Use of a Probiotic to Enhance Iron Absorption in a Randomized Trial of Pediatric Patients Presenting with Iron Deficiency

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Objective To evaluate the efficacy of low dose ferrous sulfate for the treatment of iron deficiency and if the probiotic *Lactobacillus plantarum* 299v (LP299v) enhances treatment.

Study design This randomized, double-blinded, controlled trial of the treatment of iron deficiency in children compared the use of low-dose ferrous sulfate (1-3 mg/kg/day), with or without probiotic (LP299v).

Results Serum ferritin level increased in all children from a baseline of 23.7 ng/mL to 45.4 ng/mL after 6-8 weeks of treatment. There was no significant difference in the increase in serum ferritin in children taking the probiotic LP299v compared with controls (23.2 vs 20.0 ng/mL, respectively). Additionally, an increase in ferritin level was not significantly associated with probiotic use when controlling for other factors, including child weight and dosing. Overall, the treatments were well-tolerated, with mild side effects.

Conclusions Treatment with low-dose ferrous sulfate is well-tolerated and effective in correcting iron deficiency in children. However, the probiotic LP299v did not enhance treatment. Further attention should examine the dose–response effect in children, including an alternate day dosing schedule. *(J Pediatr 2019;207:192-7)*.

Trial registration ClinicalTrials.gov: NCT01617044.

Iron deficiency is the most common micronutrient deficiency, with an estimated worldwide prevalence more than 30%.[1] Although the burden is lower in high-income, industrialized nations, iron deficiency and associated conditions remain a significant challenge for many children and their care providers in the US. The consequences of iron deficiency include anemia,[2,3] cognitive deficits,[4] attention deficit disorder,[5] impaired immunity,[6] and restless legs.[7,8] Iron is an important cofactor in many enzymatic processes in cytochrome, dopamine, and hemoglobin metabolism, and its deficiency is often the rate limiting step in these pathways.[6,9] The current recommendation for the treatment of iron deficiency in individuals in industrialized nations is oral ferrous sulfate, although the recommended dose and frequency vary widely.[3,10] Treatment with oral ferrous sulfate is associated with adverse gastrointestinal side effects of constipation, diarrhea, vomiting, and abdominal pain, which are dose related and reported in 10%-73% of children taking oral iron supplements.[3,9-11] Gastrointestinal side effects are a common reason that oral iron therapy is discontinued. In developing countries, oral iron supplementation has been associated with an increase in pathogenic gut bacteria,[12] increased morbidity from malaria,[13] and bacterial gastroenteritis.

Probiotics are a class of micro-organisms that confer benefit to the human host. In pediatric studies, there is evidence to support a beneficial effect of probiotics in the treatment of acute diarrheal disease,[14] in the prevention of antibiotic associated diarrhea disease,[15] and in the prevention of atopy associated with cow milk ingestion.[16] Probiotics have been shown to increase iron absorption in adults[17,18] and in cell culture in vitro studies,[19] and in preschool children.[20] A recent study of the probiotic *Lactobacillus plantarum* 299v (LP299v) showed an increase in iron absorption in adult women as measured by a double isotope technique.[17] However, there have been no clinical studies of probiotics in children as an adjunct in treating iron deficiency. In a randomized, controlled design, we sought to examine whether the use of a probiotic (LP299v) enhanced treatment of mild iron deficiency in patients presenting at our pediatric sleep center. We hypothesize that patients receiving a low-dose ferrous sulfate along with LP299v would have a better response to treatment compared with patients receiving low-dose ferrous sulfate alone.

Methods

Over a 3-year period (August 2012 to July 2015) children aged 5-18 years who presented to our sleep center with a complaint of insomnia or restless sleep and who had biochemical evidence of iron deficiency defined as a serum ferritin of less than 50 ng/mL (the consensus threshold in sleep medicine)[21] and no evidence of inflammation defined as a C-reactive protein of less than 0.3 were

