A *post hoc* analysis on the effects of a probiotic mixture on asthma exacerbation frequency in schoolchildren

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To the Editor:

Asthma is usually characterised by chronic airway inflammation [1]. Furthermore, respiratory infections frequently precede asthma exacerbations in children, mainly in allergic subjects [2–4]. As a result, dampening inflammation and preventing respiratory infections are essential in the therapeutical strategy. It has been underscored by the pathogenic relevance of dysbiosis, as it has been evidenced that the children with asthma present dysbiosis of the gut and lung microbiome [5]. The dysbiosis affects the immune response and, consequently, induces airways inflammation, and airflow limitation [6]. These events constituted the premise of using probiotics to modulate the immune response to restore the microbiota and immune balance [7]. Probiotics are defined as "live microorganisms which confer a beneficial effect on the host", according to the World Health Organization [8]. A significant body of evidence supports probiotics in allergic diseases, including asthma [9]. In this regard, *Bifidobacterium breve* B632 and *Ligilactobacillus salivarius* LS01 were effective in patients with atopic dermatitis and urticaria [10, 11]. The positive outcomes depend on the immunomodulatory activity, as they reduce pro-inflammatory cytokines and increase anti-inflammatory ones [12]. The PRObiotics in Pediatric Asthma Management (PROPAM) study explored the possibility of reducing asthma exacerbations in children with asthma taking the probiotic mixture containing both strains. The study was a multicentre, randomised, double-blind, placebo-controlled trial performed in a paediatric primary care setting [13]. The outcomes demonstrated the effectiveness of this probiotic mixture in preventing asthma exacerbations. However, the recruited population had a wide age range. Therefore, a *post hoc* analysis evaluated the subgroup of schoolchildren. The rationale for this *post hoc* analysis considered the asthma differences between preschoolers and schoolchildren in terms of clinical and functional characteristics [14]. In particular, the asthma diagnosis in preschoolers is dubitative, spirometry is difficult to perform and results may be unreliable. Moreover, age significantly affects the comorbidity, allergy, and lung function [15, 16].

The primary outcome was the reduction of asthma exacerbations, considering the number, duration (days), and severity of asthma attacks. The definition criteria for these outcomes were stated by the asthma guidelines provided by the Italian Society of Pediatrics in 2018 [17]. Given clinical and functional parameters, the asthma exacerbation severity was scored as mild, moderate and severe [17].

Inclusion criteria were age between 6 and 14 years and asthma diagnosis, according to Global Initiative for Asthma criteria. The exclusion criteria were severe asthma, congenital or acquired immunodeficiency, cystic fibrosis, and chronic pulmonary diseases.

The study included five visits: at baseline (T0), after 1 month of treatment (T1), 2 months (T2), 3 months (T3), and 4 months (T4).

Each sachet of the active probiotic product comprised viable strains currently used in food supplements, specifically $\geqslant 1\times10^9$ live cells of *B. breve* B632 (DSM 24706) and $\geqslant 1\times10^9$ live cells of *L. salivarius* LS01 (DSM 22775) (combined dose of $\geqslant 2\times10^9$ CFU), with maltodextrin used as a bulking agent to yield a final weight of 2 g; each placebo sachet contained 2 g of maltodextrin only (Probiotical S.p.A., Novara, Italy). The placebo powder was indistinguishable from the probiotic powder in appearance, taste, smell, and packaging. Participants were instructed to dissolve the powder in water or cold milk and drink it for 4 months in the morning and evening. The probiotic mixture or placebo was taken twice daily (one sachet in the morning and one in the evening) for 8 weeks and subsequently once daily for a further 8 weeks.

Shareable abstract (@ERSpublications)
The present randomised, placebo-controlled, double-blind study showed that a probiotic mixture significantly reduced the number of asthma exacerbations in schoolchildren https://bit.ly/382LYKV

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The Ethics Committee of the ASL Napoli 3 Sud approved the study procedure on 12 April 2017 (N. 45/21/04/2017). The study was registered at clinicaltrials.gov with identifier number: NCT04289441.

11 Italian primary care paediatricians participated in the study, each having to enrol 46 children. All of them resided in the Campania (South Italy) region.

The statistical analysis included a descriptive analysis of collected data, summarised as counts within a group for categorical variables and with mean±SD and median with interquartile range for continuous variables. Univariate logistic regression models were applied to predict the outcomes' likelihood (presence of asthma exacerbation during the treatment period). Results were quantified by odds ratio (OR) together with a 95% confidence level (95% CI). The significance level was set at 0.05. The analyses were computed using SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA).

The characteristics of the subgroup of schoolchildren are reported in table 1. Globally, 262 children were evaluated: 139 in the placebo arm and 123 in the active treatment arm. The two subgroups were well matched.

