBW neonates randomly received bLF (100 mg/day; group A1), bLF + *Lactobacillus rhamnosus* GG (10^6 colony-forming units per day; group A2), or placebo (group B) for 6 weeks. Here we analyze the incidence rates of fungal colonization, invasive fungal infection (IFI), and rate of progression from colonization to infection in all groups.

RESULTS: This study included 472 neonates whose clinical, nutritional, and demographical characteristics were similar. Overall, the incidence of fungal colonization was comparable (17.6%, 16.6%, and 18.5% in A1, A2, and B, respectively; $P = .89$ [A1] and .77 [A2]). In contrast, IFIs were significantly decreased in A1 and A2 (0.7% and 2.0%, respectively) compared with B (7.7%; $P = .002$ [A1] and .02 [A2]), and this was significantly true both in <1000 g (0.9% [A1] and 5.6% [A2], vs 15.0%) and in 1001 to 1500 g infants (0% and 0% vs 3.7%). The progression rate colonization-infection was significantly lower in the bLF groups: 3.7% (A1) and 12% (A2), vs 41.9%; $P < .001$ (A1) and $P = .02$ (A2). No IFI-attributable deaths occurred in the treatment groups, versus 2 in placebo. No adverse effects or intolerances occurred.

CONCLUSIONS: Prophylactic oral administration of bLF reduces the incidence of IFI in preterm VLBW neonates. No effect is seen on colonization. The protective effect on IFI is likely due to limitation of ability of fungal colonies to progress toward invasion and systemic disease in colonized infants. *Pediatrics* 2012;129:116–123

Preterm infants in NICUs are at great risk for fungal sepsis due to a number of specific risk factors, such as immature immunity, prolonged need for intensive care, and disorders in gut microecology with proliferation of saprophytes and pathogens including *Candida* spp.

Invasive fungal infections (IFIs) in preterm infants are caused mainly by the various *Candida* spp, and are responsible for considerable short- and long-term morbidity and high attributable mortality.[1]

Fungal colonization is a first step occurring in up to 46%[2] of preterm very low birth weight (VLBW) infants during their first month of life in a NICU. Progression from colonization to systemic infection occurs in 10% to 30% of the colonized infants,[3] depending on several factors including characteristics of colonization and of the host.

As diagnosis of fungal infection in premature infants is difficult, and prompt recognition and treatment does not avoid long-term disabilities, the best strategy is to prevent the disease.

Fluconazole has been shown to be safe and effective in a number of randomized controlled trials (RCTs) and it is recommended in settings of patients with high incidences of IFIs.[4] Despite reassuring reports[5,6] some concerns still exist related to long-term safety and modification of the fungal ecology induced by this azole with emergence of resistant strains.

Lactoferrin (LF) is a glycoprotein of mammalian milk that is involved in innate immunity mechanisms with several documented anti-infective properties, including antifungal activity.[7,8] This last mechanism is related to both fungistatic effects, and the activity of the N-terminal, 11 aminoacidic peptide of LF called lactoferricin [*hLF(1-11)*]. Lactoferricin's potent candidacidal activity occurs through stimulation of an increase in the mitochondrial membrane's potential and permeability, resulting in the synthesis and secretion of adenosine triphosphate and the production of reactive oxygen species, thereby leading to *Candida albicans* cell death.[9] Noteworthy, bovine and human LF shares a high structural homology, as well as the same antimicrobial properties.[7,8]

We recently reported in a multicenter RCT how bovine LF (bLF) supplementation since birth prevented late-onset sepsis in preterm VLBW neonates in the NICU.[10]

Our aim for this current study is to assess whether bLF, alone or in combination with the probiotic *Lactobacillus rhamnosus* GG (LGG), is also able to prevent fungal colonization and infection in such population.

