Randomized Controlled Trial Evaluating Probiotics in Children with Severe Acute Malnutrition.

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SUMMARY

This randomized, double-blind, placebo-controlled trial was conducted involving 400 children hospitalized with severe acute malnutrition (SAM). Patients received one daily dose of a blend of *Bifidobacterium animalis subsp lactis* and *Lactobacillus rhamnosus* (10 billion colony-forming units, 50:50) or placebo during hospitalization followed by an 8- to 12-week outpatient treatment period, depending on patients' recovery rate. The primary outcome was number of days with diarrhea during hospitalization. Secondary outcomes included other diarrhea outcomes, pneumonia, weight gain, and recovery. There was no difference in number of days with diarrhea between the probiotic (*n*=200) and placebo (*n*=200) groups during inpatient treatment (adjusted difference +0.2 days, 95% confidence interval -0.8 to 1.2, *P*=0.69); however, during outpatient treatment, probiotics reduced days with diarrhea (adjusted difference -2.2 days 95% confidence interval -3.5 to -0.3, *P*=0.025). Twenty-six patients died in the probiotic *versus* 20 in the placebo group (*P*=0.38). The authors concluded that these probiotics had no effect on diarrhea in children with SAM during hospitalization, but reduced the number of days with diarrhea in outpatient treatment by 26%.

COMMENTARIES

Evidence-based Medicine Viewpoint

Relevance: Severe malnutrition is a clinically significant problem with implications for affected individuals, healthcare system(s), and society at large. The problem is worse in settings with low-income, poor literacy and other social challenges. A group of researchers working in Uganda, collaborated with Danish scientists to examine whether a specific probiotic preparation, could have beneficial effects on children hospitalized with severe acute malnutrition (SAM), both during the acute treatment phase, as well as recovery phase [1]. Although a clear research question has not been stated in the publication [1], the study was designed to examine the effect(s) of a combination of two probiotic strains (I=Intervention), compared to placebo (C=Comparator), on diarrhea, lower respiratory infection (pneumonia), and nutritional recovery (O=Outcomes), in children hospitalized for severe malnutrition (P=Population), over a period of 8-12 weeks (T=time-frame), using a randomized controlled trial design (S=Study design).

Critical appraisal: ***Table* I** summarizes an outline of the trial. ***Table* II** presents a critical appraisal of the methodological characteristics of the RCT using the Cochrane Risk of Bias tool [2]. Overall, the trial had low risk-of-bias.

The investigators introduced several methodological refinements. Four sets of numbered packages (two each of probiotic or placebo) were prepared and administered to enrolled children. Although this method is inferior to administering packages labeled with the randomization number; it is still better than having only two sets of packages and administering them by allocation group. The investigators used standard definitions for most outcomes. They refined outcome assessment by introducing validated scoring systems for diarrhea severity. This is important because this outcome hinged on parental reporting (through a symptom diary). Therefore parents were trained to use the diary. Follow-up after discharge was strengthened through weekly telephone calls.

During the course of the study, the primary outcome was changed from "duration of diarrhea episodes" to "duration of diarrhea". Although this was done with due approval of the Ethics Committee, the timing of the change is unclear. Nevertheless, it appears that this change was not driven by mid-term data analysis.

