Changes in Sputum Microbiocenosis and Clinical Pattern Under Different Vaccination Protocols for Pneumococcal Infection in Patients with Bronchial Asthma

A.D. Protasov[1,*], M.P. Kostinov[2,3], A.V. Zhestkov[1], D.O. Gorbachev[1], A.M. Kostinov[4], M.E. Elner[1] and T.A. Kozina[1]

[1]*Samara State Medical University, 443099, Samara region, Samara, 89 Chapaevskaya str.*

[2]*Mechnikov Moscow Research Institute for Vaccines and Sera, 105064, Moscow, 5a Maly Kazenny Lane*

[3]*Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University), 119991, Moscow, 8 bld. 2 Trubetskaya str.,*

[4]*Lomonosov Moscow State University 119991, Russian Federation, Moscow, 1 Leninskiye Gory*

Abstract: Bronchial asthma is a serious public health issue. It is important to reduce the frequency of attacks for BA patients. Vaccination for pneumococcal infection is an important complementary tool that can improve the clinical pattern of bronchial asthma. The study involved 102 patients with bronchial asthma, who were monitored over 4 years. The patients were divided into 4 groups based on the vaccination scheme used for pneumococcal infection: PCV13, PPV23, PPV23/PCV13, PCV13/PPV23. Sequential vaccination with pneumococcal conjugate and polysaccharide vaccines leads to a decrease in the isolation of pneumococcus from sputum in BA patients, which has a pronounced positive effect on the clinical pattern of this disease, namely, it leads to a decrease in the frequency of attacks, the requirement for courses of antibacterial chemotherapy and hospitalizations, and therefore this vaccination protocol should be included in the standards of BA patient management.

Keywords: Bronchial asthma, Pneumococcal vaccination, PCV13, PPV23, Microbiological Examination of Sputum.

INTRODUCTION

Bronchial asthma (BA) is a common chronic bronchopulmonary disease, which, with insufficiently effective treatment, can significantly worsen the health-related quality of life and may lead to death. Currently, the prevalence of BA among the population is increasing everywhere (Chuchalin A.G., Avdeev S.N., Aisanov Z.R., Arkhipov V.V., Belevsky A.S., Nenasheva N.M., 2015; Protasov A.D., 2013; Protasov A.D., 2014; Edris, A., 2022; GINA, 2022).

Bacterial allergens, including pneumococcal, can act as a sensitizing agent in patients with asthma, along with sensitization to the most etiologically significant allergens (pollen, indoor, epidermal, fungal, etc.). On the one hand, microorganisms sensitize the body of a BA patient, and on the other, bacterial infection caused, among other things, by *S. pneumoniae* may lead to the development of chronic inflammation of the bronchi, increasing their hyperreactivity (Lukachev I.V., Kostinov M.P., Shabalina S.V., 2004; Pelton S.I., Weycker D., Farkouh R.A., Strutton D.R., Shea K.M., Edelsber J., 2014; Patel, G. B., 2019).

High prevalence of BA in the world and the social and economic burden of this disease, create a challenge for the medical community to develop new methods of therapy in addition to basic treatment. One of these methods is trying to influence sputum microbiocenosis in BA patients via vaccination for pneumococcal infection (Kostinov MP, Protasov AD, Zhestkov AV, Yastrebova NE, Kostinov AM *et al*, 2021).

Vaccination directly and positively affects the activity of the epidemic process in cases of pneumococcal infection. Until recently, only one polysaccharide vaccine was used for vaccination associated with this disease. Currently, in order to vaccinate adults for pneumococcal infection, 2 vaccine preparations are available in the Russian Federation, as well as worldwide: 23-valent polysaccharide pneumococcal vaccine (PPV23) and 13-valent conjugated polysaccharide pneumococcal vaccine (PCV13). It should be noted that these vaccines are used mainly for the prevention of pneumococcal infection, while their therapeutic properties remain poorly understood (Vaccination and emergency

*Address correspondence to this author at the Department of General and Clinical Microbiology, Immunology and Allergology, Head of the section for International Cooperation and Education of the Department of International Relations, Samara, Russian Federation; Tel: +79277444126;
E-mail: crosss82@mail.ru

immunoprophylaxis of infectious diseases in pregnant and lactating women: a guide for doctors / under. ed. M.P. Kostinova, L.V. Adamyan, A.P. Cherdantseva, N.A. Ozeretskovsky (ed. 1st), 2022).

The Global Strategy for the Treatment and Prevention of Bronchial Asthma (GINA, 2018) states that there is insufficient data to justify routine vaccination of BA patients for pneumococcal infection.

The use of pneumococcal vaccines can reduce the spread of antibiotic-resistant strains of pneumococcus resulting from uncontrolled use of antibiotics (WHO updates, 2017). The idea of using preventive vaccination in the fight against antibiotic resistance is also described in the Decree of the Government of the Russian Federation dated 25 September, 2017 N 2045-p "Strategy for preventing the spread of antimicrobial resistance in the Russian Federation for the period up to 2030".

