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Beneficial effects of pleuran on asthma control and respiratory tract-infection frequency in children with perennial asthma

Milos Jesenak[1,2]✉, Martin Hrubisko[3], Jerzy Chudek[4], Jaromir Bystron[5], Zuzana Rennerova[6], Zuzana Diamant[7,8,9], Juraj Majtan[10,11]✉ & Andrzej Emeryk[12]

The aim of this study was to evaluate the effects of pleuran (β-glucan isolated from *Pleurotus ostreatus*) on asthma control and respiratory morbidity in children on conventional GINA-based asthma treatment who had partially controlled perennial asthma. A double-blind, placebo-controlled multicentre clinical trial with a 2-arm, parallel design was conducted across three countries; 230 children aged 7 to 17 years were randomised (1:1) into an active group (receiving a pleuran/vitamin C combination) or a placebo group (receiving vitamin C only). This study consisted of 24 weeks of treatment (2 capsules a day) and then 24 weeks of follow-up. The primary endpoints included the effects of active treatment versus placebo on asthma control and respiratory tract infections (RTIs). Secondary endpoints included changes in the following measures: number of asthma exacerbations, with or without respiratory infection; quality of life of both asthmatic children and their caregivers; spirometric indices; fractional exhaled nitric oxide (FeNO) levels; safety after 24 weeks of treatment and also after the full 48-week study period. Overall, 206 children completed this study; 113 of these children were in the active group and received a pleuran/vitamin C combination for 24 weeks. After the 24-week treatment period, children below 12 years of age who were in the active group achieved significant improvements in asthma control compared to those in the placebo group (21.8 ± 3.5 vs. 20.3 ± 4.0; $P = 0.02$); while children at least 12 years old who were in the active group reported lower numbers of RTIs (0.7 ± 1.0 vs. 1.9 ± 1.7; $P = 0.002$) compared to children of this age in the placebo group. In addition, children below 12 years of age in the active group showed a significant decrease in asthma exacerbations compared to those in the placebo group (2.5 ± 1.6 vs. 3.3 ± 1.9; $P = 0.05$). At the end of the 48-week trial, a statistically significant improvement in asthma control was observed in 84.7% of children who received pleuran/vitamin C treatment compared to 67.0% of children who received vitamin C only ($P = 0.01$). The pleuran/vitamin C combined treatment was safe and well-tolerated, and no related serious adverse events were reported. This study highlights the favourable safety profile of pleuran/vitamin C supplementation and demonstrates positive effects of this treatment on asthma control and RTI incidence in children with allergic perennial asthma that was partially controlled by conventional therapy.

Keywords Beta-glucan, Polysaccharide, Asthma control, Respiratory tract infections, Asthmatic children, Tolerability

[1]Department of Paediatrics, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, University Teaching Hospital in Martin, Martin, Slovakia. [2]Institute of Clinical Immunology and Medical Genetics, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, University Hospital Martin, Martin, Slovakia. [3]Department of Allergy and Clinical Immunology, St. Elisabeth's Oncology Institute, Bratislava, Slovakia. [4]Department of Internal Diseases and Oncological Chemotherapy, Faculty of Medicine in Katowice, Medical University of Silesia, Katowice, Poland. [5]Department of Allergy and Clinical Immunology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic. [6]Department of Paediatric Pulmonology and Phthisiology, Faculty of Medicine, Slovak Medical University, National Institute of Children's Diseases, Bratislava, Slovakia. [7]Department of Clinical Pharmacy and Pharmacology, University of Groningen, Groningen, The Netherlands. [8]Department of Respiratory Medicine, First Faculty of Medicine, Charles University and Thomayer Hospital, Prague, Czech Republic. [9]Department of Microbiology, Immunology and Transplantation, KU Leuven, Catholic University of Leuven, Leuven, Belgium. [10]Department of Microbial Genetics, Institute of Molecular Biology, Slovak Academy of Sciences,

Bratislava, Slovakia. [11]Department of Microbiology, Faculty of Medicine, Slovak Medical University, Bratislava, Slovakia. [12]Department of Lung Diseases and Rheumatology, Medical University of Lublin, Lublin, Poland. ✉email: jesenak@gmail.com; juraj.majtan@savba.sk

Bronchial asthma is one of the most common non-communicable respiratory diseases, globally affecting up to 300 million children, adolescents, and adults, and imposing substantial burdens on individual patients, their families, and healthcare systems. The Global Asthma Report 2022 highlights that 1 in 10 children have asthma symptoms[1].

Asthma is a heterogeneous disease that is characterised by chronic airway inflammation and airway hyperresponsiveness. In cases of asthma, there is usually a history of respiratory symptoms—including wheezing, shortness of breath, chest tightness, cough—that vary over time and in intensity, together with variable expiratory-airflow limitation to a range of stimuli and irritants[2]. Early-onset asthma starts during childhood or adolescence and is often associated with allergies and/or diseases associated with Type 2 inflammation (such as allergic rhinitis and atopic dermatitis), often in the context of the so-called atopic march[3].

Asthma is associated with a typical pattern of cytokine production, specific profiles of inflammatory cells (such as eosinophils), an overproduction of mucus, and dysfunction in structural cells within the airways, including epithelial cells, smooth muscle cells, and nerves[4].

The long-term goals of asthma management are to achieve good control of symptoms and to minimize future risk of asthma-related mortality, exacerbation, persistent airflow limitation, and treatment side effects. Recently, the achievement of disease remission has been proposed as a new treatment goal[5]. Each patient's own goals for their asthma and its treatment should also be identified[2]. Asthma treatment strategies in the twentieth century (such as oral corticosteroids (OCSs), inhaled corticosteroids (ICSs), short-acting bronchodilators (SABAs), long-acting bronchodilators (LABAs), and leukotriene receptor antagonists (LTRAs)) have been intended for use in a uniform therapeutic scheme for all patients with a diagnosis of "asthma". Since the advent of highly effective biologic treatments for specific asthma phenotypes in the twenty-first century, this concept has fundamentally changed towards the individually tailored treatment of chronic airway diseases based on treatable traits ("precision medicine")[4]. For safety, GINA does not recommend the treatment of asthma with SABAs alone in adults, adolescents, or children aged 6–11 years[6]. Instead, they should receive ICS-containing treatment to reduce their risk of serious exacerbations and to control symptoms and preserve lung function over time. Reduction in severe exacerbations is a high priority across treatment steps, which aim to reduce the risks to patients, the burdens on patients and health systems, and the need for OCSs, which have cumulative long-term adverse effects[2].

Patients and the parents of children with asthma are turning to complementary therapies because of various concerns about conventional asthma treatment modalities (i.e., primarily concerns about long-term medication use, especially for steroids) and because of attractive and promising aspects of complementary therapies[7]. Complementary therapies are usually used alongside, and not instead of, conventional asthma treatments. Their usage reflects patients' and parents' underlying desires for greater self-care and shows the need for opportunities to address some of their concerns regarding asthma care[8].