METHODS

This is a secondary analysis of the data obtained during a multicenter RCT developed in Italy in the years 2006–2007; its original protocol, including structured criteria for inclusion/exclusion, was previously published.[10]

Briefly, preterm VLBW neonates from 11 tertiary NICUs were enrolled before 72 hours of life and randomly assigned to receive either bLF alone (100 mg/day, group A1; n = 153) or in combination with LGG (10^6 colony-forming units per day, group A2; n = 151) or placebo (group B, n = 168) from birth to the 30th day of life (DOL; 45th for those $<$1000 g at birth). The drugs and placebo were administered orally, once a day. Neonates not feeding in the first 48 hours received the drug(s)/placebo by orogastric tube.

Prophylaxis with antifungal drugs already instituted was an exclusion criterion and was not allowed per the original protocol.

In the current study, we focused the analysis on the data regarding fungal colonization and infection, having as a primary end point the assessment of bLF supplementation effects on the incidence rates of fungal colonization and of IFI. The secondary end points in the current study were as follows: intensity of fungal colonization; rate of progression to infection in colonized infants; frequencies of single fungal species in all groups; and IFI-related deaths.

Per the original protocol, clinical surveillance for detection of sepsis was performed, along with complete laboratory and microbiology workout in case of suspected episodes of late-onset sepsis and/or IFI.

Systematic clinical surveillance for adverse effects was also performed.

Nutritional and feeding policies were stable during the study and consistent among centers, following common guidelines. Administration of fresh, expressed maternal milk was encouraged. Each mother supplied milk only for her infant. When needed, feeding supplementations were made with a formula not supplemented with bLF.

Minimal enteral feeding with small amounts of maternal milk or formula ($<$10 mL/kg per day) was initiated at DOL 2. Cautious volume advancements were performed by adding 15 mL/kg per day. Parenteral nutrition was started at DOL 2 and continued until enteral feeding reached 150 mL/kg per day.

The criteria for hospital discharge were weight of $\geq$1800 g, full oral feeding, and resolution of acute medical conditions.

Definitions

The focus of this study was on the first episode of fungal sepsis in each infant and on the occurrence of fungal colonization.

Systematic surveillance for detection of fungal colonization was performed through clinical and weekly surveillance cultures ($\geq$3 cultures per week)

during the study period. The following surveillance cultures were performed: ear canal swab; umbilical catheter at birth; stool, gastric aspirate, rectal swab, or pharyngeal swab: at least 3 of them weekly (DOL 0, 7, 14, 21, 28 [35, 42]). In addition, cultures were obtained from surgical devices after removal and from any sites indicated by the physician. Fungal colonization was defined as the detection of at least 1 culture positive for fungi during the stay in the NICU.

IFI was defined as occurring ≥4 days after birth and included clinical signs and symptoms consistent with sepsis together with isolation of a fungal causative organism from blood (drawn from peripheral sites), urine (collected by suprapubic puncture or bladder catheterization, with growth of >10 000 fungi/mL),[10] or cerebrospinal or peritoneal fluid.

Isolation of fungi from specimens other than those listed above was considered as colonization.

Intensity of fungal colonization was assessed by calculating the number of concomitant positive cultures for fungal isolates retrieved from noncontiguous sites.[11,12]

Progression from colonization to infection was defined as the occurrence of IFI in a previously colonized patient.

IFI-related death was defined as death within 3 days after the last positive culture from any site without other causes or isolation of fungal pathogens at autopsy.

IFI episodes were treated with intravenous antifungal agents (liposomal amphotericin B in all cases) for ≥7 days. Presumed fungal sepsis (clinical presentation consistent for IFI but no microorganisms isolated) was not considered as IFI.

Diagnostic criteria relied on the existing literature, on guidelines from international consensus documents,[13] and on the Italian Neonatology Society's Fungal Infections Task Force recommendations.[14]

Blinding was not broken to guide therapy.

Microorganism Isolation and Identification

For the identification of fungi, all specimens were inoculated onto chromogen culture plates (Albicans ID; Biomerieux Inc, Durham, NC), which allow for rapid *C albicans* identification through the blue staining of the colonies after 48 hours of incubation at 37°C. Differently stained colonies were speciated through a miniaturized system of biochemical tests (Vitec Yeast; Biomerieux Inc). Isolates were tested for sensitivity to fluconazole with standardized microbroth dilution assays (ATB-Fungus-2-Int; Biomerieux Inc) according to the National Committee for Clinical Laboratory Standards recommendations.[15]

Statistical Analysis

All primary and secondary outcomes were represented by dichotomous variables (presence/absence) and analyzed by intention to treat.

