Lek. Katarzyna Wzorek-Łyczko

Skuteczność pleuranu w leczeniu ostrej biegunki infekcyjnej u dzieci - badanie z randomizacją, kontrolowane placebo, prowadzone metodą podwójnie ślepej próby.

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki medyczne

Promotor: prof. dr hab. n. med. Ernest Kuchar

Promotor pomocniczy: dr n. med. Anna Piwowarczyk

Klinika Pediatrii z Oddziałem Obserwacyjnym

Warszawski Uniwersytet Medyczny

Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych

Warszawskiego Uniwersytetu Medycznego

Warszawa, 2025 r.

Słowa kluczowe:

Biegunka infekcyjna,

Beta-glukan,

Leczenie ostrej biegunki,

Odporność śluzówkowa,

Odporność nieswoista,

Badanie interwencyjne.

Key words:

Infectious diarrhoea,

Beta-glucan,

Management of acute gastroenteritis,

Mucosal immunity,

Innate immunity,

Interventional trial.

Badanie prowadzone w ramach grantu badawczego Fundacji Nutricia nr RG3-2018

Dedykacje i podziękowania

Prof. dr hab. n. med. Ernestowi Kucharowi

Pragnę wyrazić głęboką wdzięczność za okazaną życzliwość, wsparcie merytoryczne oraz nieustanną mobilizację do podejmowania wyzwań naukowych. Pańska otwartość, entuzjazm i umiejętność dostrzegania potencjału we współpracownikach były dla mnie nieocenionym źródłem inspiracji w trakcie realizacji niniejszej pracy.

Dr n. med. Annie Piwowarczyk

Z całego serca dziękuję za niezachwianą wiarę we mnie, niezwykłą determinację i nieustępliwość, które niejednokrotnie motywowały mnie do dalszego działania. Dziękuję także za wsparcie i opiekę w czasie mojej drogi do uzyskania specjalizacji w dziedzinie pediatrii oraz za bezcenną pomoc przy opracowywaniu niniejszej rozprawy.

Dr Zbigniewowi Lewandowskiemu

Dziękuję za życzliwość i cenne wskazówki na etapie projektowania badania. Pańska wiedza i doświadczenie pomogły mi przełożyć założenia teoretyczne na rzetelny i spójny plan badawczy.

Pracę tę z głęboką wdzięcznością dedykuję mojej Rodzinie

Przede wszystkim mojemu Mężowi – za nieustające wsparcie, cierpliwość i zrozumienie.
Moim Dzieciom – za radość i motywację, którą codziennie mi dajecie.
Moim Rodzicom – za fundament wartości, którymi kieruję się w życiu i pracy.
I Rodzeństwu – za obecność i słowa otuchy w kluczowych momentach.

Dziękuję Wam za to, że mogłam realizować swoje zawodowe ambicje, nie rezygnując z tego, co w życiu najważniejsze.

Wykaz publikacji stanowiących pracę doktorską

1. **Wzorek-Łyczko K,** Woźniak W, Piwowarczyk A, Kuchar E. The anti-infective effect of β-glucans in children. Int J Vitam Nutr Res. 2024 Jun;94(3-4):296-307. doi: 10.1024/0300-9831/a000793.

Punktacja MNiSW: 100 pkt,

Impact Factor: 2

2. **Wzorek-Łyczko K,** Piwowarczyk A, Kuchar E. Protocol of the study: the effectiveness of pleuran in the treatment of acute gastroenteritis in children-a randomised, placebo-controlled, double-blind trial (EPTAGE). BMJ Open. 2021 Mar 11;11(3):e042370. doi: 10.1136/bmjopen-2020-042370.

Punktacja MNiSW: 100 pkt,

Impact Factor: 3,006

3. **Wzorek-Łyczko K, Piwowarczyk A,** Woźniak W, Kuchar E, Szymański H. A randomised trial of pleuran in peadiatric acute gastroenteritis. Scientific Reports 2025 May 15;15(1):16912. doi: 10.1038/s41598-025-94893-3.

Punktacja MniSW: 140 pkt,

Impact Factor: 3,8

Łącznie punktacja MNiSW: 340 pkt

Łącznie Impact Factor 8,8

Spis Treści

1. Wykaz stosowanych skrótów

AGE (ang. acute gastroenteritis)- ostra biegunka infekcyjna

PRISMA - Preferred Reporting Items for Systematic reviews and Meta-Analyses

SPIRIT - Standard Protocol Items: Recommendations for Interventional Trials

CONSORT - Consolidated Standards of Reporting Trials

RCT (ang. randomised controlled- trial)- badanie z randomizacją

ORS (ang. oral rehydration solution)- doustny płyn nawadniający

ESPGHAN - European Society for Pediatric Gastroenterology, Hepatology and Nutrition

ESPID - European Society for Pediatric Infectious Diseases

IDSA - Infectious Diseases Society of America

NICE - National Institute for Health and Care Excellence

TLR - ang. Toll-like receptor

CR3 - ang. complement receptor 3

NK - ang. natural killer cell

2. Streszczenie w języku polskim

Niniejsza rozprawa obejmuje trzy publikacje dotyczące zastosowania β-glukanów w leczeniu ostrej biegunki infekcyjnej u dzieci.

Ostra biegunka infekcyjna (ang. *acute gastroenteritis*, AGE) stanowi istotny problem zdrowia publicznego na świecie i jest jedną z głównych przyczyn zachorowalności i śmiertelności w populacji dziecięcej. Chociaż doustne i dożylne nawodnienie skutecznie zmniejsza śmiertelność związaną z AGE, nie wpływa na przebieg samej choroby. W związku z tym istnieje pilna potrzeba opracowania nowych strategii terapeutycznych, które nie tylko złagodzą objawy, ale również wpłyną na czas trwania choroby. W tym kontekście wybraliśmy pleuran – β-(1,3/1,6)-D-glukan – jako potencjalny środek terapeutyczny w leczeniu biegunki. Pleuran należy do grupy β-glukanów, czyli polisacharydów o wysokiej masie cząsteczkowej, występujących w różnych naturalnych źródłach, takich jak grzyby, bakterie czy zboża, które wykazują szereg korzystnych efektów, w tym właściwości immunomodulujące. Pleuran znany jest ze swoich właściwości immunomodulujących, wzmacniających naturalną odpowiedź immunologiczną organizmu oraz korzystnego profilu bezpieczeństwa. Dotychczasowe badania wskazywały na jego skuteczność w leczeniu infekcji wirusowych i bakteryjnych, jednak jego znaczenie w leczeniu AGE nie zostało zbadane.

Celem tej serii artykułów była ocena działania przeciwinfekcyjnego β-glukanów, na przykładzie pleuranu w leczeniu AGE u dzieci.

Pierwszą pozycję cyklu publikacji stanowi przegląd literatury typu scoping review, w którym podsumowaliśmy aktualną literaturę dotyczącą skuteczności β-glukanów w leczeniu infekcji u dzieci. Do przygotowania przeglądu użyto listy kontrolnej PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses, Extension for Scoping Reviews). Przeszukano bazy danych PubMed, Embase i Cochrane do maja 2021 r., identyfikując 6232 potencjalne publikacje, z których dwanaście zostało ostatecznie włączonych do analizy. Heterogeniczność analizowanych badań, obejmująca zarówno rodzaje stosowanych preparatów, ich dawki, jak i protokoły badań, uniemożliwiła przeprowadzenie przeglądu systematycznego zgodnego z przyjętymi standardami

metodologicznymi. W związku z tym podjęto decyzję o zastosowaniu przeglądu typu scoping review, który pozwala na szerszą eksplorację dostępnych danych w warunkach znacznej różnorodności badanych zmiennych. W dziesięciu badaniach wykazano skuteczność β-glukanów w zmniejszeniu częstości infekcji dróg oddechowych lub łagodzeniu objawów infekcji. Dziesięć badań potwierdziło dobrą tolerancję i profil bezpieczeństwa. Wysunęliśmy wniosek, że dobra tolerancja β-glukanów wskazuje na pozytywny bilans korzyści i strat tego typu interwencji w leczeniu ostrych infekcji dróg oddechowych u dzieci. Chociaż wiadomo, że β-glukany działają przede wszystkim poprzez stymulację odporności w obrębie jelitowego układu chłonnego, dotychczasowe badania nie analizowały ich skuteczności w leczeniu ostrych zakażeń przewodu pokarmowego u dzieci. Przeprowadzona analiza literatury pozwoliła nie tylko zidentyfikować tę istotną lukę badawczą, ale również pomogła w określeniu odpowiedniej dawki β-glukanu zastosowanej w naszym badaniu.

Druga publikacja jest to protokół badania mającego na celu ocenę skuteczności pleuranu w skracaniu czasu trwania i łagodzeniu objawów biegunki. Badanie było realizowane w ramach grantu badawczego Fundacji Nutricia nr RG3-2018. Badanie zostało zaprojektowane jako w pełni zaślepione badanie z randomizacją, kontrolowane placebo. Opracowując protokół wykorzystano listę kontrolną SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials). Przed rozpoczęciem rekrutacji badanie zostało zarejestrowane w bazie Clinical Trials. Grupę badaną stanowiły dzieci w wieku od 2 do 10 lat, które zgłosiły się do szpitala z biegunką trwającą od 24 do 72 godzin. Dzieci spełniające kryteria włączenia zostały losowo przydzielone do grupy leczonej preparatem pleuranu albo do grupy placebo. Wszyscy uczestnicy otrzymywali badany preparat codziennie aż do ustąpienia biegunki. Pierwotnym punktem końcowym było określenie czasu trwania biegunki – od jej początku do momentu unormowania konsystencji i częstotliwości stolców. Rekrutacja do badania rozpoczęła się w czerwcu 2018 r. i zakończyła w grudniu 2022 r. Z powodu niezadowalającego tempa i nieprzewidzianych ograniczeń w rekrutacji pacjentów, protokół był trzykrotnie modyfikowany w trakcie realizacji badania. Wprowadzone zmiany obejmowały rozszerzenie kryterium wieku uczestników z pierwotnego zakresu 2–6 lat do 2–10 lat oraz dodanie dwóch nowych

ośrodków rekrutujących. Każda z modyfikacji została zatwierdzona przez Komisję Bioetyczną Warszawskiego Uniwersytetu Medycznego.

Wyniki badania przedstawiono w trzecim artykule. Badanie zostało opisane zgodnie z wytycznymi CONSORT. Wzięło w nim udział 27 dzieci w wieku 2-10 lat, które losowo przydzielono do grupy eksperymentalnej (grupa A, 13 pacjentów) albo kontrolnej (grupa B, 14 pacjentów). Jedno dziecko zostało wykluczone z analizy z powodu utraty kontaktu z opiekunami. Punktami końcowymi były: czas trwania biegunki, konieczność nawodnienia dożylnego, długość hospitalizacji oraz nasilenie objawów. Nie zaobserwowano istotnych różnic między grupami w zakresie punktów końcowych. Zgłoszono jedno łagodne działanie niepożądane (wysypkę) w grupie eksperymentalnej. W toku badania wykazano, że pleuran nie skraca czasu trwania AGE ani nie łagodzi jej objawów u dzieci. Zgodnie z naszą wiedzą, była to pierwsza i jedyna randomizowana, kontrolowana placebo próba kliniczna oceniająca skuteczność pleuranu lub innych β-glukanów w leczeniu ostrej biegunki infekcyjnej u dzieci. Silnymi stronami badania był jego randomizowany, w pełni zaślepiony i kontrolowany placebo charakter. Wdrożenie listy kontrolnej SPIRIT i wytycznych CONSORT zapewniło prawidłową metodykę badania i raportowania wyników. Zastosowanie blokowej randomizacji ze stratyfikacją zapewniło równomierne rozmieszczenie uczestników z krócej, jak i dłużej trwającą biegunką pomiędzy badanymi grupami. Pomimo niewielkiej liczby uczestników, badanie to może stanowić punkt wyjścia dla przyszłych badań. Na podstawie zdobytego doświadczenia i piśmiennictwa zidentyfikowaliśmy kluczowe obszary wymagające dalszych poszukiwań w zakresie leczenia AGE.

3. Streszczenie w języku angielskim.

The effectiveness of pleuran in treatment of acute gastroenteritis in children- a randomised, placebo-controlled, double-blind trial

The present thesis comprises three publications on the use of β-glucans in the treatment of acute infectious diarrhoea in children.

Acute gastroenteritis (AGE) is a significant global health concern, representing one of the foremost causes of morbidity and mortality in paediatric population. While oral and intravenous rehydration have proven effective in reducing mortality associated with AGE, they do not address the progression of the disease itself. Consequently, there is an urgent need for new therapeutic strategies that can not only alleviate symptoms, but also impact the course of the illness. In this context, pleuran, a β-(1,3/1,6)-D-glucan, has been selected as a potential therapeutic agent for diarrhoea. Pleuran belongs to β-glucans, a group of high-molecular-weight polysaccharides found in various natural sources such as fungi, bacteria, and cereals, which have demonstrated a range of beneficial effects, including immunomodulatory properties. Pleuran is recognized for its immunomodulatory properties, which have been shown to enhance the body's natural immune response. It is also known to present a strong safety profile. Previous studies have demonstrated its efficacy in treating viral and bacterial infections. Notwithstanding its extensive range of beneficial effects, the effectiveness of pleuran in the treatment of AGE has not yet been investigated. Presented series of articles aims to evaluate the anti-infective activity of β-glucans, basing on pleuran in gastrointestinal infections in children.

The initial publication was a scoping review on anti- infective properties of β-glucans in children. We summarized the current literature on the topic focusing exclusively on clinical trials. We used the PRISMA-ScR (Preferred Reporting Items for Systematic reviews and Meta-Analyses, Extension for Scoping Reviews) to prepare the review. We performed an electronic search of PubMed, Embase, and Cochrane databases up to May 2021, identifying 6,232 relevant studies, of which twelve were ultimately included in the analysis. The type of preparation, doses and study designs varied between studies, which was the reason for summarizing results in design of scoping review instead

of systematic review. Ten out of twelve trials demonstrated the effectiveness of β-glucans in reducing the incidence of respiratory tract infections or alleviating symptoms of upper respiratory tract infections. Ten out of twelve studies have reported a good tolerance and safety profile. We concluded that good tolerance of β-glucans shows a favourable benefit-risk ratio of this type of intervention in the treatment of acute respiratory infections in children. Although it is known that β-glucans primarily act by stimulating the immune system within the gut-associated lymphoid tissue, previous studies have not evaluated their effectiveness in the treatment of acute gastrointestinal infections in children. Our literature review not only identified this important research gap but also supported determining the appropriate dose of β-glucan used in our study.

