Original Article

Polyvalent Mechanical Bacterial Lysate Administration Improves the Clinical Course of Grass Pollen–Induced Allergic Rhinitis in Children: A Randomized Controlled Trial

Kamil Janeczek, MD, PhD[a], Andrzej Emeryk, MD, PhD, DSc[a], Marta Rachel, MD, PhD[b], Dariusz Duma, PharmD, PhD[c], Łukasz Zimmer, PharmD, PhD[d], and Ewa Poleszak, PharmD, PhD, DSc[d] *Lublin and Rzeszów, Poland*

What is already known on this topic? Bacterial lysates have been used for many years to prevent respiratory tract infections in children and adults. In addition, they show beneficial effects in children with asthma and atopic dermatitis.

What does this article add to knowledge? To our knowledge, our study is the first prospective, randomized, double-blind, placebo-controlled, parallel-group study assessing the effect of polyvalent mechanical bacterial lysate therapy on the clinical course of seasonal allergic rhinitis in children sensitized to grass pollen allergens.

How does this study impact current management guidelines? The use of polyvalent mechanical bacterial lysate in children with grass pollen–induced allergic rhinitis may reduce the severity of the disease symptoms.

BACKGROUND: Recent studies highlight the immunoregulatory potential of bacterial lysates, indicating their potential use in the prevention and treatment of allergic diseases.
OBJECTIVE: To investigate the clinical efficacy of polyvalent mechanical bacterial lysates (PMBLs) in children with grass pollen–induced allergic rhinitis.
METHODS: Seventy children with seasonal allergic rhinitis were enrolled to this study and were randomly assigned to the PMBL and placebo groups. Severity of seasonal allergic rhinitis symptoms was assessed by the total nasal symptom score, total ocular symptom score, and visual analogue scale. During 3 visits, peak nasal inspiratory flow was measured, and nasal smears for the presence of eosinophils and nasal lavage fluids for the presence of allergen-specific IgE against timothy grass pollen allergens were sampled.
RESULTS: A statistically significant decrease in total nasal symptom score ($P = .001$), total ocular symptom score ($P = .04$), and visual analogue scale score for nasal and eye symptoms ($P < .001$ and $P < .001$, respectively) and an increase in peak nasal inspiratory flow ($P = .04$) were observed in the PMBL group versus the placebo group. During the grass pollen season, an increase and then a decrease in the number of eosinophils in nasal smears was observed in both groups; however, the number of eosinophils was significantly lower in the PMBL group versus the placebo group. No significant changes in allergen-specific IgE concentrations were observed in the PMBL group, whereas in the placebo group a statistically significant increase in allergen-specific IgE concentration was observed.
CONCLUSIONS: Sublingual administration of PMBLs during the grass pollen season offers significant efficacy in alleviating seasonal allergic rhinitis symptoms in children sensitized to grass pollen allergens. PMBLs probably affect mucosal immunity, weakening the response of T_H2 cells. © 2020 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;■:■-■)

Key words: *Allergic rhinitis; Seasonal allergic rhinitis; Children; Grass pollen season; Bacterial lysate; PMBL sublingual tablet; T_H2-type inflammation; Eosinophils; Specific IgE*

INTRODUCTION

Allergic rhinitis (AR) is an inflammatory process of the nasal mucosa, most often IgE-dependent, caused by environmental allergens. Typical symptoms of the disease are rhinorrhea, nasal

[a]Department of Pulmonary Diseases and Children Rheumatology, Medical University of Lublin, Lublin, Poland
[b]Chair and Department of Human Physiology and Pathophysiology, University of Rzeszów, Rzeszów, Poland
[c]Department of Laboratory Diagnostics, Medical University of Lublin, Lublin, Poland
[d]Department of Applied and Social Pharmacy, Medical University of Lublin, Lublin, Poland
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Corresponding author: Kamil Janeczek, MD, PhD, Department of Pulmonary Diseases and Children Rheumatology, Children's University Hospital, Medical University of Lublin, Profesora Antoniego Gębali 6, 20-093 Lublin, Poland. E-mail: kamil.janeczek@umlub.pl.
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Abbreviations used
AR- allergic rhinitis
asIgE- allergen-specific IgE
BL- bacterial lysate
MCID- minimal clinically important difference
PAR- perenial allergic rhinitis
PCBL- polyvalent chemical bacterial lysate
PMBL- polyvalent mechanical bacterial lysate
PNIF- peak nasal inspiratory flow
SAR- seasonal allergic rhinitis
TNSS- total nasal symptom score
TOSS- total ocular symptom score
VAS- visual analogue scale

congestion, itching of the nose, and sneezing, which disappear spontaneously or under the influence of treatment. AR is the most common inflammatory disease in the pediatric population and the most common allergic disease among schoolchildren, with a constantly increasing prevalence in many countries.[1-4]

AR in children is an important risk factor for such diseases as asthma (including preschool asthma), ear infections, or chronic inflammation of nasal and paranasal sinuses mucosa.[5] Moreover, numerous data indicate the negative impact of AR on children's quality of life, cognitive functions, sleep, school performance, learning outcomes, child's behavior, and the functioning of other organs and systems.[6-9]

Seasonal allergic rhinitis (SAR) is usually caused by allergens of wind-pollinated plants. In Poland and other Central and Eastern European countries, these are pollen of grasses, grains, trees, weeds, and shrubs.[10,11] Very often (in >90% of cases), symptoms of AR are accompanied by symptoms of allergic conjunctivitis.[12-14]

The treatment algorithm for SAR consists of[15-17]

- avoiding the allergen (whenever possible),
- educating the patient, his or her family, and carers in kindergarten and school (always),
- pharmacotherapy that is effective, safe, and easy to use, and
- allergen immunotherapy.