Therefore, the issue of the optimal protocol for the use of pneumococcal vaccines in BA patients remains unresolved. There are also no studies involving a comprehensive assessment of clinical and microbiological indicators, there is no evidence base characterizing the advantages of a certain vaccination protocol for pneumococcal infection in BA patients with separate or sequential administration of polysaccharide and conjugated polysaccharide vaccines. Taking into account all of the above, there is a need to develop an optimal vaccination protocol for pneumococcal infection in BA patients, taking into account changes in microbiological and clinical indicators.

MATERIALS AND METHODS

The study was conducted from September 2012 to September 2016 at the Department of General and Clinical Microbiology, Immunology and Allergology of the Samara State Medical University (Rector — Professor of the Russian Academy of Sciences, MD, Professor A.V. Kolsanov), as well as at the Mechnikov Moscow Research Institute for Vaccines and Sera (Director — Corresponding Member of the Russian Academy of Sciences, MD. O.A. Svitich). The study was prospective, multicenter, national, randomized.

The study is registered on the international website *ClinicalTrials.gov* (Clinical and Immunological Efficacy of Bacterial Vaccines at Adult Patients With Bronchopulmonary Pathology, registration number NCT02787863, US National Institutes of Health).

102 patients who met the certain inclusion/exclusion criteria were examined and followed up for 4 years.

Criteria for inclusion in the study group:

- persons of both sexes aged between 18 and 80 years with an established diagnosis of BA of any severity;
- rejection of flu vaccination for the next 4 years;
- signed and dated form proving informed consent to participate in the clinical trial;
- ability to comply with protocol requirements;
- for women of childbearing age, a negative pregnancy test result before vaccination (human chorionic gonadotropin hormone).

Exclusion criteria:

- age under 18 and over 80;
- a history of vaccination for pneumococcal infection;
- planned flu vaccination over the next 4 years;
- the use of immunoglobulin preparations or blood transfusion over the three months preceding the start of the clinical trial;
- long-term use (over 14 days) of immunosuppressive drugs 6 months prior to the start of the trial;
- any confirmed or suspected immunodeficiency condition;
- the presence of respiratory distress, cardiovascular insufficiency, liver and kidney dysfunction, established during physical examination during visit No. 1;
- severe birth defects or serious chronic diseases in the acute stage, including any clinically important acute stages of chronic diseases of the lungs, liver, kidneys, cardiovascular, nervous systems, mental diseases or metabolic disorders, confirmed by the patient history or via objective examination;
- a history of severe allergic reactions, autoimmune diseases;
- acute infectious and/or non-communicable diseases during the month preceding the start of the study;

- a history of chronic alcohol abuse and/or drug use;
- breast-feeding;
- pregnancy;
- participation in another clinical trial over three months prior to the trial;
- a history or presence of oncohematological and other oncological diseases;
- HIV positive status, viral hepatitis B and C.

For the study, all patients were divided into 4 groups:

I gr. – BA patients (33 people, age 52.5 (15.3) years [19; 55; 78], 11 men), PCV13 vaccination;

II gr. – BA patients (25 people, median age 53 years, interquartile range [45.5; 60], 6 men), PPV23 vaccination;

III gr. – BA patients (18 people, age 52.5 years [34; 59], 6 men), vaccination with PPV23, and after 12 months – PCV13 (PPV23/PCV13);

IV gr. – BA patients (27 people, age 51 years [35,8; 64], 9 men), PCV13 vaccination, and after 2 months — PPV23 (PCV13 /PPV23).

The purpose of the trial was thoroughly explained to all patients, and they signed the required informed consent form.

Medical histories of the patients were rigorously scrutinized. To verify the diagnosis of BA, a test of external respiration function and a standard bronchodilator test (400 mcg of salbutamol) were conducted for all patients. Patients who met the inclusion/exclusion criteria received the vaccine corresponding to the randomization protocol (sequential randomization) on an outpatient basis. Patients were assigned one after another first to the PCV13 group, and then to the PPV23, PPV23/PCV13 and PCV13/PPV23 groups.

Of all the patients included in the study, 102 completed the study in accordance with the protocol. One participant could not be observed further due to withdrawal of informed consent. For this reason, the data of this patient were not included in the analysis.

The time points of data collection in the study are shown in Table **1**.

General characteristics of the studied patients by age and severity of the disease are presented in Table **2**.

Tables **3**-**4** show the comparability of patients according to the basic therapy received for the main disorder.

