Immunomodulatory effect of **p**leuran (β-glucan from *Pleurotus ostreatus*) in children with recurrent **r**espiratory **t**ract **i**nfections

IPRRTI study

Study Protocol

NCT number: NA

Basic information about the study

Title of the study:	Immunomodulatory effect of pleuran (β-glucan from *Pleurotus ostreatus*) in children with recurrent respiratory tract infections (RRTI).
Study abbreviation:	IPRRTI
Principal investigator and site:	
Co-investigators and sites:	
Study design:	Multicenter, prospective, randomized, double-blind, placebo-controlled study with a food supplement.
Study population:	250 patients aged 3-18 years enrolled by investigators at 36 centers, randomized to the active and placebo groups (1:1 ratio)
Primary endpoints:	To evaluate the effect of pleuran administration (Imunoglukan P4H® chewable tablets) compared to the placebo group on: • reduction in the number of RTIs episodes (total number)
Secondary endpoints:	To evaluate the effect of pleuran administration (Imunoglukan P4H® chewable tablets) compared to the placebo group on: • reduction in the number of episodes of RTI subtypes • reduction in the duration of RTI episodes (total duration, RTI subtypes) • reduction of the need for antibiotic (ATB) therapy • reduction of the number of missed days at school/nursery due to RTI • reduction of the number of missed working days due to RTIs • reduction of the number of emergency department visits due to RTI • reduction of the number of physician visits due to RTI • tolerability and safety

Timeline

Pre-screening of patients: July-September 2023
Date of enrolment of the first patient: 15.09.2023
Date of enrolment of the last patient: 31.12.2023
Study completion date: 31.03.2024

Study duration and visit plan: 3 months, 4 visits in total: V0 – initial visit, V1 – control visit after 30±7 days from enrolment, V2 – control visit after 60±7 days from enrolment and V3 – control visit after 90±14 days from enrolment (completion).

Introduction

Respiratory tract infections (RTIs) are the most common cause of morbidity at every age category, especially in childhood. At the same time, they are also the most common cause of consultations and visits in clinical practice of every pediatrician. Upper respiratory tract infections have a higher prevalence, and about 10-30% of patients develop lower respiratory tract infections. The most common cause are viruses (90-95%).
Respiratory infections are characterized by both microbial load, respiratory epithelium response, and activation of inflammation and immune responses. These aspects lead to structural and functional changes in the respiratory tract. There is an overgrowth of goblet-shaped glands, which leads to excessive production of hyperviscous mucus. There is a weakening of mucociliary clearance, either due to the direct toxic influence of microorganisms on cilia and their oscillation, but also indirectly through an increase in the viscosity of mucus in the lumen of the respiratory tract. Respiratory epithelial cells form many pro-inflammatory and chemotactic compounds that lead to the development of inflammatory infiltrate in the airways. This is accompanied by oxidative stress, which impairs both the structure and function of biomolecules. Inflammation subsequently increases the reactivity of cough receptors. All these factors cause clinical signs of respiratory infections, whether from the respiratory tract (cough, expectoration, obstruction with wheezing, bronchial hyperreactivity, congestion and runny nose) or at the level of the whole organism (increased body temperature, weakness, myalgia and fatigue).

Recurrent respiratory tract infections (RRTIs)

RRTIs are a separate category of RTIs and represent a typical medical problem of preschool and school age. They can be defined on the basis of several aspects as infectious respiratory diseases, in which other more serious causes have been excluded (cystic fibrosis, primary immune disorders, congenital ciliary dyskinesias, respiratory malformations). Most children with RRTIs do not suffer from severe immunodeficiency and the high frequency of respiratory infections is due to a combination of various factors (immaturity of the immune system, high exposure to infectious agents in the family or children's collective, environmental factors, adenoid hypertrophy, etc.). In addition, allergic inflammation in the airways may also contribute to increased susceptibility to RTIs.

According to data from epidemiological studies, approximately 6-10% of children between 2-6 years of age suffer from RRTIs. Due to their high incidence, RRTIs have an important socio-economic impact (affecting quality of life, missed school days, missed days at work, repeated medical examinations, hospitalization, as well as increased antibiotic prescription).

However, it is equally important to define what the term RRTIs means, as the parent’s opinion and a doctor's point of view can differ significantly. When assessing RRTIs, in addition to their frequency, their course, duration, response to pharmacological treatment, cultivation of the pathogen as well as the development of complications are much more important. At the same time, the so-called "physiological morbidity of the child" must also be taken into account, which is considered to be:

- **Children 3-5 years**: 6 – 8 RTIs with mild course, good response to treatment, predominantly during the autumn and winter months
- **Children 6-12 years:** 2 – 4 RTIs with mild course, good response to treatment, predominantly during the autumn and winter months

The treatment of RRTIs consists not only from the treatment of each infection, but should be mainly aimed at their prevention. Therapeutic and preventive strategies should aim to support weakened immune system functions, e.g. by **immunomodulation**. Immunomodulation can be defined as therapeutic or preventive intervention in the activity of the immune system in order to achieve homeostasis.