The basic groups of drugs used according to specific SAR therapy regimens include[18,19] oral H1-antihistamines, intranasal H1-antihistamines, intranasal corticosteroids, antileukotrienes, anticholinergics, alpha-mimetics, and cromones. However, because of the high incidence of SAR, the adverse impact of the disease on the quality of life, and the incomplete effectiveness of the therapeutic methods available so far, new treatments are being sought, also in children. Recent data indicate possible clinical benefits from the use of probiotics,[20,21] vitamin D,[22] and prostaglandin D2 receptor 1 antagonist[23] during the pollen season. Bacterial lysates (BLs) also seem to be one of the possible therapeutic options for allergic diseases in children. They show such immunoregulatory potential that they can be used in the prevention and treatment of certain allergic diseases.[24-36]

BLs are a mixture of antigens that are extracted from inactivated bacteria, which are the most common etiological factors of respiratory infections. Two methods are used to obtain a mixture of BLs: the mechanical lysis method (to obtain mechanical BL) and the chemical lysis method (to obtain chemical BL). The lysis process is a key element in the production of these preparations because it determines their immunomodulatory properties. Mechanical lysates are characterized by much less bacterial antigen damage and less chemical impurities. They are obtained by sonication, that is, inactivation of the cell wall of bacterial cells by means of ultrasound. Mechanical BLs show higher immunogenicity compared with chemical BLs and thus may have higher clinical effects.[37,38]

BLs reduce the frequency of respiratory infections in children and adults.[38] When justifying the use of BLs in children with allergic diseases, it should be noted that allergic disorders are characterized by T_H2 polarization, and hence physiological T_H1-dependent mechanisms for fighting respiratory infections may be defective. Therefore, allergic children have more numerous and more severe respiratory infections than nonallergic children.[39]

In addition, *in vivo* studies show that BLs:

- can be effective in the prevention of atopic dermatitis in newborns at risk of allergies and in the treatment of this disease in children,[31,32,40]
- may improve the clinical course of AR in adults when BLs are added to standard therapy,[25,36,41] and
- reduce the number of asthma exacerbation in school children allergic to house-dust mite allergens when BLs are added to the existing chronic treatment.[42]

So far, no randomized, double-blind, placebo-controlled study with BLs in children's SAR therapy has been conducted. Therefore, the aim of this study was to evaluate the effect of polyvalent mechanical bacterial lysate (PMBL) on the clinical course of SAR caused by grass pollen allergens in children during the grass pollen season.

METHODS

Study design

The presented study was a multicenter, prospective, randomized, double-blind, placebo-controlled study in parallel groups (PMBL vs placebo). The study was approved by the Bioethics Committee of the Medical University of Lublin (resolution no. KE-0254/41/2018 of February 22, 2018), and the study was conducted according to the Declaration of Helsinki. The project was financed by the authors of this article. The study was conducted in 3 clinical centers in south-eastern Poland between April and August 2018.

The primary study objective was to assess the efficacy of 3-month PMBL therapy in improving the clinical course of SAR caused by grass pollen allergens in children during the grass pollen season. Nasal and ocular SAR symptoms were recorded by parents of children in the daily patient diary according to 4-point standard scoring systems (total nasal symptom score [TNSS]; total ocular symptom score [TOSS]), and their intensity was also evaluated during 3 visits using a visual analogue scale (VAS).[13,43] At each visit, peak nasal inspiratory flow (PNIF) was also measured by Youlten Peak Flow Meter (Clement Clarke International, Harlow, UK).[44] To assess the clinical significance of the obtained results, it was assumed that the minimal clinically important difference (MCID) for TNSS is 0.55 units and for PNIF is 5 L/min.[45]

The secondary objectives were to assess the effect of PMBL therapy on the number of eosinophils in nasal smears and on the level of allergen-specific IgE (asIgE) against timothy grass pollen allergens in nasal lavage fluid, and to compare the mean number of days of use of oral H1-antihistamines and intranasal corticosteroids for relief from SAR symptoms over the whole study period.

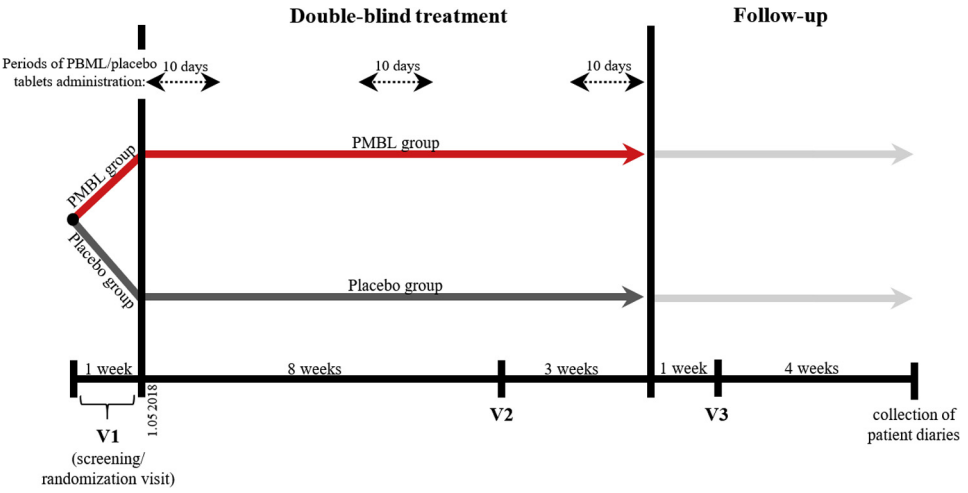

FIGURE 1. Study design.