The variables analyzed were as follows: IFI incidence; fungal colonization; all-cause death before discharge; IFI-related death; and rate of progression of fungal colonization to IFI.

Groups A1 and A2 were compared separately to group B. The data were analyzed for all groups and separately for neonates <1000 g, 1001 to 1500 g, and <750 g at birth.

Proportions and continuous variables were compared by using Fisher's exact 2-tailed test and the *t* test, respectively.

Risk ratios (RRs) and 95% confidence intervals (CIs) were calculated to compare cumulative between-group incidences by using Stata software. All tests were 2-tailed, and $P < .05$ was considered to indicate statistical significance.

Sample size analysis predicted a number of patients needed for each group of 134 for IFI and 100 for fungal colonization, on the basis of 2-sided type I error rates ≤.05 and a power of 80% to detect absolute differences between treated and nontreated infants of at least 75% (decrease from 12% to 3%, given a pretrial incidence of 12%) for IFI and of at least 60% (decrease from 25% to 10%, given a pretrial incidence of 25%) for fungal colonization. Given the low incidence of IFI in the bLF groups, the study was underpowered to detect significant differences for this outcome between A1 and A2. Assuming incidence rates of 2% in A1, 2319 infants would have been needed to reach a power of 80% to detect an absolute difference of 50%.

Power calculations were performed by using S-plus software, version 2000 (MathSoft).

RESULTS

In the original study, 494 VLBW neonates survived ≥3 days and were assessed for eligibility. Twenty-two were ineligible (data reported in the original study's consort flow diagram, see Fig 1). Four-hundred seventy-two neonates were randomly assigned to bLF ($n = 153$), bLF + LGG ($n = 151$), and control ($n = 168$).

One infant discontinued treatment (receiving only 8 drug doses) and 9 infants (2 in bLF, 4 in bLF + LGG, and 3 in control) had incomplete data on some variables that were included in the protocol (complete blood count, C-reactive protein, platelet count, and blood glucose) but were not subsequently analyzed. All 472 randomly assigned infants were therefore included in the analysis both in the original study and in the present one.

Table 1 lists demographics, neonatal characteristics, and major risk factors for IFI. There were no significant baseline differences between groups in risk factors for sepsis, management, and nutritional characteristics.