The second manuscript is a protocol for a study that aimed to evaluate the efficacy of pleuran in reducing the duration and symptoms of diarrhoea. The present study has been designed as a fully blinded, placebo-controlled, randomised clinical trial. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT checklist) was used when writing the protocol. Before recruitment, the trial was registered in the Clinical Trials database. The study group comprised children aged between 2 and 10 years who presented to the hospital with diarrhoea lasting between 24 and 72 hours. Children eligible for the study were randomised to the active treatment group, which received the pleuran preparation, and the placebo group, which received a placebo preparation. All participants received daily study preparation until the diarrhoea subsided. The primary endpoint was defined as the duration of diarrhoea, measured from the onset to the normalization of stool consistency and frequency. The study's recruitment started in June 2018 and was completed in December 2022. Due to the unsatisfactory pace of patient recruitment, the study protocol was modified three times during the course of the trial. The changes included expanding the age range of eligible participants from the original 2–6 years to 2–10 years, as well as adding two additional recruitment centres. Each modification was approved by the Bioethics Committee of the Medical University of Warsaw.

The results of this study were presented in the third manuscript. The study was reported in accordance with the CONSORT Statement. We have enrolled 27 children, who were randomly assigned to either the experimental group (Group A, 13 patients) or the control

group (Group B, 14 patients). One patient was excluded from the analysis due to a loss of contact with caregiver. The study assessed the duration of diarrhoea, intravenous rehydration, hospitalization, and symptoms improvement but did not find any significant differences in clinical outcomes between the two groups. One mild adverse event (rash) was reported in the experimental group. We demonstrated that pleuran was ineffective in shortening the duration of AGE and alleviating its symptoms in a paediatric population. To the best of our knowledge, this was the first and only randomised controlled trial (RCT) to evaluate the efficacy of pleuran or other β-glucans in the treatment of acute gastroenteritis in children. The strength of this trial lies in its randomised, fully blinded, placebo-controlled design. The implementation of both the SPIRIT Checklist and the CONSORT Statement ensured the correct methodology of study commencement and result reporting. The stratified randomisation process was utilised to ensure an equal distribution of children with shorter and longer durations of diarrhoea between the experimental and control groups. Despite the small sample size, this study serves as a pilot for future research. Based on our experience and existing literature, we have identified key areas for future research on the management of acute gastroenteritis.

4. Wstęp

Ostra biegunka infekcyjna pozostaje ważnym problemem zdrowotnym, istotną przyczyną hospitalizacji, a w krajach rozwijających się jedną z wiodących przyczyn umieralności dzieci (1). W ciągu każdego roku ostra biegunka dotyka blisko 500 milionów dzieci na świecie i jest przyczyną około 3,2 miliona zgonów dzieci poniżej 5 roku życia (2). Ryzyko zachorowania, ciężkiego odwodnienia oraz zgonu w przebiegu biegunki jest największe w grupie najmłodszych pacjentów, zwłaszcza < 5 roku życia.

Choć śmiertelność z powodu biegunki infekcyjnej w krajach o wysokich dochodach jest niewielka, choroba ta jest jedną z najczęstszych przyczyn konsultacji pediatrycznych oraz hospitalizacji, przez co generuje istotne koszty obsługi systemu opieki zdrowotnej. Zgodnie z raportem Narodowego Instytutu Zdrowia Publicznego w 2023 roku liczba zachorowań na ostrą biegunkę infekcyjną w grupie dzieci < 2 roku życia w Polsce wynosiła 20796, z czego aż 12269 pacjentów hospitalizowano (59%) (3). W Stanach Zjednoczonych liczba hospitalizacji z powodu biegunki u dzieci < 5 roku życia wynosi 150 000 rocznie, co stanowi 13% wszystkich hospitalizacji w tej grupie wiekowej (1). W krajach Unii Europejskiej 6-11% hospitalizacji dzieci < 5 roku życia spowodowanych jest biegunką infekcyjną (4). Przedłużająca się hospitalizacja, poza ogromnymi kosztami, wiąże się również z innymi zagrożeniami dla dziecka, m.in. ekspozycją na inne czynniki infekcyjne, stres, utrzymywanie dojść dożylnych związane z ryzykiem zakażenia odcewnikowego. Czas trwania choroby dziecka wpływa również na długość absencji opiekuna w pracy.

Leczenie ostrej biegunki u dzieci

Zdecydowana większość hospitalizacji związanych z ostrą biegunką infekcyjną jest spowodowanych niepowodzeniem nawadniania doustnego. Udokumentowaną podstawę leczenia i jednocześnie jedyny środek ograniczający śmiertelność w przebiegu ostrej biegunki stanowi nawadnianie dziecka, preferencyjnie doustnymi płynami nawadniającymi (ORS, ang. *oral rehydration solution*). ORS nie skracają jednak czasu trwania biegunki oraz stopnia jej nasilenia (5). Probiotyki mogą skracać czas hospitalizacji i łagodzić objawy AGE u dzieci, jednak zalecenia dotyczące ich stosowania są niespójne. Europejskie Towarzystwo Gastroenterologii, Hepatologii i Żywienia Dzieci (ESPGHAN,

ang. *European Society for Pediatric Gastroenterology, Hepatology, and Nutrition*) oraz Europejskie Towarzystwo Chorób Zakaźnych Dzieci (ESPID, ang. *European Society for Pediatric Infectious Diseases),* a także Amerykańskie Towarzystwo Chorób Zakaźnych (IDSA, ang. *Infectious Diseases Society of America*), obecnie rekomendują stosowanie czterech szczepów probiotycznych w AGE: *Lacticaseibacillus rhamnosus GG* i *Saccharomyces boulardii* (zalecenie silne) oraz *Limosilactobacillus reuteri DSM 17938* i *Lacidophilus LB* (zalecenie słabe) (6–8). Autorzy tych wytycznych ocenili jakość dowodów jako niską lub bardzo niską. W przeciwieństwie do powyższego, brytyjski instytut NICE (ang. *National Institute for Health and Care Excellence*) uznał, że dostępne dane są niewystarczające, by rutynowo zalecać probiotyki w leczeniu AGE (9). Zgodnie z wytycznymi ESPGHAN/ESPID, diosmektyt i racekadotryl mogą być stosowane w leczeniu AGE zarówno u dzieci hospitalizowanych, jak i leczonych ambulatoryjnie (5,8,9). Dieta bez laktozowa może skrócić czas trwania biegunki, ale tylko u pacjentów hospitalizowanych (10). Większość zaleceń nie popiera rutynowej suplementacji cynku w krajach o wysokim dochodzie, choć może ona przynieść korzyści dzieciom w krajach rozwijających się (5,7,11). Brakuje wystarczających dowodów, by rutynowo zalecać stosowanie innych terapii, takich jak: symbiotyki, prebiotyki, tanina, węgiel aktywowany czy kaolin-pektyna. Konieczne są próby identyfikacji innych metod terapeutycznych skutecznych w skróceniu i zmniejszeniu nasilenia objawów biegunki (5).

Działanie pleuranu

Pleuran (β-(1,3/1,6)-D-glukan) jest nierozpuszczalnym polisacharydem należącym do grupy polimerów glukozy powszechnie nazwanych β-glukanami (12). Jest otrzymywany z popularnego grzyba boczniaka ostrygowatego (*Pleurotus ostreatus).* Podobnie jak inne β-glukany, pleuran wykazuje działanie immunomodulujące poprzez aktywację komórek odpornościowych w kępkach Peyera w jelicie krętym. Boczny łańcuch β-1,3-glukanu wiąże się z glikoproteinowymi receptorami Dectin-1, TLR (ang. *Toll-like receptor*), CR3 (ang. *complement receptor 3*) na powierzchni makrofagów, monocytów oraz komórek dendrytycznych, pobudzając ich zdolności fagocytarne oraz stymulując je do produkcji cytokin zapalnych, głównie TNFα, IL-2, IL-10, IL-12 (13). Cytokiny te aktywują humoralną odpowiedź immunologiczną organizmu poprzez mobilizowanie

limfocytów B, jak również wzmagają mechanizmy odporności komórkowej stymulując komórki NK (ang. *natural killer*) (13). Bezpośrednie działanie komórek dendrytycznych na limfocyty T oraz β-glukanów na receptory powierzchniowe neutrofili potęguje odpowiedź komórkową (13).

Zastosowanie pleuranu w dotychczasowych badaniach

Dotychczas, w grupie dzieci, przeprowadzono jedno wieloośrodkowe, podwójnie zaślepione badanie z randomizacją, oceniające skuteczność przewlekłego stosowania preparatu pleuranu (przez okres 6 miesięcy) w zapobieganiu nawrotom infekcji dróg oddechowych. W grupie 175 pacjentów w wieku 2-10 lat wykazano zmniejszenie zapadalności na infekcje dróg oddechowych w grupie eksperymentalnej w porównaniu do grupy kontrolnej (otrzymującej placebo). Odnotowano również modulację zarówno komórkowej jak i humoralnej odpowiedzi immunologicznej w grupie przyjmujących pleuran (zwiększenie poziomu immunoglobulin G oraz M, przyrost całkowitej liczby komórek NK) (14). Wyniki te należy jednak traktować z ostrożnością z uwagi na ograniczenia metodologiczne badania. Autorzy wieloośrodkowego badania obserwacyjnego typu open-label wykazali statystycznie istotne ograniczenie liczby zakażeń dróg oddechowych u dzieci po co najmniej 3 miesiącach suplementacji pleuranem, w porównaniu do tej samej grupy dzieci w analogicznym okresie roku poprzedzającego badanie (n = 1030) (15). W kolejnym badaniu zaobserwowano skrócenie czasu trwania infekcji wirusem opryszczki po krótkotrwałym stosowaniu pleuranu, a także redukcję czasu trwania objawów infekcji górnych dróg oddechowych po dłuższej suplementacji – zarówno u dzieci powyżej 6. roku życia, jak i u osób dorosłych (16). W 2016 roku opublikowano wyniki wieloośrodkowego badania typu „Split-body", oceniającego skuteczność kremu z pleuranem w łagodzeniu objawów i dolegliwości związanych z łagodnym i umiarkowanym atopowym zapaleniem skóry u dorosłych. Wykazano skrócenie czasu trwania zaostrzeń, zmniejszenie obszaru zmian skórnych oraz ograniczenie świądu w obrębie skóry traktowanej pleuranem (17). Kolejne dwa badania przeprowadzono w grupie dorosłych sportowców, wykazując istotne zmniejszenie liczby infekcji dróg oddechowych u badanych w porównaniu z grupą kontrolną przy przewlekłym

stosowaniu pleuranu (18,19). Oceniając wybrane parametry immunologiczne, wykazano wzrost liczby komórek NK oraz działanie regulujące proces fagocytozy (19).

Tolerancja pleuranu

W powyższych badaniach kontrolowano również skutki uboczne stosowania pleuranu. Nie zanotowano żadnych istotnych działań niepożądanych substancji, zarówno u dzieci (14,15), jak i u dorosłych (19).

5. Cel i uzasadnienie struktury pracy doktorskiej

Niniejsza rozprawa doktorska została zorganizowana w formie cyklu trzech powiązanych publikacji naukowych, które wzajemnie się uzupełniając, kompleksowo omawiają immunomodulujące działanie β-glukanów - naturalnych polisacharydów pochodzących z grzybów o potwierdzonej aktywności biologicznej w ostrych zakażeniach przewodu pokarmowego u dzieci. Wybór powyższej struktury wynika z potrzeby systematycznego podejścia do problematyki, które obejmuje zarówno aspekty teoretyczne, metodologiczne, jak i praktyczne zastosowania kliniczne badanych związków.

Nadrzędne cele badawcze i hipotezy

Głównym celem tej pracy jest ocena potencjału terapeutycznego pleuranu w leczeniu chorób infekcyjnych przewodu pokarmowego u dzieci. Badania zostały zaprojektowane w oparciu o hipotezę, że pleuran wykazuje korzystne działanie immunomodulujące, które może być wykorzystane w terapii wspomagającej dzieci z ostrą biegunką - może skrócić czas trwania biegunki i złagodzić jej objawy, a tym samym stanowić wartościowe uzupełnienie obecnie stosowanych terapii w ostrym nieżycie żołądkowo-jelitowym.

Metodologia integrująca poszczególne publikacje

Wszystkie trzy publikacje łączy racjonalna metodologia badawcza - od przeglądu piśmiennictwa (Publikacja I) i opracowania protokołu badania (Publikacja II), do przeprowadzenia badania klinicznego z randomizacją (Publikacja III). Punktem wyjścia była hipoteza o immunomodulujących właściwościach β-glukanów, które mogą okazać się korzystne w ostrym nieżycie żołądkowo-jelitowym. Następnie przeanalizowano aktualny stan wiedzy i opracowano protokół badania klinicznego, by finalnie przetestować hipotezę w badaniu klinicznym randomizacją.

Charakterystyka poszczególnych publikacji i ich wzajemnych relacji

Pierwsza publikacja przedstawia podsumowanie mechanizmów przeciwinfekcyjnego działania β-glukanów, opartych na wynikach badań prowadzonych zarówno *in vitro*, jak i *in vivo*. Następnie, przeprowadzony zgodnie z zaleceniami PRISMA przegląd literatury pełni funkcję kluczowego ogniwa łączącego podstawy teoretyczne z ich

potencjalnym zastosowaniem klinicznym, dostarczając informacji na temat efektywności terapeutycznej oraz profilu bezpieczeństwa analizowanej substancji czynnej (20). Uzyskane wyniki stanowiły podstawę do określenia dawki zastosowanej w projekcie badania klinicznego.

Druga publikacja stanowi fundamentalne opracowanie protokołu randomizowanego, podwójnie zaślepionego badania klinicznego, które definiuje standardy metodologiczne dla całego projektu. Publikacja ta nie tylko przedstawia szczegółowy plan badawczy, ale również uzasadnia wybór populacji docelowej oraz kryteria włączenia i wyłączenia pacjentów. Protokół ten był podstawą dla realizacji badania klinicznego opisanego w trzeciej publikacji i został opracowany zgodnie z wytycznymi SPIRIT oraz zatwierdzony przez uczelnianą Komisję Bioetyczną (21).