Patients

Eligible participants were children aged 5 to 17 years with AR recognized and treated according to current Allergic Rhinitis and its Impact on Asthma recommendations,[18] with seasonal sensitization to grass pollen allergens defined as a positive skin prick test result (Allergopharma-Nexter Sp. z o. o., Przyszowice, Poland) with wheal diameter (that was) at least 3 mm more than that of the negative control or asIgE level equal to or higher than class 2 (Polycheck, Biocheck GmbH, EMMA MDT Sp. z o. o., Poland), who demonstrated clinical symptoms of the disease (rhinorrhea, nasal congestion, nasal itching, sneezing, redness of the eyes, watery eyes, itching of the eyes) in at least 2 recent grass pollen seasons in Poland before inclusion in the study. In addition, patients should not have been treated with BLs in the last year and allergen immunotherapy in the last 3 years. The exclusion criteria also included vaccination performed within 3 months before the beginning of the study, systemic immunologic disorders, and intercurrent systemic corticosteroid treatment for the past 6 months. All patients were recruited for the study at the end of April 2018, that is, before the beginning of the grass pollen season in Poland. Written informed consent was obtained from parents of patients and from all patients before enrollment in the study.

Interventions

Children from the study group received a PMBL sublingual tablet (Ismigen, Lallemand Pharma AG, Massagno, Switzerland) containing 7 mg of BLs from the following bacteria: *Staphylococcus aureus*, *Haemophilus influenzae* serotype B, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Neiserria catarrhalis*, *Streptococcus viridans*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* (6 strains: TY1/EQ11, TY2/EQ22, TY3/EQ14, TY5/EQ15, TY8/EQ23, and TY47/EQ24).

Parents gave children 1 PMBL sublingual tablet per day in the fasting state, on the first 10 days of each month, for 3 consecutive months (according to the Summary of Product Characteristics of Ismigen). The placebo group received a placebo with PMBL-matching shape following the same regimen as the PMBL group.

Randomization and masking

Participants were assigned to the study groups by using simple randomization. The randomization list was generated by pharmacists from the Department of Applied and Social Pharmacy using Random Allocation Software (it was assumed that 70 children will be enrolled in the study). Pharmacists prepared identical packages containing 30 tablets of PMBL or placebo, and then each study drug kit was labeled by them with a unique code according to the randomization list. Patients reporting for a randomization visit and meeting all the inclusion criteria and none of the exclusion criteria received the pack of tablets marked with the next free unique code on the randomization list. Patients and investigators were blinded to allocation. Unblinding was performed in September 2018.

Study protocol

The study protocol included 3 visits, the dates of which were set on the basis of retrospective measurements of grass pollen concentration in the atmospheric air and forecast grass pollen concentrations for south-eastern Poland (data obtained from Allergen Research Center in Warsaw; the time frame of the pollen season was determined using the 95% method) (Figure 1)[46,47]:

1. visit 1 (V1)—a screening/randomization visit; before the start of the grass pollen season (April 22-30, 2018),
2. visit 2 (V2)—in the peak grass pollen season (June 18-22, 2018), and
3. visit 3 (V3)—3 weeks before the end of the grass pollen season (July 16-20, 2018).

From May 1, 2018, parents filled in patient record cards (TNSS, TOSS) and administered either PMBL sublingual tablet or placebo to children. In addition, patients could use oral H1-antihistamine (desloratadine) and intranasal corticosteroid (mometasone furoate) on demand at any time during the study for relief from SAR symptoms. The first-choice medication was oral H1-antihistamine, and in case of lack of improvement, patients could have added intranasal corticosteroid for 10 to 14 days.[16,18]

On each visit (V1, V2, V3), the PNIF was measured and the severity of SAR symptoms was assessed using a VAS. In a subgroup of 38 children (54.3%), serum and nasal lavage fluid were taken on each of the 3 visits for the presence of asIgE against timothy grass pollen allergens, and nasal smears for the presence of eosinophils.

