CLINIC OF THE STATE SCIENTIFIC CENTER – INSTITUTE OF IMMUNOLOGY MH RF

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REPORT

on the clinical trial of preventive efficacy of 6 mg Polyoxidonium injections and suppositories in aged patients with frequent aggravations of chronic inflammatory diseases

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Report
on the clinical trial of preventive efficacy of 6 mg Polyoxidonium injections and suppositories in aged patients with frequent aggravations of chronic inflammatory diseases

Type: open controlled study

Phase: IV

Number of patients: 61
Product Lot №: 170701, 120601
Trial sponsor: Scientific-Production Union Petrovax Pharm
Address: 115598, Moscow, Zagorievskaya str., 10/4; phone/fax number: 7442969

Scientific-Research Institution:
Clinical Scientific-Consultative Department of State Scientific Research Center of Immunology of Russian Ministry of Health
Address: 115598, Moscow, Kashirskoe shosse str., 24/2

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Regulative Standards:
Trial is carried out in accordance with Russian Ministry of Health Pharmacology Committee requirements.

State Standarts: OST 42-511-99 “Rules for Good Clinical Trials Conduction in Russian Federation” approved by Ministry of Health on 29.12.1998;
“Rules of Clinical Practice in Russian Federation” (approved by Russian Ministry oh Health on 19.06.2003, №266).

1. Introduction

The studies aimed on investigating of specific features of immune system function in elderly people has recently become of particular importance as this category represents the most rapidly growing part of total population. People older than 60 years already exceed 20% of the population of Russia.

Regardless of the considerable number of studies, directed at elucidating the link between the immunity and senescence as well as accumulating knowledge on the immunity in aged and senescent people, the problem of age-related changes in immune system remains to be solved. Laboratory data on specific characteristics of the immune status in people older than 60 years are controversial. Considerable variation in immune status characteristics can be observed in aged and senescent people, especially in conditions of concomitant somatic disorder, therefore particular approach is required for the interpretation of dynamic changes in immunogramms of these patients and to the analysis of immunomodulation treatment effectiveness.

The high possibility of clinical manifestation of the secondary immune deficiency (SID) in aged people arise the question of evaluation of possibility and perspectives of immunomodulation therapy application to control the impaired immune functions and to prevent the chronic inflammatory diseases and concomitant somatic disorders.

The eligibility of immunomodulator Polyoxidonium for immunocorrection in elderly patients is determined by the data, which confirms the wide spectrum of its activity and efficiency in combined treatment of secondary immunodeficiency. Polyoxidonium has been used in the medical practice since 1996 (Registration certificate 96/302/9).

2. **Immunopharmacologic properties of preparation**

Polyoxidonium displays immunomodulatory activity, increases the resistance of organism both to local and general infections. Together with immunomodulatory properties, Polyoxidonium has a pronounced detoxic and antioxidative activities that are determined by its structural and high-molecular nature. The application experience has demonstrated the considerable clinical effectiveness in therapy of chronic, recurrent, indolent infectious-inflammatory disorders of skin, bronchopulmonary apparatus, gastrointestinal and urogenital tracts, as well as in the treatment of severe bacterial and viral infections.

Most data on effective use of Polyoxidonium gathered, mainly, for intramuscular injections. Availability of novel formulation – rectal suppositories gives an opportunity of wider and more convenient application of polyoxidonium, especially in aged patients.

The majority of previous trials have demonstrated Polyoxidonium' effectiveness in young and mature-aged patients. Experience of efficient application in aged patients is limited, thereby, determining the priority of Polyoxidonium effectiveness investigation in aged and senescent patients on two different routes of medication administration.

3. Research aims and objectives

Hence, the present research was aimed to investigate Polyoxidonium impact on clinical-immunological values as well as to evaluate preventive effect of the medication on the incidence of aggravations of chronic inflammatory diseases in aged patients.

The following tasks were undertaken to achieve the research goals:

1. To evaluate immune status characteristics and quantitative distribution of immunopathological syndromes in aged patients.
2. To investigate influence of Polyoxidonium given parenterally or per rectum on immune status values in aged patients with clinical manifestations of secondary immune deficiency.
3. To assess the clinical effectiveness of Polyoxidonium and the possibilities of preseasonal use of medication in order to prevent from the exacerbations of chronic inflammatory diseases in aged patients.
4. To develop optimal schedules of Polyoxidonium administration for preventive immunomodulating treatment purposes in aged patients with immune insufficiency.

4. General research plan

Research was accomplished on the basis of scientific-consultative department, clinic of the Institute of Immunology, Federal Administration "Medbioextrem", MH of RF. Immunological testing of patients were performed at the laboratory of clinical immunology, Institute of Immunology (Head of the lab., professor, Dr. of Medicine Pinegin B.V.). Clinical-laboratory, immunological and statistical methods of investigation were used during research.

4.1. Patient inclusion criteria

Aged patients older than 60 years were included in the study using the random sampling method. Aged patients who had applied to scientific-consultative department of Institute of Immunology undertook screening-questioning in order to display clinical manifestations of secondary immune deficiency. Screening questionnaires were filled in and were analyzed by study participants during visit to the physician.

High risk-group was comprised of aged patients suffering from the immune deficiency manifested by the predominant infective syndrome - recurrent, frequently repeating or persistent course of chronic bacterial, viral, fungal infections as follow:

Frequently recurring chronic bronchitis that represents either disorder complicating chronic infections of ear, nose and larynx or the "mono-disease" coupled with the medical history of isolated instances of pneumonia; Chronic bronchitis in combination with frequent acute respiratory viral infections; Recurrent pneumonia; Infectious-allergic bronchial asthma; purulent infections of the skin and hypodermal tissue (pyodermia, furunculosis, abscesses, flegmon and etc); Recurrent purulent conjunctivitis; Recurrent herpetic infection.