LP299v *Lactobacillus plantarum* 299v

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invited to participate in a randomized, double-blind, placebo-controlled trial to assess the effect of a probiotic (LP299v) plus low-dose iron plus vitamin C on restless sleep. There is evidence that iron deficiency may be important in the pathophysiology of restless sleep.[5,22] All children had a comprehensive medical history gathered at the time of their enrollment, of which none presented with a history of chronic blood loss or menorrhagia. Children with obstructive sleep apnea were not invited to participate in the study. All enrolled children were prescribed the iron treatment of 3 mg/kg/day of elemental iron up to a maximum daily dose of 65 mg elemental iron with generic ferrous sulfate 325 mg (provided by Major Pharmaceuticals, Livona, Michigan) or ferrous sulfate 15 mg elemental iron/mL (provided by Akorn Inc, Lake Forest, Illinois), and vitamin C, (generic ascorbic acid 250 mg for children >5 years of age, 125 mg for children <5 years of age; provided by Major Pharmaceuticals). In children weighing less than 20 kg, the dose of iron was 3 mg/kg/day, but in children heavier than 20 kg the dose of iron ranged from 0.4 to 3.0 mg/kg/day. The children were randomly assigned to receive either 10 × 10 colony-forming units of the probiotic LP299v (provided by Jarrow Formulas, Inc, Los Angeles, California), or an identical appearing placebo, in addition to the iron and vitamin C. The study was double blinded. The manufacturer states that each capsule contains a minimum of 10 billion LP299v viable cells. This is the same dose that was used by Hoppe et al.[18] This study was approved by the Institutional Review Board at Children's Hospitals and Clinics of Minnesota. The study is registered at ClinicalTrials.gov (NCT01617044).

At the first study visit, the study was explained, and the parents and children, age 7 or older signed a consent and assent to participate. The parent was provided with all of the study medication. Parents were instructed to not give their child milk or food within 2 hours of giving the medication. No recommendation was given regarding the timing of iron administration. Parents were provided with a medication log sheet to record daily that the medication was given. Each family was queried by telephone weekly about adverse side effects. The descriptions and grading scales of side effects found in the revised National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 were used for all adverse event reporting.[23] The parents were instructed to bring leftover medication to their second study visit, which was scheduled between 6 and 8 weeks after entrance into the study. At that visit, the drug log was reviewed and the leftover medication was counted; this provided two estimates of the amount of medication the child was given. The serum ferritin level and C-reactive protein were redrawn, and the child was given a $10 gift certificate.

Statistical Analyses

Basic descriptive statistics including mean, SD, and frequency (ie, percent) were used to describe all demographic and clinical characteristics. After meeting assumption for normality, differences between groups (ie, probiotic vs control patients) were assessed using Student t tests for continuous measures (age, weight, dose, mediation taken, and ferritin level) and χ^2 tests for categorical variables (eg, psychiatric diagnosis and adverse events and side effects). Pearson correlation coefficients were calculated to examine specific relationships between amount of medication taken, dose, and changes in ferritin level). Differences in mean ferritin level at baseline and follow-up were also compared using paired t tests. Finally, the effect of using a probiotic on change in ferritin level was examined using linear regression models adjusted for age, weight, psychiatric/mental health diagnosis, iron dose, and mediation taken. All analyses were performed using SAS 9.4 (SAS, Cary, North Carolina).

Results

Of the 112 patients screened, 65 children were enrolled and randomized for this study (**Figure 1**; available at www.jpeds.com); 33 children were randomized to the probiotic group, 32 children randomized to the placebo group. After accounting for dropouts and missing data, 27 and 25 participants, respectively, were used for analyses. No children/parents described a history of chronic blood loss or menorrhagia at intake.

The mean age across all patients was 10.2 years and the average weight was 20.0 kg (**Table I**). Nearly 58% of all patients had a psychiatric and/or mental health diagnosis. The average iron dose prescribed to patients was 1.9 mg/kg per dose. Because the highest dose of iron given was 65 mg, the dose range was 0.4 to 3.0 mg/kg/day. When comparing the 2 treatment groups, there were no differences between demographic and clinical characteristics observed.

Table I. Demographic and clinical characteristics of 52 patients presenting with iron deficiency and restless sleep

	All patients	Probiotic group	Control group	
No.	52	27	25	P*
Age, years	10.2 ± 4.2	10.7 ± 3.6	9.6 ± 4.8	.3914
Weight, kg	20.0 ± 11.3	17.4 ± 7.1	22.8 ± 14.1	.0909
Psychiatric diagnosis, %	57.7	59.3	56.0	.8121
Iron dose, mg/kg/d	1.8 ± 0.8	1.9 ± 0.7	1.7 ± 0.9	.3542
Medication taken (no. of pills)†	43.3 ± 7.2	43.5 ± 6.8	43.0 ± 7.8	.8439

Values are mean ± SD or number (%) unless otherwise noted.
*Student t test for continuous variables and χ^2 test for categorical variables, significance at $P < .05$.
†Missing data for 7 participants.