Nasal lavage fluid sample collection. The research material was collected with the use of RinoFlow (Markos-Mefar, Bovezzo, Italy) during 3 visits. Five milliliter of saline heated to body

temperature was introduced each time into the nostrils of the examined patients. The nasal lavage fluid was immediately aspirated back into the RinoFlow container. Approximately 60% of the inhalation fluid was obtained. The obtained lavage fluid was centrifuged for 5 minutes at 3000 revolutions per minute, and then the resulting supernatant was poured and frozen at −80°C. Concentrations of asIgE against timothy grass pollen allergens were assessed by ELISA immunoenzymatic method (EUROIMMUN Medizinische Labordiagnostika AG, Lűbeck, Germany).[48]

Determination of eosinophil counts in nasal smears. Nasal smears were collected from the surface of inferior turbinate by cotton swabs dampened with physiological saline. The collected material was distributed on a microscope slide and left to dry for about 24 hours. Then, the slide with the smear dried was dipped twice in May-Grunwald reagent and then in Giemsa reagent (after each staining, the slide was washed with distilled water). After appropriate staining of the material, the number of eosinophils in the light microscope under immersion (100× magnification) was identified and evaluated. The number of cells in 10 fields of vision was determined, from which the average number of eosinophils in the field of vision was determined.[49,50]

Sample size

For sample size calculation, in the grass pollen season in 2017, we conducted a pilot study with a sample size of 38 (20 in the PMBL group and 18 in the control group). In this study, the mean difference in TNSS change between groups was 2.98.[51] TNSS reduction in the PMBL group in our pilot study was 1.54, higher than the MCID (ie, 0.55).[45] Therefore, the result of our pilot study showed the potential of PMBL treatment and supported the need for the present trial. The SD of the pilot study was adjusted for better application to the true population. To achieve this adjustment, we multiplied the SD from the pilot data by a correction factor of 1.171. This adjustment provides a 90% probability that the resulting estimate of SD will be at least as large as the true population SD.[52] We established a sample size for an independent t test using the adjusted SD, and the difference in TNSS change between the PMBL group and the control group with a power of 85% and an alpha value of 2.5%. The results indicated that the number of subjects in the PMBL group and in the placebo group was 29. The total sample size calculation required 70 patients to be enrolled in anticipation of a dropout rate of 20%.

Statistical analysis

Statistical analysis of the obtained results was performed using SPSS Statistics 25 package (IBM Corp., Armonk, NY). The Mann-Whitney U test was used to check whether there are statistically significant differences between the 2 independent groups. The Wilcoxon test analysis was used to assess the occurrence of significant differences between the 2 study periods. In case of more periods, the Friedman test was used. McNemar test allowed to check whether there were statistically significant differences between the 2 examined periods within the range of the variable measured on the nominal scale. Spearman rank correlation analysis was used to assess the occurrence of a statistically significant relationship between the variables studied. The χ^2 test was used to check whether there was a significant relationship between the nominal variables. All calculated P values were 2-tailed. P value of less than .05 was considered statistically significant.

Weekly average TNSS and TOSS values from the following periods were used for statistical analysis (the average weekly concentrations of grass pollen grains in the atmospheric air are given in parentheses—data obtained from Allergen Research Center in Warsaw) (Figure 2):

1. May 1-7, 2018—T0, beginning of the grass pollen season (15 grains/m^3—possible first symptoms of SAR),
2. May 25-31, 2018—T1 (103 grains/m^3—significant intensification of SAR symptoms),
3. June 18-24, 2018—T2, peak grass pollen season (111 grains/m^3—significant intensification of SAR symptoms),
4. July 14-20, 2018—T3, 3 weeks before the end of the grass pollen season (20 grains/m^3—the symptoms of SAR still persist and may show an intensity comparable to that of the period with a much higher concentration of grass pollen grains—priming effect).[53]

Analyses were performed on the intent-to-treat population, defined as all patients who received at least 1 tablet and had at least 1 postbaseline assessment.

RESULTS

Participant flow

A total of 76 children were enrolled in the study. After excluding 4 children who failed to fulfill inclusion criteria and 2 children whose parents did not agree to participate in the study, 35 children were randomized to receive PMBLs and 35 to receive placebo (Figure 3). There were no significant differences between randomized groups in age, sex, place of residence, and type of sensitizing allergens (Table I).

Primary outcome

In the PMBL group, a statistically significant decrease in TNSS ($P = .001$), TOSS ($P = .04$), VAS score for nasal symptoms ($P < .001$), and VAS score for eye symptoms ($P < .001$) and an increase in PNIF ($P = .04$) values during the grass pollen season were noted, which was not observed in the placebo group (Tables II and III). TNSS reduction in the PMBL group was 1.00; therefore, it was higher than the MCID (ie, 0.55). The increase in PNIF also turned out to be greater than the MCID (ie, 5 L/min) and amounted to 18.54 L/min.

Children taking PMBLs showed much less intensity of nasal symptoms of pollinosis as compared with children receiving placebo; no such observation was recorded in terms of ocular symptoms of pollinosis. The severity of the nasal symptoms of SAR on the TNSS was found to be significantly lower in the PMBL group versus the placebo group at measuring point T2 and T3 ($P < .001$). Children from the PMBL group reached a statistically significantly lower mean severity of nasal symptoms of SAR on the VAS compared with the placebo group on V2 and V3 ($P = .009$ and $P < .001$, respectively). The average PNIF value from V3 turned out to be significantly higher in the PMBL group than in the placebo group ($P = .02$). The compared groups do not show significant differences in the severity of ocular symptoms assessed on the TOSS and VAS at individual measuring points (Figure 4, *A-E*).