The following two patient groups were formed based on the presence of secondary immune deficiency manifestations that were exposed by screening-questioning of aged patients.

Main group – was composed of 44 aged patients with clinical manifestations of secondary immune deficiency that undertook immunological testing and subsequent preventive course of immunomodulatory therapy by Polyoxidonium.

Control group – contained 17 aged patients without clinical signs of secondary immune deficiency. Control group patients did not require immunomodulatory treatment and they were subjected merely to the dynamic evaluation of immune status values.

Patients suffering from the decompensated severe somatic disorders and those with oncological diseases were not included in the study as these conditions can in itself provoke the clinical manifestations of secondary immune deficiency as well as the deviations in immune status values that would impede the evaluation of clinical - immunomodulatory effects of the medication.

4.2. Treatment schedules

Polyoxidonium treatment course was performed on preventive purpose in autumn. The main cohort of patients was divided into two treatment groups.

First group – 20 patients applied rectal suppositories containing 6mg of Polyoxidonium on alternate days, 10 suppositories per course.

Second group – 24 patients received intramuscular injections of 6mg Polyoxidonium bid week, 5 injections per course.

4.3. Description of medicinal formulations of test preparation - "Polyoxidonium"

The following two medicinal formulations of Polyoxidonium produced by "Immapharma" Ltd. (18, Gamalei str., Moscow) were used during research:

1. Polyoxidonium for injections 0.006g. Serial number 170701. Date of release 16.07.01.

Medicine description: Porous light-yellow substance in flasks. The medication is soluble in isotonic sodium chloride solution and in novocaine.

2. Rectal suppositories containing 0.006g Polyoxidonium. Serial number 12062001. Date of release 05.07.01.

Medicine description: (on cacao oil base) torpedo-shaped light-yellow suppositories.

Storage: Polyoxidonium should be stored under dry, covered from the light conditions at 4-8^{0}C. Period of validity – 2 years.

4.4.Patient status evaluation criteria

4.4.1. Clinical values

Polyoxidonium recipients remained under dynamic medical observation. Medication tolerance and development of adverse effects during treatment period were evaluated carefully.

4.4.2. Clinical-laboratory findings

Aged and senescent patients from the Polyoxidonium therapy groups were subjected to laboratory testing on the day of treatment commencement and 7-10 days after it's completion:

- Complete blood count (hemoglobin level, numbers of erythrocytes, reticulocytes, leukocytes and thrombocytes, ESR, differential white blood cell count implying estimation of basophil, eosinophil, band neutrophil, segmented neutrophil, lymphocyte and monocyte counts)
- Biochemical blood test (total protein, creatinine, total bilirubin, AST, ALT, glucose)
- Urine test.

4.4.3. Immunological parameters

The analysis of immune status indexes in patients treated by Polyoxidonium (the main group) was performed twice – on the day of treatment commencement and 7-10 days after the immunomodulation therapy completion.

Irrespective to the immunomodulator agent application the possibility of variation in immune status characteristics in aged patients during the period of two weeks could not be excluded. Therefore, aged patients, enrolled into the control group, underwent the analysis of immune status twice within the testing interval of 3 weeks.

The following immune status characteristics were evaluated

- Leukocyte count
- Percentage and absolute count of lymphocyte

- Percentage and absolute count of lymphocyte subpopulations
- CD3+ - receptor with the marker of mature T-lymphocytes
- CD4+ - the marker of helper/inducer T-cell subpopulations
- CD8+ - the marker of cytotoxic T –cell subpopulation
- CD4+/CD8+ lymphocyte ratio (immunoregulation index)
- CD19+ - common B-lymphocyte marker
- The ability of peripheral blood monocytes and granulocytes to phagocytose of St. aureus was also evaluated

Data analysis was performed using Flow Cytometer "Facscalobur"

The serum class A, M, and G immunoglobulin (IgA, IgM, IgG) levels were assayed using Manchini method.

6. **Medicine safety evaluation criteria**

The evaluation of medicine safety was based first of all on the rate and severity of adverse effects of Polyoxidonium treatment. In addition the dynamic changes in clinical and biochemical values of blood and urine tests were taken into account.

7. Effectiveness evaluation criteria

Medical history data of patients treated with Polyoxidonium were also used during evaluation of the clinical effectiveness of the given immunomodulatory therapy. Aged patients remained under medical observation during a year after the Polyoxidonium course completion. Incidence and durability of aggravations of chronic infections, duration of remission as well as the hospitalization rate per year before Polyoxidonium treatment and during the year after course completion were studied. Treatment effectiveness appraisal by patients was analyzed as well. Patients were offered to characterize their state of health in accordance with following four categories: significant improvement, improvement, without change, worsening.

Dynamic changes in immune status values of patients treated with Polyoxidonium and of those from control group were analyzed in order to assess immunomodulatory effect of the medication.

8. Results

8.1. Patient characteristics

61 tested patients (age range from 62 to 90 years) were randomized in two treatment groups based on the data acquired by screening-questioning of patients regarding the secondary immune deficiency manifestations.

44 patients who had turned out to suffer from the secondary immune deficiency manifestations were enrolled in the **main group**. These patients undertook immune status testing and subsequent preseasonal preventive course of immunomodulatory treatment with Polyoxidonium.