Additionally, there was no difference in the average dose of iron prescribed.

The daily medication logs and leftover medication collected at follow-up provided two measures of medication taken, which correlated well with each other (Pearson coefficient of 0.68; $P < .0001$) for estimating the number of doses of iron each child was given during the course of treatment. Figure 2 displays the mean ferritin levels at baseline and follow-up across all patients and by treatment group. There was a significant increase in ferritin level at follow-up in all patients (from 23.7 to 45.4 ng/mL) and by treatment groups, from 25.4 to 48.7 ng/mL in the probiotic group and from 21.9 to 41.9 ng/mL in the control group (all $P < .0001$). There was also a significant positive correlation between the dose of iron prescribed and change in ferritin level (Pearson coefficient of 0.32; $P = .0226$; data not shown). However, there was not a significant correlation between the number of doses of medication taken and change in ferritin level (data not shown).

The values and change in ferritin level from baseline to follow-up are presented in Figure 3. In the probiotic group, there was a mean change in the ferritin level of 23.2 ng/mL. In the control group, the mean ferritin level change was 20.0 ng/mL. However, when comparing the differences in means by treatment group, no significant difference was observed ($P = .4265$).

A series of linear regression models were performed to examine change in serum ferritin level as the dependent variable and treatment group and other demographic and clinical variables as independent variables (Table II). In separate models, where each independent variable was a predictor of change in ferritin level, weight ($\beta = -0.16$; $P = .0408$) and iron dose ($\beta = 5.82$; $P = .0226$) were significantly associated with change in ferritin level. The use of probiotic or not was not significantly associated with change in ferritin level. When accounting for all variables, no significant associations were detected.

Finally, adverse side effects from treatment were minimal (Table III; available at www.jpeds.com). The side effects, if any, were generally mild (32%-33%) and almost exclusively gastrointestinal pain associated with abdominal pain, constipation, vomiting, or diarrhea (data not shown). Side effects were described by parents as moderate or severe in only 3 children. There was no difference in side effects between the probiotic and the placebo groups.

Discussion

Our findings that, low dose iron therapy with ferrous sulfate increased the serum ferritin levels, is consistent with standard practice for treating children with iron deficiency in industrialized nations.[3,10,24] At the end of 6-8 weeks of therapy the median ferritin level was 41 ng/mL, which is greater than the current threshold for correcting iron-deficient erythropoiesis,[25] although below the goal of greater than 50 ng/mL for the treatment of children with restless sleep.[21] That the probiotic LP299v was well-tolerated but did not enhance treatment complicates the limited evidence suggesting the benefits of probiotics with iron treatment. Hoppe et al found, in a population of women ages 21-40 years, that consumption of a capsule containing iron plus LP299v increased iron absorption compared with only iron.[17] A study of children receiving probiotic- and prebiotic-fortified milk showed lower iron deficiency in intervention than the control group,[20] although these children were not receiving iron supplements. Although much research has been conducted on benefits of probiotics in children,[26-29] these studies have not focused on the effects on iron absorption. More research