Trzecia publikacja prezentuje wyniki zaprojektowanego badania klinicznego z randomizacją, które jako pierwsze oceniło skuteczność pleuranu w leczeniu ostrych zakażeń przewodu pokarmowego u dzieci. Została przygotowana zgodnie z zaleceniami CONSORT (22). Badanie to stanowi kulminację całego cyklu prac.

Oryginalny wkład naukowy i znaczenie praktyczne

Cykl publikacji wnosi istotny wkład do aktualnej wiedzy na temat zastosowania β- glukanów w zakażeniach w populacji dziecięcej, skupiając się na ostrej biegunce infekcyjnej. Na tle dotychczasowej wiedzy, która koncentrowała się głównie na immunomodulacyjnym działaniu pleuranu w profilaktyce zakażeń dróg oddechowych, jako pierwsi przeprowadziliśmy badanie z randomizacją oceniające skuteczność tego preparatu w leczeniu ostrego nieżytu żołądkowo-jelitowego. Praca wyróżnia się nie tylko innowacyjnym zastosowaniem znanej substancji w nowym wskazaniu klinicznym. Uzyskane wyniki dostarczają nowych, oryginalnych danych na temat potencjału terapeutycznego pleuranu i mogą stanowić podstawę do dalszych badań oraz aktualizacji zaleceń klinicznych dotyczących wspomagającego leczenia AGE u dzieci.

6. Kopie opublikowanych prac

Review

The anti-infective effect of β-glucans in children

A scoping review

Katarzyna Wzorek-Łyczko, Weronika Woźniak, Anna Piwowarczyk, and Ernest Kuchar

The Department of Pediatrics with Clinical Assessment Unit, Medical University of Warsaw, Poland

Abstract: *Background:* β-glucans are bioactive β-D-glucose polysaccharides of natural origin, presenting antimicrobial and immunomodulation properties, with a low risk of toxicity. *Objectives:* This scoping review aims to present the current knowledge on the anti-infective properties of β-glucans in the pediatric population. *Methods:* We used the PRISMA Extension for Scoping Reviews Checklist to prepare this review. Studies were identified by electronic searches of Pubmed, Embase, and Cochrane databases up to May 2021. *Results:* The primary search allowed us to find 6232 studies, twelve of which were finally included in the analysis. Eight studies were designed as randomized, placebo-controlled trials, while in four studies the intervention outcome was compared with the pre-intervention period in the same group. The type of preparation and doses varied between studies: in five trials pleuran was administered (in dose 10 mg/5 kg of body weight/day), and in one study baker's yeast β-glucan was used (in two doses: 35 mg/day and 75 mg/day). In six other studies, the analyzed preparation comprised β-glucan and other substances. The shortest study lasted seven days, while the most prolonged intervention lasted six months, followed by six months of follow-up. Ten out of twelve trials demonstrated the effectiveness of β-glucans in reducing respiratory tract infection incidence or alleviation of upper respiratory tract infection symptoms. Ten out of twelve studies have reported a good tolerance and safety profile. *Conclusions:* Good tolerance of β-glucans shows a favorable benefit-risk ratio of this type of intervention. Nevertheless, further monitoring of their efficacy and safety in high-quality research is necessary.

Keywords: pleuran, immunomodulation, bioactivity, polysaccharides

Introduction

β-glucans belong to a bioactive β-D-glucose polysaccharides of fungal, bacterial, yeast or oat origin. Depending on their biochemical structure, they can trigger non-specific reactions of different groups of cells, stimulating an immune response to tumor cells, and infective agents. Besides antitumor, immunomodulatory, hypocholesterolemic, and hypoglycemic effects, β-glucans also demonstrate anti-infective properties [1, 2]. They appear to be attributed to the immunomodulating effect, but β-glucans' direct antimicrobial and antiviral activity have also been suggested. The source of β-glucans is edible mushrooms and cereals, making them easily obtainable, and reducing concerns about their safety [2]. The antimicrobial and immunomodulating properties of β-glucans, together with no known signs and symptoms of their toxicity, make them perfect candidates for an anti-infective product. Several trials concerning the effectiveness of β-glucans in the treating and preventing infections have been conducted [3–36]. In this article, we focus on the pediatric population, as children belong to the patients with one of the highest incidences of infections. Due to the limited data on this topic, the high heterogeneity of the design, and the type of intervention, it is impossible to quantitatively summarize data or make specific recommendations to practitioners, as with a systematic review. Therefore we present a scoping review to summarize the current literature concerning the anti-infective efficacy of β-glucans in children and to identify the direction for further research.

Background

The properties of β-glucans strongly depend on their source, structure and physicochemical characteristics. Linear β-glucans (without branches) are found mainly in bacteria and cereal grains (oats and barley), while branched β-glucans usually occur in mushrooms (short β-1,6-side chains) and yeasts (long β-1,6-side chains) [37]. Only β-1,3-glucans with β-1,6-side chains are recognized by immune cells. Therefore they are critical features of immunomodulating polymers, determining their bioactivity [38, 39]. Medium or large molecular weight polymers present higher immunomodulating activity [1, 40]. On the other hand, low molecular weight of β-glucan is associated with better solubility, which makes the compound easier to disperse in food. In contrast to double helix and random coil, a triple helix conformation in the aquatic environment

has been correlated with the highest immunostimulatory activity [1, 40, 41]. The most active polysaccharides generally have a small degree of branching (between 20% and 33%) [40]. The type and composition of sugar or non-sugar components influence β-glucans' bioactivity [1]. The difference in immunomodulatory properties between water-soluble and insoluble β-glucans suggests, that they may act through different mechanisms: insoluble β-glucans bind preferably to the Dectin-1. In contrast, soluble β-glucans stimulate immune response via the CR3 receptor [42]. Among the best known and described bioactive β-glucans are lentinan (from *Lentinus edodes*), schizophyllan (from *Schizophyllum commune*), scleroglucan (from *Sclerotium glucanicum*), ganoderan (from *Ganoderma lucidum*), pleuran (from *Pleurotus ostreatus*), and zymosan (from *Saccharomyces cerevisiae*) [1].

The immunomodulatory mechanisms of β-glucans rely on their ability to activate innate and adaptive immune responses. Since β-glucans are molecules of bacterial, fungal, yeast or oat origin, and never naturally occur in the human body, they are recognized through pattern recognition receptors (PRRs) as pathogen-associated molecular patterns (PAMPs) and trigger a group-specific innate immune response [43]. Studies have shown various glucan-binding sites on immune cells, the most studied being the Dectin-1 receptor [44, 45, 46]. Dectin-1, a transmembrane protein receptor expressed on macrophages, granulocytes and dendritic cells' surface, plays a crucial role in triggering an innate immune response by β-glucans, enhancing both phagocytic process and pro-inflammatory factors production [42, 43, 47, 48, 49, 50]. A signalling pathway follows Dectin-1 activation and leads to the release of cytokines, including interleukin (IL)-12, IL-6, tumor necrosis factor (TNF)-α, and IL-10 [42]. Cytokine release and direct contact of β-glucan's fragments with the CR3 receptor on the surface of NK cells activate them [1, 50]. Furthermore, the stimulating effect of β-glucans on B lymphocytes in terms of pro-inflammatory cytokine production, immunoglobulins expression and induction of neutrophil chemotaxis has been described [1, 51]. Another interesting mechanism of β-glucans is immunopotentiation of mucosal immunity, discovered as an increase in epithelial lymphocyte count in the digestive system and stimulation of interferon-ϒ production in mice after a 1-week course of orally administered β-glucan [52]. The next exciting hypothesis on β-glucans concerns their capability to induce innate immune memory called Trained Immunity. Based on it, after contact with a certain stimulus, the innate immune cells go through metabolic, mitochondrial and epigenetic transformations, followed by a phenotype memory of enhanced immune responses when exposed to a secondary, heterologous agent [53]. Regarding adaptive immunity, some studies showed that β-glucans could stimulate T helper cells by binding to the histocompatibility complex class II on antigen-presenting cells. Further observation revealed increased T cell activity and interferon-ϒ production following its interaction with the β-glucan – dendritic cell complex [54].

Antiviral properties of β-glucans

Several *in vitro* and animal model studies have shown the antiviral properties of β-glucans against hepatitis type B virus [15], herpes simplex virus type 1 (HSV-1) [16, 17, 20, 21], influenza virus [18], hepatitis type C virus [19], western equine encephalitis virus, measles virus, mumps virus [21], rubella virus [22], rabies virus [23]. Although some of these studies have demonstrated the direct antiviral effect of glucans, in others the observed result may be due to the immunomodulatory activity of those polysaccharides.

Objectives

This review aims to present current literature on clinical trials investigating the anti-infective effect of β-glucans in the pediatric population and answer the research question: Are β-glucans effective in preventing and treating infectious diseases in children? We have focused on studies that reported at least one clinical outcome.

Methods

To prepare this article we have used The Preferred Reporting Items for Systemic Reviews and Meta-Analysis, an extension for Scoping Reviews (PRISMA-ScR) Checklist [55].

The study protocol was prepared before the study commencement, but it still needs to be published. However, it is available from the corresponding author on request.

Studies for this review were identified by conducting electronic searches of PubMed, Embase, and Cochrane databases, from their inception to May 2021. A trained librarian developed the search strategy, which can be found in this article's Electronic Supplementary Material (ESM) 1. For the primary search, we have used rather general keywords, due to the heterogeneous terminology applied in research on the anti-infective effects of β-glucans. We have removed duplicates. We compared authors, locations, dates, and sample sizes to identify identical studies in case of uncertainty. Despite different publications, we considered the studies identical if their sample sizes and exact results matched. After reviewing the titles and abstracts, we have selected only clinical trials assessing the anti-infective effect of β-glucans in children. Studies investigating other aspects of β-glucans, based on the adult population, and those assessing only laboratory outcomes with no

clinical ones, were excluded from the review. The reference lists of the selected articles and relevant reviews were checked for additional sources that were not initially found in the search. The most recent research was performed on the 7th of May 2021. Studies were selected based on the following inclusion criteria:

1. Studies demonstrate an anti-infective effect of β-glucans (including treatment and preventive properties).
2. Clinical trials with at least one clinical outcome.
3. Studies on children population (until 18 years of age).
4. Studies published up to May 2021.

The search was not limited by language or study design.

Two researchers (WW, KWŁ) independently reviewed the selected articles for studies that met the inclusion criteria. Discrepancies over study selection or data extraction were discussed between all the authors (WW, KWŁ, AP, EK) and resolved through consensus. KWŁ developed a data-charting form. The two reviewers independently charted the data from each eligible article and discussed the results. Any disagreements were resolved through discussion between the two reviewers. We have abstracted data on country of origin, study design, study population, used intervention, treatment indication, treatment duration, follow-up, and primary outcomes. The data extracted from the source of evidence are presented in Table 1, and in a narrative form.

Results

The primary search allowed us to find 6232 studies (2126 in PubMed, 3869 in Embase, and 237 in Cochrane) to be retrieved and assessed for eligibility. After removing duplicates we selected 4842 studies. In the titles and abstracts revision, 20 studies met inclusion criteria and were referred for full-text reading. After full-text reading nine articles were excluded (reasons for exclusion are presented in Flow Chart (Figure 1). The analysis of reference lists of selected articles, and relevant reviews has revealed one additional study, that was not initially found in the search. Therefore, we considered 12 studies to be eligible for this review. Additionally, we have identified one currently ongoing study concerning the anti-infective effect of β-glucans [56]. Characteristics of included studies are presented in Table 1. The selection process is presented in Flow Chart (Figure 1).

Studies included

Twelve studies were considered to be eligible for this review. Accordingly, we have identified six double-blind, placebo-controlled, randomized trials (RCT) [3, 4, 9, 10, 13, 14], four open-label prospective studies [5, 6, 7, 8], and two randomized, placebo-controlled, open-label trials investigating the anti-infective effect of β-glucans [11, 12].

Study population

The patients' age varied in all the studies, but only in one participants were younger than one year [14]. In 11 of 12 enrolled studies, the study population included only children, with a maximum age of 12. One trial included children older than six years old, and adults [13]. In 7/12 trials, the inclusion criterion was the history of recurrent respiratory infections in the previous year [3, 6, 7, 8, 12], season [5], or months preceding the study [4]. The remaining 5/12 studies included healthy individuals only, with no history of recurrent respiratory tract infections (RRTI). RRTI definitions varied between studies.

Type of preparation and the route of administration

In six trials, β-glucans were administered as a single preparation. In five of them, pleuran [3, 5, 6, 7, 13], and in one baker's yeast β-glucan was used [4]. In six other studies, the analyzed preparation was composed of β-glucan and other substances: a fortified formula with docosahexaenoic acid, a prebiotic blend of polydextrose and galactooligosaccharides [9, 10], a herbal mixture with Echinacea angustifolia, Arabinogalactan, Acerola (Vitamin C), Zinc [8]; Carboxymethyl-β-glucan combined with Resveratrol [12, 14] or Colloidal silver [11]. In three trials, a topical (intranasal) β-glucan preparation was used [11, 12, 14]. In the remaining nine studies, the route of administration was oral.

Outcomes

The incidence of respiratory tract infection (RTI) was the primary outcome assessed in 10/12 studies, but endpoints and outcome measures were variable. Among those trials, 8 demonstrated a positive effect of β-glucan on RTI incidence reduction [3, 4, 5, 6, 7, 8, 9, 12]. In two studies, there was no significant difference between active and placebo groups regarding RTI prevalence [10, 13]. Two studies demonstrated the efficacy of β-glucan in upper respiratory tract infection (URTI) symptom alleviation [11, 14].