Table 1: Study Schedule (Data Collection Time Points)

Indicator	1st visit (screening, vaccination)	2nd visit (6 months)	3rd visit (12 months)	4th visit (48 months)
Signing of informed consent	+			
Assessment of compliance with inclusion/exclusion criteria	+			
Collection of demographic and medical data of patients	+			
Clinical examination, registration of objective symptoms of the disease	+	+	+	+
Registration of blood pressure, respiration rate and heart rate	+	+	+	+
The number of patients with no BA attacks, the average number of attacks per 1 patient	+		+	+
The number of patients who did not take ABH, the average number of ABH courses per 1 patient	+		+	+
Number of patients who didn't require hospitalizations, average number of hospitalizations per 1 patient	+		+	+
Sputum	+	+	+	+

Table 2: Characteristics of Patients with BA by Age and Severity of the Disease (Groups I, II, III, IV)

Severity of BA	I gr. (PCV13)			II gr. (PPV23)			III gr. (PPV23/PCV13)			IV gr. (PCV13/PPV23)		
	Age, years	Total		Age, years	Total		Age, years	Total		Age, years	Total	
		abs.	%		abs.	%		abs	%		abs.	%
Light intermittent pattern	49,3 (14,9) [37;45;66]	3	9	20	1	4	21	1	6	-	-	-
Mild persistent pattern	53,7 (21,3) [19;61,5;78]	10	30	56,1 (8,8) [37;54;67]	11	44	54 [54; 65]	6	33	47,9 (16,1) [22;51;70]	18	67
Moderate severity	53,6 (14) [22;58;73]	14	43	46,3 (12,7) [27;48,5;64]	8	32	48,4 (12,6) [28;51;65]	10	55	40,8 (16,3) [24;40,5;58]	4	15
Severe pattern	49,5 (7,9) [40;49;61]	6	18	44,8 (13) [27;52;57]	5	20	28	1	6	57,2 (10,8) [44;64;66]	5	18
Total	52,5 (15,3) [19;55;78]	33	100	53 [45,5; 60]	25	100	52,5 [34; 59]	18	100	51 [35,8; 64]	27	100

Note: the data of normally distributed variables are presented as the mean (standard deviation (CO)) [min; median; max]. Values with a distribution other than normal are represented as the median [interquartile range].

The analysis of Tables **3**-**4** indicates the initial comparability of the studied groups of BA patients according to the basic therapy received for the underlying disease. Throughout the study, the basic therapy for the underlying disease in the study groups did not fundamentally change.

The following drugs were used for vaccination:

- 23-valent pneumococcal polysaccharide polyvalent vaccine (PPV23, series J0186-5, Sanofi Pasteur, France), which contains purified capsular polysaccharides of *S. pneumoniae* 23 serotypes. One

Table 3: Characteristics of Broncholytic and Anti-Inflammatory Therapy in BA Patients from Groups I (n=33, PCV13) and II (n=25, PPV23)

Medication group	PCV13 (n=33)		PPV23 (n=25)		P, FET
	Abs.	%	Abs.	%	
Short- or long-acting bronchodilator (β_2-agonist, M-cholinolytic)	19	58	16	64	0.7777
Combined bronchodilator	19	58	9	36	0.0861
Inhalated glucocorticosteroids (IGCS)	13	39	6	24	0.937
Combination therapy (IGCS + long-acting bronchodilator)	14	42	14	56	0.224
Antileukotriene drugs	0	0	1	4	0.431

P is the value of.
FET - Fisher's exact test.

Table 4: Characteristics of Broncholytic and Anti-Inflammatory Therapy for BA Patients from Groups III (n=18, PPV23/PCV13) and Group IV (n=27, PCV13/PPV23)

Medication group	PPV23/PCV13 (n=18)		PCV13/PPV23 (n=27)		P, FET
	Abs.	%	Abs.	%	
Short- or long-acting bronchodilator (β_2-agonist, M-cholinolytic)	14	78	14	52	0.0731
Combined bronchodilator	4	22	13	48	0.0731
Inhalated glucocorticosteroids (IGCS)	5	28	15	56	0.062
Combination therapy (IGCS + long-acting bronchodilator)	9	50	6	22	0.0538
Antileukotriene drugs	1	6	1	4	0.4909

P is the value of.
FET - Fisher's exact test.

dose of the vaccine is 0.5 ml. The vaccine was administered I.M. once.

- 13-valent pneumococcal conjugated polysaccharide adsorbed vaccine (PCV13, F96122 series, Pfizer, USA), contains capsular polysaccharides of 13 serotypes of *S. pneumoniae,* individually conjugated with diphtheria protein CRM_{197} and adsorbed on aluminum phosphate. One dose of the vaccine is 0.5 ml. The vaccine was administered I.M. once.

Microbiological Examination of Sputum

The study used the classical microbiological method used at the Department of General and Clinical Microbiology, Immunology and Allergology of the Mechnikov Moscow Research Institute of Vaccines and Sera.

Primary material was collected in accordance with the requirements of the Order of the Ministry of Health of the USSR No. 535 dated 22 April, 1985. Sputum was collected into disposable sterile containers in compliance with the rules of aseptics and was delivered to the microbiological laboratory within 30 minutes. No more than 60 minutes passed from the moment of material collection to the plating on the nutrient medium.

Before the procedure, the oral cavity was cleaned (via brushing of teeth and thorough rinsing with boiled water). Sputum was collected before meals. Patients were instructed about the need to expectorate the contents of the lower respiratory tract, not the oronasopharynx [Dobrikh V.A., 2013]. Whenever possible, sputum was collected under the supervision of a doctor.