Secondary outcome

In both the PMBL and placebo groups, an increase and then a decrease in the average number of eosinophils in nasal smears was observed during the grass pollen season. The analysis of Spearman rank correlation showed a statistically significant relationship between the grass pollen season and the number of eosinophils in the nasal smears (placebo group: $\rho = 0.51$;

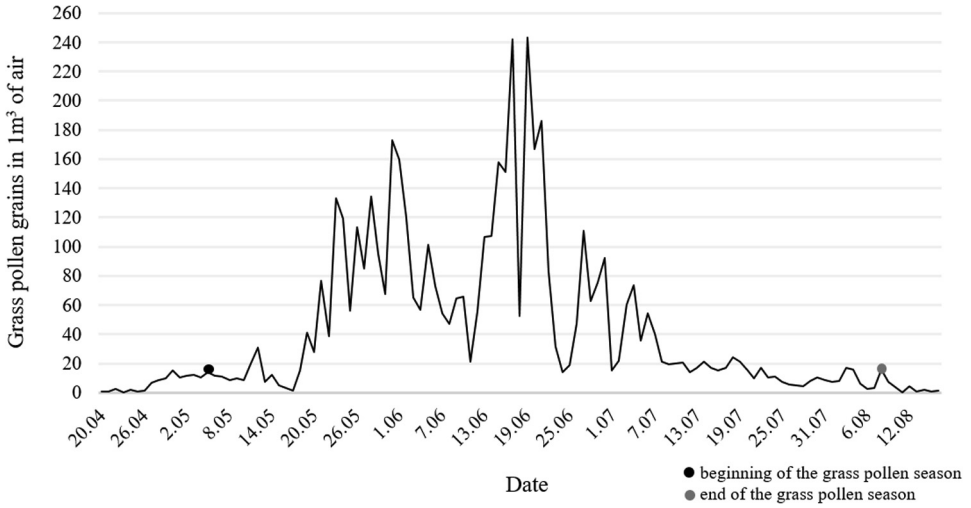

FIGURE 2. Grass pollen concentration in the atmospheric air for south-eastern Poland.

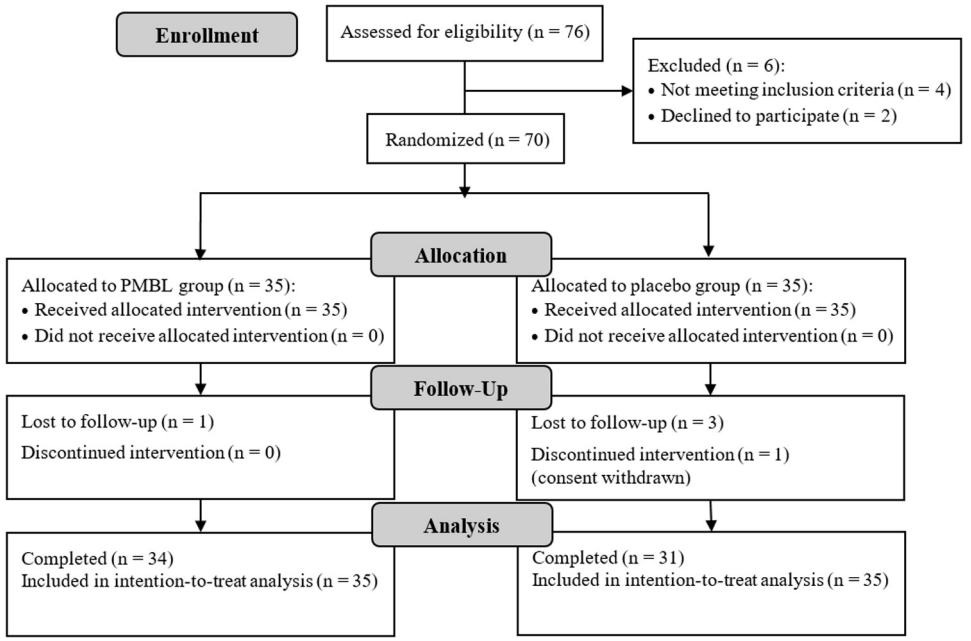

FIGURE 3. Flowchart showing progress of participants through the study (presented in accordance with the Consolidated Standards of Reporting Trials guidelines).

$P < .001$; PMBL group: $\rho = 0.57$; $P < .001$) (Table IV). However, the mean number of eosinophils in nasal smears taken on V2 and V3 was statistically significantly higher in the placebo group versus the PMBL group ($P = .01$ and .02, respectively) (Figure 4, *F*).

In the placebo group, a statistically significant increase in asIgE concentrations against timothy grass pollen allergens was observed in nasal lavage fluid during the grass pollen season ($P = .03$). No significant changes in asIgE concentrations in the PMBL group were demonstrated ($P = .89$) (Table IV). Both groups did not differ in the range of the tested variable at individual measuring points (V1, $P = .09$; V2, $P = .71$; V3, $P = .13$) (Figure 4, *G*).

The mean number of days of use of oral H1-antihistamines and intranasal corticosteroids per patient was respectively 35% and 37% lower in the PMBL group versus the placebo group.

No serious adverse reactions were reported, with comparable and good tolerability for PMBLs and placebo. One patient in the PMBL group reported abdominal pain as an adverse drug reaction. However, its intensity was reported to be mild and did not lead to treatment discontinuation.