Apart from RTI incidence, other outcomes were also described. One study demonstrated a shorter duration of HSV-1 infection symptoms in patients treated with β-glucan [13]. 4/12 trials demonstrated fewer missed days in daycare

Table 1. Characteristic of trials addressing the anti-infective effect of β-glucans in children

Country of study	Study design	Study population	Intervention and comparison	Route of administration	Duration of treatment and observation	Indication for treatment	Main clinical outcomes	Reference
Slovak Republic	DBPCRT	n=175 Age 2–10 y	Pleuran 10 mg/5kg of bw/d vs. placebo	Oral	6 months+6 months follow-up	RRTI	– Reduced RTI incidence (in the active group 36% of children did not suffer from any RTI compared to 21% in the placebo group; p<0.05). – Reduced number of flu and flu-like episodes (p<0.05). – Reduced number of LRTI (p<0.05). – Positive pediatricians' assessment of pleuran's effectiveness in respiratory morbidity reduction. – No adverse events observed.	Jesenak et al., 2013 [3]
China	DBPCRT	n=174 Age 1–4 y	BYBG 35 mg/d vs. BYBG 75 mg/d vs. Placebo	Oral	3 months	>2 URTI in previous 3 months	– Higher concentration of IgG, IgM, and more pronounced increase in IgA concentration in the active group compared to placebo. – A transient increase in NK cells in the active group that returned to baseline levels at the end of the follow-up. – Decreased proportion of patients with at least one infectious disease during cold season (p<0.001). – Reduced number and duration of URTI (p<0.0001). – Reduced number and duration of common childhood illness (p<0.0001). – One episode of vomiting considered as adverse event of product supplementation. No serious adverse events observed.	Meng et al., 2016 [4]
Poland	OLP	n=194 Age 1–10 y	Pleuran 10 mg/5kg of bw/d	Oral	3 months+3 months follow-up	RRTI	– Reduced RTI incidence compared to the previous year in both age groups (younger and older than 5 y) (p<0.001). – Reduced particular RTI types incidence: common cold, otitis media, laryngitis, bronchitis (p<0.001). No significant differences in pneumonia and tonsillitis. – Reduced number of days-off kindergarten or school (p<0.001). – No significant adverse events observed.	Pasnik et al., 2017 [5]
Slovak and Czech Republic	OLP	n=215 Age 3–7 y	Pleuran 10 mg/5kg of bw/d	Oral	Mean 6.8 months (minimum 3 months)	RRTI	– More than 50% decline in the RTI incidence in 153 enrolled children (71.2%). – Lower median annual frequency of respiratory infections in the group of subjects who responded positively to pleuran (p<0.001). – No adverse events observed.	Jesenak et al., 2010 [6]

(Continued on next page)

Table 1. (Continued)

Country of study	Study design	Study population	Intervention and comparison	Route of administration	Duration of treatment and observation	Indication for treatment	Main clinical outcomes	Reference
Spain	OLP	n=166 Age 1–12 y	Pleuran 10 mg/5kg of bw/d	Oral	3 months+6 months follow-up	RRTI	– Reduced RTI incidence compared to the previous year in both age groups (younger and older than 3.5 y) including: otitis, laryngitis, common cold and bronchitis ($p<0.001$). – Reduced number of visits in the emergency department compared to the previous year ($p<0.001$), but no differences in the total amount of hospitalizations ($p=1.000$). – Lower use of the pharmacology treatment ($p=0.035$). – Reduced number of missed days in school ($p=0.04$). – Positive parental opinion on the study preparation and on the child condition after the supplementation. – 5 cases of adverse cases were observed, but only one of them was considered as connected with supplementation.	Sapena Grau et al., 2015 [7]
Italy	OLP	n=37 Age 3–10 y	Herbal mixture: Echinacea angustifolia, Arabinogalactan, Acerola (Vitamin C), Zinc, and 50 mgBeta-Glucan/ per serving	Oral	6 months	RRTI (tonsillitis and/or otitis media)	– Reduced mean RTI number ($p=0.04$). – Reduced number of antibiotic use episodes ($p=0.03$). – An improvement (defined as a reduction in the number of RTI by a minimum of 1 compared to the previous period) observed in 77% of participants. – Positive parental opinion about the treatment measured in VAS scale. – No adverse events observed.	Minetti et al., 2011 [8]
China	DBPCRT	n=310 Age 3–4 y	Follow-up formula with 25 mg DHA, 1.2 g PDX/ GOS, and 8.7 mg yeast β-glucan per serving vs. placebo	Oral	7 months	Healthy individuals	– Fewer episodes ($p=0.04$), and shorter average duration ($p=0.007$) of RTI. – Lower use of antibiotics ($p=0.01$). – Lower percentage of children who missed at least one day at day care ($p=0.001$). – No episodes of LRTI or diarrhoea were observed in both groups. – No differences both in stool pattern and growth were reported. – Higher concentration of serum IL-10 during study in active vs. control group. – No changes in fecal secretory immunoglobulin A, serum TGF-b1, TGF-b2, IL-4, ferritin, Zinc, Hgb, Hct, RBC between groups. – Higher WBC level counted at 28 weeks in active group vs. placebo.	Li et al., 2014 [9]

(Continued on next page)

Table 1. (Continued)

Country of study	Study design	Study population	Intervention and comparison	Route of administration	Duration of treatment and observation	Indication for treatment	Main clinical outcomes	Reference
Brasil	DBPCRT	n=265 Age 1–4 y	Follow-up formula with 25 mg DHA, 1.2 g PDX/GOS, and 8.7 mg yeast β-glucan per serving vs. placebo	Oral	7 months	Healthy individuals	– No difference in the incidence of RTI or diarrheal disease between groups. – Fewer episodes of AM (p=0.021). – More frequent occurrence of thrush in active group than in placebo one. – Serious adverse events reported more frequent in placebo group: 10 participants in the placebo group vs. 2 in the active group. – No growth differences between groups. – Softer stool in active group vs. placebo (p<0.024) – No changes in fecal secretory immunoglobulin A, serum TGF-b1, TGF-b2, IL-10, IL-4, ferritin, Zinc, Hgb, Hct, RBC, WBC between groups.	Pontes et al., 2016 [10]
Italy	ROLT	n=100 Age 1–12 y	Colloidal silver and carboxymethyl-β-glucan drops (3 drops in each nasal fossa/d) vs. saline	Nasal	7 days	URTI/nasal obstruction	– Alleviation of common cold symptoms according to post-treatment CARIFS score analysis (p<0.001) and CARIFS VAS after 7-day long treatment (p=0.012). – Higher recovery rate after 7 days of treatment (90% vs. 66% in the active group vs. placebo, respectively). – Lower incidence of nasal therapy complications. – Higher incidence of nasal secretion absence after treatment: 70% in active group vs. 32% in placebo (p<0.001). – Lower incidence of RTI complications: 10% in active group vs. 34% in placebo (p=0.04). – Mild adverse events observed with no differences between groups.	Damiani et al., 2011 [11]
Italy	ROLT	n=82 Age 3–12 y	Resveratrol and carboxymethyl-β-glucan (12 drops diluted in 4 ml of saline 2 times per day) vs. saline	Nasal	20 days+90 days follow-up	RRTI	– Reduced RTI symptoms duration after 60 and 90 days of treatment: nasal obstruction (p<0.0001), rhinorrhea (p<0.0001), sneezing (p<0.0001), cough (p<0.0001), fever (p<0.0001). – Reduced number of days with medication use (p<0.0001). – Reduced number of missed days at school (<0.001). – No adverse events observed.	Varricchio et al., 2014 [12]

(Continued on next page)

Table 1. (Continued)

Country of study	Study design	Study population	Intervention and comparison	Route of administration	Duration of treatment and observation	Indication for treatment	Main clinical outcomes	Reference
Italy	DBPCRT	n=100	Resveratrol 0.05% and carboxymethyl-β-glucan 0.01% (3 drops in each nostril, 4 times a day) vs. saline	Nasal	7 days+21 days follow-up	URTI	- Reduced number of sneezing and cough episodes after 7 days ($p<0.005$). - No difference of Rhinovirus detection or cytokine expression in nasal swabs after 48 h or 7 days. - No difference in rhinorhoea, nasal congestion, snoring, fever, night wakening, infantile colic, appetite between groups.	Baldassare et al., 2020 [14]
Slovakia	DBPCRT	n=90 Patients (adults and children over 6 y)	*Acute phase:* Pleuran 300 mg, and zinc 10 mg vs. placebo *Preventive phase:* Pleuran 100 mg vs. placebo	Oral	10 days of acute treatment and 120 days of preventive phase	HSV-1 infection	*Acute phase:* - Reduced duration of HSV infection symptoms ($p=0.046$). - The higher number of patients without symptoms on day 5 ($p=0.027$) and day 6 ($p=0.013$). - No significant difference in terms of efflorescence size and HSV symptoms severity between groups after 10 days of treatment. *Preventive phase:* - No difference in RTI and intercurrent diseases (abdominal pain, aphthous stomatitis, labial herpes infection, skin rash) incidence. - Reduced duration of rhinitis and cough symptoms, as well as lower cough intensity. - No difference in adverse events incidence between groups.	Urbancikova et al., 2020 [13]

Abbreviations: DBPCRT: double blind, placebo controlled, randomized trial; OLP: open-label prospective study; ROLT: randomized placebo-controlled open-label trial; y: years old; d: daily; 3x/d: three times a day; RRTI: recurrent respiratory tract infections; URTI: upper respiratory tract infections; LRTI: lower respiratory tract infections; RTI: respiratory tract infections; AM: allergic manifestations; bw: body weight; BYBG: baker's yeast β-glucan; DHA: docosahexaenoic acid; PDX/GOS: prebiotic blend of polydextrose; CARIFS: Canadian Acute Respiratory Illness and Flu Scale); CARIFS VAS: Visual Analogic Scale; HSV-1: herpes simplex virus type 1; RBC: red blood cells; WBC: white blood cells; PLT: platelets; Hgb: haemoglobin; Hct: hematocrit.

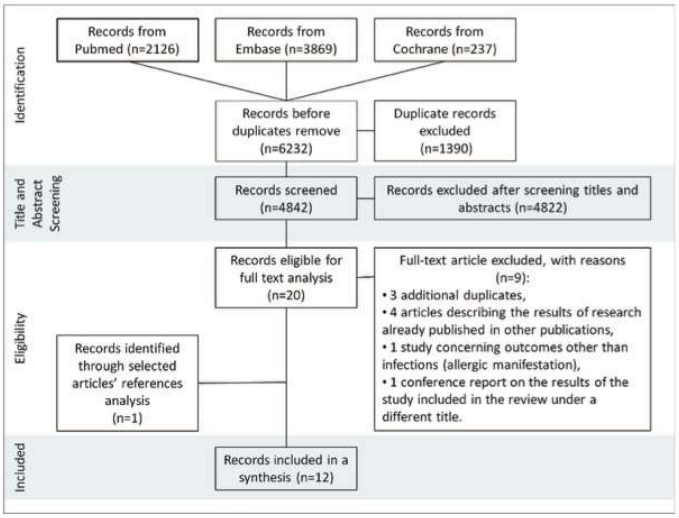

Figure 1. Flow chart.

after treatment with β-glucan [5, 7, 9, 12]. The reduction of complementary treatment use was described in 4/12 studies: antibiotics [8, 9, 12], anti-inflammatory, and antipyretic drugs [12]. In one trial, the kind of medication was not specified [7]. The pediatricians' opinion about the supplementation effectiveness was considered in one study [3]. Two studies described the positive parents' opinions about the product and their satisfaction with the improvement after supplementation [7, 8]. One trial considered Rhinovirus detection and cytokine expression in nasal swabs after 48 h and seven days of URTI treatment. However, the authors found no difference between the study, and control groups [14].

Adverse events (AE)

10/12 studies reported a good safety profile of the intervention. In 8 trials, no adverse events were observed [3, 5, 6, 8, 12, 14] or no statistically significant differences between active and placebo groups [11, 13]. In 3 studies, mild, episodic adverse events were observed: one episode of vomiting [4], five episodes of thrush [10], and one non-specified episode, classified as 'with a possible relationship with supplement' [7]. In one study, 10 participants in the placebo group and 2 in the active group experienced at least one serious adverse event. However, the authors did not specify their character [10]. One trial provided no information on adverse events or product tolerability [9].

Intervention and follow-up duration

The shortest study lasted seven days [11], while the most prolonged intervention lasted six months, followed by six months of follow-up [3]. In one study, the intervention period was divided into active (10 days) and preventive phases (120 days) [13].

Discussion

Eleven out of twelve included studies have shown a positive effect of β-glucans in treating or preventing infectious diseases in the pediatric population. However, each trial focused on different outcome measures. Among those, 8 studies showed a positive effect of β-glucans on RTI incidence reduction, 2 demonstrated their efficacy in URTI symptoms alleviation and 1 study indicated β-glucans effectiveness in HSV-1 infection treatment. Respiratory infections were discussed in all 12 studies. However, some of them aimed to assess the effectiveness of β-glucans in treating or preventing other infections as well: diarrhea [9, 10], childhood infectious diseases in general [4], and HSV-1 infection [13]. However, it is worth mentioning, that the rates of non-RTI infections in these studies were very low. Thus, conclusions from those trials could be limited only to respiratory infections. The preventive properties on RTI were of most interest and were addressed in 10/12 studies.

In 3 trials authors focused on the treatment of acute infection (one study considered both treatments in the acute phase and preventive properties of β-glucan in infections) [11, 13, 14].

To our knowledge, the anti-infective properties of β-glucans as a single substance in the pediatric population have been analyzed in two RCTs so far [3, 4]. Subjective outcomes assessment (self-reporting of RTI symptoms) was a limitation in both studies. Nevertheless, they are the first double-blind, placebo-controlled, randomized trials that showed the preventive effect of β-glucans on the morbidity of respiratory infections in the pediatric population. Four studies in our review showed a positive effect of β-glucans on the number of RRTI compared to the previous season in the same group [5, 6, 7, 8]. A certain limitation of those studies was their open-label design. Additionally, with increasing patients age, the respiratory tract infections frequency may also decrease spontaneously and naturally. It is worth noting that three prospective open-label trials had similar protocols, allowing for a common conclusion [5, 6, 7]. The results were inconsistent in another group of studies using the same preparation (follow-up milk formula fortified with β-glucan) and study protocol [9, 10]. Li observed the reduction of RTI prevalence and duration, which Pontes did not demonstrate. The authors find the reason for differences between their results in distinct study populations. In contrast to other studies, the assessment of endpoints did not depend on subjective self-reporting, but each episode was consulted with a pediatrician. Three studies assessed the effects of intranasal carboxymethyl-β-glucan application in children in comparison to placebo (saline): two on the treatment of acute symptoms of infection [11, 14], and the other on RRTI prevention [12]. The use of saline solution may be a source of bias, as it can benefit patients with rhinitis symptoms, and affect the study results. Patients were randomly assigned to the experimental and control groups in two studies, but neither they nor their physicians were blinded to the allocation [11, 12]. Despite those limitations, the studies show other possibilities for using β-glucans in local, intranasal administration. The antiviral activity of pleuran in treating HSV-1 infection in children was described in a multicenter, double-blind, placebo-controlled, randomized trial in Slovakia [13]. The results showed that fewer drugs were used in the active group, yet the duration of symptoms was significantly shorter. However, the outcomes for children and adults were not separately analyzed. Additionally, patients in the acute and preventive phase of the disease could but did not have to use antiherpetic medications, increasing the risk of bias.