The criteria for diagnosing pneumonia are not

TABLE I SUMMARY OF THE TRIAL

Study design	Double blind, placebo-controlled, Randomized controlled trial (RCT)
Study setting	Tertiary care, national-level, referral hospital based in Kampala, Uganda.
Study duration	Participant enrolment from March 2014 to July 2015 (16 consecutive months) and follow-up for 3 months beyond enrolment.
Sample size	*A priori* sample size calculation was done to detect a difference in diarrhea duration of 0.3 SD (equivalent to 1 day) between the two trial arms; with alpha error 5% and beta error 15%. The calculated sample size was 178 in each arm; hence enrolment was continued will 200 were recruited in each arm.
Inclusion criteria	Children in the age range 6-59 mo, hospitalized with SAM, which was defined by either (*i*) weight for height/length z score <-3.0, or (*ii*) mid upper arm circumference <11.5 cm, or (*iii*) pitting pedal edema.
Exclusion criteria	Children with critical illness (shock, severe respiratory distress), extreme malnutrition (weight < 4 kg), disability, malignancy, and those with a prior admission for the same problem.
Intervention and Comparison groups	Intervention (Probiotic group): Combination of *Bifidobacterium animalis subspecies lactis* (BB-12) and*Lactobacillus rhamnosus* (LCG) (dose 10 billion colony forming units, in a 1:1 ratio). The probiotics were delivered in 1 g Maltodextrin.Comparison (Placebo):1 g Maltodextrin.
Outcomes	*Primary outcome*: Duration of diarrhea during hospitalization (diarrhea was defined as >3 loose/watery stools per day). *Secondary outcomes*: **In-patient phase of treatment:** (*i*) Diarrhea: Incidence, Severity; (*ii*) Pneumonia: Incidence, Duration and Severity; (*iii*) Nutritional recovery: Weight gain; (*iv*) Days with fever, Days with vomiting; (*v*) Duration of hospitalization; (*vi*) Mortality; (*vii*) Other adverse events. **Out-patient phase of treatment:** (*i*) Diarrhea: No. of days, Incidence, Severity; (*ii*) Pneumonia: Incidence; (*iii*) Nutritional recovery: Weight gain, weight recovery; (*iv*) Days with fever, Days with vomiting; (*v*) Mortality; (*vi*) Other adverse events.
Statistical analysis	Modified intention-to-treat (ITT) analysis was undertaken; i.e patients with available data were included in the analysis (rather than all who were randomized). Missing values for the primary outcome were imputed from children with available data, matched for diarrhea pattern, and days of hospitalization. Statistical models were adjusted for age, gender, HIV status, presence of edema, and weight for height/ length z scores.
Main results (Probiotic *vs* Placebo)	*Primary outcome:* Duration of diarrhea during hospitalization (d): 6.9+6.0 (*n*=187) *vs* 6.5+6.4 (*n*=182) *Secondary outcomes*: **In-patient phase of treatment** • Diarrhea: Incidence: 89% *vs* 85% (NS); episodes: 299 *vs* 293; severity score: 10.2±3.8 *vs* 9.9±3.7 • Pneumonia: Incidence: 39% *vs* 41%; Duration (d): 1.9±3.6 *vs* 2.0 ±3.7; Severity score: 2.6±5.5 *vs* 2.7±5.3 • Nutritional recovery: Weight gain (g/kg/day): 6.5±4.7 *vs* 6.1±4.2 • Days with fever: 7.0±5.1 *vs* 6.7±4.4 • Days with vomiting: 2.1±3.3 *vs* 2.0±4.4 • Duration of hospitalization (d): 18.3+9.1 *vs* 18.0+9.3; Mortality: 23 *vs* 16 • Other adverse events: None reported **Out-patient phase of treatment** • Diarrhea: Duration (d): 6.0+8.2 (n=147) *vs* 8.5+10.9 (*n*=145); Incidence: 70% *vs* 76% (NS); episodes: 354 *vs* 398; severity score: 4.3+1.7 *vs* 4.4+1.7 • Pneumonia: Incidence: 5% *vs* 10% • Nutritional recovery: weight gain (g/kg/day): 3.0+2.1 *vs* 3.2+2.3; weight recovery: 66% *vs* 64% • Days with fever: 1.6+2.9 *vs* 2.0+3.9 • Days with vomiting: 1.2+2.3 *vs* 1.6+6.0; Mortality: 3 *vs* 4 • Other adverse events: None reported