Respiratory tract infections (RTIs), mainly of viral aetiology, are significant factors that exacerbate the clinical course of asthma in children, and various viruses are simultaneously involved in the complex pathogenesis of asthma in early life. There are several approaches to preventing and/or treating children suffering from recurrent RTIs using immunomodulating preparations, biologically active polysaccharides, bacterial lysates, probiotics, and vitamins, as well as complementary and alternative medicinal products such as herbal and bee products. A recently presented international consensus on products used for the prevention of RTIs in susceptible children, including those who are atopic/allergic or asthmatic, concluded that, among the analysed approaches and products, only OM-85 has a sufficient number of well-conducted clinical trials with an adequate safety profile[9].

β-glucans, which are biologically active polysaccharides, are promising candidates for treatments to prevent recurrent RTIs based on their pluripotent biological activities (which are immunomodulatory, anti-allergic, or anti-inflammatory) and their favourable safety and tolerability profiles. Although the basic structures of β-glucans from different sources are similar, they often show differences in bioactivity[10]. A recent review by Wzorek-Łyczko et al.[11] has suggested a positive anti-infective effect of β-glucans in the treatment and prevention of infectious diseases in the paediatric population.

Regarding the prevention of recurrent RTIs in children, the most clinically studied β-glucan is insoluble beta-(1,3/1,6)-D-glucan pleuran isolated from *Pleurotus ostreatus*[12–15]. In a double-blind, placebo-controlled study performed in children with recurrent RTIs, β-glucan pleuran significantly decreased respiratory morbidity, the frequencies of flu and flu-like diseases, and the number of lower RTIs[13], which were followed by a significant reduction in peripheral blood eosinophilia and stabilised levels of total serum IgE[16]. These data suggest that pleuran has immunomodulatory and potentially anti-allergic effects[16,17].

Based on these data, we hypothesised that the oral administration of β-glucan in asthmatic children as an immunomodulatory adjuvant therapy may improve the clinical course of asthma, and even disease control, through complex immunomodulatory effects and a decline in RTI frequency. Sarinho et al.[18] showed that subcutaneous injection of particulate beta-1–3-glucan extracted from *Saccharomyces cerevisiae* increased serum IL-10 levels and relieved asthma symptoms in children between 6 and 12 years of age who had mild to moderate persistent asthma and inadequate disease control. The possibility of glucan being able to modulate allergic sensitisation and exert beneficial actions in restoring immune homeostasis should be assessed, as this may represent a new therapeutic strategy. To our knowledge, there are currently no clinical data that have evaluated the clinical course of asthma in children treated with orally administered β-glucans.

Taking into consideration the high levels of requests for complementary natural therapies from the parents of asthmatic children, and the absence of well-designed clinical trials with β-glucans, we performed this

international, multicentre, randomised, double-blind, and placebo-controlled study to evaluate the effects of pleuran (β-glucan isolated from *Pleurotus ostreatus*) on asthma control and respiratory morbidity in asthmatic children after a 24-week treatment period and a complete 48-week study period.

Materials and methods

Patients

This study included children of both sexes between the ages of 7 and 17 years with partially controlled perennial asthma (based on asthma control test score) that have been diagnosed and treated according to the Global Strategy for Asthma Management and Prevention (GINA 2016) for at least 12 months before enrolment[19]. The study was conducted between 2017 and 2020 in 12 centres in the Czech Republic, Slovakia, and Poland.

Patients were recruited consistently all year round upon meeting the following criteria: (a) a positive allergy test (skin prick test with at least a 3 mm difference to negative control and/or specific-IgE test results of at least class 2) to house dust mite (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*) and/or another perennial allergen (cat, dog, *Alternaria*, *Cladosporium*, or cockroach) at the time of enrolment or documented within the 12-month period before enrolment; (b) partially controlled perennial asthma (defined as Asthma Control Test score > 15 points and ≤ 19 points) already being treated with ICS, ICS + LABA, or ICS + LTRA in stable doses and with SABA or ICS + LABA as needed within the 3 months prior to enrolment; and (c) at least 2 respiratory infection-induced acute asthma exacerbations within the 12-month period before the initial visit as determined based on the patient's medical records.

The following exclusion criteria were used: diagnosis of primary immunodeficiency syndromes; diagnosis of chronic diseases (including cystic fibrosis, bronchiectasis, alpha-1 antitrypsin deficiency, malignancy, endocrine diseases); product intolerance or allergy to any of the ingredients of the study product; a history of near-fatal asthma (e.g., brittle asthma); pregnancy or planned pregnancy; breastfeeding; any significant medical history and/or treatment for acute cardiac, renal, neurological, hepatic, or endocrine diseases; or any laboratory abnormality indicative of a significant underlying condition that may interfere with a patient's safety, compliance, or study evaluation according to the investigator's opinion. Children who were still receiving or had received any oral or subcutaneous allergen-immunotherapy, bacterial lysates, or ribosomal preparations within the 6 months prior to enrolment, or who had received β-glucans, transfer factors or inosine pranobex in the 3 months prior to enrolment, were excluded from the study. Children whose parents/guardians declined to give consent for participation were also excluded.

Ethics statement

This study was conducted in accordance with the legal and regulatory requirements and the general principles outlined in the fourth International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), the ICH Guidelines for Good Clinical Practice, and the 1964 Declaration of Helsinki and its later amendments. The study protocol was evaluated and approved by a local ethics committee according to their regulations (The Medical University in Lublin: No. KE-0254/242/2017; The Bratislava Self-Governing Region: No. 07999/2017/HF; The University Hospital and Faculty of Medicine Palacky University in Olomouc: No. 153/17; and The Hospital Agel of Prostejov). The parents/guardians of patients were informed about the implications and practical details of the clinical trial and provided signed informed consent for their child's participation.

Study design

This placebo-controlled clinical trial consisted of a 24-week treatment period and a 24-week follow-up period. Patients who met the inclusion criteria were simply randomised by in a 1:1 ratio to the active and placebo groups.

They were required to take either two capsules of Imunoglukan P4H® (containing 100 mg of pleuran and 100 mg of vitamin C per capsule) or two capsules of placebo (containing vitamin C 100 mg only per capsule) in the morning on an empty stomach for 24 weeks. The active substance within the administered natural product is a complex of biologically active polysaccharides consisting in beta-1,3/1,6-D-glucan pleuran isolated from mushroom *Pleurotus ostreatus* using patented technology; this active substance was previously identified and chemically characterised by Karacsonyi and Kuniak[20].