Most studies have reported a favorable safety profile, reporting only episodic, mild adverse events. In one study, 2 cases of serious AE in the active and 10 cases in the placebo group were observed, suggesting no relationship to the intervention [10]. Considering the natural occurrence of β-glucans as a popular diet ingredient, their good tolerance reported in studies, also in chronic use, as well as rarely occurring serious AE, it seems that these substances are safe, with a favorable benefit-risk ratio. Nevertheless, further monitoring of their safety and precise reporting in research is necessary.

Results observed in this review in children are consistent with multiple trials demonstrating the anti-infective properties of β-glucans in adults. In two I/II phase studies, lentinan extracted from the popular Shiitake mushroom (*Lentinus edodes*) was shown to increase the number of CD4(+) cells in HIV-positive adults, compared to placebo [24, 25]. In another RCT, Biobran (a polysaccharide that contains ß-1,3-glucans and activated hemicellulose) had been demonstrated to significantly lower viremia in chronic hepatitis type C adult patients [26]. In a few studies, oral intake of β-glucans was associated with a lower incidence of common cold and upper respiratory tract infection episodes in healthy adult volunteers [27, 28], the elderly [29], high-performance athletes [30], and chronically stressed adults [31, 32], as well a fewer lost workdays or less severe respiratory infection [33, 34]. Another RCT has revealed a significantly lower prevalence of nosocomial pneumonia, sepsis, and reduced mortality in adult patients with severe multiple traumas after β-glucans administration than placebo [35]. There are also a few studies indicating the benefits of curdlan (β-glucan produced by Agrobacterium species) administration in patients with severe cerebral malaria, resulting from the ability of this β-glucan to disrupt the cytoadherence of parasites in the tissue [36]. All those studies showed good tolerance and no toxicity of β-glucans.

At the turn of 2019 and 2020, the extremely rapidly spreading Severe Acute Respiratory Syndrome Coronavirus Type 2 (SARS-CoV-2) pandemic accelerated the search for effective strategies to reduce morbidity and mortality. Taking into account the antiviral and immunomodulatory properties of β-glucans, together with the possibility of oral ingestion, a favorable safety profile, and the possibility of application without contact with healthcare professionals, the hypothesis, that these polysaccharides could be beneficial in COVID-19 treatment and prophylaxis has been formulated [57]. β-glucans are one of the most studied factors inducing TRIM [53]. It has been shown, that β-glucans use leads to innate immune cell epigenetic reprogramming, causing immune cell activation, increased cytokine production, and changes in metabolic function. Following those alterations cells can produce an enhanced immune response after contact with heterologous secondary stimuli [53, 58]. It has been suggested, that their ability to enhance antiviral cytotoxic immunity could help reduce SARS-CoV-2 infection severity [58, 59]. It has also been speculated, that the

ability of β-glucans to activate macrophages and their role in cytokine production regulation could be profitable for patients with cytokine storm and hyperinflammation observed during COVID-19 [39, 58, 59, 60]. According to the literature, the hypotensive properties of β-glucans could also benefit people suffering from COVID-19 [58]. Finally, β-glucans were suggested to be a potential SARS-CoV-2 vaccine adjuvant [61, 62]. Further research on this issue is necessary to confirm these hypotheses.

Limitations

The main limitation of this review is the high heterogeneity of included studies, making it impossible to draw clear conclusions. A further limitation is the inclusion of trials with the intervention consisting of several substances besides β-glucans, which makes it difficult to assess the effect of a single substance. Our decision to restrict the analysis to the studies with at least one clinical outcome could be another source of bias. However, laboratory tests and biomarkers alone do not allow us to conclude about the effectiveness and safety of the intervention. Nevertheless, there are multiple data on the impact of β-glucans on various laboratory markers of immune system stimulation in children [63, 64, 65, 66, 67]. Three studies described in this review assessed various laboratory parameters. An RCT by Jesenak reported an increase in IgA, IgM, and IgG immunoglobulin blood levels, a slower decline of T-cytotoxic lymphocytes, and an increase in the NK cell number in children receiving pleuran [3]. The trial performed by Li demonstrated an increase in IL-10 levels in the active group compared to the placebo [9]. However, no similar effect was observed in an analogous study by Pontes [10]. The significance of these observations is yet unknown, but they are consistent with the potential mechanisms of action of β-glucans described so far [1, 42, 51, 52, 53, 54].

Conclusions

Most of presented studies demonstrated the positive effect of β-glucans on RTI incidence, URTI symptom alleviation, and good substance tolerance. Nevertheless, the limitations of available evidence make it impossible to draw a combined conclusion. There is a strong need for further, high-quality RCTs to clarify the effect of β-glucans in this field.

Electronic Supplementary Material

The electronic supplementary material (ESM) is available with the online version of the article at https://doi.org/10.1024/0300-9831/a000793

ESM 1. Pubmed search strategy (PDF).

References

1. Giavasis I. Bioactive fungal polysaccharides as potential functional. Curr Opin Biotech. 2014;26:162–73.
2. Giavasis I. Production of microbial polysaccharides for use in food. In: Archer D, Giavasis I, Harvey L, McNeil B, ed. Microbial production of food ingrediens, enzymes and nutraceuticals. Woodhead Publishing. 2013;413–68.
3. Jesenak M, Majtan J, Rennerova Z, Kyselovic J, Banovcin P, Hrubisko M. Immunomodulatory effect of pleuran (beta-glucan from *Pleurotus ostreatus*) in children with recurrent respiratory tract infections. Int Immunopharmacol. 2013;15(2):395–9.
4. Meng F. Baker's yeast beta-glucan decreases episodes of common childhood illness in 1- to 4-year-old children during cold season in China. J Nut Food Sci. 2016;6(4):133–4.
5. Pasnik J, Ślemp A, Cywinska-Bernas A, Zeman K, Jesenak M. Preventive effect of pleuran (β-glucan from *Pleurotus ostreatus*) in children with recurrent respiratory tract infections – Open-label prospective study. Curr Paediatr Res. 2017;21(1):99–104.
6. Jeseňák M, Sanislo L, Kuniaková R, Rennerová Z, Buchanec J, Bánovčin P. Imunoglukan P4H in the prevention of recurrent respiratory infections in childhood. Czech-Slovak Paediatr. 2010;65:639–47.
7. Sapena Grau J, Sirvent LP, Ingles MM, Urgell MR. Beta-glucans from *Pleurotus ostreatus* for prevention of recurrent respiratory tract infections. Acta Paediatr Esp. 2015;73:186–93.
8. Minetti AM, Forti S, Tassone G, Torretta S, Pignataro L. Efficacy of complex herbal compound of *Echinacea angustifolia* (Imoviral® Junior) in recurrent upper respiratory tract infections during paediatric age: preliminary results. Minerva Paediatr. 2011;63(3):177–82.
9. Li F, Jin X, Liu B, Zhuang W, Scalabrin D. Follow-up formula consumption in 3- to 4- year-olds and respiratory infections: an RCT. Paediatr. 2014;133(6):1533–40.
10. Pontes MV, Ribeiro TCM, Ribeiro H, de Mattos AP, Almeida IR, Leal VM, et al. Cow's milk-based beverage consumption in 1- to 4-year-olds and allergic manifestations: an RCT. Nutr J. 2016;15:19.
11. Damiani V, Di Carlo M, Grappasonni G, Di Domenico R, Dominici P. Efficacy of a new medical device based on colloidal silver and carbossimetyl beta glucan in treatment of upper airways disease in children. Minerva Paediatr. 2011;63(5):347–54.
12. Varricchio AM, Capasso M, Della Volpe A, Malafronte L, Mansi N, Varricchio A, et al. Resveratrol plus carboxymethyl-beta-glucan in children with recurrent respiratory infections: a preliminary and real-life experience. Ital J Paediatr. 2014;40:93.
13. Urbancikova I, Hudackova D, Majtan J, Rennerova Z, Banovcin P, Jesenak M. Efficacy of pleuran (β-glucan from *Pleurotus ostreatus*) in the management of herpes simplex virus type 1 infection. Evid Based Complement Alternat Med. 2020;8562309.
14. Baldassarre ME, Di Mauro A, Labellarte G, Pignatelli M, Fanelli M, Schiavi E, et al. Resveratrol plus carboxymethyl-β-glucan in infants with common cold: A randomized double-blind trial. Heliyon. 2020;6(4):e03814.
15. Gu CQ, Li JW, Chao FH. Inhibition of hepatitis B virus by D-fraction from *Grifola frondosa*: Synergistic effect of combination with interferon-alpha in HepG2 2.2.15. Antiviral Res. 2006;72(2):162–5.
16. Gu CQ, Li JW, Chao F, Jin M, Wang XW, Shen ZQ. Isolation, identification and function of a novel anti-HSV-1 protein from *Grisofa frondosa*. Antiviral Res. 2007;75(3):250–7.

17. Marchetti M, Pisani S, Pietropaolo V, Seganti L, Nicoletti R, Degener A. Antiviral effect of a polysaccharide from *Sclerotium glucanicum* towards herpes simplex virus type 1 infection. Planta Med. 1996;62(4):303–7.
18. Obi N, Hayashi K, Miyahara T, Shimada Y, Terasawa K, Watanabe M, et al. Inhibitory effect of TNF-alpha produced by macrophages stimulated with *Grifola frondosa* extract (ME) on the growth of influenza A/Aichi/2/68 Virus in MDCK cells. Am J Chin Med. 2008;36(6):1171–83.
19. Matsuhisa K, Yamane S, Okamoto T, Watari A, Kondoh M, Matsuura Y, et al. Anti-HCV effect of Lentinula edodes mycelia solid culture extracts and low-molecular-weight lignin. Bioechom Biophys Res Commun. 2015;462(1):52–7.
20. Sarkara S, Kogab J, Whitleya RJ, Chatterjeea S. Antiviral effect of the extract of culture medium of Lentinus edodes mycelia on the replication of herpes simplex virus type 1. Antiviral Res. 1993;20(4):293–303.
21. Sorimachi K, Niwa A, Yamazaki S, Toda S, Yasumura Y. Antiviral activity of water-solubilized lignin derivatives in vitro. Agr Biol Chem. 1990;54(5):1337–9.
22. Mastromarino P, Petruzziello R, Macchia S, Rieti S, Nicoletti R, Orsi N. Antiviral activity of natural and semisynthetic polysaccharides on the early steps of rubella virus infection. J Antimicrob Chemother. 1997;39(3):339–45.
23. Pietropaolo V, Seganti L, Marchetti M, Sinibaldi L, Orsi N, Nicoletti R. Effect of natural and semisynthetic polymers on rabies virus infection in CER cells. Res Virol. 1993;144(2):151–8.
24. Gordon M, Bihari B, Goosby E, Gorter R, Greco M, Guralnik M, et al. A placebo-controlled trial of the immune modulator, lentinan, in HIV-positive patients: A phase I/II trial. J Med. 1998;29(5–6):305–30.
25. Gordon M, Guralnik M, Kaneko Y, Mimura T, Goodgame J, DeMarzo C, et al. A phase II controlled study of a combination of the immune modulator, lentinan, with didanosine (ddI) in HIV patients with CD4 cells of 200–500/mm3. J Med. 1995;26(5–6):193–207.
26. Salama H, Medhat E, Shaheen M, Zekri AN, Darwish T, Ghoneum M. Arabinoxylan rice bran (Biobran) suppresses the viremia level in patients with chronic HCV infection: A randomized trial. Int J Immunopathol Pharmacol. 2016;29(4):647–53.
27. Graubaum HJ, Busch R, Stier H, Gruenwald J. A double-blind, randomized, placebo-controlled nutritional study using an insoluble yeast beta-glucan to improve the immune defense system. Food Nutr Sci. 2012;3(6):738–46.
28. Auinger A, Riede L, Bothe G, Busch R, Gruenwald J. Yeast (1,3)-(1,6)-beta-glucan helps to maintain the body's defence against pathogens: a double-blind, randomized, placebo-controlled, multicentric study in healthy subjects. Eur J Nutr. 2013;52(8):1913–8.
29. Fuller R, Moore MV, Lewith G, Stuart BL, Ormiston RV, Fisk HL, et al. Yeast-derived β-1,3/1,6 glucan, upper respiratory tract infection and innate immunity in older adults. Nutr. 2017;39–40:30–5.
30. Talbott S, Talbott J. Effect of BETA 1, 3/1, 6 GLUCAN on upper respiratory tract infection symptoms and mood state in marathon athletes. J Sports Sci Med. 2009;8(4):509–15.
31. Talbott SM, Talbott JA, Cox D. Beta-glucan supplement reduces URTIs (upper respiratory tract infections) and improves mood state in healthy stressed subjects. FASEB J. 2010;24:922–911.
32. Talbott SM, Talbott JA. Baker's yeast beta-glucan supplement reduces upper respiratory symptoms and improves mood state in stressed women. J Am Coll Nutr. 2012;31(4):295–300.
33. Feldman S, Schwartz HI, Kalman DS, Mayers A, Kohrman HM, Clemens R. Randomized phase II clinical trials of wellmune WGP[R] for immune support during cold and flu season. J Appl Res. 2009;9:1–2.
34. Dharsono T, Rudnicka K, Wilhelm M, Schoen C. Effects of yeast (1,3)-(1,6)-beta-glucan on severity of upper respiratory tract infections: a double-blind, randomized, placebo-controlled study in healthy subjects. J Am Coll Nutr. 2019;38(1):40–50.
35. de Felippe Júnior J, da Rocha e Silva Júnior M, Maciel FM, Soares Ade M, Mendes NF. Infection prevention in patients with severe multiple trauma with the immunomodulator beta 1–3 polyglucose (glucan). Surg Gynecol Obstet. 1993;177(4):383–8.
36. Havlik I, Looareesuwan S, Vannaphan S, Wilairatana P, Krudsood S, Thuma PE, et al. Curdlan sulphate in human severe/cerebral *Plasmodium falciparum* malaria. Trans R Soc Trop Med Hyg. 2005;99(5):333–40.
37. Barsanti L, Passarelli V, Evangelista V, Frassanito AM, Gualtieri P. Chemistry, physico-chemistry and applications linked to biological activities of β-glucans. Nat Prod Rep. 2011 Mar;28(3):457–66.
38. Zhang H, Xiong Y, Bakry AM, Xiong S, Yin T, Zhang B, et al. Effect of yeast β-glucan on gel properties, spatial structure and sensory characteristics of silver carp surimi. Food Hydrocol. 2019;88:256–64.
39. Mirończuk-Chodakowska I, Kujawowicz K, Witkowska AM. Beta-glucans from fungi: biological and health-promoting potential in the COVID-19 pandemic era. Nutrients. 2021;13(11):3960.
40. El Enshasy HA, Hatti-Kaul R. Mushroom immunomodulators: unique molecules with unlimited applications. Trends Biotechnol. 2013;31(12):668–77.
41. Meng Y, Lyu F, Xu X, Zhang L. Recent advances in chain conformation and bioactivities of triple-helix polysaccharides. Biomacromolecules. 2020;21:1653–77.
42. Chan G, Chan W, Sze D. The effects of β-glucan on human immune and cancer cells. J Hematol Oncol. 2009;2:25.
43. Schorey JS, Lawrence C. The pattern recognition receptor Dectin-1: from fungi to mycobacteria. Curr Drug Targets. 2008;9(2):123–9.
44. Vetvicka V, Thornton BP, Ross GD. Soluble beta-glucan polysaccharide binding to the lectin site of neutrophil or natural killer cell complement receptor type 3 (CD11b/CD18) generates a primed state of the receptor capable of mediating cytotoxicity of iC3b-opsonized target cells. J Clin Investig. 1996;98(1):50–61.
45. Brown GD, Gordon S. A new receptor for beta-glucans. Nature. 2001;413(6851):36–37.
46. Vereschagin EI, van Lambalgen AA, Dushkin MI, Schwartz YS, Polyakov L, Heemskerk A, et al. Soluble glucan protects against endotoxin shock in the rat: the role of the scavenger receptor. shock. 1998;9(3):193–8.
47. Taylor PR, Brown GD, Reid DM, Willment JA, Martinez-Pomares L. The beta-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. J Immunol. 2002;169(7):3876–82.
48. Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S. Dectin-1 mediates the biological effects of beta-glucans. J Exp Med. 2003;197(9):1119–24.
49. Gantner BN, Simmons RM, Canavera SJ, Akira S, Underhill DM. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp Med. 2003;197(9):1107–17.