Before the start of the microbiological study, Gram staining of the smear was performed in order to assess the "representativeness" of the sputum sample for further study. If there were less than 25 leukocytes and more than 10 epithelial cells in the smear while viewing at least 8-10 visual fields with low magnification, subsequent sputum inoculation was not performed, since in this case the studied material was most likely the contents of the oral cavity, and the diagnostic significance of the study would be extremely low [Palange P., Simonds A.K., 2013].

Isolation and identification of microorganisms were carried out based on the standard method [Hof H., Dorries R., 2009; Satzke C., Turner P., 2013].

To isolate and identify pneumococcus, the test material was inoculated on agar with the addition of 5% defibrinated human blood. A petri dish with the seeding was incubated at 37 ^{0}C for 24 hours in an atmosphere with a high content of CO_2. To create an increased concentration of CO_2, a desiccator was used, in which a lighted candle was placed, which used up the oxygen while burning. When the candle goes out, the concentration of CO_2 reaches 3% [Krechikova O.I., 2000].

Identification of pneumococci was performed on the basis of morphological growth and phenotype characteristics. Pneumococci, when growing on blood agar (BA), may produce several types of colonies, which depends on how pronounced the capsule is. Colonies with a highly developed capsule, such as serotype 3, may be several millimeters in diameter and be so slimy that they resemble a drop of oil on the surface of agar. Their identification does not pose any significant problems. Colonies of strains with a less pronounced capsule are small in size, and their isolation is associated with certain difficulties.

To identify pneumococcus, characteristic morphological growth characteristics were sought — a grayish hue of the colonies, a convex surface and a moist "creamy" consistency.

Further identification of *S. pneumoniae* was carried out via standard phenotypic methods, the main ones are sensitivity to optochine and lysis in the presence of bile salts.

The method for determining sensitivity to optochin is based on the ability of optochin (ethylhydrocuprein hydrochloride) to selectively suppress the growth of pneumococcus, unlike other alpha-hemolytic streptococci. For this method, agar with the addition of 5% defibrinated blood and discs containing 5 micrograms of optochin (optochin test, "bioMerieux") were used.

1 colony of alpha-hemolytic streptococcus, suspected of pneumococcus morphology, was streaked on the blood agar sector. Then the disk with optochine was placed on the surface and incubation was performed for 18-24 hours at a temperature of 35 ° C in an atmosphere with 5-7% CO_2. Inhibition zone > 14 mm (disk with a diameter of 6 mm) or > 16 mm (disk with a diameter of 10 mm) indicates the presence of *S. pneumoniae*. The inhibition zone < 14 mm (< 16 mm) means a confirmation is required that the test culture is indeed *S. pneumoniae* via the lysis test in the presence of bile salts.

Bile salts (especially sodium deoxycholate and sodium taurocholate) have the ability to selectively lyse *S. pneumoniae* colonies on agar or in bouillon The method is based on the activation of pneumococcal autolysins — enzymes involved in cell wall lysis. Bile salts activate autolysins of most strains, which leads to visual lysis of *S. pneumoniae* within 0.5–2 hours.

To perform the lysis test in the presence of bile salts, a suspension of the strain under study was prepared in 1-2 ml of sterile distilled water (or 0.9% sodium chloride solution) to achieve McFarland standard turbidity 1. Half of the resulting suspension was transferred to another tube equal in diameter.

3-4 drops of 10% sodium deoxycholate solution were added to the test tube labeled "Test", and 3-4 drops of 0.9% sodium chloride solution were added to the test tube labeled "Control". The test tubes were thoroughly shaken and incubated for 0.5–2 hours at a temperature of 35 ° C, after which the turbidity of the microbial suspension in 2 test tubes was visually compared. Clearing of the liquid in the Test tube in comparison with the Control tube indicates that the culture belongs to *S. pneumoniae* [Ford M., 2010].

Cultivation of *Hemophilus influenzae* requires the presence of X and/or V growth factors in nutrient media. X factor is a group of thermally stable tetrapyrrol compounds that are part of iron-containing pigments (for example, hemin, hematin). Species requiring the X factor are unable to synthesize protoporphyrin from δ-aminolevulinic acid, which is used for one of the identification tests.

Most types of *Hemophilus influenzae* also need a thermolabile V factor – nicotinamide adenine dinucleotide (NAD, co-enzyme I) or nicotinamide adenine dinucleotide phosphate (NADP, co-enzyme II), which participates in redox reactions.

X and V factors are present in blood. However, in native sheep and human blood there are enzymes (NADases) that destroy the V factor. Therefore, V-dependent types of Hemophili do not grow well or not at all on blood agar (BA) prepared on the basis of sheep or human blood.