The generation of randomization lists, allocation concealment, and the implementation (enrolment of patients/treatment assignment/intervention) were performed independently by different parties. Randomization was conducted by a third-party administrator using Microsoft Excel ensuring equal distribution in both study groups. The randomization list was delivered to each site by blinded clinical monitors. Study products were allocated to a patient by a site investigator or another qualified and trained person according to the randomization list. Concealment of allocation was achieved by opaque sealed envelopes containing kit numbers pre-assigned to a corresponding randomisation number. Kit numbers were unique non-sequential 6-digit numbers. Algorithm for scheduling kit numbers was linked to 4 batch numbers. The study coordinators, investigators, outcome assessors, and participants remained blinded with respect to treatment allocation (Imunoglukan P4H® or placebo) throughout the study. Study product and placebo were blinded by identically packed and labelled bottles identified only by a unique trial identifier (6-digit kit number). Opaque bottles were of equal appearance and weight. Similarly, capsules in sealed bottles were of similar appearance and taste. Thus, it was not possible to distinguish them at any time during the study period, neither by investigators nor by patients.

Throughout the 48-week study period, there were a total of 5 study visits (V1–V5 and 1 telephone call conversation (TC) six weeks after enrolment) (Fig. 1). At the time of inclusion in the study, each study investigator collected data on relevant demographic and anthropometric data, vital signs, physical examination measures, comorbidities, results from skin prick tests and/or specific-IgE tests performed or documented within

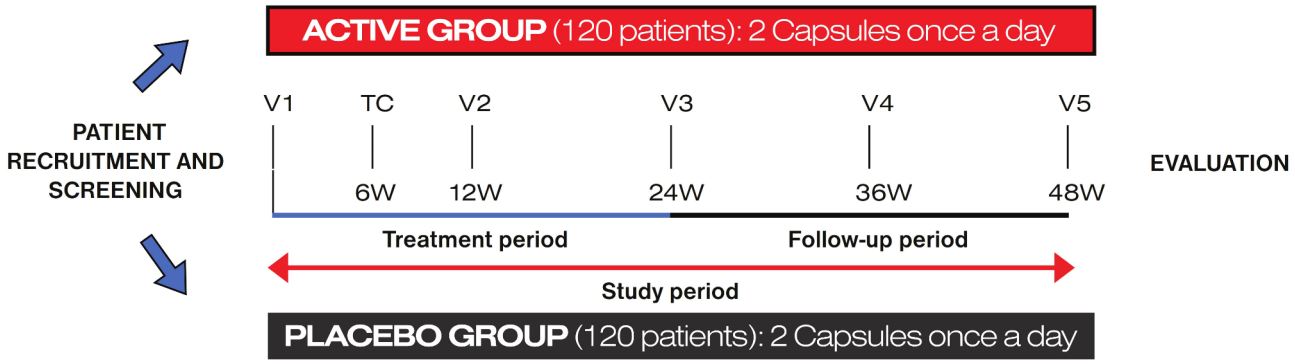

Fig. 1. Study outline.

the 12 months prior to enrolment, asthma history, asthma medication usage, RTI incidence, and antibiotic usage within the 12 months prior to enrolment (pre-treatment period).

The primary endpoints were to evaluate the effects of pleuran on asthma control and RTI incidence. At each study visit, patients or their parents/guardians completed the Asthma Control Test (ACT for children aged at least 12 years)[21,22] or the Childhood Asthma Control Test (C-ACT for children aged 7 to 11 years)[23,24], which have been validated for assessing asthma control in paediatric asthma. Asthma control was assessed based on mean ACT/C-ACT scores. The composite variable of improvement in asthma control was defined by fulfilling one of the following categories: an increase in ACT/C-ACT score of 3 or greater, a decreased rate of emergency/urgent care visits, a decreased rate of oral corticosteroid courses, or a decrease in rescue-SABA usage in the previous month[25]. The number of RTIs experienced and their treatments were recorded in the patient diary and reviewed by physicians at each study visit.

Secondary endpoints that were monitored throughout the study period included differences in the number of asthma exacerbations, differences in the number of infection-induced asthma exacerbations, differences in spirometry indices, and differences in FeNO levels at three timepoints: at enrolment, after the 24-week treatment period, and after the complete 48-week study period. Changes in the quality of life of patients and their caregivers were assessed at each study visit using the Paediatric Asthma Quality of Life Questionnaire (PAQLQ)[26] or the Paediatric Asthma Caregiver's Quality of Life Questionnaire (PACQLQ)[27] addressing asthma-related functional impairments within the following domains: symptoms, activity limitation, and emotional function. During the whole study period, patient compliance and the occurrence of adverse events (AEs) were monitored.

Statistical analysis

The calculation of the size of study groups assumed obtaining statistical significance with 20% difference in primary endpoint of the study between parallel groups. The study planned to enroll approximately 100 subjects in each arm. Assuming a 10% dropout rate during the study we aimed to enroll and randomize approximately 220 subjects.

Quantitative data are presented using frequency tables displaying absolute and relative numbers N (%). Continuous data are shown as mean and standard deviation (SD). Differences between the treatment groups were identified using chi-squared tests for categorical data and t-tests or ANOVA tests for continuous data. The tests were two-sided with significance defined as $P < 0.05$. There was no correction due to multiple testing, and the P values are presented in an exploratory fashion. Statistical analyses were performed using the STATISTICA 11.0 PL (TIBCO, Palo Alto, CA, USA).

Results

A total of 230 eligible patients were enrolled and randomised in this double-blind, placebo-controlled, international multicentre clinical trial, which was conducted between 2017 and 2020. From the total 230 patients, 125 patients were assigned to the active (Imunoglukan P4H®) group and 105 patients were assigned to the placebo group. A total of 24 patients were excluded from the final analysis (12 from the active group and 12 from the placebo group). Drop-out cases included 8 cases of loss of contact, 11 cases of non-compliance, and 5 premature discontinuations of active/placebo treatment before TC. Data from 206 patients was included in the analysis (113 patients in the active group and 93 patients in the placebo group) (Fig. 2).

As shown in Table 1 (pre-treatment period), the active and placebo groups, respectively, were characterised by similar ages (10.3 ± 2.9 years vs. 10.4 ± 2.9 years), proportions of males (71, 62.8% vs. 69, 74.2%), percentages of participants aged < 12 years (69.0% vs. 69.9%), time since asthma diagnosis (6.1 ± 3.5 years vs. 5.8 ± 3.3 years), levels of asthma severity (78.8% vs. 81.7% of individuals with moderate asthma according to GINA 2016), and levels of asthma control assessed by the C-ACT (17.2 ± 2.9 vs. 17.5 ± 2.7) or ACT (17.8 ± 3.4 vs. 18.6 ± 3.4) scores. Allergic rhinitis was the most frequent comorbidity, which was reported in 95.6% of active group patients and 96.8% of placebo group patients (Table 1). The mean numbers of RTIs (4.6 ± 1.8 vs. 4.4 ± 2.4) and antibiotic treatments (2.2 ± 1.3 vs. 2.1 ± 1.3) reported during pre-treatment period were similar between the active and placebo groups.