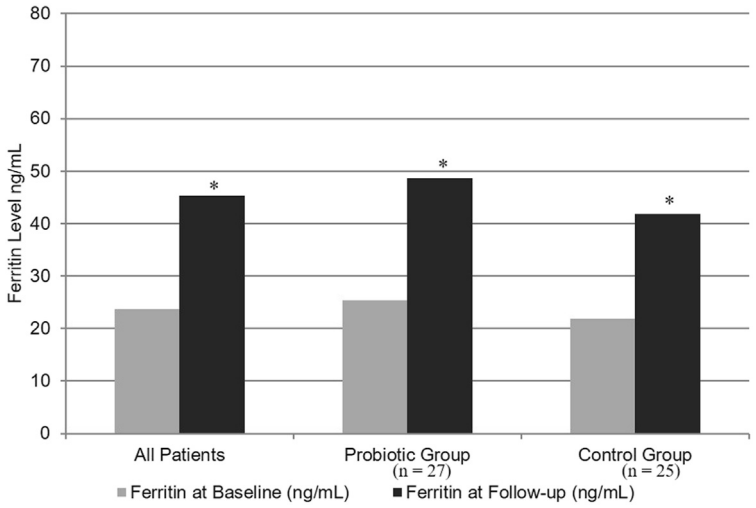

Figure 2. Mean change in ferritin levels of 52 patients presenting with iron deficiency and restless sleep between baseline and follow-up. *Paired *t* test; significance at $P < .0001$.

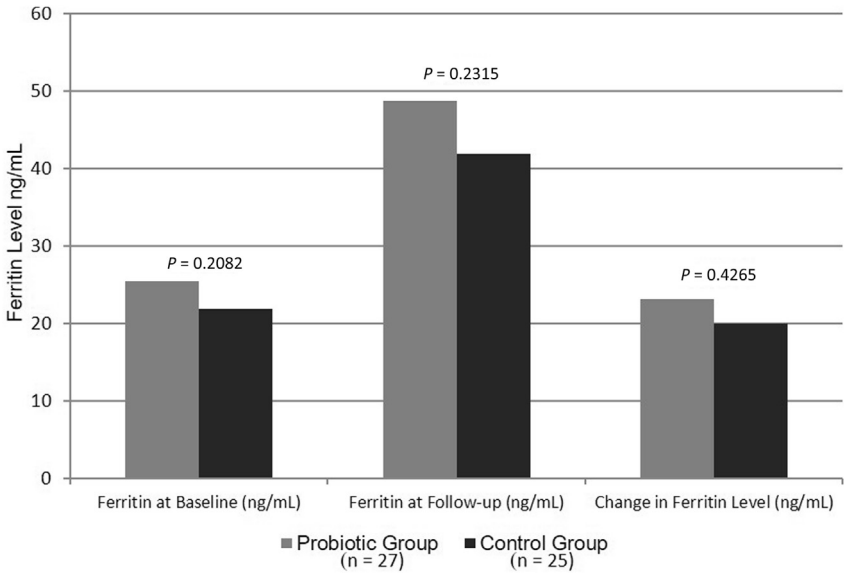

Figure 3. Mean difference in ferritin levels of 52 patients presenting with iron deficiency and restless sleep by treatment group. No significant difference between groups using Student *t* test at $P < .0001$.

should be conducted on the effects of probiotics on absorption of iron supplements. Additionally, comparisons between different probiotics may be warranted, in addition to the possible synergistic effect of combining prebiotics and probiotics, as suggested by Sazawal et al.[20]

Iron absorption is a complex process, influenced by factors such as bioavailability,[30] the presence of dietary enhancers and/or inhibitors of iron absorbtion,[30] as well as the time of day.[31] Based on this complexity, limitations in this research exist. For instance, although parents in this study were asked not to provide milk or food with the iron supplement, we cannot be sure of adherence with this requirement, nor was a detailed dietary history taken. Future research on this topic would benefit from a more rigorous dietary assessment.[17] Generalizability of our study was limited somewhat by the study including only patients at a sleep center with restless sleep/restless legs symptoms. Additionally, the treatment dose of elemental iron ranged in both groups from 0.4 to 3.0 mg/kg/day, because the maximum dose by the study design was set at 65 mg/day. The increase in the ferritin level correlated with the dose of iron taken in milligrams per kilogram per day, but did not correlate with the actual number of doses of iron taken between 24 and 60 doses. In regression analyses, patient weight and dosing were found to be significant contributors to changes in ferritin level. After accounting for weight, dose, and other covariates assessing the relationship between probiotic vs controls, the effect was no longer significant. However, the finding regarding dosing is notable, and should be followed with future research in iron-deficient children. Finally, the findings of this study are limited by a small sample size. Although our results suggest a trend toward an enhancement of ferritin elevation in the probiotic group, we did not find a significant association in our analyses. This could be due to the small number of participants. Further research using a larger population, sufficiently powered to detect a medium to small effect size, is warranted.