To identify *H. influenzae*, we used chocolate agar, which was prepared by adding blood to an enriched agar base at a temperature of about 80 ° C in order to destroy red blood cells and release X and V factors. Commercial discs with bacitracin (10 units) were used to selectively isolate Hemophili from clinical material. Naturally resistant to bacitracin Hemophili will grow around the disc. The seeding dishes were incubated in an exsiccator with a lit candle at a temperature of 37 ° C for 18-24 hours [Bogdanovich T.M., 2000].

To isolate and identify *M. catarrhalis*, sputum inoculation was performed on blood and chocolate agar and incubated at 37 ° C in an atmosphere with a high carbon dioxide content for 24 hours. Identification was carried out using commercial biochemical panels (Gonochek-II, quardFERM+) via generally accepted tests for key characteristics: absence of hemolysis, presence of "hockey-puck effect" (gliding motility), absence of agar corrosion, oxidase- and catalase-positivity, acid not formed from carbohydrates in aerobic (oxidation) and anaerobic (fermentation) conditions, nitrates are restored.

Bacteria of the genus *Staphylococus spp.* were identified using standard microscopic and microbiological methods of laboratory diagnostics without subsequent identification of the species.

Differentiation of gram-negative bacteria into microorganisms of the *Enterobacteriaceae* family and non-fermenting gram-negative bacteria was carried out using the Hugh-Leifson medium. Further identification of the species of microorganisms of the *Enterobacteriaceae* family and non-fermenting gram-negative bacteria was carried out using appropriate paper indicator systems (Microgen, Russia) in accordance with the instructions attached to the kits.

Determination of the Clinical Efficiency of Vaccination

The clinical efficiency of vaccination against pneumococcal infection in BA patients was evaluated according to the following criteria:

1. The number of patients who experienced attacks, as well as the average number of BA attacks per 1 patient over one year before vaccination and during the first and fourth years after vaccination.

2. The number of patients who took antibacterial chemotherapy drugs (ABH), as well as the average number of ABH courses per 1 patient one year before vaccination and during the first and fourth years after immunization.

3. The number of patients hospitalized with BA attacks, as well as the average number of hospitalizations per 1 patient during the year

before vaccination and during the first and fourth years after immunization.

The BA attack was understood as the appearance or intensification of chest wheezing, feeling of chest compression, which required medical help and modification of the therapy, which was evident from the patient records.

The study took into account the number of ABH courses taken by patients the year before vaccination and during the first and fourth years after vaccination due to BA attacks, as well as for any other reasons unrelated to BA. This information was also confirmed by the patient records.

Hospitalization is understood as admission of the trial participant to a hospital for BA treatment, both planned and emergency. Confirmation of hospitalization was an extract from the medical history, or an entry in the outpatient card. In the course of the trial, information was collected on all hospitalizations for BA during the year before vaccination and during the first and fourth years after vaccination.

STATISTICAL ANALYSIS

The description of quantitative characteristics corresponding to the normal distribution is presented in the form of the mean value (standard deviation) [min; median; max]; characteristics that differ from the normal distribution — in the form of a median [interquartile range]. Qualitative characteristics are presented as % and absolute numbers.

The choice of the statistical evaluation criterion depends on the type of data distribution and the fulfillment of the condition of equality of variances. The hypothesis of the normality of the data distribution was tested (Shapiro-Wilk test). If the data of each sample are distributed normally, a comparison is made for the equality of variances (Levene's test). If both conditions are met, the Student's t-test is applied to independent samples, if not, its nonparametric alternative (the Mann-Whitney U test) is applied. The same applies to paired criteria when comparing characteristics in dynamics (Student's paired t-test or Wilcoxon's test for paired samples). Comparative analysis of qualitative variables was carried out using Fisher's exact test for independent samples and McNemar's test for dependent samples. The differences were considered statistically significant at $p < 0,05$.

Statistical processing of the results was carried out using specialized licensed software StatPlus Pro 6.2.0.0 (license No. 2883) using appropriate methods. The percentage of decrease in the value was calculated using the following formula: (final value – initial value)/initial value × 100 % (Lang T.A., Sesik M., 2011).

RESULTS

The clinical effect of vaccination for pneumococcal infection was evaluated separately for BA patients in accordance with the distributed groups. Table **5** shows the number (%) of patients that didn't experience BA attacks, didn't take ABH and were not hospitalized during the year before vaccination, as well as in the 1st and 4th years after vaccination for pneumococcal infection of the study groups.

Analysis of the data in Table **5** indicates that 1 year after vaccination, the number of patients who didn't experience BA attacks was significantly higher than the

Table 5: Dynamics of the Clinical Pattern of BA in Various Vaccination Protocols

Preparation and administration protocol	Number of patients in the group	Number of patients who didn't experience attacks (%)			Number of patients who did not take ABH (%)			Number of patients that didn't require hospitalization (%)		
		before vaccination	during the 1st year	during the 4th year	before vaccination	during the 1st year	during the 4th year	before vaccination	during the 1st year	during the 4th year
		With BA								
PCV13	33	5 (15,2)	28 (84,8)	11 (33,3)	12 (36,4)	29 (87,9)	21 (63,6)	18 (54,5)	32 (96,9)***	27 (81,8)*
PPV23	25	0 (0)	**14 (56)*** **	4 (16)	6 (24)	16 (64)	8 (32)	15 (60)	24 (96)***	16 (64)
PPV23/PCV13	18	1 (5,6)	**7 (38,8)****	5 (27,8)	4 (22,2)	9 (50)	7 (38,9)	11 (61,1)	17 (94,4)**	14 (77,8)
PCV13/PPV23	27	2 (7,4)	**20 (74,1)****	**13 (48,1)****	7 (25,9)	16 (59,3)	18 (66,7)	20 (74,1)	21 (77,8)	26 (96,3)