17 patients without clinical signs of immune deficiency were included in the control group. Patients from the given group did not require immunomodulating therapy and they undertook immunological tests later on.

The majority of examined patients were older than 65 years. The mean ages of patients from both study groups were 70 years. Women prevailed that was in general agreement with essential demographical tendencies. Hence, women comprised 70% of total cohort of study participants, 90% of patients older than 75 years were women (Table 1).

Table 1

Gender and age dependent distribution of patients included in the trial

	Main group – aged patients with SID manifestation (n=44)		**Control** group – aged patients without SID manifestations (n=17)	
Age	Male	Female	Male	Female
60-65yr	3	5	2	4
65-70 yr	4	9	1	0
71-75 yr	5	8	2	3
≥ 76 years	1	9	-	4
In total	13 (30%)	31 (70%)	5 (29%)	12 (71%)

Taking into account the age category of study participants, undoubtedly, the concomitant somatic pathologies were found in all patients. Data retrieved by processing of medical histories and out-patient records contributed to the occurrence rate assessment of diverse somatic disorders in both study groups – patients with and without manifestations of secondary immune deficiency (Table 2).

The majority of aged and senescent patients displayed compensated or subcompensated concomitant somatic disorders. The most prevalent somatic disorders were cardiovascular diseases as well as disorders of respiratory system and intestinal tract.

Table 2 **Concomitant somatic disorders in aged patients enrolled in the study**

Diseases	**Main group** – aged and senescent patients with SID manifestations (n=44)		**Control group** – aged and senescent patients without SID manifestations (n=17)	
	Number of patients	%	Number of patients	%
Hypertension	25	60%	10	58%
Myocardial ischemic diseases	19	43%	7	41%
NK 1-2 grade	3	8%	3	17%
Dyscirculatory encephalopathy	9	20%	3	17%
Bronchial asthma	14	32%	4	24%
Chronic bronchitis	29	67%	-	-
Lung emphysema	16	36%	2	12%
Pneumosclerosis	16	36%	2	12%
Nasal pollinosis	3	8%	1	6%
Chronic antritis	10	23%	-	-
Vasomotor rhinitis	7	16%	4	24%
Gastric ulcer	2	5%	2	12%
Duodenal ulcer	2	5%	2	12%
Chronic pancreatitis	14	32%	2	12%
Chronic gastroduodenitis	15	34%	3	17%
Cholelithiasis	7	16%	2	12%
Chronic cholecystitis	17	37%	4	24%
Diabetes mellitus II type	3	8%	1	6%
Hypothyroidism	5	11%	1	6%
Chronic prostatitis	5	11%	-	-

Urolithiasis	3	8%	-	-
Osteochondrosis	19	43%	7	41%
Rosacea	-	-	2	12%
Herpes Zoster in anamnesis	4	9%	-	-
Postherpetic neuralgia	2	5%	-	-

The main and control groups appeared similar in gender, age and in rates of concomitant somatic disorders. Moreover, main group patients showed evident clinical manifestations of secondary immunodeficiency.

Characteristics of clinical manifestations of secondary immune deficiency in aged patients are enumerated in Table 3.

Table 3.

Clinical manifestations of secondary immune deficiency in aged patients (Main group) n=44

Disease	Patient number	%
Chronic recurrent mucous-purulant bronchitis, in combination with frequent acute respiratory viral infectious disease	19	43%
Infectious-allergic bronchial asthma	10	22%
Chronic antritis	10	22%
Recurrent herpetic infection, labial form	9	20%
Herpetic infection, genital form	1	2%
Suppurative infections of the skin and hypodermal tissue	2	4,5%
Medical history of Herpes Zoster manifestations (after 60 years of age)	4	9%

Secondary immune deficiency in aged patients was manifested by recurrent bacterial and viral infections. The most frequently observed inflammatory diseases included those of respiratory tract (chronic recurrent bronchitis, sinusitis, frequent acute respiratory viral infections), recurrent herpetic infection. The main group patients underwent preventive preseasonal course of immunomodulatory treatment with Polyoxidonium in accordance with two different schedules of medication administration.

First group – 20 aged and senescent patients were treated with rectal suppositories of Polyoxidonium given at 6 mg single dose on alternate days (10 suppositories per course).

Second group – 24 aged and senescent patients undertook intramuscular injections of 6mg Polyoxidonium bid week (5 injections per course).

8.2. Therapy safety evaluation

Medication was well tolerated by the majority of patients. However, significant increase in blood pressure values up to 170/100 mm Hg. accompanied by headache and exacerbation of overall state of health was observed on the day following Polyoxidonium injection in a 69 years old patient, who had suffered from hypertension and had taken the administered hypotensive treatment irregularly. Blood pressure decreased shortly. It is most likely that the development of complication in this case was not related to Polyoxidonium injection. Nevertheless, the patient refused from treatment continuation.

Six patients out of those treated with intramuscular injections of Polyoxidonium complained of slight soreness on the site of injection that did not require medication discontinuation. Polyoxidonium on either routes of administration was well tolerated by the rest patients.

Statistical changes in blood test findings were not observed following treatment completion (Table 4).