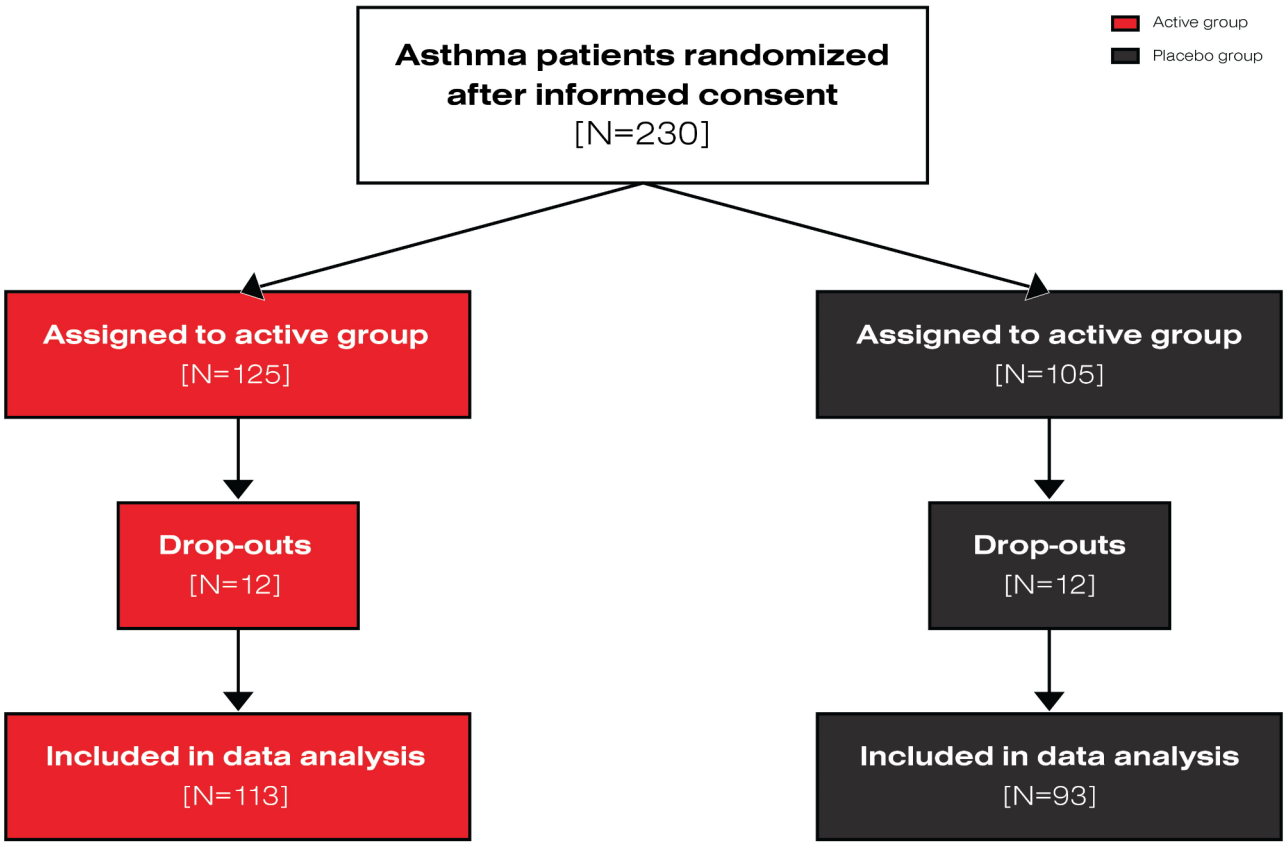

Fig. 2. Flow diagram of patient inclusion.

	Active group (N = 113)	Placebo group (N = 93)
Gender		
Male [N; %]	71; 62.8	69; 74.2
Female [N; %]	42; 37.2	24; 25.8
Age (years)	10.3 ± 2.9	10.4 ± 2.9
< 12 years [N; %]	78; 69.0	65; 69.9
≥ 12 years [N; %]	35; 31.0	28; 30.1
Body weight (kg)	39.3 ± 14.1	41.5 ± 15.4
Body height (cm)	143.5 ± 16.5	145.1 ± 15.0
Period of time since asthma diagnosis (years)	6.1 ± 3.5	5.8 ± 3.3
Asthma bronchial classification (GINA 2016)		
Mild [N; %]	22; 19.5	16; 17.2
Moderate [N; %]	89; 78.8	76; 81.7
No data [N; %]	2; 1.8	1; 1.1
Asthma control test (C-ACT/ ACT)		
< 12 years [C-ACT score; pts]	17.2 ± 2.9	17.5 ± 2.7
≥ 12 years [ACT score; pts]	17.8 ± 3.4	18.6 ± 3.4
Comorbidities		
Atopic eczema [N; %]	15; 13.3	12; 12.9
Allergic rhinitis [N; %]	108; 95.6	90; 96.8
Food allergy [N; %]	8; 7.1	3; 3.2
Pre-treatment period		
Number of RTIs [N]	4.6 ± 1.8	4.4 ± 2.4
Number of antibiotic treatments [N]	2.2 ± 1.3	2.1 ± 1.3

Table 1. Basic demographic characteristics of patients included in the study.

Primary outcomes: changes in asthma control

Comparison of asthma control between the two treatment groups, which was based on the ACT and C-ACT tests, was the primary endpoint of the clinical trial. No differences in asthma control, as assessed by the C-ACT or ACT scores, were observed at the initial visit between the active and placebo groups. However, after the 24-week treatment period, asthma control was significantly better in children under 12 years old in the active group compared to those in the placebo group (21.8 ± 3.5 vs. 20.3 ± 4.0; $P = 0.02$) (Fig. 3A) but was similar in children at least 12 years old from the two groups (Fig. 3B). No differences in asthma control at the end of the 48-week study period were found between the active and placebo groups either in children under 12 years old (Fig. 3A) or in children at least 12 years old (Fig. 3B).

After the 24-week treatment period, an improvement in asthma control (defined as an increase in ACT/C-ACT score of 3 or greater, a decreased rate of emergency/urgent care visits, a decreased rate of oral corticosteroid courses, or a decrease in rescue-SABA usage in the previous month), was observed in both the active and placebo groups. An improvement in asthma control was recorded in 64.9% of the active group and 56% of the placebo group. After the 48-week study period, a statistically significant improvement in asthma control was observed in 84.7% of patients receiving pleuran/vitamin C combination compared to 67.0% of patients receiving placebo ($P = 0.01$) (Fig. 3C). Likewise, subgroup-analysis of children at least 12 years old revealed significant improvement in asthma control in the active group compared to the placebo group (91.9% vs. 64.7%; $P = 0.01$) after 48 weeks (Fig. 3C).