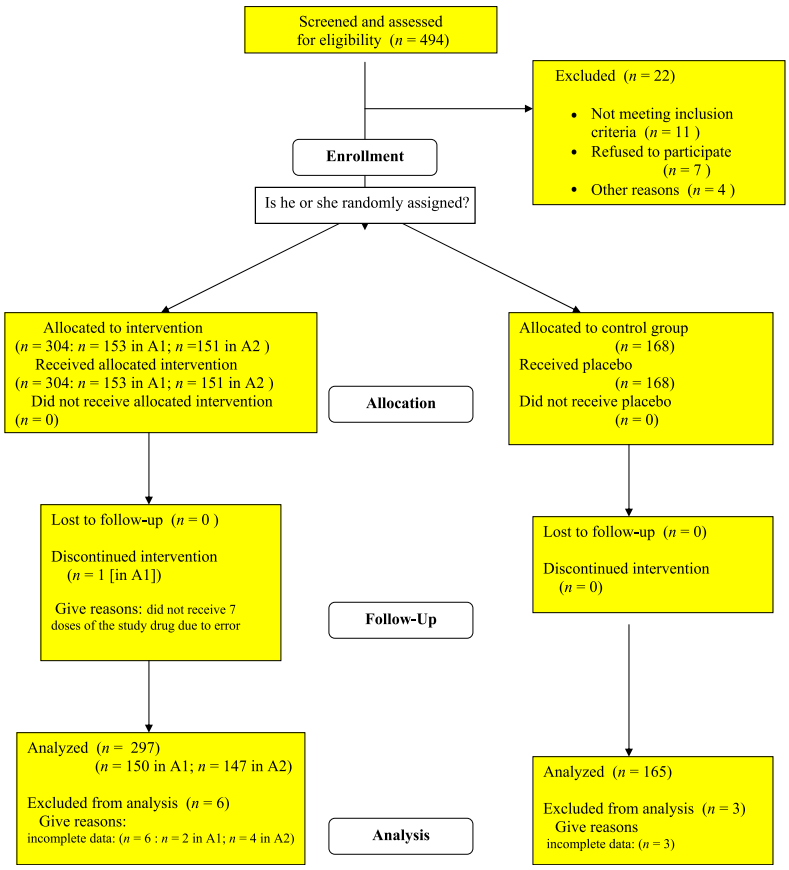

FIGURE 1
Consort E flowchart. bLF supplementation prevents invasive fungal infections in preterm VLBW neonates: data from a multicenter, randomized, double-blind, placebo-controlled study. Adapted from Manzoni et al[10].

Tables 2 and 3 show the main results and distribution of fungal species with infection sites, respectively. There were 17 infants each having an episode of IFI with 20 causative fungal isolates.

Overall, the incidence of fungal colonization (at least 1 site) was similar in the 3 groups (17.6%, 16.6%, and 18.5% in A1, A2, and B, respectively; $P = .89$ for A1 and 0.77 for A2; Table 2).

The proportion of infants with high intensity of colonization (ie, >3 different sites concomitantly colonized) was lower in the bLF-treated groups (4.4% and 3.3% in A1 and A2, respectively, compared with 7.7% in B), but the difference was not significant ($P = .10$).

The overall incidence of IFI was lower in groups A1 and A2 (0.7% and 2.0%, respectively) compared with B (7.7%; RR = 0.08 [95% CI: 0.01–0.64]; $P = .002$ for A1; RR = 0.26 [95% CI: 0.07–0.88]; $P = .02$ for A2). The rate of progression from colonization to infection was significantly lower in both bLF groups (3.7% and 12%, vs 41.9%; $P < .001$ and $P = .02$ in A1 and A2, respectively; Table 2).

In bLF-treated infants, IFIs were less frequent both in extremely low birth weight (0.9% and 5.6%, vs 15.0% for A1 and A2, respectively) and in 1001 to 1500 g infants (0% vs 3.7% for both A1 and A2; data not shown in the tables).

Colonizing and infecting isolates are shown in Table 3. *C albicans* was the most frequent one, followed by *Candida parapsilosis* and some others. Due to the small numbers of isolates, only for *C albicans* was it possible to detect a significant difference between groups, with 60% and 80% reduction in the treatment groups.

In both treatment groups, the incidence of fungal urine infections was 0.7% compared with 2.4% in the placebo group ($P = .37$ in both cases). No differences in any of the primary and secondary outcomes (Tables 2 and 4) were detected in the treatment and placebo groups when comparing infants fed exclusive maternal milk with infants fed exclusive formula milk (data not shown in the tables).

No deaths attributable to IFI occurred in the treatment groups compared with 2 in the placebo group.

No adverse effects or intolerances attributable to bLF and/or LGG occurred.

DISCUSSION

IFIs pose a major problem to viability of the most immature neonates, with increasing attributable and related short- and long-term morbidity.

bLF can exert a specific antifungal activity for its peculiar ability to bind to the fungal cell wall's receptors thus producing cell wall disruption.[9,16] In addition, bLF is synergistic with antifungal drugs such as fluconazole.[16,17]

Consistent with the experimental literature, this secondary analysis of the data from a large multicenter RCT shows that administration of bLF since the early moments of life is effective in preventing infections caused by *Candida* spp in preterm VLBW infants.

Two different points in the process of the fungus–host interaction may be identified and may be targeted for preventative strategies. The first possible step is to prevent the onset of colonization; the second is to prevent the colonizing yeast resulting in infection throughout multiplication and systemic dissemination. In both cases, the ultimate result of targeting those 2 different steps is the same (ie, decreasing the incidence of fungal infections).