Table 4

Dynamic changes in blood test values of aged patients treated with Polyoxidonium, n=44

Parameters (normal values)	**First group**, patients treated with rectal suppositories of Polyoxidonium (n=20)		**2nd group**, patients treated with intramuscular injections of Polyoxidonium (n=24)	
	Before therapy	After therapy	Before therapy	After therapy
	M ± δ	M ± δ	M ± δ	M ± δ
Hemoglobin (110-165 g/l)	137,9±12,0	138,3±11,1	136,6±12,2	138,4±13,8
Erythrocytes (3,8-5,8*10^{12}/l)	4,5±0,4	4,4±0,37	5,8±6,4	4,5±0,4
Leukocytes (3,5- 10,0*10^{9}/l)	6,7±2,1	6,2±2,06	7,52±3,3	6,7±1,5
Band neutrophils % (1-6)	3,4±2,3	3,5±2,56	4,04±2,9	3,6±2,8
Segmented neutrophils % (47-72)	57,5±8,8	53,7±8,65	60,15±10,6	58,2±6,2
Eosinophils % (0,5-5)	2,7±2,7	1,9±2,49	3,00±5,3	2,5±3,1
Basophils % (0-1)	0,1±0,3	0,1±0,2	0,26±0,5	0,1±0,4
Lymphocytes % (19-37)	30,7±6,2	33,6±10,11	26,9±7,1	28,4±5,1
Monocytes % (3-11)	7,5±3,5	7,3±3,34	5,26±2,1	7,0±3,0
ESR mm/hr (2-15)	11,1±8,7	9,5±7,63	14,39±9,2	11,9±9,4

Table 5. **Dynamic changes in biochemical blood test values of aged patients treated with Polyoxidonium, n=44**

Parameters (normal values)	**First group**, patients treated with rectal suppositories of Polyoxidonium (n=20)		**2nd group**, patients treated with intramuscular injections of Polyoxidonium (n=24)	
	Before therapy	After therapy	Before therapy	After therapy
	M± σ	M ± δ	M± δ	M± δ
Glucose (3,5-5,8 mmol/l)	5,2±0,7	4,8±0,7	5,6±2,0	5,2±0,9
AST (5-37 U/l)	24,5±6,5	25,6±7,1	25,7±6,7	24,8±6,2
ALT (5-40 U/l)	20,1±8,7	20,4±10,7	21,6±9,0	20,6±7,3
Total bilirubin (0-22,2 μmol/l)	11,9±5,3	14,2±6,4	13,5±5,2	11,6±4,7
Creatinine (44-106 mmol/l)	95,8±33,6	90,8±9,7	85,4±8,6	87,9±10,0
Total protein (62-83 g/l)	76,7±3,5	74,8±3,9	76,2±3,4	74,3±3,6

Biochemical blood test findings as well as clinical urine test values met the physiological ranges in a majority of patients. Significant alterations in laboratory findings were not revealed after the completion of the immunomodulatory treatment course (Table 5). The retrieved data provided evidence for renal and hepatic safety of Polyoxidonium treatment in aged patients.

8.3. Clinical effectiveness evaluation

Medical history data of patients treated with Polyoxidonium were used for clinical effectiveness evaluation of the immunomodulatory therapy. Aged patients remained under medical observation during a year following Polyoxidonium course completion. Incidence and durability of aggravations of chronic infections, remission duration as well as hospitalization rate per year before Polyoxidonium treatment and during a year following course completion have been studied.

Treatment efficacy evaluation criteria showed significant improvement – double prolongation of remission durability, decrease in incidence and duration of chronic disease exacerbations as well as in hospitalization rates were observed in the prevalent majority of aged patients treated either with intramuscular injections or with rectal suppositories of Polyoxidonium.

Table 6.

Clinical effectiveness of preventive course of Polyoxidonium applied preseasonally in aged patients, n=44

Parameters	**First group** patients treated with rectal suppositories of Polyoxidonium (n=20)		**2nd group** patients treated with intramuscular injections of Polyoxidonium(n=24)	
	Before therapy	After therapy	Before therapy	After therapy
	M± δ	M± δ	M± δ	M± δ
Remission duration (Months)	2,1±0,7	4,2±1,7	1,9±0,8	4,0±2,0
Aggravation duration (Days)	16,6±4,6	8,6±6,9	17,3±5,5	9,0±7,7
Aggravation rate (per year)	4,5±1,1	2,3±1,7	5,5±1,9	2,6±1,9
Hospitalization rate (per year)	1,0±0,8	0,6±0,5	1,1±0,8	0,7±0,5

Nevertheless, immunomodulating therapy proved ineffective in 6 (30%) patients treated with rectal suppositories of Polyoxidonium (**first** group) and in 6 (27%) patients from the **second** group (intramuscular injections) as they did not show considerable changes in pointed parameters following treatment course. Hence, taking into account the finding that Polyoxidonium therapy did not yield clinical effects in almost 30% of patients, most of which were older than 70 years, one may surmise that Polyoxidonium effectiveness decreases with age.

Polyoxidonium therapy given either rectally or intramuscularly resulted in prolongation of chronic infection remission up to 4-6 months in 60% of patients. However, almost 30% of both study group participants, in other words every third patient either failed to achieve the disease remission or remission duration did not exceed 3 months (Table 7).

Table 7

Duration of remission of chronic infections following preventive preseasonal treatment course with Polyoxidonium in aged patients displaying clinical manifestations of SID, n=44

	First group patients treated with rectal suppositories of Polyoxidonium (n=20)		**2nd group** patients treated with intramuscular injections of Polyoxidonium (n=24)	
Duration of remission	Number of patients	**%**	Number of patients	**%**
6 months	2	**10**	3	**14**
5 months	6	**30**	6	**27**
4 months	5	**25**	5	**23**
3 months	1	**5**	2	**9**
Without remission	6	**30**	6	**27**

Thus, majority of aged patients, who suffered from clinical manifestations of SID showed prolongation of chronic infection remission as well as decline in exacerbation rate and durability following preseasonal completion of preventive treatment course with Polyoxidonium.