TABLE II METHODOLOGICAL APPRAISAL OF THE TRIAL

Similarity of groups at baseline	The authors reported similarity of multiple parameters including age, gender, anthropometric measurements, HIV status, maternal HIV status, symptoms at presentation, and treatment sought.
Sequence generation	A person not associated with the study, used a computer program to generate the randomization sequence (Adequate).
Allocation concealment	The sequence was available only to a few personnel including head of the Unit, and staff packaging the products. None of these personnel were associated with the study procedures (Adequate).
Blinding	Enrolled children, their caregivers, study investigators and staff; were all blinded. The intervention and placebo were delivered in similar appearing, similar tasting, and similar smelling formulations (Adequate).
Incomplete outcome data	The authors used a modified ITT analysis. Rather than including all randomized children in the denominator, they included those who had at least some data. Thus the single outcome that showed statistical significance had less than 75% of the originally enrolled participants in each arm. The impact of this attrition is unclear.
Selective outcome reporting	No evidence of selective outcome reporting.
Other sources of bias	The probiotic manufacturer was one of the two funding agencies; and 'suggested' the intervention including dosage and strains of probiotics, but did not have any further role in the conduct of the study.
Overall assessment	Low risk of bias

described; diagnosis was made by the clinical judgment of individual pediatricians. Likewise, the severity grading criteria are described differently in the text and the table of the report [1]. Further, even though the severity grading had a maximum score of 2, it was reported as the sum of daily scores during the hospitalization period. This makes it difficult to interpret as children with similar scores need not have similar severity.

The authors acknowledged some limitations, including the inability to examine stools for the microbiological etiology of diarrhea. This is somewhat surprising as the study protocol on the Trial Registry website [3] shows that additional samples of blood and stool were to be collected at admission, discharge, and at 8 weeks follow-up to identify gut microbiota changes and immune response markers. In fact, the protocol also mentions thymus ultrasonography at these three time-points. However, these data are not presented.

There was significant attrition of study participants from both arms, although the inter-arm drop-out rate was comparable overall. However, one of the reasons for attrition is stated as lack of data on HIV status. This is again surprising as the table of baseline parameters clearly shows maternal and child HIV status.

Although the data analysis is described as intention-to-treat, it was a modified form wherein only those participants with some data were included. Therefore, the primary outcome is reported only in about 90% of the enrolled cohort. For participants with missing data, the method of imputing data have been clearly described.

Perhaps the most important issue in the critical appraisal of this RCT is whether the single statistically significant result favoring probiotic (*viz* reduction in duration of diarrhea by about 2 days, during out-patient follow-up) is clinically meaningful. Careful analysis of a figure provided as a Supplementary file to the article [4] shows that both arms had a comparable percentage of patients with diarrhea duration upto 9 days, suggesting lack of benefit of probiotic for acute diarrhea. In contrast, the probiotic arm had slightly more patients with diarrhea duration between 10 and 19 days. However, the percentage of children with total duration of diarrhea ranging from 20 to >40 days was significantly less in the probiotic group. This means that the overall statistically significant reduction in diarrhea duration is not due to decrease in the duration of acute diarrhea (as the study suggests) but because children in the probiotic group were less likely to have long(er) duration of diarrhea. This could be either because of reduction in the number of diarrhea episodes, or reduced number of days of chronic diarrhea. Since acute diarrhea episodes last 3-5 days (on average) [5-7], children with total duration of 20-40 days during 8 weeks follow-up would have had multiple (5 to 10 episodes). A reduction of 2.2 days across this large number of episodes is not clinically significant. If the children were suffering from persistent or chronic diarrhea, a reduction of 2.2 days is clinically insignificant. Therefore, it would have been very helpful if the investigators had shown the data of diarrhea duration in terms of number of episodes in each arm, and the duration as well as incidence of episodes each week

after discharge. Otherwise it is difficult to understand the true meaning of the statistically significant difference reported [1]. It must be noted that an older trial in Malawi that compared probiotic (lactic acid bacteria) *versus* placebo, also did not find any significant impact of probiotic on diarrhea in severely malnourished children [8].

Although all the children in the study fulfilled the definition for SAM, their weight (or length) for age Z-scores suggest that they were also chronically undernourished. In fact, the enrolled children had acute-on-chronic malnutrition rather than SAM.