Primary outcomes: RTIs

The data analysis showed similar mean numbers of RTIs in the active and placebo groups during the 24-week treatment period and during the 24-week follow-up period, as well as over the whole 48-week study period (Fig. 4A). Similarly, subgroup-analysis of patients under 12 years old did not show any significant differences in the mean numbers of RTIs after either the active treatment period or the whole study period (Fig. 4B). Notably, subgroup-analysis of patients at least 12 years old showed a statistically significant decrease (0.7 ± 1.0 vs. 1.9 ± 1.7; $P = 0.002$) in the mean number of RTIs per patient in the active group compared to the placebo group during 24 weeks of treatment as well as during the whole study period (1.2 ± 1.5 vs. 3.0 ± 3.1; $P = 0.003$) (Fig. 4B).

Secondary outcomes: asthma exacerbations

The mean numbers of asthma exacerbations during the treatment period, during the follow-up period, and during the whole study period were similar in the active and placebo groups (Fig. 5A). Subgroup-analysis of patients under 12 years old showed a statistically significant difference in the mean number of asthma exacerbations between pleuran/vitamin C combination vs. placebo (2.5 ± 1.6 vs. 3.3 ± 1.9; $P = 0.05$) after 24 weeks of active treatment and this difference was clinically meaningful and important (Fig. 5B). In the case of asthma exacerbations induced by infections, no differences were observed between the active and placebo groups after the 24-week treatment period (0.4 ± 0.9 vs. 0.4 ± 0.9; $P = 0.48$), neither in children < 12 years (0.4 ± 1.0 vs. 0.6 ± 1.0; $P = 0.53$) nor in children ≥ 12 years (0.1 ± 0.4 vs. 0.2 ± 0.4; $P = 0.73$).

Secondary outcomes: changes in spirometric parameters, FeNO levels, and quality of life

No significant differences in the changes in selected spirometric parameters were found between the active and placebo groups at the end of the 24-week treatment period or at the end of the 48-week study period (Table 2). In addition, FeNO levels were also stable at these two timepoints in both treatment groups.

Changes in PAQLQ and PACQLQ parameters were also analysed to assess patients' and caregivers' quality of life. There was a greater improvement in the quality of life for caregivers than for patients; no difference in quality of life between treatment groups was found at either the 24- or 48-week timepoint (Table 3).

Secondary outcomes: overall tolerability and safety

Compliance to treatment was similar in both treatment groups (94.3 ± 7.9% vs. 93.8 ± 7.2%, respectively). A total of nine AEs were reported by investigators within this clinical trial, including three episodes of acute infectious diseases of viral origin, two episodes of acute gastritis, one episode of vomitus, two episodes of skin and subcutaneous tissue disorders, and one episode of headache. None of these AEs caused discontinuation of the active or placebo treatment, and they were not reported as being attributed to the study products. The incidence rate was similar in both treatment groups (N = 4; (3.2%) in the active group and N = 5 (4.8%) in the placebo group; P = ns).

Discussion

Bronchial asthma is the most common chronic respiratory disease in children, having a significant impact on all the spheres of life of the affected patient and his/her family. Therefore, any tools for improving asthma control could have a significant impact on the prognosis of affected children. Complementary medicine is highly requested and is preferred by many parents; however, not all of the available natural compounds also possess sufficient evidence for their efficacy. The most important prerequisite for this trial was the complex biological effects of pleuran and its possible effects on chronic allergic inflammation and RTI incidence. In this study, pleuran-based supplement applied daily for a period of 24 weeks to children with partially controlled perennial asthma significantly and positively changed asthma control in children under 12 years old and decreased the mean number of RTIs in children at least 12 years old compared to the placebo group. The supplement also significantly decreased the mean number of asthma exacerbations in children under 12 years old compared to the placebo group after 24 weeks of treatment. Evaluating the whole study period shows that there was a significant improvement in asthma control in children receiving pleuran-based supplement with vitamin C in comparison