Appraisal of treatment efficacy by patients was also taken into account. After 6 months following Polyoxidonium therapy the majority of patients from both study groups reported about the improvement of their overall health state (Table 8).

Table 8.

Self-evaluation by aged patients at a lapse of 6 months following Polyoxidonium treatment, n=44

Parameters	**First group** patients treated with rectal suppositories of Polyoxidonium (n=20)		**2nd group** patients treated with intramuscular injections of Polyoxidonium (n=24)	
	Number of patients	**%**	Number of patients	**%**
Significant improvement	0	**0**	4	**18%**
Improvement	13	**65%**	11	**50%**
Without change	6	**30%**	6	**28%**
Worsening	1	**5%**	1	**4%**

However, approximately one third of patients failed to notice health status improvement. Moreover, the clinical effects of Polyoxidonium therapy were not revealed in the most of these patients either.

Data analysis proved application preseasonal of Polyoxidonium for preventive purposes to exert positive clinical effects in 70% of patients treated either with rectal or with intramuscular formulations of medication.

Rectal suppositories and intramuscular injections of Polyoxidonium showed to produce similar clinical effects in aged patients suffering from the secondary immune deficiency manifestations. Though, better tolerance, absence of any adverse effects and simplicity of application are considered to be the advantages of rectal suppositories over another medicinal formulation of Polyoxidonium.

8.4. Results of immunological testing of aged patients

Preliminary evaluation of baseline immune status in aged patients was required for assessment of immunomodulatory effect of Polyoxidonium. Immunological testing of 61 aged patients showed that practically, all values of immune status met physiological ranges (Table 9).

However, diminution of CD3+ lymphocyte level was revealed in 20% of patients, decrease in CD4+ lymphocytes was found in 40% and CD19+ lymphocyte level was lowered in one third of aged and senescent patients. Serum level of IgA was elevated in almost 30% of patients.

Statistically significant difference in immunogram values of aged patients with clinical manifestations of secondary immune deficiency and of those without clinically apparent SID was not found. Moreover, control group patients exhibited higher rate of the characteristic age-related decrease in CD4+, CD8+ T-cell and CD19+ lymphocyte counts in comparison with those demonstrating clinical manifestations of secondary immune deficiency that is consistent with the observation that recurrent infections as well as other manifestations of SID trigger immune system activation, thereby, grading the age-related alterations of pointed findings.

Hence, immune status properties characteristic to aged and senescent participants of the study were as follow: decrease in T-lymphocytes bearing CD3+, CD4+ immunophenotype, decline in B-lymphocytes (CD19 positive cells) and enhancement in serum IgA level. There was not found the correlation between the profundity of immune status alterations and the severity of SID manifestations. This observation corroborates the presumption that explored changes, first of all are related to the senescence process and are rarely accompanied by the development of clinical signs of immune deficiency.

Table 9. **Immune status findings of aged patients, n=61**

Parameters (normal values)	**Main group**, patients with SID (n=44)	**Control group**, patients without SID (n=17)
	M ± σ	M ± σ
Age	70,0±5,3	70,5±7,2
Leukocyte count, abs. (4000-9000)	7113,6±2791,9	6523,5±2055,9
Lymphocyte % (19-37)	28,8±6,8	28,2±7,2
Lymphocyte count, abs. (1200-3000)	1931,4±498,4	1867,2±853,0
CD3+ % (55-80)	63,6±10,4	61,4±8,8
CD3+ Abs. (800-2200)	1231,3±380,8	1167,1±605,8
CD4+ % (31-49)	35,6±9,9	36,2±7,6
CD4+ Abs (600-1600)	693,5±266,0	703,9±399,7
CD8+ % (19-37)	28,1±8,0	25,2±7,4
CD8+ Abs (300-800)	538,7±188,1	454,9±217,8
CD4+/CD8+ (1-2,5)	1,4±0,6	1,6±0,7
CD19+ %(5-19)	7,6±4,3	6,4±2,3
CD19+ abs (100-500)	142,1±86,2	116,1±69,2
Phagocytosis - neutrophils (70-95)	87,5±10,4	89,0±6,4
Phagocytosis - monocytes (60-85)	74,1±11,6	72,7±10,1
IgA mg% (100-350)	238,1±100,0	257,9±105,6
IgG mg% (900-1800)	1203,3±278,0	1308,5±276,9
IgM mg% (80-250)	154,5±62,2	127,9±61,1

8.5. Evaluation of immunomodulatory effect of Polyoxidonium

Analysis of immune status exploration data in patients treated with Polyoxidonium either rectally or intramuscularly failed to demonstrate significant difference in mean values of study groups. Therefore, parameters of both study groups were subjected to the repeated handling in order to explore carefully the dynamic changes in immune status findings compared to baseline values. The findings that met lower than average level of the given group were considered to be "low" as well as values exceeding average level were meant to be "high" (subsequently mentioned as "high" and "low" levels).

Authentical increase in absolute count and percentage of CD3+ CD4+ lymphocytes, enhancement of immunoregulatory index (CD4+/CD8+) and elevation of serum IgG and IgA levels following treatment with rectal suppositories of Polyoxidonium were achieved in patients exhibiting the low initial values of given parameters. Statistically significant decrease in phagocytic capacity of neutrophils and monocytes was revealed in patients with high values at baseline (Table 10).