Last but not the least, the study setting has a reported SAM mortality rate of around 20%; whereas the study population had an overall mortality rate of less than 10% during hospitalization. This suggests that the highly controlled research study environment could be very different from the real-life scenario; thereby limiting generalizability even within the study setting.

Extendibility: The study setting is notably different from the setting in our country, in terms of the profile of enrolled children (two-thirds had kwashiorkor, and 1 in 6 infants were HIV positive). Further, the baseline local in-hospital morality rate of over 20% in SAM suggests that the children in that setting present with serious life-threatening illnesses. The anthropometric measurements also suggest severe wasting and stunting. For these reasons, it may not be appropriate to directly extrapolate the study results to other settings even if clinically significant results had been demonstrated.

Conclusion: This well designed placebo-controlled randomized trial did not find significant beneficial effects of administering a specific formulation of probiotics to children during and after hospitalization for severe malnutrition.

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Pediatrician's Viewpoint

Relevance: Diarrhea is one of the most common co-morbidity with which SAM children may present to a health facility. The relationship between malnutrition and diarrhea is bidirectional – while malnutrition predisposes children to a greater incidence and longer duration of diarrhea, it is also true that malnutrition can be triggered or worsened by significant diarrhea due to reduced nutrient and fluid absorption [1].

Critical appraisal: Probiotics especially *Lactobacillus rhamnosus* GG and *Saccharomyces boulardi* have been reported to decrease duration of acute diarrhea and need of IV rehydration in well nourished children [2]. Probiotics and prebiotics have potential to promote healthy gut flora, reduce pathogenic gut bacteria and immune modulation. Since large proportion of children with severe acute malnutrition have bacterial overgrowth, it was largely believed that probiotics may help in improving their outcome in terms of weight gain and recovery. However in a randomized controlled trial in Malawi (PRONUT STUDY), 795 SAM children were assigned to ready-to-use therapeutic food either with or without Synbiotic 2000 forte , nutritional cure rates were similar in both the groups (54% *vs* 51%) [3]. Further, there is concern regarding probiotics causing invasive infections in presence of increased intestinal permeability.

In present study, authors assessed the effect of probiotics on diarrhea during in- and outpatient treatment of children with severe acute malnutrition. Patients received one daily dose of a blend of *Bifidobacterium animalis subsp lactis* and *Lactobacillus rhamnosusGG* (10 billion CFU,50:50) or placebo. There was no difference in number of days with diarrhea in probiotics group during inpatient period. Although probiotics reduced days with diarrhea during outpatient treatment, there was no effect of probiotics on diarrhea incidence, severity of pneumonia, weight gain or recovery in both inpatient and outpatient treatment [4].

Conclusions: Present study results do not support use of probiotics in children with SAM. Both PRONUT study from Malawi and present study could not find any significant difference in weight gain and recovery, which are key outcome indicators of any SAM management program.

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Pediatric Gastroenterologist's Viewpoint

In this study, the authors report the result of a randomized controlled trial on the effects of probiotics administration on the incidence and severity of diarrhea among hospitalized children with severe acute malnutrition (SAM) [1]. They randomized 400 children between the ages of 6 months to 59 months admitted in hospital with severe acute malnutrition to receive one daily dose of a blend of *Bifidobacterium animalis subsp lactis* and *Lactobacillus rhamnosus* (10 billion colony-forming units, 50:50) or placebo during hospitalization, followed by an 8- to 12-week outpatient treatment period. The management of SAM was as per WHO guidelines and all inpatients received antibiotics. The authors conclude that probiotics did not affect incidence severity and duration of diarrhea among inpatients with SAM as these children had more severe gut dysfunction and were receiving antibiotics thus off-setting the ability of the probiotics to act. Mortality across groups was similar.

While other probiotic studies have demonstrated a reduction in days of acute diarrhea in outpatients, most have been in a developed world setting. This study demonstrates its efficacy in children with SAM who are more vulnerable to the adverse effects of prolonged diarrhea. The benefit is still modest, and more research to identify efficacious probiotics combination and doses are needed before a recommendation of the use of probiotics in all children with SAM can be made.

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