Table II. Linear regression examining relationship between change in ferritin level and treatment group (n = 52)

	Ferritin change as outcome			
	Separate linear regression models*		Full linear regression model†	
	β (SE)	P	β (SE)	P
Independent variables				
Treatment group (probiotic vs control)	3.18 (4.00)	.4305	1.13 (3.82)	.7685
Age, years	−0.87 (0.47)	.0672	−0.17 (0.60)	.7856
Weight, kg	−0.36 (0.17)	.0408	0.10 (0.33)	.7725
Psychiatric diagnosis	−3.84 (4.04)	.3459	−5.73 (3.74)	.1325
Iron dose, mg/kg/d	5.82 (2.47)	.0226	4.45 (5.30)	.4064
Medication taken (no. of pills)	−0.21 (0.25)	.3916	−0.26 (0.26)	.3147

*Regression model represents each independent variable as predictor of ferritin change.
†Regression model of all independent variables as predictors of ferritin change.

At first glance, the absence of correlation between the number of doses of iron and the increase in serum ferritin level seems surprising. However, studies in adults have shown that a dose of oral iron leads to an increase in hepcidin levels,[32] which in turn leads to a decrease of the fractional iron absorption, which persists into the next day. Very little difference in iron absorption was found between iron treatment twice a day

and once a day in these studies. This finding suggests a possible benefit of iron dosing every other day, which would be one-half of the doses received with every day treatment, and one-quarter of the doses received with twice a day treatment. Studies of alternate day dosing in adults suggest this regimen may optimize iron absorption over consecutive day dosing.[11] Examination of alternating day dosing of oral iron for children would be warranted for future research. The increase in ferritin level with treatment in the group of children with the lowest ferritins, less than 15 ng/mL, was no greater than the increase in ferritin level in children whose baseline ferritin was 15-30 ng/mL or 30-50 ng/mL (data not shown). This is a surprising finding. Low ferritin levels would be expected to lead to low hepcidin levels, which should lead to better iron absorption.[33,34] Hepcidin levels were not assessed during this study, and our study was not designed or powered to address these questions.

The adverse side effects of low-dose of ferrous sulfate, 3 mg/kg/day up to a maximum of 65 mg/day were infrequent and generally mild. An elevated C-reactive protein was found in 5 children (8%), and did significantly elevate serum ferritin levels, a reminder of the importance of checking a marker of inflammation when checking ferritin.

Although our study found no significant effect of probiotics on absorption of oral iron, negative findings are important for clarifying practice. Thus, even though there was no significant enhancement in the treatment of iron deficiency, our study did demonstrate that the probiotic could be tolerated and present minimal harm to a cohort of pediatric patients. Our secondary findings with regard to dosing raise compelling questions about optimal dose timing for pediatric patients with iron deficiency. ■

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50 Years Ago in *The Journal of Pediatrics*

The Use of Acid-Base Measurements in the Clinical Evaluation and Treatment of the Sick Neonate

Behrman RE. J Pediatr 1969;74:632-7.

Acid-base measurements are a key part to the management of sick neonates, be they asphyxiated term newborns or the most immature preterm neonates. One of the most important developments in newborn medicine in the last 50-60 years was the introduction of measurements of acid-base balance in the blood. In the 1960s, such measurements became common practice and led to a much improved understanding of neonatal physiology and pathophysiology, followed by improved care for newborns.[1] However, as outlined by Behrman in *The Journal* 50 years ago, there were pitfalls in the methodology; the equipment, competency of the personnel, buffers used, and maintenance of the reference electrodes were all potential sources of errors in the results obtained.

Although the sample analysis methods available in the 1960s were much less automated than those of today, with a higher risk of errors in results, the principles remain much the same (pCO_2 was initially measured indirectly because pCO_2 electrodes were not available). Behrman's points regarding temperature and how the patient's temperature at the time of sampling influence the results of pH and pCO_2 are still valid and important regarding the interpretation of the results from neonates undergoing therapeutic hypothermia.

Behrman formulated clinical guidelines urging the clinician to use clinical skills to interpret results from blood sampling to avoid delays in analysis, to regularly perform equipment checks, and to use caution when interpreting the results of an acid-base measurement. The physiology behind the results of an acid-base measurement, and the considerations regarding the interactions among HCO_3^-, CO_2, BE, and pH are just as valid today as they were 50 years ago.