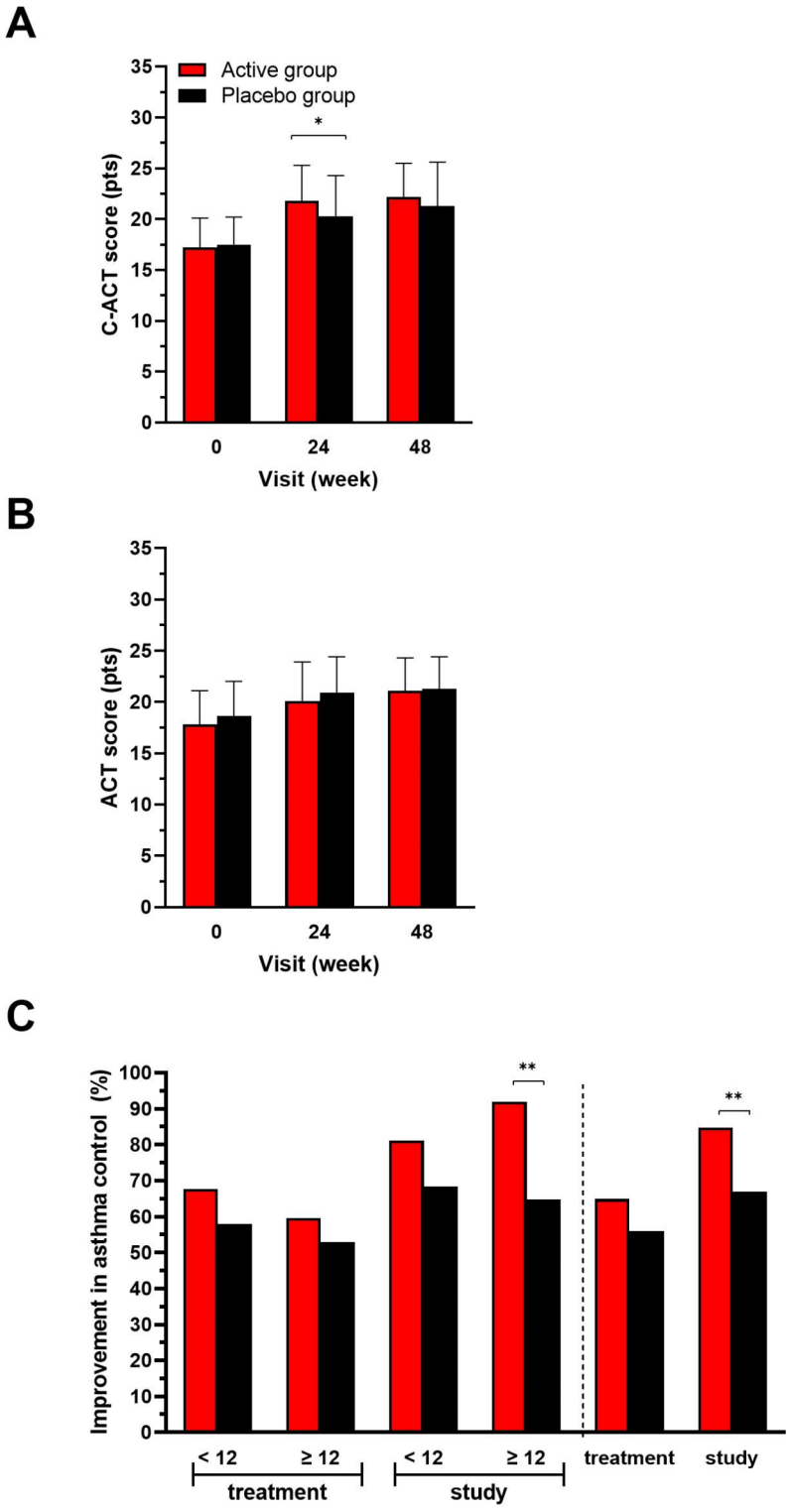

Fig. 3. Changes in asthma control (assessed by C-ACT/ACT scores) in children younger than 12 years (**A**), at least 12 years old (**B**), and improvement in asthma control (defined as an increase in ACT/C-ACT score of 3 or greater, decreased rate of emergency care visits, a decreased rate of oral corticosteroid courses, or a decrease in rescue-SABA usage in the previous month) in the whole study population and age subgroups (**C**). $^{*}P=0.02$; $^{**}P=0.01$.

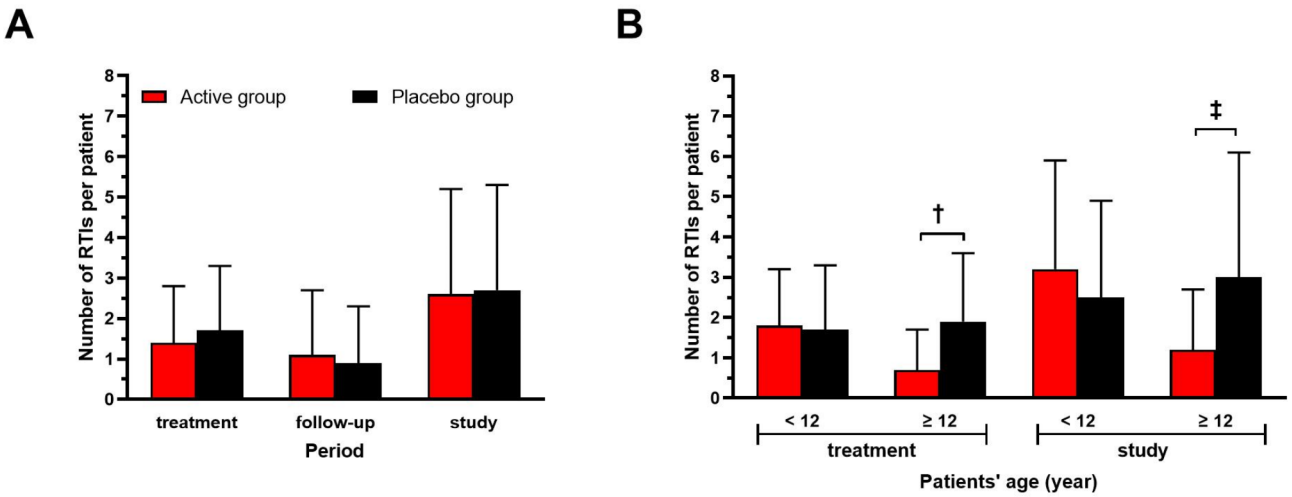

Fig. 4. Number of respiratory tract infections (RTIs) in the whole study population (**A**) and in subgroups according to age (**B**) during the treatment and study period. $^{\dagger}P = 0.002$, $^{\ddagger}P = 0.003$.

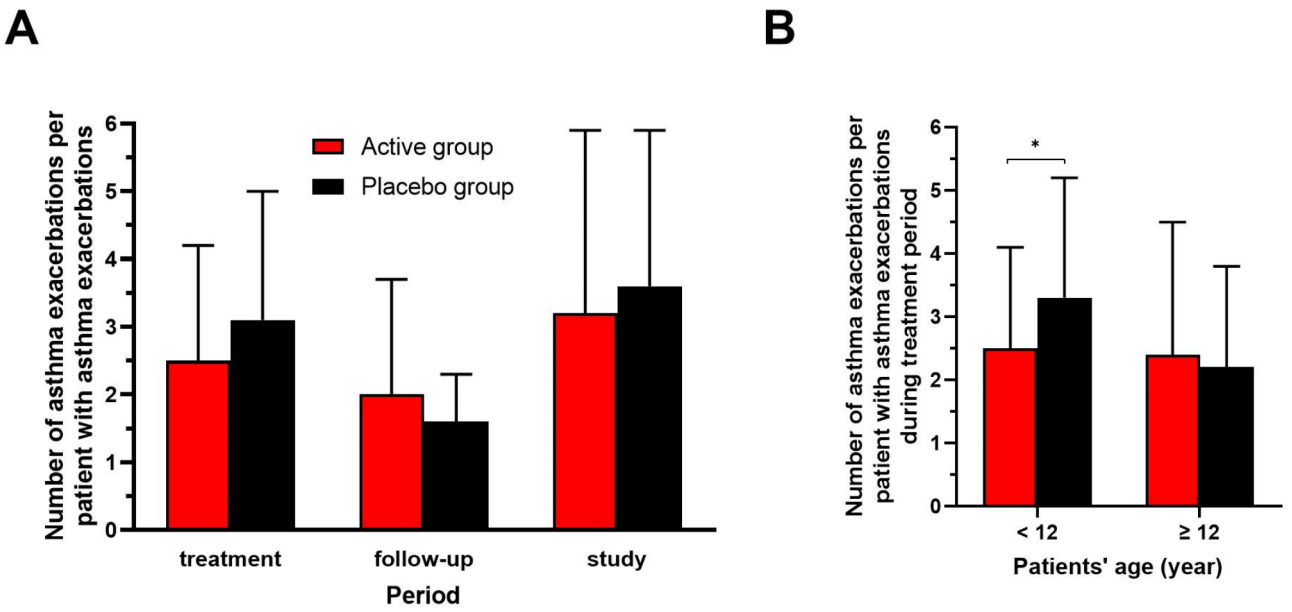

Fig. 5. Changes in the number of asthma exacerbations in the whole study population (**A**) and in the subgroups according to the age (**B**). $^{*}P = 0.05$.

to placebo (vitamin C only), suggesting a persistence of the biological effect of the applied immunomodulator even after the end of its application.