50. Hong F, Yan J, Baran JT, Allendorf DJ, Hansen RD, Ostroff GR, et al. Mechanism by which orally administered beta-1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. J Immunol. 2004;173(2): 797–806.
51. Ali MF, Driscoll CB, Walters PR, Limper AH, Carmona EM. β-glucan–activated human B lymphocytes participate in innate immune responses by releasing proinflammatory cytokines and stimulating neutrophil chemotaxis. J Immunol. 2015;195(11):5318–26.
52. Tsukada C, Yokoyama H, Miyaji C, Ishimoto Y, Kawamura H, Abo T. Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of beta-glucan. Cell Immunol. 2003;221(1):1–5.
53. Netea MG, Joosten LA, Latz E, Mills KHG, Natoli G, Stunnenberg HG, et al. Trained immunity: A program of innate immune memory in health and disease. Science. 2016;352: 6284.
54. Lin YL, Liang YC, Lee SS, Chiang BL. Polysaccharide purified from *Ganoderma lucidum* induced activation and maturation of human monocyte-derived dendritic cells by the NF-kappaB and p38 mitogen-activated protein kinase pathways. J Leukoc Biol. 2005;78(2):533–43.
55. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation. Ann Intern Med. 2018;169(7):467–73.
56. Wzorek-Łyczko K, Piwowarczyk A, Kuchar E Protocol of the study: the effectiveness of pleuran in the treatment of acute gastroenteritis in children-a randomised, placebo-controlled, double-blind trial (EPTAGE). BMJ Open. 2021;11(3):e042370.
57. Brueggeman JM, Zhao J, Schank M, Yao ZQ, Moorman JP Trained immunity: an overview and the impact on COVID-19. Front Immunol. 2022;13:837524.
58. Geller A, Yan J. Could the induction of trained immunity by β-glucan serve as a defense against COVID-19? Front Immunol 2020;11:1782.
59. Rao KS, Suryaprakash V, Senthilkumar R, Preethy S, Katoh S, Ikewaki N, et al. Role of immune dysregulation in increased mortality among a specific subset of COVID-19 patients and immune-enhancement strategies for combatting through nutritional supplements. Front Immunol. 2020;11:1548.
60. Murphy EJ, Masterson C, Rezoagli E, O'Toole D, Major I, Stack GD, et al. β-Glucan extracts from the same edible shiitake mushroom Lentinus edodes produce differential in-vitro immunomodulatory and pulmonary cytoprotective effects – Implications for coronavirus disease (COVID-19) immunotherapies. Sci. Total Environ. 2020;732:139330.
61. Ikewaki N, Iwasaki M, Kurosawa G, Rao KS, Lakey-Beitia J, Preethy S, et al. Beta-glucans: wide-spectrum immune-balancing food-supplement-based enteric (Beta-WIFE) vaccine adjuvant approach to COVID-19. Hum Vaccin Immunother. 2021;17(8):2808–13.
62. Rodriguez JAM, Bifano M, Goma ER, Plasencia CM, Torralba AO, Font MS, et al. Effect and tolerability of a nutritional supplement based on a synergistic combination of β-glucans and selenium- and zinc-enriched *Saccharomyces cerevisiae* (ABB C1®) in volunteers receiving the influenza or the COVID-19 vaccine: a randomized, double-blind, placebo-controlled study. Nutrients. 2021;13(12):4347.
63. Vetvicka V, Richter J, Svozil V, Rajnohova Dobiasova L, Kral V. Placebo-driven clinical trials of transfer point glucan #300 in children with chronic respiratory problems: antibody production. Am J Immunol. 2013;9(2):43–7.
64. Richter J, Svozil V, Král V, Rajnohová Dobiášová L, Stiborová I, Vetvicka V. Clinical trials of yeast-derived β-(1,3) glucan in children: effects on innate immunity. Ann Trans Med. 2014; 2(2):15.
65. Sarinho E, Medeiros D, Schor D, Silva AR, Sales V, Motta ME, et al. Production of interleukin-10 in asthmatic children after beta-1-3-glucan. Allergol Immunopathol. 2009;37(4):188–92.
66. Henao SLD, Urrego SA, Cano AM, Higuita EA. Randomized clinical trial for the evaluation of immune modulation by yogurt enriched with β-glucans from lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (Agaricomycetes), in children from Medellin, Colombia. Int J Med Mushrooms. 2018;20(8):705–16.
67. Vetvicka V, Richter J, Svozil V, Rajnohova Dobiasova L, Kral V. Placebo-driven clinical trials of yeast-derived β-(1,3) glucan in children with chronic respiratory problems. Ann Transl Med. 2013;1:26.

History
Received September 16, 2022
Accepted September 3, 2023
Published online October 2, 2023

Acknowledgements
We want to thank the Medical University of Warsaw authorities, without whose support this study could not have been conducted.

Conflict of interest
All authors certify that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject discussed in this manuscript.

Authors' contribution
All authors meet The ICMJE Recommendations for Authorship. KWŁ has initially conceptualized the study and created a data charting form. KWŁ and WW performed the database search and data extraction. KWŁ, WW, AP and EK synthesized of the results and provided a general interpretation of the results. All authors have contributed to and approved the final manuscript.

ORCID
Katarzyna Wzorek-Łyczko
https://orcid.org/0000-0003-0353-8909
Weronika Woźniak
https://orcid.org/0000-0002-9069-0695
Anna Piwowarczyk
https://orcid.org/0000-0002-8764-7442
Ernest Kuchar
https://orcid.org/0000-0002-7883-2427

Katarzyna Wzorek-Łyczko
The Department of Pediatrics with Clinical Assessment Unit
Medical University of Warsaw
63A Żwirki & Wigury Street
02-091 Warsaw
Poland
katarzynawzorek@vp.pl

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)

Katarzyna Wzorek-Łyczko, Anna Piwowarczyk, Ernest Kuchar

To cite: Wzorek-Łyczko K, Piwowarczyk A, Kuchar E. Protocol of the study: the effectiveness of pleuran in the treatment of acute gastroenteritis in children—a randomised, placebo-controlled, double-blind trial (EPTAGE). *BMJ Open* 2021;**11**:e042370. doi:10.1136/bmjopen-2020-042370

► Prepublication history and additional materials for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2020-042370).

Received 02 July 2020
Revised 07 January 2021
Accepted 25 January 2021

The Department of Pediatrics with Clinical Assessment Unit, Medical University of Warsaw, Warsaw, Poland

Correspondence to
Anna Piwowarczyk;
apiwowarczyk@wum.edu.pl

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.

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.

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]

The mortality due to AGE in developed countries is much more lower than in developing ones[2]; 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 effloresce 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 Scale[26]) 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 Gatsroenterology, Hepatology and Nutrition - ESPGHAN recommendations[4]), 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 scale[29] 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 Scale[26]) 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 CDS[30] and WHO[29] 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 Scale[26]). 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 Scale[28] 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 nomogram[31] 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 ClinicalTrails.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.

Acknowledgements The authors thank the research team and clinicians at all participating sites, and The Medical University of Warsaw authorities, without whose support this study could not be conducted. They also thank Dr Zbigniew Lewandowski for his expert advice in the field of statistics.

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.

Funding This work was supported by the Nutricia Foundation's research grant (No. RG3-2018), The Medical University of Warsaw (WUM) and PLEURAN s.r.o. The Nutricia Foundation's research grant covered the recruitment, equipment and administrative costs. The statutory resources of the Department of Paediatrics with Clinical Assessment Unit, WUM, were acquired to finance the researcher's voluntary liability insurance. The active product and placebo were donated to the study by PLEURAN s.r.o., Slovakia. Both the Nutricia Foundation and the PLEURAN s.r.o. had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data or decision to submit results. An agreement was signed between WUM and PLEURAN s.r.o., substantially limiting the right of PLEURAN s.r.o., in particular: no financial obligations between the parties, and no rights to documentation created as a result, in the course of or in connection with the study for PLEURAN. WUM committed to sharing with PLEURAN unprocessed data (not including the personal data of patients) obtained during the study, provided that in case of PLEURAN concluded individual results using this database, and at the same time PLEURAN would like to refer to the study, these results should be agreed with WUM.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iD

Katarzyna Wzorek-Łyczko http://orcid.org/0000-0003-0353-8909

REFERENCES

1 Fitzwater S, Chandran A, Kosek M. Infectious diarrhea. In: Selendy JMH, ed. *Water and sanitation-related diseases and the environment: challenges, interventions, and preventive measures*. John Wiley and Sons, 2011: 47–70.
2 World Health Organization. The global burden of disease: 2004 update, 2008. Available: www.who.int/healthinfo/global_burden_disease/2004_report_update/en/ [Accessed Jun 2020].
3 Pediatric ROTavirus European CommitTee (PROTECT). The paediatric burden of rotavirus disease in Europe. *Epidemiol Infect* 2006;134:908–16.
4 Guarino A, Ashkenazi S, Gendrel D. ESPGHAN/ESPID Evidence-based guidelines for the management of acute gastroenteritis in children in Europe: update 2014. *J Pediatr Gastroenterol Nutr* 2014;59:132–52.
5 Freedman SB, Ali S, Oleszczuk M, *et al*. Treatment of acute gastroenteritis in children: an overview of systematic reviews of interventions commonly used in developed countries. *Evid Based Child Health* 2013;8:1123–37.
6 NICE. Diarrhoea and vomiting caused by gastroenteritis in under 5S: diagnosis and management. NICE clinical guidelines. Available: www.nice.org.uk/guidance/cg84 [Accessed Jun 2020].
7 Hojsak I, Fabiano V, Pop TL. European guidance on paediatric use of probiotics states that benefits are limited to several conditions and urges caution with specific vulnerable groups. *Acta Paediatr* 2018;107:927–37.
8 Szajewska H, Guarino A, Hojsak I, *et al*. Use of probiotics for management of acute gastroenteritis: a position paper by the ESPGHAN Working group for probiotics and prebiotics. *J Pediatr Gastroenterol Nutr* 2014;58:531–9.
9 van den Berg J, Berger MY. Guidelines on acute gastroenteritis in children: a critical appraisal of their quality and applicability in primary care. *BMC Fam Pract* 2011;12:134.
10 Pérez-Gaxiola G, Cuello-García CA, Florez ID, *et al*. Smectite for acute infectious diarrhoea in children. *Cochrane Database Syst Rev* 2018;4:CD011526.
11 Liang Y, Zhang L, Zeng L, *et al*. Racecadotril for acute diarrhoea in children. *Cochrane Database Syst Rev* 2019;12:CD009359.
12 Lazzerini M, Ronfani L. Oral zinc for treating diarrhoea in children. *Cochrane Database Syst Rev* 2012;6:CD005436.
13 Florez ID, Veroniki A-A, Al Khalifah R, *et al*. Comparative effectiveness and safety of interventions for acute diarrhea and gastroenteritis in children: a systematic review and network meta-analysis. *PLoS One* 2018;13:e0207701.
14 MacGillivray S, Fahey T, McGuire W. Lactose avoidance for young children with acute diarrhoea. *Cochrane Database Syst Rev* 2013;10:CD005433.
15 Di Luzio NR. Update on the immunomodulating activities of glucans. *Springer Semin Immunopathol* 1985;8:387–400.
16 Chan GC-F, Chan WK, Sze DM-Y. The effects of β-glucan on human immune and cancer cells. *J Hematol Oncol* 2009;2:25.
17 Tsukada C, Yokoyama H, Miyaji C, *et al*. Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of beta-glucan. *Cell Immunol* 2003;221:1–5.
18 Jesenak M, Majtan J, Rennerova Z, *et al*. Immunomodulatory effect of pleuran (β-glucan from Pleurotus ostreatus) in children