DISCUSSION

As mentioned earlier, this study was designed to assess the effect of PMBLs on improvement of the clinical course of SAR caused by grass pollen allergens in children, as well as to gain

TABLE I. Demographic characteristic and types of sensitizing allergens in the study population

Characteristic	PMBL group (n = 35)	Placebo group (n = 35)	*P* value
Sex, n (%)			
Male	20 (57.1)	18 (51.4)	.81
Female	15 (42.9)	17 (48.6)	
Age (y), mean ± SD	9.17 ± 3.63	9.29 ± 3.03	.52
Place of residence, n (%)			
Village	16 (45.7)	19 (54.3)	.63
City	19 (54.3)	16 (45.7)	
Sensitizing allergen, n (%)			
Grasses	35 (100)	35 (100)	—
Cereals	30 (85.7)	28 (80)	.38
Trees	17 (48.6)	16 (45.7)	.5
Weeds	8 (22.9)	6 (17.1)	.38
House-dust mite	23 (65.7)	24 (68.6)	.5
Pet dander	8 (22.9)	6 (17.1)	.38
Molds	3 (8.6)	2 (5.7)	.5

TABLE II. TNSS and TOSS

PMBL group (n = 35)					
Score	**T0**	**T1**	**T2**	**T3**	***P* value**
TNSS	2.63 ± 1.87	3.35 ± 2.5	2.29 ± 1.93	1.63 ± 1.64	.001
TOSS	0.71 ± 1.07	0.95 ± 1.22	0.65 ± 1.14	0.33 ± 0.68	.04
Placebo group (n = 35)					
Score	**T0**	**T1**	**T2**	**T3**	***P* value**
TNSS	2.74 ± 1.74	3.81 ± 1.77	4.36 ± 1.97	3.49 ± 1.72	.09
TOSS	0.69 ± 1.18	0.68 ± 0.86	0.85 ± 0.98	0.59 ± 0.87	.90

T0, May 1-7, 2018 (beginning of the grass pollen season); *T1*, May 25-31, 2018 (high concentration of grass pollen grains in the air); *T2*, June 18-24, 2018 (peak grass pollen season); *T3*, July 14-20, 2018 (3 wk before the end of the grass pollen season).

TABLE III. VAS for nasal and eye symptoms and PINF

PMBL group (n = 35)				
	V1	**V2**	**V3**	***P* value**
nVAS	38.94 ± 20.25	31.97 ± 22.6	17.41 ± 13.93	<.001
eVAS	18.91 ± 21.16	12.63 ± 16.56	5.74 ± 10.75	<.001
PNIF (L/min)	100.14 ± 42.17	111.86 ± 43.6	118.68 ± 40.02	.04
Placebo group (n = 35)				
	V1	**V2**	**V3**	***P* value**
nVAS	34.71 ± 22.44	41.31 ± 17.19	40.1 ± 22.79	.08
eVAS	10.2 ± 10.89	13.06 ± 15.17	13.13 ± 23.51	.57
PNIF (L/min)	104.29 ± 28.85	98.03 ± 27.76	93.42 ± 33.27	.15

eVAS, Visual analogue scale for eye symptoms; *nVAS*, visual analogue scale for nasal symptoms; *V1*, visit 1 (before the beginning of the grass pollen season); *V2*, visit 2 (peak grass pollen season); *V3*, visit 3 (3 wk before the end of the grass pollen season).

insight into the potential mechanisms underlying the effect of PMBLs in this disease. These objectives were achieved by conducting both clinical observations and laboratory measurements. To our knowledge, our study represents the first clinical effort to evaluate the applicability of PMBLs to the treatment of SAR in children.

The primary end point was partly reached. It has been shown that sublingually administered PMBL improves the clinical course of SAR in children sensitized to grass pollen allergens. The PMBL group recorded a reduction in the severity of nasal symptoms of SAR expressed as a decrease in TNSS and VAS score for nasal symptoms and an increase in the PNIF value compared with the placebo group. BLs contributed to the reduction in the severity of symptoms of allergic conjunctivitis; however, the change in TOSS and VAS score for eye symptoms between the measuring points was comparable between the groups. The lack of significant differences between the groups in the severity of ocular symptoms assessed using TOSS may be explained by the insufficiency of the power of the statistical test assessed *post hoc* (50%), which may be related to the small sample

