

BMJ Open Protocol of the study: the effectiveness of pleuran in the treatment of acute gastroenteritis in children – a randomised, placebo-controlled, double-blind trial (EPTAGE)

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ABSTRACT

Introduction Acute gastroenteritis is one of the most common causes of children's morbidity and mortality globally. Oral or intravenous rehydration was proven effective in reducing the mortality rates in acute gastroenteritis, although it does not affect the course of the disease. Attempts to identify new therapeutic methods effective in reducing the symptoms of diarrhoea are of interest. Pleuran's potential immunomodulatory effect in acute gastrointestinal infection relies on the stimulation of innate immunity. The effectiveness of pleuran (β -(1,3/1,6)-D-glucan) administration to treat acute infectious diarrhoea remains unknown. This study evaluates the efficacy of pleuran in reducing diarrhoea duration and the severity of acute gastroenteritis symptoms in children.

Methods and analysis Our study is a randomised, double-blind, placebo-controlled superiority trial with two parallel groups and a 1:1 allocation ratio. A total of 120 children aged 2–10 years hospitalised or requiring a visit to the emergency department because of acute gastroenteritis will be randomly assigned to receive either pleuran oral suspension in the experimental group or matching placebo in the control group. The primary outcome measure will be the duration of diarrhoea. We will analyse the results in both intention-to-treat and per-protocol approaches.

Ethics and dissemination The Bioethics Committee of The Medical University of Warsaw approved the study protocol (approval number: KB/45/2018). Written informed consent of the patients' caregivers participating in the study will be obligatory. The results of this study will be published in a medical journal, regardless of whether they confirm or deny the research hypothesis.

Trial registration number NCT03988257; Pre-results.

INTRODUCTION

Background

Acute gastroenteritis (AGE) remains a significant problem, an important cause of hospitalisation and one of the leading causes of paediatric mortality in developing countries.^{1,2} In 2004, AGE led to around 1.8 million deaths of children below 5 years worldwide.²

Strengths and limitations of this study

- A precise clinical question has been posed to fill a gap in knowledge as to whether pleuran effectively reduces the duration and alleviates diarrhoea symptoms.
- The study design (randomised controlled trial) is the most robust methodology to assess the effectiveness of therapeutic interventions.
- The stratified randomisation using two subgroups ensures an equal distribution of confounders in the experimental and the control groups in terms of diarrhoea duration at the time of enrolment.
- Limited monitoring of the adherence to the protocol after discharge from hospital (no procedure of preparation return).
- Follow-up and endpoint data collection conducted via phone may be a source of error.

The mortality due to AGE in developed countries is much more lower than in developing ones²; however, it remains a common cause of paediatric consultations and hospitalisations. In the USA and the European Union, the number of admissions due to AGE in children aged <5 years accounts for 13% and 11% of all hospitalisations in this age group, respectively.^{1,3}

According to the PROTECT (Pediatric ROTavirus European CommitTee) report published in 2006, the median length of hospital stay (LOS) for rotaviral infection in Europe is 4.8 days.³ Besides high costs, prolonged hospitalisation carries other risks for a child, including exposure to infectious agents, stress and catheter-related bloodstream infection. Furthermore, the duration of a child's illness contributes to the loss of parental working days.³ Considering all the above factors, the reduction of LOS is a desirable goal.

Treatment of AGE in children

Appropriate rehydration is recommended as the first-line treatment in AGE, preferably with oral rehydration solution (ORS).⁴ Nonetheless, ORS does not reduce the duration of AGE and its severity.⁴ Many studies have shown the influence of specific probiotic strains on shortening the length of acute diarrhoea and alleviating its symptoms. However, their heterogeneity and other methodological limitations make it impossible to draw definite conclusions and formulate clear recommendations for the use of probiotics.^{5–9} Many other potential antidiarrhoeal drugs are currently being investigated, but the data collected so far are insufficient to introduce them to routine gastroenteritis management. Diosmectite and racecadotril have been proven to shorten the diarrhoea duration, although the quality of evidence is low.^{10–11} The use of zinc may be beneficial only in children aged above 6 months in developing countries,^{12–13} while the reduction of diarrhoea duration following the use of lactose-free products was demonstrated only in hospitalised patients and the quality of evidence was low.^{13–14} New, good-quality data on the effect of symbiotics on the diarrhoea duration are promising, but needed to be repeated.¹³ In light of the above, further attempts to identify other therapeutic methods effective in reducing the length and the severity of AGE symptoms are necessary.