Table 10.

Dynamic changes in immune status parameters of aged patients treated with rectal suppositories of Polyoxidonium (first group), regarding initial levels of findings, n=20

	Low baseline level (lower than the average level)			High baseline level (higher than the average level)		
Parameters	Before treatment	After treatment	*Index of changes*	Before treatment	After treatment	*Index of changes*
	M± δ	M± δ		M± δ	M± δ	
Leukocyte, abs	5000,0±780,3	4930,0±1220,2	*0,99*	8340,0±1484	7560±1893	*0,91*
Lymphocyte %	25,9±4,0	28,8±9,1	*1,11*	35,4±3,8	38,3±9,1	*1,08*
Lymphoc., abs	1607,3±224,8	1658,4±366,8	*1,03*	2326,1±348	2296,1±567	*0,99*
CD3+ %	52,9±7,5	**61,8±10,9***	*1,17*	71,7±6,4	73,6±9,1	*1,03*
CD3+ abs	945,8±252,5	**1189,4±507,8***	*1,26*	1496,7±235	1501,6±368	*1,00*
CD4+ %	24,7±5,3	**36,1±10,7***	*1,46*	39,7±5,7	37,0±7,9	*0,93*
CD4+ abs	437,8±126,0	**605,2±309,2***	*1,38*	850,7±182,0	846,4±190	*0,99*
CD8+ %	21,4±4,4	24,7±7,0	*1,15*	36,7±7,7	31,4±6,4	*0,86*
CD8+ abs	394,4±119,5	454,4±143,8	*1,15*	746,9±139,2	633,6±143	*0,85*
CD4+/CD8+	0,8±0,3	**1,5±0,9***	*1,90*	1,8±0,7	1,4±0,5	*0,78*
CD19+ %	5,5±1,8	5,0±2,7	*0,91*	12,3±4,2	8,3±5,2	*0,67*
CD19+ abs	103, 7±33,2	83,5±41,6	*0,81*	241,7±93,6	183,2±121	*0,76*
Phagocytosis Neutrophils	81,9±8,1	86,3±4,3	*1,05*	95,0±2,1	**89,4±7,6***	*0,94*
Phagocytosis Monocytes	67,4±7,1	70,7±10,0	*1,05*	84,0±3,6	**72,7±8,8***	*0,87*
IgA mg%	188,1±57,9	233,0±79,2	*1,24*	339,7±64,5	332,4±92,5	*0,98*
IgG mg%	1041,3±91,6	1185,3±163,0	*1,14*	1551,9±221	1443,3±314	*0,93*
IgM mg%	104,8±19,2	101,2±33,6	*0,97*	186,7±54,6	200,3±80,4	*1,07*

- Difference between the given findings and initial values bore statistically authentical character - $p<0.05$ (Wilconson criterion).

Intramuscular injections of Polyoxidonium produced similar dynamic changes in immune status parameters. Statistically significant increase in absolute count and percentage of lymphocytes as well as in absolute number of CD3+CD4+ lymphocytes, enhancement of CD4+/CD8+ ratio and elevation of serum IgG and IgA levels were found in patients with low initial levels of respective parameters. High baseline values of leukocyte number and phagocytic capacity of monocytes declined following Polyoxidonium therapy (Table 11).

Table 11

Dynamic changes in immune status findings of aged patients treated with intramuscular injections of Polyoxidonium (2nd group) regarding their initial levels, n=24

	Low baseline level (lower than the average level)			High baseline level (higher than the average level)		
Parameters	Before treatment	After treatment	*Index of changes*	Before treatment	After treatment	*Index of changes*
	M ± δ	M ± δ		M ± δ	M ± δ	
Leukocyte, abs	5727,3±538	6218,2±1461	*1,09*	9609,1±3865,6	**7236,4±1330***	*0,75*
Lymphocyte %	21,6±5,6	**27,4±5,3***	*1,26*	32,1±3,8	29,5±4,9	*0,92*
Lymphoc., abs	1481,4±229	**1674,3±370***	*1,13*	2357,5±398,9	2112,4±458	*0,90*
CD3+ %	57,5±6,3	60,7±9,0	*1,06*	71,8±5,2	68,8±9,6	*0,96*
CD3+ abs	911,9±233,1	1156,4±477	*1,27*	1597,2±213,8	**1308,8±265***	*0,82*
CD4+ %	31,2±6,2	34,6±5,5	*1,11*	45,1±7,6	44,9±6,8	*1,00*
CD4+ abs	514,0±162,8	**746,3±338***	*1,45*	959,6±173,5	768,4±209,9	*0,80*
CD8+ %	22,5±3,2	25,3±5,5	*1,12*	32,5±4,1	28,2±8,2	*0,87*
CD8+ abs	409,2±95,8	410,4±152,1	*1,00*	633,1±127,4	598,7±165,1	*0,95*
CD4+/CD8+	1,0±0,2	**1,6±0,7***	*1,58*	1,9±0,3	1,6±0,7	*0,84*
CD19+ %	4,0±1,7	7,2±2,2	*1,80*	9,5±3,2	7,5±2,3	*0,80*
CD19+ abs	65,7±34,3	125,6±52,9	*1,91*	171,6±51,4	155,2±75,6	*0,90*
Phagocytosis Neutrophils	78,8±13,2	79,6±9,9	*1,01*	94,2±2,3	92,6±4,8	*0,98*
Phagocytosis Monocytes	63,9±11,8	69,5±13,0	*1,09*	82,5±4,3	**65,0±13,2***	*0,79*
IgA mg%	144,8±35,2	**209,5±113***	*1,45*	300,0±83,6	322,9±107,7	*1,08*
IgG mg%	935,8±128,9	**1123,5±260***	*1,20*	1270,9±160,6	1287,0±285	*1,01*
IgM mg%	110,0±25,1	113,8±25,7	*1,03*	219,5±45,3	214,5±56,8	*0,98*

- Difference between the given findings and initial values bore statistically authentical nature - $p<0.05$ (Wilconson criterion).