Note: * - $p<0.05$ compared to the value before vaccination in the corresponding group.
** - $p<0.01$ compared to the value before vaccination in the corresponding group.
*** - $p<0.001$ compared to the indicator before vaccination in the corresponding group.

initial values for vaccination protocols PPV23 (56%, p < 0.001), PPV23/PCV13 (38.8%, p < 0.01) and PCV13/PPV23 (74.1%, p < 0.01). 4 years after vaccination, only in the PCV13/PPV23 group there was a significant increase in the number of patients who didn't experience BA attacks compared to the initial value, amounting to 48.1% (p < 0.01). There were no significant differences in the number of patients with BA who did not take ABH in the first and fourth years after vaccination compared to the initial values. After 1 year for BA patients, all vaccination protocols, except PCV13/PPV23, led to a significant increase in the number of patients who didn't require hospitalization, after 4 years, only in the PCV13 group, a significant increase in the number of BA patients who didn't require hospitalization was noted compared to the initial value (81.8% vs. 54.5%, p < 0.05).

Table **6** shows the results of the average number of attacks, ABH courses and hospitalizations per 1 patient initially, during the 1st and 4th years, depending on the vaccination protocol applied to BA patients.

RESULTS OF BACTERIOLOGICAL EXAMINATION OF SPUTUM

We studied the effect of vaccination for pneumococcal infection on the microbiocenosis of sputum in BA patients. Statistical processing of the results of microbiological examination of sputum of BA patients was carried out taking into account the data of all patients, including those who did not experience sputum expectoration. Table **7** shows the data of microbiological examination of sputum of BA patients initially, after 1 and after 4 years.

Table 6: The Average Number of Attacks, ABH Courses and Hospitalizations per 1 Patient Initially, during the 1st and 4th Years, Depending on the Vaccination Protocol Applied to Patients with BA

Vaccination protocol (n= number of patients in the group)	Average number of attacks per 1 patient with BA (total attacks)			Average number of ABH courses per 1 BA patient (total ABH courses)			Average number of hospitalizations per 1 BA patient (total hospitalizations)		
	Before vaccination	1st year	4th year	Before vaccination	1st year	4th year	Before vaccination	1st year	4th year
PCV13 (n=33)	2,24 (74)	0,24 (8)	0,85 (28)	1,12 (37)	0,12 (4)	0,69 (23)	0,73 (33)	0,03 (1)	0,3 (10)
PPV23 (n=25)	2,68 (67)	0,64 (16)	1,2 (30)	1,68 (42)	0,56 (14)	1,44 (36)	0,56 (14)	0,04 (1)	0,56 (14)
PPV23/PCV13 (n=18)	2,39 (43)	1,22 (22)	1 (18)	1,72 (31)	1,06 (19)	1,06 (19)	0,56 (10)	0,06 (1)	0,44 (8)
PCV13/PPV23 (n=27)	1,89 (51)	0,44 (12)	0,56 (15)	1,44 (39)	0,48 (13)	0,56 (15)	0,48 (13)	0,26 (7)	0,04 (1)

Table 7: Results of Microbiological Examination of Sputum of BA Patients in the Analyzed Groups Initially, 1 and 4 Years after Vaccination

Causative Agent	Analyzed Group (all participants)											
	PCV13 (n=33)			PPV23 (n=25)			PPV23/PCV13 (n=18)			PCV13/PPV23 (n=27)		
S. pneumoniae, n (%)	initially	1 year	4 years	initially	1 year	4 years	initially	1 year	4 years	initially	1 year	4 years
	12 (36,4)	0 (0)***	5 (15,2)**	10 (40)	4 (16)	6 (24)	5 (27,8)	0 (0)***	3 (16,7)*	12 (44,4)	0 (0)***	2 (7,4)**
H. influenzae, n (%)	3 (9,1)	5 (15,2)	6 (18,2)	7 (28)	6 (24)	5 (20)	5 (27,8)	4 (22,2)	3 (16,7)	4 (14,8)	3 (11,1)	4 (14,8)
M. catarrhalis, n (%)	7 (21,2)	6 (18,2)	10 (30,3)	5 (20)	9 (36)	7 (28)	8 (44,4)	5 (27,8)	6 (33,3)	6 (22,2)	6 (22,2)	8 (29,6)
K. pneumoniae, n (%)	2 (6,1)	2 (6,1)	3 (9,1)	1 (4)	1 (4)	2 (8)	1 (5,6)	1 (5,6)	1 (5,6)	4 (14,8)	5 (18,5)	6 (22,2)
S. spp., n (%)	-	2 (6,1)	3 (9,1)	-	2 (8)	2 (8)	1 (5,6)	2 (11,1)	2 (11,1)	-	1 (3,7)	1 (3,7)
A. baumanii, n (%)	-	-	-	-	-	-	-	-	-	-	-	-
M. odoratus, n (%)	-	-	-	-	-	-	-	-	-	-	-	-
Candida spp., n (%)	3 (9,1)	2 (6,1)	5 (15,2)	1 (4)	3 (12)	4 (16)	3 (16,7)	1 (5,6)	2 (11,1)	2 (7,4)	2 (7,4)	3 (11,1)
Bacillus spp., n (%)	-	-	1 (3)	-	-	-	-	-	-	-	-	1 (3,7)
Proteus spp., n (%)	-	-	-	-	-	-	-	1 (5,6)	1 (5,6)	-	-	-
Pseudomonas aeruginosa, n (%)	-	1 (3)	1 (3)	-	-	-	-	-	-	-	-	-