Biologically active polysaccharides such as pleuran possess a broad spectrum of biological activities that could be beneficial in the management of allergic diseases. Allergic inflammation could be influenced by immunomodulatory, antioxidant, and anti-allergic effects[28–30]. Moreover, its anti-infective effects could protect against the worsening of asthmatic symptoms during respiratory infections[11].

In our study, we observed a significant improvement in asthma control in younger children (using the C-ACT scores achieved) as well as in older children (when assessing the improvement of asthma control by an increase in ACT score of ≥ 3 points, a decreased rate of emergency/urgent care visits, a decreased rate of oral corticosteroid courses, or a decrease in rescue-SABA usage in the previous month) receiving the pleuran/vitamin C combination compared to vitamin C only. Moreover, the mean frequency of asthma exacerbations was significantly decreased in the active treatment group in children younger than 12 years. All enrolled children had partially controlled asthma at the beginning of the study, and all had house dust mite-driven allergic symptoms. Complex anti-allergic effects of β-glucans modulate allergic inflammation and thus lead to an improvement in asthma control and a decline in exacerbations[17]. Under experimental conditions, application of β-glucans modified and restored Th1 and Th2 homeostasis[31,32], increased levels of the transcription factors of Th1 and

Parameter	Active group			Placebo group			*P* value	
	Time (weeks)							
	0	24	48	0	24	48	24	48
VC (mL)	2373 ± 922	2377 ± 979	2492 ± 884	2525 ± 873	2625 ± 914	2852 ± 991	0.25	0.09
FVC (mL)	2521 ± 1041	2608 ± 1025	2687 ± 1005	2440 ± 842	2511 ± 769	2657 ± 817	0.46	0.82
PEF (L/sec)	4.3 ± 1.8	4.5 ± 1.8	4.6 ± 1.7	4.4 ± 1.5	4.6 ± 1.5	4.9 ± 1.6	0.78	0.10
FEV_1 (mL)	2179 ± 876	2239 ± 853	2287 ± 792	2160 ± 723	2266 ± 727	2421 ± 783	0.81	0.23
FEV_1 predicted (%)	97 ± 16	95 ± 15	92 ± 13	94 ± 13	94 ± 13	95 ± 13	0.85	0.07
FEV_1/FVC (%)	101 ± 10	100 ± 10	100 ± 9	100 ± 8	101 ± 7	101 ± 8	0.51	0.31
FEF_{25-75} (%)	95 ± 28	94 ± 28	91 ± 26	88 ± 22	90 ± 22	93 ± 23	0.38	0.52
FeNO (ppb)	22 ± 25	21 ± 21	21 ± 20	21 ± 23	22 ± 26	21 ± 19	0.79	0.91

Table 2. Spirometry parameters and FeNO levels at initial visit (t = 0), after 24-week treatment period with Pleuran/Vitamin C combination or Placebo (Vitamin C only) (t = 24) and after 48-week study period (t = 48). FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; FEF, Forced expiratory flow; PEF, Peak expiratory flow; VC, vital capacity.

Parameter	Active group	Placebo group	*P* value
	t = 0 week		
PAQLQ (pts)	129.6 ± 23	128.1 ± 24.4	0.64
PACQLQ (pts)	67.8 ± 16.8	65.7 ± 16.9	0.38
	t = 24 weeks		
PAQLQ (pts)	141.1 ± 21.1	140.5 ± 18.7	0.84
PACQLQ (pts)	77.6 ± 11.6	77.4 ± 12.6	0.90
	t = 48 weeks		
PAQLQ (pts)	141.1 ± 21.1	140.3 ± 19.8	0.79
PACQLQ (pts)	77.5 ± 11.4	77.9 ± 11.7	0.80

Table 3. Quality of life of patients and caregivers. PAQLQ, Paediatric Asthma Quality of Life Questionnaire; PACQLQ, Paediatric Asthma Caregiver's Quality of Life Questionnaire.

Treg cells[33], increased the production of tolerogenic and anti-allergic IL-10[34], decreased the release of CCL20 involved in the progression of allergic infiltrate[35], decreased levels of total[36] and specific IgE[33,36], and decreased the number of eosinophils in the blood and BALF[37]. Some of these results from experimental studies were even confirmed in human observations and trials. In our previous study, application of pleuran in children with recurrent RTIs decreased the number of eosinophils in the peripheral blood and prevented increases in total IgE levels in the serum[16]. Interestingly, the subcutaneous application of yeast β-glucan in asthmatic patients increased the production of anti-allergic IL-10[18]. In this clinical trial, we focused on clinical outcomes and did not perform any laboratory testing; however, the results from the other studies and experiments support the possible anti-allergic and anti-inflammatory effects of pleuran.

Only a few published studies demonstrate the alleviating effects of β-glucans on allergic or asthmatic symptoms. Administration of yeast β-glucan to ragweed-allergic patients reduced nasal and eye symptoms during ragweed pollen season[38]. Sarinho et al.[18] observed a decline in asthma symptom score at the end of their trial of subcutaneous application of β-glucan. Moreover, in another study the intranasal application of carboxymethyl-β-glucan reduced all assessed nasal symptoms, decreased the use of antihistamines in children with pollen-induced allergic rhinitis, and decreased the severity and sequelae of respiratory infections in these children[39]. In our study, certain analyses did not show significant differences between active treatment and placebo groups. However, clinical trials are also able to improve disease control through better adherence to treatment and regular clinical check-ups[40]. In both arms of our study, the rate of compliance to treatment with the study medication was over 93%, and we can suppose that high compliance was also maintained with standard anti-asthmatic treatments.

Bronchial asthma is a chronic airway disease characterised by airway inflammation, reversible airway obstruction, mucus hypersecretion, and airway hyperresponsiveness[41]. Accumulating data suggest that immune responses to pathogens may be altered in asthmatics[42–44]. Paediatric asthma has different patterns according to children's ages, and mortality and morbidity from asthma are higher in adolescents (12–17 years old) than in children (5–9 years old). According to a survey conducted over a 3-year period in the UK, asthma mortality was six times higher in adolescents than in children[45]. In fact, adolescent asthmatic patients are a distinct group of patients with different treatment requirements from paediatric patients[46]. In this study, active treatment with a pleuran/vitamin C combination decreased the number of RTIs compared to placebo (vitamin C only), especially in children at least 12 years old; this effect was seen immediately after the 24-week treatment period and persisted