with recurrent respiratory tract infections. *Int Immunopharmacol* 2013;15:395–9.
19 Jesenak M, Sanisló E, Kuniakova R. Imunoglukan P4H® in the prevention of recurrent respiratory infections in childhood. *Cesk Pediatr* 2010;65:639–47.
20 Pasnik J, Ślemp A, Cywinska-Bernas A. Preventive effect of pleuran (β-glucan from Pleurotus ostreatus) in children with recurrent respiratory tract infections – an open-label prospective study. *Curr Pediatr Res* 2017;21:99–104.
21 Grau JS, Sirvent LP, Ingles MM. Beta-glucans from Pleurotus ostreatus for prevention of recurrent respiratory tract infections. *Acta Pediatrica Espanola* 2015;73:186–93.
22 Urbancikova I, Hudackova D, Majtan J, *et al*. Efficacy of Pleuran (*β*-Glucan from *Pleurotus ostreatus*) in the Management of Herpes Simplex Virus Type 1 Infection. *Evid Based Complement Alternat Med* 2020;2020:1–8.
23 Jesenak M, Urbancek S, Majtan J, *et al*. β-Glucan-based cream (containing pleuran isolated from Pleurotus ostreatus) in supportive treatment of mild-to-moderate atopic dermatitis. *J Dermatolog Treat* 2016;27:351–4.
24 Bergendiova K, Tibenska E, Majtan J. Pleuran (β-glucan from Pleurotus ostreatus) supplementation, cellular immune response and respiratory tract infections in athletes. *Eur J Appl Physiol* 2011;111:2033–40.
25 Bobovcák M, Kuniaková R, Gabriž J, *et al*. Effect of Pleuran (β-glucan from *Pleurotus ostreatus*) supplementation on cellular immune response after intensive exercise in elite athletes. *Appl Physiol Nutr Metab* 2010;35:755–62.
26 Wojtyniak K, Szajewska H, Dziechciarz P. Translation to Polish, cross-cultural adaptation, and validation of the Bristol stool form scale among healthcare professionals and patients. *Prz Gastroenterol* 2018;13:35–9.
27 World Health Organization. The child growth standards: BMI-for-age. Available: https://www.who.int/toolkits/child-growth-standards/standards/body-mass-index-for-age-bmi-for-age
28 Schnadower D, Tarr PI, Gorelick MH, *et al*. Validation of the modified Vesikari score in children with gastroenteritis in 5 US emergency departments. *J Pediatr Gastroenterol Nutr* 2013;57:514–9.
29 World Health Organization. Cholera. fact sheet 107, 2012. Available: https://apps.who.int/iris/bitstream/handle/10665/43209/9241593180.pdf;jsessionid=D985C2545AFC4972997064A6F75EB960?sequence=1 [Accessed Jun 2020].
30 Friedman JN, Goldman RD, Srivastava R, *et al*. Development of a clinical dehydration scale for use in children between 1 and 36 months of age. *J Pediatr* 2004;145:201–7.
31 Altman DG. Statistics and ethics in medical research: III how large a sample? *Br Med J* 1980;281:1336–8.
32 Chan A-W, Tetzlaff JM, Altman DG, *et al*. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200–7.

scientific reports

Check for updates

OPEN

A randomised trial of pleuran in paediatric acute gastroenteritis

Katarzyna Wzorek-Łyczko[1✉], Anna Piwowarczyk[1], Weronika Woźniak[1], Ernest Kuchar[1] & Henryk Szymański[2]

Pleuran, as a potent immunomodulator targeting intestinal immunity with a strong safety profile, could be a potential treatment for acute gastroenteritis. This study evaluates the effect of pleuran on the duration and severity of acute infectious diarrhoea in children. This is a multi-centre, randomised, double-blind, placebo-controlled, CONSORT statement superiority trial. Children aged 2–10 years presenting to hospital with acute gastroenteritis were included. The primary outcome measure was the duration of diarrhoea. Twenty-seven children were enrolled. There were no significant differences between the experimental and control groups regarding duration of diarrhoea, hospitalisation, intravenous rehydration and symptom severity. The administration of Pleuran was well tolerated. In this study, Pleuran was ineffective in the treatment of acute gastroenteritis in children. Further studies are needed to investigate its potential as a nutraceutical in children.

***Clinical registration*: ClinicalTrials.gov ID: NCT03988257 (the date of registration: 17/06/2019). The Medical University of Warsaw Bioethical Committee Approval: KB/45/2018. Nutricia Foundation Grant: RG-3/2018.**

Keywords Infectious diarrhoea, Beta-glucan, Management of acute gastroenteritis, Mucosal immunity, Innate immunity, Interventional trial.

Acute gastroenteritis (AGE) is a major cause of childhood morbidity and mortality worldwide. Oral or intravenous rehydration has been shown to be effective in reducing mortality associated with AGE. However, it does not affect disease progression. New treatment that can effectively relieve the symptoms are therefore needed. Pleuran (β-(1,3/1,6)-D-glucan) is of particular interest in the treatment of acute gastrointestinal infections. It has potential immunomodulatory properties targeting the innate intestine immunity and a strong safety profile. However, the efficacy of pleuran in AGE treatment has not been investigated. In this manuscript, we present the results of an original randomised, double-blind, placebo-controlled trial to assess the efficacy of pleuran in the treatment of AGE in children, conducted according to the CONSORT Statement (See Supplementary information).

Background

The prevalence of AGE in children aged < 3 years in Europe is 0.5- 2 episodes per child per year[1]. AGE is the third leading cause of child mortality worldwide. It kills more than 400,000 children under the age of five each year[2]. Most deaths occur in developing countries because of limited access to safe water and health care. In developed countries, the death rate from AGE is much lower. However, infectious diarrhoea remains a common cause of paediatric emergency department visits and hospital admissions[1]. Age under 6 months, early weaning, immunodeficiency and malnutrition are risk factors for severe or persistent diarrhoea[3]. Prolonged hospitalisation is associated with exposure to infectious agents, stress, catheter-related bloodstream infections and high costs. In addition, prolonged illness contributes to lost parental working days[4]. Therefore, finding an effective way to shorten and alleviate AGE symptoms would not only benefit children's health, but also reduce social costs.

Few interventions to reduce the duration and severity of diarrhoea have been shown to be effective in AGE. Adequate rehydration is recommended as a first-line treatment, preferably with oral rehydration solutions (ORS)[3,5]. Oral rehydration reduces mortality, the need for hospitalisation and intravenous rehydration, but has no effect on the duration or intensity of AGE. Probiotics may reduce the length of hospital stay and relieve symptoms of AGE in children[3,5–7]. However, recommendations for their use are inconsistent[8,9]. European Society for Paediatric Gastroenterology Hepatology and Nutrition/ European Society for Paediatric Infectious Diseases (ESPGHAN/ESPID) and the Infectious Diseases Society of America (IDSA) currently recommend 4 probiotic

[1]The Department of Pediatrics with Clinical Assessment Unit, Medical University of Warsaw, 63A Żwirki & Wigury Street, Warsaw 02-091, Poland. [2]The Department of Pediatrics, St Hedwig of Silesia Hospital, Trzebnica, Poland. ✉email: katarzynawzorek@vp.pl

strains for AGE: *Lacticaseibacillus rhamnosus GG* and *Saccharomyces boulardii* (strong recommendation), *Limosilactobacillus reuteri* DSM 17,938 and heat-like *Lacidophilus LB* (weak recommendation). The authors of these guidelines rated the quality of the evidence as low or very low[1,3,5]. In contrast, the National Institute for Health and Care Excellence (NICE) found the evidence insufficient to recommend routine use of probiotics in this indication[10]. According to ESPGHAN/ESPID, both diosmectide and racecadotril can be considered for treating AGE in hospitalised and outpatients[3,5,10]. A lactose-free diet shortens the duration of diarrhoea, but only in inpatients[11]. Most guidelines do not support routine zinc supplementation in high-income countries, although it may be beneficial for children in developing countries[3,8,9]. Evidence for the effectiveness of other therapies (symbiotics, prebiotics, gelatine tannate, activated charcoal, kaolin-pectin) in treating and reducing the symptoms of diarrhoea is insufficient to recommend their routine use[3].

Further studies are needed to evaluate new therapeutic interventions that are effective in reducing the duration and severity of diarrhoea and the length of hospital stay.

Beta-Glucans

Beta-glucans are high molecular weight polysaccharides found in the cell walls of bacteria, fungi, marine algae and cereal grains. They exert multi-directional beneficial effects on the human body. The immunomodulatory, anti-infective, anti-tumour, hypocholesterolemic, hypoglycaemic and antihypertensive properties are the most studied[12,13]. The natural origin of β -glucans from common dietary components reduces concerns about their safety. They are well tolerated. No serious adverse events have been reported following β-glucan administration[14–18].

The anti-infective properties of β-glucans may be related to their immunomodulatory effect. However, direct antiviral and antimicrobial activity of β-glucans has also been described in several studies[12,19]. In clinical trials, oral β-glucans have been associated with reduced incidence of respiratory infections in paediatric populations[14,16,17,20], healthy adults[21], the elderly[22], athletes[23] and chronically stressed adults[24]. It has been suggested that they may help to reduce the severity of SARS-CoV-2 infection and play an important role in regulating cytokine production in patients with the cytokine storm and hyperinflammation observed during COVID-19[25,26]. Recently, it has been reported that β-glucans selectively stimulate the growth of a small number of beneficial bacteria, therefore they have prebiotic properties[27,28].

The structure and physicochemical characteristics of β-glucans strongly influence their properties. β-1,3-glucans with β-1,6 side chains are the most active immunomodulators[29]. Medium or large molecular weight, triple helix conformation in aqueous environment and a low degree of branching (between 20 and 33%) correlate with the highest immunostimulatory and antitumour activity[12,13,18,30]. Water-insoluble β-glucans have stronger immunostimulating properties than water-soluble ones[31]. Although intestinal absorption of insoluble polysaccharides is very low (systemic blood levels less than 0.5%) or undetectable, significant systemic immunostimulation is observed following oral ingestion of insoluble β-glucans. This phenomenon can be explained by the role of M cells and dendritic cell pseudopods, which transport insoluble β-glucan or its fragments from the intestinal lumen to the lymph fluid and gut-associated lymphoid tissue (Peyer's patches)[32–34].

β-glucans never occur naturally in the human body. Therefore, they are recognised by pattern recognition receptors (PRRs) as pathogen-associated molecular patterns (PAMPs). They trigger signalling pathways to stimulate the innate immune response, as well as mucosal immunity via induction of various inflammatory cytokines, chemokines and interferons[31,35]. PRRs activation initiates pathogen-specific humoral responses by activating B and T lymphocytes. It has also been suggested that β-glucans are inducers of an innate immune memory called Trained Immunity (TRIM)[26,36]. After exposure to a specific stimulus, innate immune cells undergo metabolic, mitochondrial and epigenetic changes, followed by phenotypic memory of enhanced immune responses to secondary heterologous stimulus. It remains controversial whether β-glucans can induce an adaptive immune response. However, studies have shown increased T-cell activity and interferon-ϒ production following interaction with the β-glucan-dendritic cell complex[37].

Pleuran

Pleuran is an insoluble β-(1,3/1,6)-glucan isolated from the popular dietary oyster mushroom (*Pleurotus ostreatus*). It was identified and chemically characterised by Karacsonyi and Kuniak[38]). It has a β-1,3-linked main chain with D-glucosyl side groups attached to every third backbone unit with β-1,6 links and a degree of branching of 0.25. The molecular weight of pleuran is between 600 and 700 kDa. Many studies have reported pleiotropic properties of pleuran, in particular: immunostimulatory, antitumour, hypocholesterolemic, hypoglycaemic and antidiabetic[39–42]. The immunomodulatory properties of pleuran have been demonstrated in numerous studies. Much of this research has focused on the treatment and prevention of infections in children and adults, including reducing the frequency of respiratory tract infections (RTI) in children with recurrent RTI, relieving symptoms associated with upper RTI, and treating HSV infections[14–17,20]. In all of these studies, pleuran was administered on a chronic basis, with treatment durations ranging from 3[14–16] to 6 months[17]. Pleuran is derived from a common dietary ingredient and is therefore considered to be a highly safe nutraceutical. No serious adverse events were observed in any of the above studies.

Pleuran is a promising candidate for the treatment of AGE in children due to its immunomodulatory and antiviral properties, the intestinal mucosa as the site of action, and its strong safety profile.

Objectives

This study aims to evaluate pleuran's efficacy in reducing the duration and severity of AGE. To ensure the accuracy of the results, we decided to use a placebo as a comparator.

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

Methods
Trial design
This study is a multicentre randomized, double-blind, placebo-controlled superiority trial with two parallel groups and 1:1 allocation ratio. We used the CONSORT Statement to prepare this manuscript[43].

Protocol
The protocol of this trial was registered in the Clinical Trials platform (NCT03988257) before the study commenced and published in the British Medical Journal[44].

Research ethics approval
The Bioethics Committee of The Medical University of Warsaw approved the study protocol with all the following amendments and other required documents (an information sheet for patient's caregivers, informed consent forms in the local language version, and information on personal data management). The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Study settings
We have recruited the study group from patients requiring hospitalization in the pediatric department or counselling in the emergency department (ED) due to AGE. The recruitment took place in three hospitals in Poland: The Paediatric Teaching Clinical Hospital of the Medical University of Warsaw, the Department of Pediatrics, St Hedwig of Silesia Hospital in Trzebnica, and the Department of Pediatrics of Warsaw Medical Center "KOPERNIK".

Eligibility criteria
Eligibility Criteria are presented in Table 1.

Interventions
Pre-intervention assessment
We assessed the eligibility criteria for the study by conducting physical examination and interviewing caregivers within 24 h of the patient's admission to the hospital or presentation into the ED. We provided the patient's caregiver with comprehensive information about the purpose, methods, safety, and duration of the study intervention. We have obtained written consent for the participation from the legal guardians of all patients. The patient's legal guardian had the right to withdraw the patient from the study at any time without negative consequences. We calculated body mass index (BMI) and converted it into percentiles using WHO percentile charts. Before starting the intervention, we collected stool samples to determine the aetiology of AGE (rotavirus, adenovirus and norovirus antigens from stool sample). When indicated, we also performed microbiological stool cultures.

Used scales
We used appropriate scoring systems to ensure comparability of the parameters and outcomes analysed. The severity of diarrhoea was assessed with the Modified Vesikari Score[45]. The degree of dehydration was evaluated by the WHO Scale[46] and the Clinical Dehydration Scale (CDS)[47]. The Bristol Stool Form Scale was applied to objectively assess stool consistency[48].