** - p<0.01 compared to the initial value in the corresponding group.
** - p<0.01 compared to the value before vaccination in the corresponding group.
*** - p<0.001 compared to the initial value in the corresponding group.

An analysis of the data in Table **7** indicates that in the group of BA patients after 4 years, pneumococcus was isolated much less often only in patients who were vaccinated with a conjugated polysaccharide vaccine (*i.e.*, the PCV13, PPV23/PCV13 and PCV13/PPV23 groups). There were no significant changes in the frequency of isolation of other microorganisms other than pneumococcus after 4 years in the studied groups of BA patients.

DISCUSSION

Currently, immunoprophylaxis of pneumococcal infections is recognized worldwide as the most important anti-epidemic measure capable of providing a significant reduction in the disease incidence caused by *S. pneumoniae.* Significant progress has been made in the development of vaccines, including anti-pneumococcal ones.

In chronic bronchopulmonary diseases, such as BA, one of the most important etiologically significant factors is *S. pneumoniae.* This justifies the practicality of vaccination as part of the fight against this infection.

In the conducted study, the effect of different vaccination protocols for pneumococcal infection on the pattern of BA was studied in order to choose the most optimal protocol. Evaluation of the clinical efficiency of vaccination in the analyzed groups for BA patients was carried out initially, after 1 and 4 years.

1 year after vaccination, the number of patients who didn't experience BA attacks was significantly higher than the initial value for vaccination protocols PPV23 (56%, $p<0.001$), PPV23/PCV13 (38.8%, $p<0.01$) and PCV13/PPV23 (74.1%, $p<0.01$). 4 years after vaccination, only in the PCV13/PPV23 group there was a significant increase in the number of patients who didn't experience BA attacks compared to the initial value, amounting to 48.1% ($p < 0.01$). There were no significant differences in the number of patients with BA who did not take ABH in the first and fourth years after vaccination compared to the initial values. After 1 year all vaccination protocols, except PCV13/PPV23, led to a significant increase in the number of patients who didn't require hospitalization, after 4 years, only in the PCV13 group, a significant increase in the number of BA patients who didn't require hospitalization was noted compared to the initial value (81.8% vs. 54.5%, $p < 0.05$).

The positive clinical effect of vaccination on BA patients is associated with its effect on the microbiocenosis of sputum, which is understood as the defense of the patient's body from microbe contamination, or the possibility of their eradication of microbes in case of their presence in the body before immunization. The fight against the carrier of pneumococcus is also important. In this regard, a microbiological study of sputum of BA patients was conducted in association with vaccination for pneumococcal infection.

After 1 year in BA patients, the isolation rate of *S. pneumoniae* dropped sharply to zero, only in the PPV23 group it was 16%. After 4 years, pneumococcus was isolated much less frequently only in patients who were vaccinated with a conjugated polysaccharide vaccine (*i.e.*, the PCV13, PPV23/PCV13 and PCV13/PPV23 groups). Vaccination against pneumococcal infection in patients with BA after 1 and 4 years did not lead to significant changes in the frequency of isolation of other microorganisms.

An extremely important finding of the study is the fact that in BA patients after 1 and 4 years along with a decrease in the frequency of *S. pneumoniae* isolation contamination of sputum with other pathogens was not observed.

CONCLUSION

Sequential vaccination with pneumococcal conjugate and polysaccharide vaccines leads to a decrease in the isolation of pneumococcus from sputum in BA patients, which has a pronounced positive effect on the clinical pattern of this disease, namely, it leads to a decrease in the frequency of attacks, the requirement for courses of antibacterial chemotherapy and hospitalizations, and therefore this vaccination protocol should be included in the standards of BA patient management. Moreover, BA patients should be vaccinated as early as possible after diagnosis. In our opinion, the improvement of the clinical pattern of the disease is primarily due to the etiological effect of vaccination on pneumococcal infection, which means that this medical intervention will bring the greatest benefit to those BA patients for whom pneumococcus plays an important role in the pathogenesis of the disease.