The main result showed that children taking a placebo had a significantly higher probability of having one asthma exacerbation (OR 4.3; 95% CI 1.9–9.74) than children supplemented with the probiotics mixture. Interestingly, considering the odds of having two exacerbations, the outcome was even more significant as the number of children with two asthma exacerbations was less than a tenth in the active group (OR 11.5; 95% CI 1.48–90). In addition, no children in active treatment group experienced a severe exacerbation, whereas two children in the placebo group had a severe asthma exacerbation. Finally, the median duration of exacerbations was 4 days in both groups.

The current sub-analysis demonstrated that a probiotic mixture, containing *B. breve* B632 and *L. salivarius* LS01 diminished the asthma exacerbation incidence in a cohort of schoolchildren evaluated in a primary care setting.

The occurrence of an asthma exacerbation episode is a challenge for paediatricians [18]. The leading cause of asthma exacerbation is usually an acute respiratory infection, mainly caused by viruses in childhood [19]. The pathophysiological mechanisms include airways inflammation promoting bronchial hyperreactivity and obstruction. In addition, the type 2 immune response amplifies these events.

TABLE 1 Demographic and clinical data in schoolchildren with asthma, taking (active treatment) or not taking (placebo) a probiotic mixture

Demographic characteristics	Total	Placebo	Active treatment
Subjects, n	262	139	123
Age, years	8.7±2.96	8.4±2.72	9.0±3.18
Sex			
Female	114 (43.5)	60 (43.2)	54 (43.9)
Male	148 (56.5)	79 (56.8)	69 (56.1)
Asthma severity			
Intermittent	203 (77.5)	108 (77.7)	95 (77.2)
Mild persistent	37 (14.1)	21 (15.1)	16 (13.0)
Moderate persistent	22 (8.4)	10 (7.2)	12 (9.8)
Sensitised children			
No	116 (44.3)	66 (47.5)	50 (40.7)
Yes	146 (55.7)	73 (52.5)	73 (59.3)
Patients with asthma exacerbations	**Placebo**	**Active treatment**	**OR (95% CI); p-value**
No exacerbation	107 (77.0)	115 (93.5)	4.30 (1.90–9.74); <0.001
At least one exacerbation	32 (23.0)	8 (6.5)	
Less than two exacerbations	127 (91.4)	122 (99.2)	11.53 (1.48–90.00); 0.020
Two exacerbations	12 (8.6)	1 (0.8)	
Total number of asthma exacerbations	44	9	
Clinical judgement			
Mild exacerbation	16 (36.4)	0 (0.0)	
Moderate exacerbation	26 (59.1)	9 (100.0)	
Severe exacerbation	2 (4.5)	0 (0.0)	
Duration, days	4.0 (0.0–12.0)	4.0 (0.0–8.0)	

Data are presented as mean±SD, n (%) or median (interquartile range), unless otherwise stated.

Consequently, restoring a physiological immune response is relevant in asthma management. Moreover, dysbiosis reduces microbial diversity that fosters impaired immune response in many sites, including the respiratory mucosa [20]. There is also evidence that specific probiotic strains are able to restore a physiologic type 1 immune response and reduce inflammation by inducing "eubiosis" [9–12].

The present study had some limitations, including treatment duration and lack of mechanistic information. 4-month supplementation could be a short treatment period, considering the protracted seasonality of respiratory infections, a primary trigger for asthma exacerbation. In addition, an adequate follow-up could give more information on long-term effects after supplementation discontinuation. The allergic population also deserved more attention, considering peculiar pathophysiological characteristics. These limitations could represent a bias for the correct interpretation of the findings.

However, this study suggested the possibility of using probiotics as a complementary prevention of asthma exacerbations in schoolchildren. In this regard, the supplementation with probiotics could be a fruitful aid in managing schoolchildren with asthma by primary care paediatricians. Effective and safe probiotic strains could reduce the susceptibility to respiratory infections and dampen type 2 inflammation, the main pathogenic factors involved in asthma exacerbation [21]. As standard pharmacological treatments do not allow complete asthma control in all children, probiotic supplementation could represent handy support that the primary care paediatricians can prescribe in clinical practice. The current study, characterised by a robust methodology (*i.e.*, randomisation, double-blind, placebo-control, and sample size calculation), and the primary care setting, sustained this therapeutic option.

In addition, the present *post hoc* analysis added the additional proof that the probiotic supplementation may also prevent asthma exacerbation in the subgroup of schoolchildren. Interestingly, considering the value of the odds ratio, the preventive effect seemed even more favourable than the global population (4.3 *versus* 3.17).

This PROPAM study sub-analysis suggested that supplementation with a probiotic mixture, containing *B. breve* B632 (DSM 24706) and *L. salivarius* LS01 (DSM 22775), was likely to benefit the prevention of asthma exacerbations in schoolchildren significantly.

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This study is registered at www.clinicaltrials.gov with identifier number NCT04289441. The data were collected in case report forms and were stored.

Conflict of interest: L. Drago has nothing to disclose. L. Cioffi has nothing to disclose. M. Giuliano has nothing to disclose. M. Pane is an employee of Probiotical. G. Ciprandi is an associate editor of this journal.

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