Exploration of dynamic changes in immune status findings that were acquired by repeated testing of 17 aged patients from control group (not receiving immunomodulatory treatment) over the three week interval, revealed variation of immune status findings at ranges from 10 to 15% of baseline levels of respective parameters without obvious reasons. However, statistically significant differences were not found. Repeated testing of given patients that was accomplished at a lapse of 3 weeks demonstrated changes in initially low findings of absolute counts of CD3+CD4+ lymphocytes, CD4+/CD8+ ratio and serum IgA, IgG levels though alterations did not exceed 5-10% of the baseline values of respective findings (Table 12).

Table 12.

Dynamic changes in immune status of control group patients without manifestations of SID and therefore, without immunomodulatory therapy, n=17

	Low baseline level (lower than the average level)			High baseline level (higher than the average level)		
Parameters	Before treatment	After 3 weeks	*Index of changes*	Before treatment	After 3 weeks	*Index of changes*
	M± δ	M± δ		M± δ	M± δ	
Leukocyte, abs	5300,1±441,6	5400±796,9	*1,02*	8400,1±2384	7220,1±1512	*0,86*
Lymphocyte %	23±2,5	25,2±2,2	*1,10*	34,6±4,8	32,7±8,9	*0,95*
Lymphoc., abs	1393,2±217,1	1494,2±351,1	*1,07*	2564±873,0	2196,6±903,7	*0,86*
CD3+ %	52±3,4	52,8±5,6	*1,02*	69,8±8,2	72,2±7,5	*1,03*
CD3+ abs	747,8±145,1	843,6±253,7	*1,13*	1727±534,1	1518,2±750,8	*0,88*
CD4+ %	30,0±5,1	33,60±5,9	*1,12*	42,2±5,0	36,6±9,1	*0,87*
CD4+ abs	461,6±47,7	493,0±159,9	*1,07*	1072,6±355,1	890,0±251,8	*0,83*
CD8+ %	21,6±3,0	24,8±2,8	*1,15*	28,8±3,6	27,0±6,3	*0,94*
CD8+ abs	333,4±23,7	369,6±66,0	*1,11*	662,8±208,2	573,4±409,6	*0,87*
CD4+/CD8+	1,38±0,2	1,3±0,3	*0,96*	1,9±0,3	1,5±0,5	*0,79*
CD19+ %	4,2±1,5	4,4±2,1	*1,05*	8,4±1,5	5,2±1,5	*0,62*
CD19+ abs	65,2±21,1	69,8±38,5	*1,07*	192,4±77,7	102,6±50,7	*0,53*
Phagocytosis Neutrophils	81,6±6,8	87,4±7,5	*1,07*	94±2,9	90±5,4	*0,96*
Phagocytosis Monocytes	63,8±5,4	70,2±7,3	*1,10*	77,2±5,7	78,8±11,9	*1,02*
IgA mg%	224,2±58,5	226,4±61,9	*1,01*	395±65,8	324,2±69,0	*0,82*
IgG mg%	1267,2±74,4	1151,8±123	*0,91*	1480,6±108,4	1321,6±343,6	*0,89*
IgM mg%	95,8±24,3	92,4±19,3	*0,96*	187,2±66,4	181,6±84,3	*0,97*

On the contrary to control group patients, Polyoxidonium recipients with low baseline levels of enumerated parameters exhibited more substantial increase in respective values (20-25% of initial findings) following immunomodulatory therapy. This observation proves the revealed changes in immune status parameters to result from Polyoxidonium therapy.

Alterations of phagocytic activity of peripheral blood neutrophils and monocytes that might be related to the accomplishment of immunocorrective treatment were explored following therapy. Control group patients exhibiting high baseline values of phagocytosis did not show considerable changes in respective parameters at a lapse of 3 weeks. On the contrary, Polyoxidonium recipients, whose initial parameters of phagocytosis met or even exceeded slightly the near-upper values of physiological ranges demonstrated normalization of phagocytosis findings following therapy.

Considerable variations of absolute count and percentage of CD19+ lymphocytes were found in patients treated with Polyoxidonium as well as in the control cohort. Therefore, changes in these parameters were not ascribed to the Polyoxidonium activity but were considered to mirror the immune status characteristics associated with aging process.

Hence, Polyoxidonium exerts normalizing effects on immune status parameters, namely, the medication increases absolute number and percentage of CD3+ CD4+ lymphocytes, enhances the CD4+/CD8+ ratio and serum IgG and IgA levels in aged patients. There were observed the similar activities of rectal suppositories and intramuscular injections of Polyoxidonium in respect with the nature and significance of immunomodulating impact. Moreover, direction of immunocorrection depended on the baseline levels of certain parameters. Increase in low initial values as well as normalization of enhanced parameters resulted from Polyoxidonium therapy in a majority of cases.