Mode of action of Pleuran

Pleuran (β -(1,3/1,6)-D-glucan) is an insoluble polysaccharide belonging to the group of glucose polymers commonly called β -glucans isolated from *Pleurotus ostreatus*.¹⁵ Similar to other β -glucans, pleuran presents immunostimulating effects on the organism, by activating immune cells of gut-associated lymphoid tissue in the distal jejunum and the ileum. The β -1,3 glucan side chain binds to glycoprotein receptors Dectin-1, TLR and CR3 on an immune cell surface, especially macrophages, monocytes and dendritic cells, stimulating their phagocytic capacity and inflammatory cytokine production (mainly tumour necrosis factor alpha, interleukin (IL) 2, IL-10 and IL-12).¹⁶ These cytokines activate the humoral immune response by mobilising B lymphocytes and enhancing cellular immunity by stimulating natural killer (NK) cells.¹⁶ β -glucans have also been shown to enhance mucosal immunity, causing an increase in the epithelial lymphocytes of digestive system.¹⁷

Effects of Pleuran

Recent data show that pleuran could play a significant role in complementary therapy in various diseases and pathological conditions.¹⁶ Its anti-infective properties have been assessed in several trials.

The randomised controlled trial (RCT) by Jesenak and colleagues demonstrated a significant reduction in respiratory tract infections in the experimental group compared with that in the control group.¹⁸ A group of 175 children aged 2–10 years, with a history of recurrent respiratory tract infections (RRTI), were randomly

allocated to receive the pleuran or placebo for 6 months and were followed up for another 6 months. The main result was a significant reduction in respiratory morbidity in the pleuran group. In another three open-label prospective studies, after 3 months of pleuran supplementation, a statistically significant reduction in the incidence of respiratory infections in children with RRTI, compared with the previous infection season in the same group, was observed.^{19–21}

The anti-infective activity of pleuran was demonstrated in a multicenter RCT enrolling 90 patients aged above 6 years (including adults) with acute herpes simplex facial/labial infection.²² After 10 days of pleuran administration, a reduction in the symptom duration was observed, compared with placebo. However, there was no significant difference in efflorescence size and herpes symptoms severity between groups after 10 days of treatment.

A multicenter, 'split-body' study (n=80)²³ proved the efficacy of pleuran in reducing the duration and severity of atopic dermatitis exacerbations and pruritus in adults.

In the RCT by Bergendiova and colleagues, 50 adult athletes were assigned to receive either pleuran or placebo for 3 months and were followed up for another 3 months. A significant reduction in the number of upper respiratory tract infections in the experimental group was observed.²⁴ Additional laboratory evaluation of selected immunologic parameters showed an increase in NK cells and the regulation of phagocytosis.²⁴ Another RCT of 20 healthy athletes demonstrated no reduction of NK cell activity after intensive exercises in the group receiving pleuran for 2 months, compared with the placebo group.²⁵

No study has evaluated the effectiveness of pleuran in the treatment of AGE in children. However, considering the intestinal mucosa as the site of initiation of the immunomodulatory action of β -glucan, and described mechanisms of its action, we have speculated that pleuran could facilitate the elimination of both viral and bacterial diarrhoeal pathogens, as well as infected host cells, therefore alleviate and shorten the duration of acute infectious diarrhoea in children.

Objectives

This study aims to evaluate the efficacy of pleuran in reducing diarrhoea duration and the severity of AGE symptoms in children. To ensure high reliability of results, we decided to use a placebo as a comparator. In the study, we used a commercially available preparation, that is, a combination of pleuran with vitamin C. To evaluate the effectiveness of pleuran alone in treating AGE, vitamin C is also a placebo component. To our knowledge, there are no reports of vitamin C influencing the course of infectious diarrhoea in children.

METHODS

Research hypothesis

Pleuran is more effective than a placebo in reducing the duration and severity of diarrhoea in children.

Trial design

We have designed EPTAGE as a randomised, placebo-controlled superiority trial with two parallel groups and a 1:1 allocation ratio. The WHO Trial Registration Data Set can be found as an online supplemental file 1.

Study settings

We will recruit the study participants from patients requiring hospitalisation in four paediatric departments or counselling in the emergency department (ED) due to AGE in the Paediatric Teaching Clinical Hospital, The Medical University of Warsaw. We may extend the study settings to include additional paediatric departments in Warsaw hospitals.

Eligibility criteria

Inclusion criteria

- ▶ Children aged 13–120 months, hospitalised or requiring counselling in ED due to AGE, defined as a decrease in the stool consistency (grade 6 or 7 in Bristol Stool Form Scale²⁶) or an increase in the frequency of stool evacuations >3 in 24 hours.⁴
- ▶ Duration of symptoms 24–72 hours at the time of inclusion.
- ▶ Caregiver's written, informed consent.