Of note, no effect of bLF was seen on fungal colonization, whose incidence

TABLE 1 Demographic and Nutritional Characteristics of the Patients and Major Risk Factors for Fungal Colonization and Infection

	LF	LF + LGG	Control	LF Versus Control, *P*	LF + LGG Versus Control, *P*
Demographics					
No. of patients, total = 472	153	151	168		
Birth weight, g	1142	1138	1109	.25	.31
Mean (±SD) and range	(±244) 634–1495	(±253) 550–1500	(±269) 437–1500		
Gestational age in weeks	29.6	29.8	29.5	.82	.39
Mean (±SD) and range	(±2.5) 23–36	(±2.8) 23–35	(±3.2) 23–39		
Apgar score at 5 min, Mean ± SD[a]	7.6 ± 1.4	7.5 ± 1.6	7.6 ± 1.5	.57	.42
Gender, % of boys	48.4	47.7	51.2	.66	.58
Race, % of white patients[b]	90.2	88.1	91.1	.84	.46
Vaginal delivery, %	19.0	21.2	20.2	.78	.89
Use of antenatal corticosteroids, %	71.2	68.9	73.2	.80	.45
Risk factors for IFI					
Use of TPN, d, Mean ± SD	20.2 ± 20.9	17.8 ± 18.1	18.5 ± 15.0	.35	.57
Central venous catheter(s) positioned, d, Mean ± SD	13.2 ± 10.3	13.7 ± 10.2	15.0 ± 11.5	.13	.28
Intubation, d, Mean ± SD	9.5 ± 8.39	9.8 ± 7.4	10.9 ± 10.2	.31	.40
Use of H2 blockers, d, Mean ± SD	3.1 ± 6.8	3.0 ± 8.3	2.7 ± 6.5	.60	.73
Use of third generation cephalosporins, d, Mean ± SD	0.7 ± 0.5	0.7 ± 0.6	0.9 ± 0.8	.65	.88
Use of antibiotics, d, Mean ± SD	11.6 ± 8.5	11.8 ± 9.5	13.3 ± 10.5	.12	.19
Use of postnatal steroids, d, Mean ± SD	1.0 ± 0.9	1.1 ± 1.1	0.9 ± 1.3	.80	.62
Supplemental oxygen, d, Mean ± SD	14.0 ± 9.8	14.3 ± 11.0	14.4 ± 12.5	.76	.77
Mean duration of stay in the NICU, alive infants, d, Mean ± SD	54.2 ± 24.4	54.7 ± 22.3	55.1 ± 21.7	.73	.87
Mean duration of stay in the NICU, deceased infants, d, Mean ± SD	14 ± 5.6	14 ± 7.0	27.4 ± 21.6	.25	.16
Early-onset neutropenia, %	10.2	8.6	7.2	.45	.76
Nutritional characteristics					
Time of initiation of oral feeding, DOL, Mean ± SD	2.2 ± 3.1	2.1 ± 3.8	2.4 ± 3.5	.49	.34
Time of achievement of full feeding, DOL, Mean ± SD	12.5 ± 4.1	13.4 ± 5.1	14.8 ± 4.7	.05	.21
Vol of feedings advanced daily, mL/d, Mean ± SD	10.0 ± 4.5	11.0 ± 3.9	10.6 ± 3.0	.59	.68
Infants fed with only formula, %	15.7	17.2	13.1	.53	.35
Infants fed with only maternal milk, %	27.4	21.2	22.1	.30	.89
Infants fed with both formula and maternal milk, %	56.9	61.6	64.8	.72	.84
Daily average amounts of human fresh milk intake, mL/kg, Mean ± SD	69.3 ± 41.7	65.7 ± 41.5	66.8 ± 35.5	.56	.79
Total d of human fresh milk feeding, Mean ± SD	22.3 ± 13.6	21.4 ± 13.7	22.8 ± 12.6	.71	.33

P values were calculated with the use of Fisher's exact 2-tailed test for comparing proportions and the *t* test for comparing continuous variables (eg, birth weight). d, mean days until discharge; TPN, total parenteral nutrition.