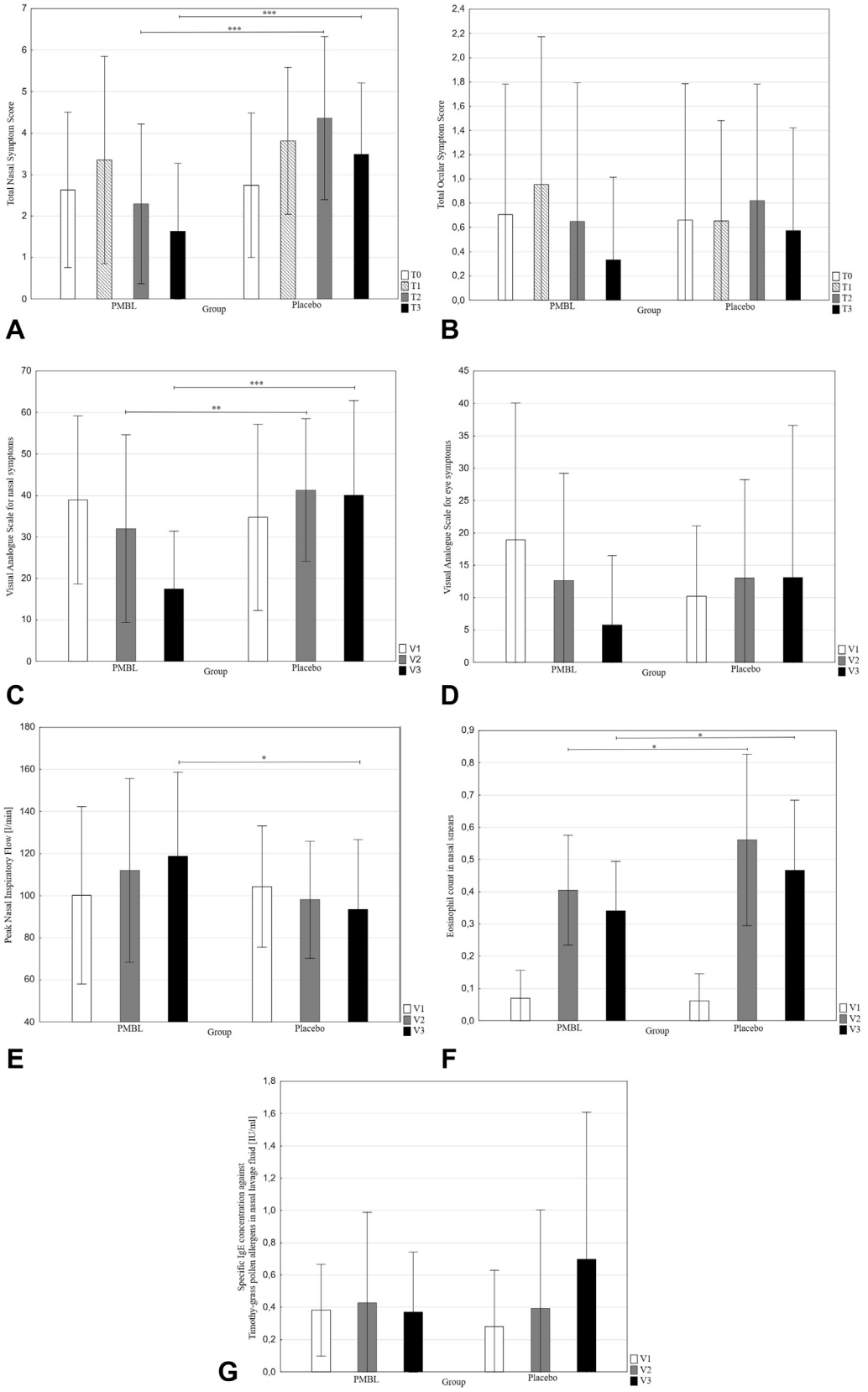

FIGURE 4. (**A**) TNSS. (**B**) TOSS. (**C**) VAS for nasal symptoms. (**D**) VAS for eye symptoms. (**E**) PNIF. (**F**) Eosinophil count in nasal smears. (**G**) Specific IgE concentration against timothy grass pollen allergens in nasal lavage fluid. The error bars represent SD. $*P < .05$; $**P < .01$; $***P < .001$.

TABLE IV. The mean number of eosinophils in nasal smears and average concentration of asIgE against timothy grass pollen allergens in nasal lavage fluid

PMBL group (n = 19)				
	V1	**V2**	**V3**	***P* value**
No. of eosinophils in nasal smears	0.07 ± 0.09	0.41 ± 0.17	0.34 ± 0.15	<.001
Concentration of asIgE against timothy grass pollen allergens in nasal lavage fluid	0.38 ± 0.28	0.43 ± 0.56	0.37 ± 0.37	.89
Placebo group (n = 19)				
	V1	**V2**	**V3**	***P* value**
No. of eosinophils in nasal smears	0.06 ± 0.08	0.56 ± 0.26	0.47 ± 0.22	<.001
Concentration of asIgE against timothy grass pollen allergens in nasal lavage fluid	0.28 ± 0.35	0.39 ± 0.61	0.7 ± 0.91	.03

V1, Visit 1 (before the beginning of the grass pollen season); *V2*, visit 2 (peak grass pollen season); *V3*, visit 3 (3 wk before the end of the grass pollen season).

size. The presented data correspond to the results obtained by the authors of this publication in 2017, when during the grass pollen season a randomized, open-label study was conducted to assess the influence of PMBL therapy on the clinical course of SAR in children.[51] In the group of children receiving PMBLs, a decrease in the severity of nasal symptoms of SAR in the second half of the grass pollen season was achieved in comparison to the group of children not receiving PMBLs. Concurrently, PMBLs have not been shown to reduce the severity of symptoms of allergic conjunctivitis. Apart from our initial report from 2017, 2 more studies are available that assessed the benefits of using BLs in AR therapy.[36,41] The first evaluated the efficacy of treatment with PMBL in the prophylaxis of AR in 41 adult patients.[36] The researchers showed that BLs were effective in the reduction or in the elimination of AR symptoms in comparison to a nonimmunostimulating treatment. The second study, which recently appeared, provides evidence of the benefits of polyvalent chemical bacterial lysate (PCBL) in perennial allergic rhinitis (PAR) therapy in adult patients.[41] Meng et al[41] have shown that PCBL contributes to the reduction in nasal PAR symptoms expressed as a reduction in TNSS value and stress that oral administration of PCBL offers remarkable and sustained efficacy in alleviating AR symptoms and may be considered as an alternative therapeutic strategy for patients with PAR. Both studies are mainly concerned with adults with PAR, and therefore our studies represent the first clinical observation on the therapeutic effectiveness of PMBLs in the treatment of SAR in children.