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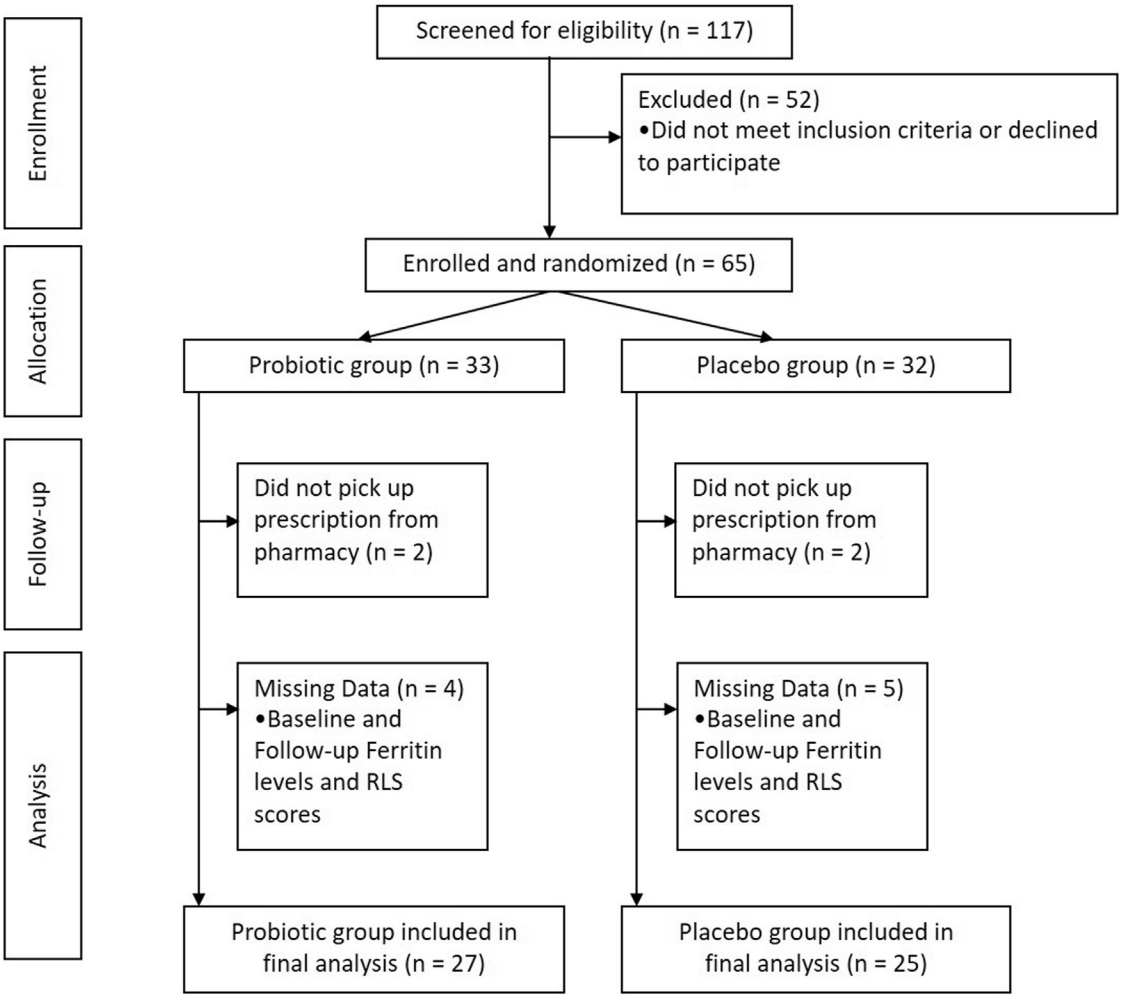

Figure 1. CONSORT diagram. RLS, restless legs syndrome.

Table III. Adverse events reported by 52 patients presenting with iron deficiency and restless sleep by treatment group

	Probiotic group	Control group	*P* value*
n	27	25	.0612
None	16 (64.0)	16 (59.3)	
Mild	8 (32.0)	9 (33.3)	
Moderate	1 (4.0)	1 (4.0)	
Severe	0 (0)	1 (3.7)	

Values are number (%) unless otherwise stated.
*Fisher exact test, significance at $P < .05$.