[a] The Apgar score ranges from 0 to 10, with higher scores indicating better functioning.

[b] Race was determined by the investigators. Percentage refers to both parents.

rates remained similar in all groups. Only a slight trend to feature lower intensity grades of colonization was seen in treated infants, as disclosed by the nonsignificant decrease in the number of infants colonized in >3 different sites.

These findings are associated with lower colonization spread to infection or suggest an ability of bLF to limit spread, but the data do not support the attributing cause. Our data disclose that bLF is not able to limit the onset of colonization after the contact of fungi with the host, but nonetheless bLF can act at a later stage in the process of the fungus–host interaction, ultimately resulting in decreased odds of progressing to infection when getting colonized.

According to the present data, bLF has the same impact in prevention of IFIs in preterm infants as prophylactic fluconazole.[18,19]

This azole prevents both colonization and infection, but its ability to prevent progression to infection in colonized patients is questioned,[19] thus suggesting that the preventive effect of fluconazole may occur by limiting colonization rather than contrasting the ability of fungal colonies to progress to infection. In contrast, bLF seems to mimic a physiologic action aiming at preventing pathogenicity of fungal colonies rather than preventing the contact with the fungi. In other words, fluconazole protects the host from the contact from fungus, whereas bLF protects the infant host from the risk that fungi may cause infection in the premature host. As breastfed, healthy neonates may often be challenged by *Candida* spp that colonize, or even infect, the mother's nipple, this last mechanism appears more natural and in line with the unfeasibility of avoiding, in real life, any neonate–fungi contact.

bLF, as well as human LF, modulate the growth and functional development of the nascent enterocytes, accelerating maturation and immune-competency and ultimately enhancing the gut barrier effect in the early ages of life.[20] As most of the fungal systemic infections arise from translocation of colonizing fungal colonies from the gut reservoir to the bloodstream,[21] the specific

TABLE 2 Main Results: IFI, Previous Colonization, Progression From Colonization to Infection, and Mortality Related to IFI in the Study Groups

	Groups			LF Versus Placebo			LF + LGG Versus Placebo		
	LF, A1, $n = 153$	LF + LGG, A2, $n = 151$	Placebo, B, $n = 168$	RR	95% CI	P	RR	95% CI	P
Total IFI, $n = 17$ (%)	1/153 (0.7)	3/151 (2.0)	13/168 (7.7)	0.08	0.01–0.64	.002	0.26	0.07–0.88	.02
IFI in extremely low birth weight neonates (%)	1/53 (1.9)	3/54 (5.6)	9/60 (15.0)	0.13	0.02–0.96	.02	0.37	0.11–1.30	.13
IFI in 1001–1500 g neonates (%)	0/100 (0.0)	0/97 (0.0)	4/108 (3.7)	—	—	.12	—	—	.12
D of onset of fungal sepsis, DOL, Mean ± SD	21.1 ± 4.4	17.1 ± 8.2	19.5 ± 12.8	—	—	.14	—	—	.05
Colonization and progression to IFI									
Overall fungal colonization, at least 1 site (%)	27/153 (17.6)	25/151 (16.6)	31/168 (18.5)	0.96	0.60–1.53	.89	0.90	0.56–1.45	.77
Infants colonized in >3 different sites (%)	7/153 (4.6)	5/151 (3.3)	13/168 (7.7)	0.59	0.24–1.44	.26	0.43	0.16–1.17	.10
Progression rate colonization/IFI, all neonates (%)	1/27 (3.7)	3/25 (12.0)	13/31 (41.9)	0.09	0.01–0.63	<.001	0.29	0.09–0.89	.02
Mortality (before discharge)									
Overall mortality, not attributable to sepsis (%)	4/153 (2.6)	6/151 (4.0)	12/168 (7.1)	0.37	0.12–1.11	.07	0.56	0.21–1.45	.24
Mortality attributable to IFI (%)	0/153 (0.0)	0/151 (0.0)	2/168 (1.2)	—	—	.50	—	—	.50