In more than 90% of cases, AR is an IgE-mediated inflammatory reaction of the nasal mucosa associated with environmental allergens involving many inflammatory cells (eosinophils, neutrophils, mast cells, lymphocytes) accumulated in the nasal mucosa and the submucosal layer.[1-4] The inflammatory process within the nose is believed to be associated with T_H2 lymphocytes, more specifically with a decrease in the T_H1/T_H2 ratio of nasal mucosa. T_H2 cells initiate an allergic inflammatory process by releasing numerous cytokines, among others: IL-3, IL-4, IL-5, IL-9, and IL-13. IL-4 and IL-13 contribute to the production of asIgE by B lymphocytes, whereas IL-3 and IL-5 contribute to nasal infiltration by eosinophils.[54,55] Therefore, in the presented study, asIgE concentration in nasal lavage fluid and eosinophil counts in nasal smear samples were assessed, which indirectly provides information on T_H2-type cytokine concentrations. During the grass pollen season, an increase and then a decrease in the number of eosinophils in nasal smears was observed in both groups; however, the number of eosinophils was significantly lower in the PMBL group compared with the placebo group. In the group receiving BLs, no significant changes were found in asIgE concentrations against timothy grass pollen allergens in nasal lavage fluid and the asIgE concentration remained at a comparable level throughout the grass pollen season. In the placebo group, however, a statistically significant increase in asIgE concentration in nasal lavage fluid was obtained during the grass pollen season. These observations indicate PMBLs' ability to inhibit local growth of eosinophil count and asIgE concentration, which is indirect evidence of the suppression of T_H2-type inflammation in nasal mucosa (inhibition of the secretion of T_H2-type cytokines). Therefore, it can be assumed that the mechanism of action of PMBLs in children with SAR is associated with the weakening of the dominant T_H2-cell response and thus with normalization and maintenance of T_H1/T_H2 balance. Similar observations are provided by the study of Meng et al,[41] who demonstrated that PCBL influences the increase in the concentration of T_H1-type cytokines (IFN-γ), the decrease in the concentration of T_H2-type cytokines (IL-4 and IL-13) in nasal lavage fluid, and the reduction in the number of eosinophils in nasal smears. In Banche et al,[36] stimulation with BLs in patients with AR resulted in a decrease in the concentration of IL-4 and an increase in the concentration of IFN-γ in the blood serum. The evidence for the above is also provided by the study conducted by Liu et al,[56] who showed that PCBL shortens the duration of bronchiolitis in infants and strengthens the immune function of T_H1 lymphocytes (increase in IFN-γ) and weakens the function of T_H2 lymphocytes (decrease in IL-4 concentration). This was also confirmed in a mouse model of IgE-dependent asthma, where BL therapy reduced T_H2 response (IL-4), decreased the number of eosinophils, and increased the T_H1 (IFN-γ) level in bronchoalveolar lavage fluid and thus weakened the inflammatory process in airways.[27] Based on the available literature, it can be assumed that the above-described immunologic effects of BLs result from their ability to stimulate immune mechanisms through Toll-like receptors.[24,34,57]

The conducted analysis allowed to establish the beginning of the noticeable effects associated with PMBL therapy. The compared groups differed in terms of the severity of nasal AR symptoms assessed according to the TNSS at measurement points T2 and T3. Thus, it can be assumed that the first effects of PMBL are noticeable approximately 4 to 6 weeks after the initiation of therapy. Similar conclusions were made by Banche et al,[36] who observed a significant reduction in the severity of AR symptoms 2 to 3 weeks at the earliest after starting PMBL therapy. Explanations for such a rapid effect of BLs can be found in the study of Lanzilli et al.[58] Researchers obtained peripheral blood from healthy human donors and then isolated

mononuclear cells from it, which they incubated with PMBLs, observing significant changes in T_H1-type and T_H2-type cytokine concentrations after 24 hours.[58] However, eosinophils and other cells involved in the allergic inflammatory process that are already present in the nasal mucosa may persist for several weeks.[59,60]

For the past few years, there has been increasing talk about the role of the respiratory mucosa in the pathophysiology of allergic diseases. BLs show an immunoregulatory effect on mucosal immunity and thus become a topic of interest for many researchers who report the benefits of lysates in the treatment of bronchitis and asthma.[33,42] It should be remembered that systemic immunity and mucosal immunity in AR are 2 independent processes.[61] It is assumed that the effects of BLs in AR are related to their influence on mucosal immunity, which is confirmed by the results of our study, because inhibition of eosinophil count and asIgE concentration was found to occur only in nasal mucosal immunity, but not in systemic immunity, according to the serum eosinophil count and asIgE concentration.

Our study confirmed the clinical effectiveness of PMBL in SAR therapy in children sensitized to grass pollen allergens, but it also has some limitations. First, a possible limitation of our study might be the small sample size (in relation to ocular symptoms), and so to confirm our findings it is necessary to conduct studies on a larger group of children. Second, the mechanism of action of PMBLs in SAR is probably more complex and involves many signaling pathways, and so determining the basis for the beneficial effect of PMBLs in SAR therapy requires further studies with the evaluation of many other immunologic parameters.

CONCLUSIONS

Sublingual administration of PMBLs during the grass pollen season offers significant efficacy in alleviating SAR symptoms in children sensitized to grass pollen allergens. PMBLs probably affect mucosal immunity, weakening the response of T_H2 cells and thus restoring T_H1/T_H2 balance.

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