Exclusion criteria

- ▶ Duration of symptoms >72 hours at the time of inclusion.
- ▶ Co-occurring other systemic infectious diseases (eg, pneumonia and sepsis).
- ▶ Diagnosed immune deficiency.
- ▶ Malnutrition (defined as the body mass index (BMI) ≤ -2 SDS (SD Scores) or <3rd percentile based on the WHO percentile charts).²⁷
- ▶ Chronic diarrhoeal gastrointestinal diseases (eg, inflammatory bowel disease, short bowel syndrome, cystic fibrosis, coeliac disease and food allergy).
- ▶ Use of antibiotics, probiotics, prebiotics or antidiarrhoeal medicines (eg, diosmectite and racecadotril) within 7 days before enrolment into the study.
- ▶ Use of probiotics, diosmectite or racecadotril during the current AGE episode.
- ▶ Use of pleuran in the 14 days before enrolment into the study.
- ▶ Parallel involvement of the patient in other clinical trials, except for observational studies.
- ▶ No legal caregivers' consent to participate in the study.

Rationale for age-group selection

The preparation used in our trial, in other studies conducted so far, has been used with good tolerance from 1 year of age (13 months of age). Our study was not intended to evaluate the formulation in infants, and this group was excluded from the study. Children above 5 years of age have a lower risk of severe illness and dehydration due to AGE. However, during the first 6 months of the study, as many as 24% of patients hospitalised due to AGE were >6 years of age; hence, we concluded that

this group could also benefit from the intervention. This was the reason for the expansion of age inclusion criteria to 10 years.

Interventions

Pre-intervention assessment

We will evaluate the study's inclusion and exclusion criteria within 24 hours of hospitalisation or ED visit. The BMI will be calculated and converted into percentile using the WHO percentile charts. To determine the aetiology of AGE, we will collect the stool samples for viral tests (rotavirus, adenovirus and norovirus markers) before the intervention. If indicated (according to the European Society for Paediatric Gastroenterology, Hepatology and Nutrition - ESPGHAN recommendations⁴), we will also perform the microbiological stool tests. The caregivers will be informed about the purpose, methods and safety of the intervention.

Scales used

We will apply appropriate scoring systems to ensure that the assessed parameters and endpoints are comparable. We will use the Modified Vesikari Score to assess the severity of diarrhoea.²⁸ The dehydration degree will be evaluated using the WHO scale²⁹ and the Clinical Dehydration Scale (CDS).³⁰ If the results of these scales are different, we will consider more severe one for dehydration management. The Bristol Stool Form Scale will help to objectify the assessment of stool consistency.²⁶

Intervention

Children allocated to the experimental group will receive Imunoglukan PH4 oral suspension (10 mg of pleuran and 10 mg of vitamin C in 1 mL of syrup) in a dose of 1 mL/5 kg body weight. In comparison, patients in the control group will receive a placebo: a vitamin C oral suspension (10 mg of vitamin C in 1 mL of syrup) in a dose of 1 mL/5 kg body weight. Both suspensions will be administered once daily in the morning before the first meal, until signs of AGE subside (<3 stools/24 hours, and normalisation of stool consistency: grades 2–5 according to the Bristol Stool Form Scale) or until the 14th day of the intervention.

Concomitant treatment

Patients in both groups will receive ORS or intravenous fluids if required. A regular diet will be delivered. Each patient will receive antipyretic, analgesic (ibuprofen or acetaminophen) and antiemetic (ondansetron) if necessary. Patients will not receive other medicines that may interfere with the disease course (probiotics, diosmectite, or racecadotril). If indications occur, the patient will receive an antibiotic according to the ESPGHAN recommendations.⁴

Intervention modification

We will repeat the dose of the study preparation in the event of vomiting up to 30 min of administration. We may precede the repeated treatment with an antiemetic

(ondansetron). In the case of emesis after 30 min of administration, we will consider the dose to be absorbed. If any severe or persistent, or bothersome adverse event (AE) occurs, we will stop the intervention. A mild allergic reaction will be treated with an antihistamine drug. The intervention will be stopped in case of severe allergic reaction. If the child does not tolerate the preparation, we may mix it with milk, tea or fruit juice.

Outcomes

Primary outcome measure

The duration of diarrhoea, defined as the time (hours) until normalisation of stool consistency (grades 2, 3, 4 or 5 according to the Stool Form Scale²⁶) and until normalisation of number of stools per day. The number of stools per day and their consistency will be assessed daily until the 14th day of intervention by the physician and patient's caregiver.