TABLE 3 Fungal Sepsis by Microorganisms and by Sites of Isolation

	All Infants[a] Total = 17	LF Total = 1	LF + LGG Total = 3	Placebo Total = 13
Isolates				
Candida albicans	13	1	2	10
C parapsilosis	3	—	1	2
C glabrata	1	—	—	1
C guillermondii	1	—	—	1
Sites of isolation of IFI				
Blood	10	—	2	8
Urine	6	1	1	4
Cerebrospinal fluid	1	—	—	1

[a] One neonate in the placebo group was infected by 2 different species of fungi, concomitantly.

ability of bLF on the enterocytes might be the key step in explaining why bLF protects from infection but not from colonization. In this view, the mechanisms of action of bLF, once more, look more similar to what occurs in nature than for fluconazole.

Probiotics, namely LGG, have been shown to be able to prevent enteric fungal colonization,[22, 23] thus we might expect that a synergistic activity with bLF can boost such effect and further decrease the rate of colonization and possibly infection in the bLF + LGG group. However, the data collected in this study do not reveal any additional benefit. We speculate that this is related to the low rates of colonization here reported compared with the original studies where fungal colonization rates in the control groups were higher than 40% compared with only 18.5% in this study. Thus, an LGG effect, provided it exists in all settings, might be relevant only when colonization rates are high.

Human milk has known anti-infective properties,[24] and is also reported to have a specific fungistatic effect attributed to LF.[25] In our study, a slight, nonsignificant trend to decrease colonization rates occurred in the treated infants; however, no effect existed on the progression rates thus concluding that human fresh milk feeding does not determine any additional preventative effect on IFI (data not shown in the tables).

The limited number of patients and events in each group, though, as well as the low rate of infections in this study, might justify why these findings do not confirm previous ones.[24]

A trend toward a protective effect was seen also on urine infections caused by fungi. This is inconsistent with a substantial lack of effect on urinary infections caused by all other pathogens in the original study.[10] Studies on larger patient samples should assess whether different mechanisms may be involved in the development of urinary tract infections by different agents, and that only some of them may be targeted by bLF.

CONCLUSIONS

Prophylactic oral administration of bLF (either alone, or in combination with LGG) is effective in reducing the incidence of IFI in preterm VLBW neonates, regardless of their birth weight. No effect is seen on colonization. The protective effect on IFI is likely due to limitation of ability of fungal colonies to progress toward invasion and systemic disease in the colonized infants.

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This is a secondary analysis of the results from an RCT (its results were published in 2009) that was partially supported by Dicofarm SpA, Rome, with a grant, besides supplying the drugs and placebo used in the original study.

TABLE 4 Other Outcomes

	LF	LF + LGG	Control	LF Versus Control, *P*	LF + LGG Versus Control, *P*
Threshold retinopathy of prematurity, requiring surgery (%)	6/153 (3.9)	13/151 (8.6)	19/168 (11.3)	.02	.46
Severe, grade 3–4, IVH (%)	6/153 (3.9)	4/151 (2.7)	2/168 (1.2)	.16	.43
Bronchopulmonary dysplasia* (%)	4/153 (2.6)	4/151 (2.7)	6/168 (3.6)	.75	.75
Infants undergoing major surgery, including ligation of PDA (%)	5/153 (3.3)	2/151 (1.3)	3/168 (1.8)	.49	.99
Death, before hospital discharge, all causes (%)	4/153 (2.6)	6/151 (4.0)	12/168 (7.1)	.08	.24
NEC, stage II or greater (%)	3/153 (1.9)	0/151 (0.0)	10/168 (6.0)	.09	.002
Death or NEC, stage II or greater (%)	7/153 (4.6)	7/151 (4.6)	18/168 (10.7)	.06	.06

IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus.

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Address correspondence to Paolo Manzoni, MD, Department of Neonatology and NICU, S. Anna Hospital, Azienda Ospedaliera Regina Margherita–S. Anna, C. Spezia 60, 10126 Torino, Italy. E-mail: paolomanzoni@hotmail.com

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