In practice, various preparations of natural origin are used to prevent RRTIs, but only few of them have scientific evidence proving efficacy with a sufficient number of relevant clinical studies and observations that elucidate individual aspects of the mechanisms of action as well as proven efficacy and safety in the management of individual diseases. Vitamins, minerals, as well as many natural immunomodulatory preparations (phytotherapy, probiotics, prebiotics, biologically active polysaccharides) are most commonly used, while individual biological effects are important – anti-infectious, immunomodulatory, anti-inflammatory, antioxidant, etc. By using these preparations or their combination, a synergy of effects can be obtained in the treatment of the selected condition.

Biologically active polysaccharides (BAP e.g., β-glucans - polymers of glucose commonly found in fungi, yeast, bacteria and cereals) represent one of the most studied natural immunomodulators with pluripotent biological effects (anti-infectious, antiviral, immunomodulatory). Selected molecules have an anti-inflammatory effect, inhibit the production of pro-inflammatory cytokines, and their preventing effect on lung damage was described in an animal model. By promoting the production of anti-inflammatory and regulatory substances, they restore balance in the cytokine network. The antiviral effect of BAP can be mediated either directly, through inhibition and/or disruption of viral particle integrity, or indirectly, by enhancing host antiviral defenses by supporting the body's immune processes. Other significant effects of β-glucans include their significant immunomodulatory effect (increasing the number of NK cells, stimulating phagocytic activity, promoting the development of antibody production, improving the post-vaccination response) and modulating effect on T-lymphocytes. The mechanism of action of β-glucans is mediated by several receptors, the most important is the Dectin-1 receptor.
Zinc application may also have a possible positive effect on the immune system, as several studies have suggested its effect in coronavirus infections. In vitro, zinc inhibits RNA-polymerase activity as well as virion replication. Vitamin D is also important for the functioning of the immune system, its positive effect has been observed in protection against infections (antibacterial effect, anti-inflammatory effect, differentiation of monocytes into macrophages), tumor diseases (anti-proliferative and antidifferentiation effect), and its deficiency may be a risk factor in the development of immunopathological reactions (autoimmune diseases).

The efficacy and safety of food supplement Imunoglukan P4H® (pleuran, β-glucan from *Pleurotus ostreatus*, enriched with vitamin C) has been proven in numerous clinical studies and observations in children and adults, and it is therefore a suitable choice for supporting weakened immunity for both short-term and long-term use. Open-label and double-blind, placebo-controlled studies in children with recurrent respiratory infections confirmed the preventive effect of Imunoglukan P4H® on the incidence and intensity of recurrent bacterial and viral infections in children from 1 year of age. Imunoglukan P4H® has also shown the ability to induce changes in specific and non-specific cellular immunity parameters, which have reflected into a reduced incidence of respiratory infections in children and adults, prevention of immunosuppression induced by intense physical stress, and reduction of the incidence of infectious exacerbations in patients with chronic inflammatory diseases.

Supplementation with Imunoglukan P4H® was also able to achieve significant changes in markers of allergic inflammation in children (total IgE, blood eosinophil count), thus confirming previous results of in vitro tests, which indicated the anti-allergic potential of this active substance. Taking Imunoglukan P4H® supports physiological functions and maturation of the immune system in children and does not

cause its overstimulation. Imunoglukan P4H® ACUTE! (pleuran + vitamin C + zinc) confirmed its immunomodulatory effect in shortening the duration of symptoms in patients with herpes simplex virus type 1 (HSV-1) infection.

The results of clinical studies have confirmed the complex immunomodulatory effect of this preparation without any significant side effects.

Study design

It is a multicenter, prospective, randomized, double-blind, placebo-controlled study with a food supplement at an outpatient healthcare provider.

The investigators will enroll a total of **250 patients** who meet the inclusion criteria. The proposed study does not imply any change in pharmacological/non-pharmacological treatment, diagnostic interventions administered to enrolled subjects. In case the respiratory tract infections occur during the study period, the patient enrolled in the study will be treated according to standard management plan.

Patients will be randomized into 2 arms (active group and placebo group):

- **Active group: Imunoglukan P4H® chewable tablets** (IMG® 50 mg + Zinc 5 mg + Vitamin D 10 µg in 1 tablet):
    - up to 25 kg of body weight 1 tablet once a day for 3 months
    - over 25 kg 2 tablets once a day for 3 months
- **Placebo group: Placebo chewable tablets** (Zinc 5 mg + Vitamin D 10 µg in 1 tablet)
    - up to 25 kg of body weight 1 tablet once a day for 3 months
    - over 25 kg 2 tablets once a day for 3 months

Discussion about design

Multicenter, prospective, randomized, double-blind, placebo-controlled study design is a perpective for obtaining clinically, scientifically and statistically significant results to elucidate the impact of the investigational product on primary and secondary endpoints. The 3-month duration of the study is justified in evaluating the modulation of the immune response and its clinical impact on such events as a reduction in the number of respiratory infections, shortening their duration, and reducing the need for ATB treatment. In addition to confirming the underlying hypothesis, the pilot study should also provide data on tolerability, safety and possible adverse effects.