Secondary outcome measure

1. The number of days with the need for intravenous rehydration. The indication for intravenous rehydration will be assessed daily until the discharge from the hospital by a physician, based on the CDS³⁰ and WHO²⁹ dehydration scales, and the possibility of oral rehydration.
2. The number of hours until normalisation of stool consistency (type 2–5 according to the Bristol Stool Form Scale²⁶). The stool consistency will be assessed daily until the 14th day of observation based on the diary completed by the physician and the caregiver.
3. The number of hours until the normalisation of number of stools per day (<3 stools/day). The number of stools per day will be assessed daily until the 14th day of observation, based on the diary completed by the physician and the caregiver.
4. The duration of hospitalisation due to AGE (number of days) in hospitalised children. The indications for discharge from the hospital will be assessed daily by the physician until the 14th day of observation.
5. The percentage of children with moderate and severe diarrhoea, assessed by the Modified Vesikari Scale²⁸ after the 14th day of observation.
6. The number of hours until the child's general condition improved according to the caregiver's daily assessment until the 14th day of observation.

Participant timeline and follow-up

During hospitalisation or ED visit, the patient will be examined by the physician, assessing dehydration status and the need for intravenous rehydration or concomitant treatment. In hospitalised children, the assessment will be performed every day. We will record the results of the evaluation in the Case Report Form (CRF). During and after hospitalisation, the caregiver will record the AGE symptoms in a diary (number of stools per day, their consistency and accompanying symptoms, for example, fever, vomiting or abdominal pain). After discharge, the

physician will contact the patient's caregiver via phone daily or every second day until the 14th day of observation to collect data on the patient's symptoms intensity. After discharge, the caregivers will receive a consultation regarding oral rehydration and potential side effects.

Sample size

We used the Altman nomogram³¹ to estimate the sample size of the experimental and control groups. To demonstrate a clinically relevant difference of 24 hours in the duration of AGE symptoms in the experimental and control groups, with 5% significance level and a power of 90%, assuming the SD of the variable in each group to be 40 hours, a sample of 110 patients will be needed (55 patients in each group). Allowing for 10% attrition of participants during the study, we estimated the sample size as 120 patients (60 patients for each experimental and control group).

Recruitment

A team of physicians working at the Department of Paediatrics with Clinical Assessment Unit (department conducting the study) will recruit study participants among patients hospitalised or requiring counselling in ED due to AGE in the Paediatric Teaching Clinical Hospital, The Medical University of Warsaw, Poland. We will evaluate the inclusion and exclusion criteria in every patient admitted to four paediatric departments or ED due to AGE.

Randomisation

The randomisation will involve randomly allocating patients to group A (experimental) or group B (placebo). We have designed the stratified randomisation using two subgroups. The subgroups will be created depending on the duration of diarrhoea at the time of enrolment: 24–48 hours (short-lasting diarrhoea) and 48–72 hours (long-lasting diarrhoea). Each subgroup will be randomly allocated to the study groups (experimental and control), in blocks of four.

Allocation and blinding

The active product and placebo will be prepared, weighed, packaged in identical bottles and signed with random numbers by the manufacturer, according to the company's internal procedure (standard operating procedure), with the blinded meaning of numbers. The randomisation list delivered by the manufacturer will be deposited in a sealed envelope in a safe place in the central part of the department, maintained by an independent person not involved in the study. The study product will be delivered to the principal researcher in boxes of four, ensuring the contest of two active and two placebo samples in each box. We will use subsequent boxes to allocate consecutive patients within the subgroups. By opening each subsequent box, the bottles from the box will be randomly assigned to the next four patients in a subgroup by the researcher. The contents of the bottles will look and taste the same. Researchers, caregivers,

patients and a person responsible for the statistical analysis will be blinded to the intervention until completing the study. The unblinding will be permissible if a serious adverse reaction is suspected. In that case, the allocated intervention will be revealed based on the randomisation list. Any such situation will be documented and reasoned in an appropriate report.

Data collection, data management and confidentiality

We will create a coded, paper CRF containing all the scales and symptoms diaries for each patient. After completing the study, two independent researchers will transfer data from the CRFs to two electronic databases, to confirm the accuracy of the data. In the case of dropouts, data and endpoints will be collected until the dropout day, with a documented cause and dropout date. We will prepare electronic databases and CRFs using the Statistical Analysis System software. The collected data will be stored in areas with limited access. Confidentiality of data, including the personal data of the study participants, will be maintained.