Conclusion

The given research represents **open** study of Polyoxidonium effectiveness. Medical history data of participating patients were used for the clinical efficacy verification. Dynamic testing of immune status parameters in aged and senescent patients that did not display SID manifestations and therefore, escaped immunomodulatory therapy (control group patients) contributed to the assessment of variations in immunological findings that took place in this category of aged patients over 3 weeks period. The retrieved results provided evidence for the clinical effectiveness and immunomodulating impact of Polyoxidonium in aged and senescent patients.

Availability of two medicinal formulations of Polyoxidonium – solution for injections and rectal suppositories, had determined patient distribution into two different treatment groups. Those enrolled in the first group were treated with rectal suppositories and the second group participants were subjected to intramuscular injections of Polyoxidonium. Present research has shown that Polyoxidonium administered either intramuscularly or per rectum is well tolerated by the majority of aged patients. Besides, absence of worsening of biochemical blood and urine test findings corroborates the medication safety regarding liver and kidneys. Adverse effects secondary to medication use were not observed.

There was revealed the similar effectiveness of preseasonal preventive application of Polyoxidonium – either 6mg rectal suppositories given on alternate days, 10 suppositories per course, or intramuscular injections given at 6 mg single dose twice a week, 5 injections per course - in aged and senescent patients with clinical manifestations of SID. Aged and senescent patients from the both treatment groups achieved redoubled durability of chronic infection remission following preseasonal preventive course of Polyoxidonium. The remission lasted 4-5 months in the most patients. The exacerbation rate and duration lessening as well as decrease in hospitalization incidence were observed as well.

Hence, 30% of patients from each treatment group could not achieve clinical effects of Polyoxidonium. The most of these patients were older than 70 years whereas, only one third of Polyoxidonium responders had reached 70 years of age. Thus, the inverse correlation between the age and Polyoxidonium effectiveness was supposed.

The present study has demonstrated Polyoxidonium influence on immune status parameters of aged patients suffering from the clinical manifestations of secondary immune deficiency. Different medicinal formulations – injection solution and suppository – have produced the similar results in respect with the nature and the profundity of immunomodulatory influence.

Characteristics of Polyoxidonium impact on the immune status parameters of aged and senescent patients with the clinical manifestations of secondary immune deficiency claim the immunomodulatory effect of the medication: increase in lessened immune status values and normalization of enhanced findings. The increase in absolute count and percentage of T lymphocytes with CD3+ CD4+ immunophenotype, enhancement of CD4+/CD8+ ratio as well as elevation of serum immunoglobulin – IgA and IgG levels – especially, in patients with low initial levels of the respective parameters appeared to be the most pronounced effects exerted by the probationer medication. Polyoxidonium therapy resulted in achievement of normal values of differential white blood cell count and of phagocytic capability of monocytes in the cases with increased baseline findings of mentioned parameters.

It is conceivable that the observed changes are related to the Polyoxidonium therapy as the control group patients did not exhibit significant changes in pointed immunological parameters on repeated testing at a lapse of 3-4 weeks period.

Preseasonal preventive course of Polyoxidonium on different routes of medication administration (intramuscular injections or rectal suppositories) in aged and senescent patients produced identical effects regarding the nature and the profundity of clinical and immunomodulatory influence of the medication. The retrieved results promote to recommend Polyoxidonium treatment in aged and senescent patients on the purposes of prevention of chronic inflammatory disease aggravations. However, the immunomodulatory effect of Polyoxidonium use might decrease with age as it has been described in concern with other immunocorrective therapies. Thus, 70 years and older patients can exhibit the least efficiency of Polyoxidonium treatment in comparison with younger patients.

The present research has demonstrated that changes in certain immune status parameters take place quite frequently in aged and senescent patients and that these alterations are not always accompanied by the development of secondary immune deficiency symptoms. Therefore, exploration of variations in immune status values of aged patients should not be considered as the indication of Polyoxidonium use. Though, advent of clinical manifestations of secondary immune deficiency in aged and senescent patients has proved to be the incentive for the preseasonal preventive administration of Polyoxidonium either rectally (6mg suppositories on alternate days, № 10 per course) or intramuscularly (6 mg injections intramuscularly bid week, № 5 per course) in order to hinder from the aggravation of chronic infectious process. Individual peculiarities of the patient's overall status, possibility to visit a doctor and patient's willingness to undertake treatment must be taken into account during the selection of the medicinal formulation of Polyoxidonium.

Thus, the preseasonal administration of preventive immunomodulatory therapy with Polyoxidonium to aged and senescent patients with clinical manifestations of secondary immune deficiency contributes to the significant decrease in the exacerbation rates of chronic infections and to the improvement of quality of life in this category of patients.

9. Conclusions:

Results of the clinical effectiveness investigation of two different medicinal formulations (solution for injections and rectal suppositories) of Polyoxidonium given at 6mg single dose to aged patients on purposes of prevention from the seasonal exacerbations of chronic inflammatory diseases have yielded the following conclusions:

- Good tolerance of both pharmacological formulations of the medication and absence of treatment-related adverse effects;
- Similar clinical effectiveness and improvement of immunogramm values on intramuscular as well as rectal application of Polyoxidonium;
- Redoubling of duration of chronic infection remission, significant (two times) decrease in the rate and durability of aggravations as well as in hospitalization incidence.

The present research data encourage to recommend Polyoxidonium use in accordance to abovementioned schedules – 6mg suppositories (№ 10) given on alternate days or intramuscular injections (№ 5-10) at 6 mg single dose bid week on purposes of preseasonal prevention from the worsening of chronic infections in aged patients.

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