Subjects enrolled in the study complete an anamnestic data collection and fill out relevant questionnaires during control visits.

Study endpoints

Primary endpoints – to evaluate the effect of pleuran administration (Imunoglukan P4H® chewable tablets) compared to the placebo group on reduction in the number of RTIs episodes (total number)

Secondary endpoints – to evaluate the effect of pleuran administration (Imunoglukan P4H® chewable tablets) compared to the placebo group on:

- reduction in the number of episodes of RTI subtypes
- reduction in the duration of RTI episodes (total duration, RTI subtypes)
- reduction of the need for antibiotic (ATB) therapy
- reduction of the number of missed days at school/nursery due to RTI
- reduction of the number of missed working days due to RTIs
- reduction of the number of emergency department visits due to RTI
- reduction of the number of physician visits due to RTI
- tolerability and safety

Inclusion criteria

- signed informed consent
- age 3-18 years
- history of recurrent respiratory infections:
    - **Age 3 to 5 years** (< 6 years): 6 or more upper or lower respiratory tract infections from October 2022 until the end of March 2023
    - **Ages 6 to 18 years** (≥ 6 years): more than 3 upper or lower respiratory tract infections from October 2022 until the end of March 2023

Exclusion criteria

- refused informed consent
- bronchopulmonary dysplasia
- primary immunodeficiency
- cystic fibrosis
- chronic diarrhoea
- chronic diseases of the lungs, kidneys and liver
- malformations of the cardiovascular system
- oncological disease
- malnutrition
- intolerance to any of the ingredients in the study product
- taking preventive immunomodulatory therapy (beta-glucans, bacterial lysates, isoprinosine) less than 14 days prior enrolling the patient into the study

List of data collected from study subjects

Basic identification data	○ Patient ID ○ Patient Initials ○ Patient's Date of birth ○ Date of completion of CRF / Date of visit
Medical history and demographics	○ Age ○ Sex ○ Current comorbidities ○ Current treatment of comorbidities
Diagnostic procedures	○ Patient diary (Respiratory infections)
Primary endpoints	○ RTIs incidence (total)
Secondary endpoints	○ RTIs subtypes incidence ○ Duration of RTIs (total duration, RTIs subtypes) ○ The need for ATB therapy for the treatment of RTIs

	○ Number of missed days in school/nursery due to RTIs ○ Number of missed working days due to RTIs of the child ○ Number of Emergency department visits due to RTIs ○ Number of Physician visits due to RTIs ○ Tolerability ○ Safety (occurrence of adverse events)
Other data	○ Compliance

Study visit and evaluation plan

Visit number	**V0**	**V1**	**V2**	**V3**
Day of visit	Initial examination	after 1st month (30 ±7 days)	after 2nd month (60 ±7 days)	after 3rd month (90 ±14 days)
Informed consent	X			
Inclusion / exclusion criteria	X			
Demographics	X			
Medical history	X			
Comorbidities	X	X	X	X
Dispensing of study product	X			
Incidence of respiratory tract infections (Patient diary)		X	X	X
Adverse events		X	X	X
Returning the study product, product accountability and patient compliance				X

Required diagnostic procedures

Patient diary "Respiratory tract infections"

Parents or legal representatives of study subjects will record up-to-date data (incidence of infectious morbidity, symptoms, need for ATB therapy, physician or emergency department visits, number of missed days at school/nursery and work due to RTIs) throughout the study period. The filled "Patient diary" will be handed over to the treating physician at each visit.

Adverse event form

The physician fills out the Adverse event form when adverse events occur and records the occurrence of adverse events also in the CRF.

Compliance

Study subjects will return the container with remaining study medication at V3 (visit after 3 months) to their treating physician, who will count the number of remaining tablets. The physician will record the result in CRF. Patients with <75% compliance will be excluded from the study due to lack of collaboration.

Appendices to the study protocol, other changes in the conduct of study

Any amendments to the Protocol will be made in the form of an Appendix to the Protocol. Changes to the study are not permitted. Any unexpected and sudden changes in the conduct of study will be recorded in the clinical report.

Privacy Policy

All patients in this study will be identified with a unique ID. Identification of the patient through this ID will not be possible. Only the monitor can validate the source data. The unique ID of a particular patient will only be known to the investigator or monitor. The personal identification data entered into this study will be: date of birth, gender and initials. The nature of personal data identifiers is governed by all relevant legislative requirements.