to the end of study. Moreover, the number of RTIs decreased significantly during the study period compared to the pre-treatment period only in children at least 12 years old from the active treatment group. A plethora of studies have investigated the anti-infective effects of different types of β-glucans in healthy participants and athletes as well as in paediatric patients[11,47,48]. Indeed, β-glucan may provide effective prevention against infections and enhance the host's immune resistance to microbial pathogens, particularly in paediatric populations. However, one of the important factors determining β-glucan anti-infective properties is a child's age. Young children under 5 years old are more sensitive to RTIs due to higher rates of breathing, narrow airways, developing lungs, and specific characteristics of the maturating immune systems[49]. We have recently shown that the total number of RTIs in 1030 children—with an average age of 3.5 years—within a 6-month study period (3 months of pleuran/vitamin C supplementation then 3 months of follow-up) was significantly decreased compared to the same period of previous year[50]. The reduction of RTIs could be achieved by a β-glucan-mediated improvement of innate mucosal immunity. Mucosal immune cells function reaches the level observed in adults around the age of 4 to 6 years[51]. This provides protection against pathogenic viruses and bacteria, and it is mainly composed of salivary immunoglobulin A and antimicrobial peptides. In our previous double-blind placebo-controlled clinical trial, application of pleuran decreased the number of RTIs and increased the proportion of healthy children. Moreover, a decline in flu and flu-like diseases was also observed along with the positive changes in cellular immunity[13]. Many other studies have confirmed the potential preventive effects of various β-glucans on the prevention of RTIs[52–54]; however, it seems that pleuran is the most potent β-glucan with regard to preventive capacity[11,48].

The potential mechanisms involved in the anti-infectious effects of β-glucans could include the decreased expression of intercellular adhesion molecule 1 (ICAM-1, entrance receptor for human rhinovirus)[55], the modulation of cellular immunity[13], the induction of trained immunity[56], and its modulating effects on the cytokine microenvironment[54]. Currently, trained immunity and its modulation were hypothesised to be involved in the pathogenesis of bronchial asthma[57] and can serve as potential therapeutic targets[58]. Certain inconsistencies in decreases in respiratory infections, prevention of asthma exacerbations, and improvements in asthma control can be explained by the evolution of asthmatic phenotypes in different age categories and by different triggering factors for asthma exacerbations (from allergen exposure to infections)[59–61]. The incidence of allergic asthma is highest in early childhood and subsequently steadily declines with increasing age[62]. In younger children, a greater improvement in asthma control and a significant decline in asthma exacerbations could be attributed mainly to the anti-inflammatory and anti-allergic effects of pleuran. In older children and adolescents, a significant decline in asthma exacerbations was not confirmed. Nevertheless, significant decrease in the number of RTIs in the subgroup of children at least 12 years old was accompanied by a positive effect on asthma control. The improvement of asthma control in older children despite the lack of significant changes in the parameter of asthma exacerbation frequency could be explained by the more complex domains evaluated through the ACT. It is possible that with increasing age, especially during adolescence, other environmental factors and triggers that are not directly influenced by immunomodulatory therapy are important and contribute to asthma exacerbations (e.g., hormonal changes, exercise, stress, smoking, perfumes, risky behaviour and unhealthy life-style, and problems with compliance to the anti-asthmatic treatment) alongside infectious and allergic triggers[63–65]. Moreover, the prevalence of comorbidities complicating the achievement of asthma control is higher in adolescence[66–68]. Interestingly, several studies are trying to provide a more detailed and precise division of paediatric asthma into various phenotypes or "clusters"[60,69] and, more importantly, to reveal possible "reshuffling" from an initial phenotype into another one[70]. Our results also showed another important clinical issue—the onset of the clinical effects of immunomodulators can differ between age categories and requires the regular application of the active compound for at least several months. This was shown even in our previous studies[13,16].

A few limitations must be acknowledged in the present study, including the use of vitamin C in the placebo group and the absence of data describing immunological parameters. The evidence has recently been reviewed for vitamin C having preventive and/or relieving therapeutic benefits in respiratory diseases, including asthma[71]. In addition, the absence of laboratory immunological data in our study does not allow us to explain the exact mechanisms of action of the pleuran and vitamin C combination. The interaction of beta-glucans and vitamin C has mainly been investigated in fish and livestock animals, in which enhanced immunological responses were reported (especially on non-specific immunity); however, no human clinical study has supported these pre-clinical outcomes.

In conclusion, this is the first double-blind placebo-controlled trial that has shown potential beneficial effects of pleuran (β-glucan isolated from *Pleurotus ostreatus*) on asthma control and asthma exacerbation rate in children with partially controlled, house dust mite (HDM)- and/or another perennial allergen-induced bronchial asthma. The observed effect could be attributed to the immunomodulatory, anti-infective, anti-allergic, anti-inflammatory, and antioxidant biological activities of pleuran. The application of pleuran seems to be a safe way to prevent infections, decrease exacerbation rates, improve disease control, and modulate allergic inflammation in allergic asthmatic children.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request and with the approval of Pleuran, s. r.o.

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Author contributions

All authors contributed to the study conception and design. The data were collected by Jerzy Chudek. The first draft of the manuscript was written by Juraj Majtan and Milos Jesenak and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

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Declarations

Competing interests

MJ has received consulting fees (AstraZeneca, GlaxoSmithKline, Chiesi, Pleuran); honoraria for lectures, presentations (AstraZeneca, GlaxoSmithKline, Chiesi, Novartis, Sanofi, TEVA); support for attending meetings and/or travel (Sanofi, Chiesi, Zentiva) and honoraria for participation on Advisory Boards (AstraZeneca, GlaxoSmithKline, Chiesi). ZD has received speaker or consultant honoraria and/or served on advisory boards at: Antabio, Arcede, Biosion, Foresee Pharmaceuticals, Galenushealth, GlaxoSmithKline, Hippo-Dx, QPS-Netherlands, Sanofi-Genzyme-Regeneron, all outside the submitted work. During the last 3 years of her assignment as Research Director Respiratory and Allergy, QPS-Netherlands received an European grant from ERA4TB and funding from Foresee Pharmaceuticals for early clinical studies. She serves as associate editor for Allergy and Respiratory Medicine and acts as Chair of the Asthma Expert Panel at EUFOREA (2020–2023). ZR has received honoraria for lectures, presentations (Berlinchemie/A.Meranini Slovakia, Danone, Pleuran, Ewopharma, Chiesi, Angelini Pharma, Novartis, Zentiva); support for attending meetings and/or travel (Stallergenes, AstraZeneca, Berlinchemie/A.Meranini Slovakia, ALK, Danone, Angelini Pharma, Sanofi-Aventis) MH has received honoraria for lectures, presentations (Novartis, AstraZeneca, GlaxoSmithKline, Pleuran). JM, JB, JC and AE have no relevant financial or non-financial interests to disclose.

Ethics approval

The study protocol was evaluated and approved by a local ethics committee according to their regulations (The Medical University in Lublin: No. KE-0254/242/2017; The Bratislava Self-Governing Region: No. 07999/2017/HF; The University Hospital and Faculty of Medicine Palacky University in Olomouc: No. 153/17; and The Hospital Agel of Prostejov).

Consent to participate

Written informed consent was obtained from the parents/guardians, and verbal consent was obtained from all participating children.

Additional information

Correspondence and requests for materials should be addressed to M.J. or J.M.

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