REFERANCES

[1] Chuchalin A.G., Avdeev S.N., Aisanov Z.R., Arkhipov V.V., Belevsky A.S., Nenasheva N.M. Approved recommendations on the use of the long-acting anticholinergic drug tiotropium in the treatment of bronchial asthma. Pulmonology. 2015; 25(2): 143-150. https://doi.org/10.18093/0869-0189-2015-25-2-143-150

[2] Protasov A.D. Vaccination against pneumococcal infection in patients with chronic bronchopulmonary pathology (literature review). Bulletin of Modern Clinical Medicine. 2013; 6(2): 60-65. https://doi.org/10.20969/VSKM.2013.6(2).60-65

[3] Protasov A.D. Comparative evaluation of the effectiveness of vaccination against pneumococcal infection in patients with bronchial asthma using 13-valent conjugate and 23-valent polysaccharide vaccines. Pulmonology. 2014; 5: 52-56.

[4] Edris, A. Pharmacogenetics of inhaled corticosteroids and exacerbation risk in adults with asthma / A. Edris, E. W. de Roos, M. J. McGeachie // Clin. Exp. Allergy. - 2022; 52(1): 33-45. https://doi.org/10.1111/cea.13829

[5] Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2022. Available from: www.ginasthma.org.

[6] Lukachev I.V., Kostinov M.P., Shabalina S.V., Magarshak O.O., Zhirova S.N. "PNEUMO-23" vaccine in the complex treatment of children with bronchial asthma. Epidemiology and preventive vaccination. 2004; 3(16): 41-44.

[7] Pelton S.I., Weycker D., Farkouh R.A., Strutton D.R., Shea K.M., Edelsber J. Risk of Pneumococcal Disease in Children With Chronic Medical Conditions in the Era of Pneumococcal Conjugate Vaccine. Clinical Infectious Diseases. 2014; 59(5): 615-623. https://doi.org/10.1093/cid/ciu348

[8] Patel, G. B. Comorbidities associated with severe asthma / G. B. Patel, A. T. Peters // J. Precis. Respir. Med. 2019; 2(1): 5-9. https://doi.org/10.2500/jprm.2019.190006

[9] Kostinov MP, Protasov AD, Zhestkov AV, Yastrebova NE, Kostinov AM, Vlasenko AE, Polyshyuk VB, Kostinova AM, Korovkina ES, Ryzhov AA, Shmitko AD. A Randomized Safety Study of 13-Valent Pneumococcal Conjugated Vaccine and Post-Vaccination Immune Response to Streptococcus pneumoniae Serotypes in Adult Patients with Bronchial Asthma and Chronic Obstructive Pulmonary Disease. Annals of the Russian Academy of Medical Sciences. 2021; 76(3): 324-334. https://doi.org/10.15690/vramn1342

[10] Vaccination and emergency immunoprophylaxis of infectious diseases in pregnant and lactating women: a guide for doctors / under. ed. M.P. Kostinova, L.V. Adamyan, A.P. Cherdantseva, N.A. Ozeretskovsky (ed. 1st). - M.: Group MDV, 2022. 98 p.

[11] Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2018. Available from: www.ginasthma.org.

[12] WHO updates Essential Medicines List with new advice on use of antibiotics, and adds medicines for hepatitis C, HIV, tuberculosis and cancer. 2017. Available at: http://www.who.int/mediacentre/news/releases/2017/essential-medicines-list/en /

[13] Dobrikh V.A., Mun I.E., Kovaleva O.A., Digor A.A., Uvarova I.V., Makarevich A.M. Diagnostic significance of cytological examination of effusion of the lower respiratory tract. Far Eastern Medical Journal. 2013; 1: 125-129.

[14] Palange P., Simonds A.K. ERS Handbook of Respiratory Medicine. 2nd ed. UK: Hermes; 2013. https://doi.org/10.1183/9781849840415-hba02

[15] Hof H., Dorries R. Medizinische Mikrobiologie. 2009. https://doi.org/10.1055/b-001-2124

[16] Krechikova O.I. Isolation, identification and determination of antibiotic sensitivity of Streptococcus pneumoniae. Clinical microbiology and antimicrobial chemotherapy. 2000; 2(1): 88-98.

[17] Ford M. Medical Microbiology / M. Ford // Medical Microbiology. - Oxford University Press. - 2010. - P. 365.

[18] Bogdanovich T.M. Isolation, identification and determination of sensitivity to antibiotics of Haemophilus influenza. Clinical microbiology and antimicrobial chemotherapy. 2000; 2(2): 93-109.

[19] Lang T.A., Sesik M. How to describe statistics in medicine. A guide for authors, editors and reviewers. M.: Practical Medicine; 2011.

Received on 19-11-2022 Accepted on 15-12-2022 Published on 28-12-2022

DOI: https://doi.org/10.12974/2312-5470.2022.08.05