Statistical methods

We will use descriptive statistics to summarise baseline characteristics. We aim to present the results of the study through intention-to-treat and per-protocol analyses. Comparing groups A and B in terms of primary endpoint (duration of diarrhoea) will be performed with Student's t-test if the p value of the Shapiro-Wilk test is >0.05 . Otherwise, we will use the Mann-Whitney U test. In the case of dropouts or not-completed observations before the 14th day of the study, the log-rank test will be performed. We will use the χ^2 test or Fisher's exact test to compare groups A and B in terms of incidence of AEs and diarrhoea severity. Statistical significance will be based on two-tailed tests with a p value of 5%. We will present all estimates with a 95% CI.

Data monitoring

The establishment of the Data Monitoring Committee or auditing was not considered in the study design. Based on the grant financing agreement, the Scientific Council of the Nutricia Foundation is authorised to inspect and control the study documentation. The study design does not include interim analysis. In case an unfavourable safety profile is observed, the principal investigator, in consultation with the researchers' team, will terminate the study prematurely, regardless of its stage.

Harms

In our study, an AE will be defined as any untoward medical occurrence in a participant during the study, without regard to the possibility of a causal relationship. According to a pre-established procedure, we will record all AEs occurring after the entry into the study and during the observation period in CRF and immediately report them to the manufacturer. AE will be considered as severe if any of the following occurs: death, life-threatening condition, severe or permanent disability, prolonged

hospitalisation or a significant hazard. The incidence of AEs in the experimental and control groups, as well as their characteristics, will be analysed and described after completion of the study.

Patient and public involvement statement

Patients and the public were not involved in the study commencement, design, recruitment, conduction or dissemination.

Research checklist

We used the Standard Protocol Items: Recommendations for Interventional Trials - SPIRIT checklist when writing our report.³²

ETHICS AND DISSEMINATION

Research ethics approval

The Bioethics Committee of The Medical University of Warsaw approved the study protocol and other requested documents (an information sheet for patient's caregivers, informed consent forms in local language version and information on personal data management).

Protocol amendments

Any modifications to the protocol which may affect the conduct of the study, a potential benefit for the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures or significant administrative aspects will require a formal amendment to the protocol. Such an amendment will be submitted to the Bioethics Committee of The Medical University of Warsaw for approval and reported to the platform ClinicalTrials.gov.

Consent or assent

Before recruiting a patient into the trial, all the information about the study, including the study's objective, design, methodology, the possibility of AEs, possible risks and benefits of taking part in the study, will be presented to the patient's caregiver by a trained physician. The patient's caregiver will receive an information sheet, including the study's main aspects, and the investigator's contact information. The physician will answer any questions regarding the study. The informed written consent will be collected from the patient's caregiver by the same physician. The patient's legal caregiver will have the opportunity to withdraw the patient from the study without consequences.

Dissemination policy

The results of this study will be published in a medical journal, regardless of whether they confirm or deny the null hypothesis. The study founders will not have any role in the decision to submit results. Information on completing the trial and its results will be indicated in ClinicalTrials.gov, where the study is already registered. After completing the study, patients' caregivers who have

expressed such a desire at the recruitment stage will receive information about the study results via email.

AMENDMENT NO 1

The presented protocol contains amendments approved by the Bioethics Committee on 11 May 2020.

The main reason for those changes was an unsatisfactory recruitment pace in the first 11 months of study commencement. A detailed analysis revealed the reduction in the number of hospitalisations due to AGE in our department compared to the previous year and difficulties in meeting the inclusion criteria. In the following months, the COVID-19 pandemic significantly limited the number of patients admitted to our hospital. By the time the changes were made, we had recruited only three patients into the study. For the listed reasons, we have introduced the following changes to the protocol:

1. The first version of the protocol assumed the recruitment of patients among hospitalised children only. The amendment added the possibility of recruitment also among children requiring counselling in the ED.
2. Initially, patients were recruited only at the Department of Paediatrics with Clinical Assessment Unit (the department initiating and conducting the study). The amendment introduced four other departments of the Paediatric Teaching Clinical Hospital, The Medical University of Warsaw, including the ED.
3. The age group of recruited patients, initially 13–60 months (up to 6 years) of age, was extended to older children of 13–120 months (up to 10 years) of age.

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Contributors All authors initiated the study design and its implementation, wrote the protocol, and have contributed to and approved the final manuscript. AP initially conceptualised the study. KW-Ł conducted the study. KW-Ł and AP provided statistical expertise and analysed the data with blinded statistician supervision. EK is a grant holder.

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Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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