Inclusion criteria
• Children aged 13–120 months, hospitalized or requiring counselling in ED due to AGE, defined as a decrease in the stool consistency (grade 6 or 7 in Bristol Stool Form Scale[46]) or an increase in the frequency of stool evacuations > 3 in 24 h.
• The duration of symptoms: 24–72 h at the time of inclusion.
• A caregiver's written, informed consent.
Exclusion criteria
• The duration of symptoms > 72 h at the time of inclusion.
• Co-occurring other systemic infectious diseases (e.g., pneumonia, sepsis).
• Diagnosed immune deficiency.
• Malnutrition (defined as the body mass index (BMI) <-2 SDS (Standard Deviation Scores) or < 3rd percentile based on the World Health Organization (WHO) percentile charts.
• Chronic diarrhoeal gastrointestinal disease (inflammatory bowel disease, short bowel syndrome, cystic fibrosis, celiac disease, food allergy).
• The use of antibiotics, probiotics, prebiotics, or anti-diarrhoeal medicines (diosmectite, racecadotril) within seven days before enrolment into the study.
• The use of probiotics, diosmectite, and racecadotril during the current AGE episode.
• The use of pleuran in the 14 days before enrolment into the study.

Table 1. Eligibility criteria.

Intervention
Children in the experimental group received Imunoglukan PH4 oral suspension (10 mg of pleuran and 10 mg of vitamin C in 1 ml of syrup) at a dose of 1 ml per 5 kg of body weight. Patients in the control group received a placebo (10 mg of vitamin C in 1 ml of syrup) at a dose of 1 ml per 5 kg of body weight. Both suspensions were administered daily in the morning before the first meal. Treatment continued until symptoms of AGE resolved, as indicated by fewer than 3 stools in 24 h and normalisation of stool consistency (Bristol Stool Form Scale grade 2–5), or until day 14.

Concomitant treatment
Patients in both groups received an ORS or intravenous fluids as needed, based on their daily fluid requirements, to maintain adequate hydration. Regular diet was recommended. Each patient received antipyretic and analgesic (ibuprofen, acetaminophen) and anti-emetics (ondansetron) as needed. No other medications that could potentially interfere with the course of the disease, such as probiotics, diosmectide, or racecadotril, were administered. When indicated, antibiotics were prescribed according to the ESPGHAN/ESPID guidelines[3].

Intervention modification
If vomiting occurred within 30 min after administration of the study drug, the dose was repeated. However, if vomiting occurred more than 30 min after administration, we considered the dose to be absorbed. In the event of severe, persistent or troublesome adverse events (AEs), we were prepared to discontinue the intervention. Mild allergic reactions were treated with antihistamine medication. All adverse events were documented in the study records and reported to the manufacturer according to the established procedure. If a child had difficulty tolerating the preparation, it could be mixed with milk, tea or fruit juice.

Outcomes
All outcome measures were evaluated daily until the 14th day of intervention, using the Symptoms Diary completed by the physician and the caregiver. Each day in Symptoms Diary was divided into 8-hour intervals. The number and consistency of stools were assessed at each time interval. Outcomes are presented in Table 2. An example of a Symptoms Diary, accompanied by a comprehensive demonstration illustrating the calculation of endpoints, can be found in the Supplementary Information.

Participant timeline and Follow-up
During the hospitalisation or the emergency department visit, the physician examined the patients to assess their dehydration status, and the need for intravenous rehydration, or concomitant treatment. For hospitalised children, this assessment was performed daily. The evaluation results were documented in the Case Report Form (CRF). The patient's caregiver recorded AGE symptoms, such as the number of stools per day, their consistency, and any accompanying symptoms (fever, vomiting, abdominal pain), in a Symptom Diary during and after the hospitalisation. Following discharge, the physician contacted the caregiver via phone, daily during their treatment and every second day after the treatment ended (after the diarrhoea subsided), until the 14th day of observation. They recorded informations on the intensity of symptoms in CFR. After discharge, caregivers could contact the physicians if they needed advice.

Sample size calculation
We used the Altman nomogram to determine the sample size[47]. To demonstrate a clinically significant difference of 24 h in the duration of AGE symptoms in experimental and control groups, with a 5% significance level and a power of 90%, assuming the standard deviation of the variable in each group of 40 h, a sample of 110 patients was established as needed. To account for 10% of potential dropouts or attrition during the study, we increased the sample size to 120 patients (60 patients in each group).

Recruitment
Study participants were recruited from patients admitted to hospital or consulted in the ED due to AGE in three centres: The Paediatric Teaching Clinical Hospital of the Medical University of Warsaw, the Department

Primary outcome
The duration of diarrhoea is defined as the time (hours) until the stool consistency normalization (grade 2, 3, 4, or 5 according to the Stool Form Scale[46]) and until normalization of the number of stools per day.
Secondary outcomes
1. The number of days with the need for intravenous rehydration. A physician used CDS[49] and WHO[48] dehydration scales and physical examination to assess the indication for intravenous rehydration and the possibility of oral rehydration.
2. The number of hours until normalization of stool consistency (defined as the beginning of the first 8-hour time interval when Bristol Stool Form Scale was 2–5 [46]).
3. The number of hours until the normalization of a number of stools per day (defined as the beginning of the first 24 h period (3 consecutive time intervals) when the total number of stools did not exceed 3).
4. The duration of hospitalization due to AGE (number of days) in hospitalized children.
5. The percentage of children with moderate and severe diarrhoea was evaluated with the Modified Vesikari Scale[47] after the 14th day of observation.
6. The number of hours until the child's general condition improved according to the caregiver's assessment.

Table 2. Outcome measures.

of Pediatrics of Warsaw Medical Center "KOPERNIK", and the Department of Pediatrics, St Hedwig of Silesia Hospital in Trzebnica. The inclusion and exclusion criteria were assessed in every patient admitted to paediatric departments or ED due to AGE.

Randomization

We implemented a stratified randomization, using two subgroups based on the duration of diarrhoea at enrolment: 24–48 h (referred to as short-lasting diarrhoea - SLD) and 48–72 h (referred to as long-lasting diarrhoea - LLD). The participants of each subgroup were randomly allocated to either group A (experimental) or B (control) in blocks of four.

Allocation and blinding

The manufacturer prepared and packaged the active product and the placebo in identical bottles. These bottles were then labelled with random numbers, the meaning of which was kept blind. The randomisation list prepared by the manufacturer was placed in a sealed envelope in the administrative part of the department and kept by an independent person not involved in the study. The study product was delivered to the principal investigator in boxes of four, ensuring that each box contained two active and two placebo samples. Subsequent boxes were used to allocate consecutive patients within the subgroups. On opening each subsequent box, the researcher randomly assigned the numbers on the bottles to the next four patients in a subgroup. The contents of all bottles looked and tasted the same to maintain blinding. The researchers, carers, patients and the person responsible for statistical analysis were blind to the intervention until the end of the trial.

Data collection, data management and confidentiality

A coded paper CRF was completed to record each patient's scales and Symptom Diaries. Two independent researchers transferred the data from the CRFs into two electronic databases at the end of the study to ensure data accuracy. In case of drop-out, data and endpoints were collected up to the date of dropout, with the reason and date of dropout documented. Confidentiality of data, including personal data of study participants, was maintained.

Statistical methods

The analyses were performed using R V. 4.1.3 (2022-03-10) and Rstudio V.2022.12.0 + 353 (2022-12-0) with the help of "rstatix"[49], "tidyverse"[50], and "flextable"[51] packages. The Shapiro-Wilk W test was employed to ascertain whether the sample exhibited non-normality evidence. The Student's t-test was used to compare the means of continuous variables that conformed to a normal distribution. In contrast, the Mann-Whitney U test was used for non-normally distributed variables. The mean difference (M_{diff}) with a 95% CI was calculated for normally distributed variables, while the Hodges-Lehmann estimator was used to estimate the median difference (Me_{diff}) and its confidence interval for non-normally distributed variables. The χ^2 test, together with Phi or Cramer's V effect size, as appropriate, was used to compare proportions for the categorical variables. The difference between study groups was deemed significant when the 95% CI for mean/median difference did not include 0 (equivalent to $p < 0.05$) and the p-value for χ^2 was smaller than 0.05. All statistical analyses were conducted using a two-tailed test with a significance level of 5%. The IQR in Tables 3 and 4 is defined as the difference between the 25th percentile (Q1) and the 75th percentile (Q3). These percentiles were calculated using R's default Type 7 quantile method, which follows the interpolation approach recommended by Hyndman and Fan (1996). We have decided to use interquartile range as one of several established methods for computing sample quantiles.

Harms

We carefully recorded all AEs on the CRF after enrolment and throughout the observation period. These AEs were promptly reported to the manufacturer according to a predefined procedure. An AE was considered serious if it met one of the following criteria: death, life-threatening condition, serious or permanent disability, prolonged hospitalisation, or significant risk.

Results

Recruitment started in June 2019 and ended in December 2022. Twenty-seven children were enrolled and randomly assigned to one of the two groups – 13 patients in group A (experimental) and 14 in group B (control). Due to a lost contact with the caregivers and therefore inability to collect measurements, it was necessary to exclude one patient from the analysis of the duration of diarrhoea, the need for antibiotics, the severity of diarrhoea according to the Vesikari scale, the time to normalisation of stool consistency, the time to normalisation of the number of stools, and the time to improvement of the child's general condition according to the caregiver's assessment. For these outcomes, 26 patients were included in the analysis (12 and 14 in groups A and B, respectively). All 27 children were included in the assessment of intravenous rehydration duration (13 and 14 in groups A and B respectively). We excluded children who only had an emergency department visit from the analysis of length of hospitalisation ($n = 9$), because the intervention did not affect the length of hospital stay. Therefore, we evaluated 18 patients (8 and 10 from groups A and B, respectively) for this outcome. All analyses were performed in the groups as originally assigned.

After the study was completed, we were able to contact the caregiver of the lost patient from group A - the reason for not answering the phone was a difficult family situation; they did not observe any adverse events and the patient stopped taking the study medication after leaving the hospital.

Baseline demographic and clinical characteristics are shown in Table 3. Group A had a higher proportion of female participants, more prolonged diarrhoea, and more episodes of vomiting and abdominal pain. In group B, the aetiology of diarrhoea was more often related to rotavirus. However, all observed differences were deemed

Characteristics	Group A $N=13$	Group B $N=14$	P values
Duration of diarrhoea at the time of the intervention (hours), mean (SD)	42.46 (13.62)	41.71 (12.62)	0.804
Duration of diarrhoea at the time of the intervention (hours), median (IQR)	48.0 (24)	40.0 (14)	
Age (months), mean (SD)	32.85 (28.05)	38.29 (31.45)	0.481
Age (months), median (IQR)	30.0 (20)	26.5 (23)	
Sex n (%) (W)	8 (61.5)	4 (28.6)	0.182
Duration of diarrhoea at the time of enrolment (strata SLD/ LLD n (%) (LLD)	5 (38.5)	3 (21.4)	0.585
Vomiting n (%)	9 (69.2)	5 (35.7)	0.175
Stomach Pain n (%)	12 (92.3)	8 (57.1)	0.100
Fever n (%)	8 (61.5)	7 (50)	0.830
Need for antibiotic use n (%)	2 (16.7)	2 (14.3)	1.00
Etiology of acute gastroenteritis n (%)			0.566
Norovirus	1 (7.7)	0 (0)	
Rotavirus	3 (23.1)	6 (42.9)	
Salmonella	1 (7.7)	1 (7.1)	
Unknown	8 (61.5)	7 (50)	
Dehydration level before enrolment according to the Clinical Dehydration Scale (no/some/moderate-severe) n (%)			1.000
Mild	9 (69.2)	9 (64.3)	
Not present	4 (30.8)	5 (35.7)	
Dehydration level before enrolment according to the WHO scale (no/some/severe) n (%)			0.560
Mild	3 (23.1)	4 (28.6)	
Not present	9 (69.2)	10 (71.4)	
Severe	1 (7.7)	0 (0)	

Table 3. Baseline demographic and clinical characteristics. W- woman, SLD- short-lasting diarrhea, LLD- long-lasting diarrhea, WHO- World Health Organisation, SD- standard deviation, IQR- interquartile range.

Outcomes	Group A	Group B	*P* values	MD/V	95% CI
Duration of diarrhoea (hours), median (IQR)	96.0 (70.0)	116.0 (32.0)	0.836	-8.00	-48 to 40
Duration of hospitalization (days), mean (SD)	3.5 (1.8)	3.4 (1.1)	0.891	0.10	-1.47 to 1.67
Duration of intravenous rehydration (days), median (IQR)	1.0 (1.0)	2.0 (1.8)	0.545	0.00	-1 to 1
Time until normalization of stool consistency (hours), median (IQR)	96.0 (70.0)	92.0 (64.0)	0.485	8.00	-24 to 64
Time until normalization of the number of stools (hours), median (IQR)	56.0 (52.0)	84.0 (52.0)	0.226	-24.00	-64 to 24
Time until the improvement of the child's general condition according to the caregiver's assessment (days), median (IQR)	2.5 (2.5)	1.5 (3.0)	0.598	0.00	-1 to 2
Severity of diarrhoea according to the Vesikari Scale, mean, mean (SD)	10.6 (1.6)	9.6 (2.9)	0.311	0.94	-0.94 to 2.82
Duration of diarrhoea since intervention (hours), median (IQR)	56.0 (76.0)	84.0 (44.0)	0.605	-8.00	-48 to 40
Time until normalization of stool consistency since intervention (hours), median (IQR)	56.0 (76.0)	56.0 (66.0)	0.757	8.00	-32 to 56
Time until normalization of the numbers of stools since intervention (hours), median (IQR)	12.0 (54.0)	32.0 (70.0)	0.268	-24.00	-72 to 24
The severity of diarrhoea according to the Vesikari Scale (mild/moderate/severe) (n), %			0.653	0.18	
Mild	1 (8.3%)	3 (21.4%)			
Moderate	8 (66.7%)	8 (57.1%)			
Severe	3 (25.0%)	3 (21.4%)			
Adverse events (n), %			0.970	0.01	
No	12(92.3%)	14(100.0%)			
Yes	1 (7.7%)	0 (0.0%)			

Table 4. Clinical endpoints measures summary. MD- mean or median difference, as appropriate; NA- not applicable; V- Cramer's V or Phi, as appropriate; SD- standard deviation, IQR- interquartile range.

statistically insignificant which means that the two groups were similar in terms of baseline demographics and clinical characteristics.

Clinical endpoints

A summary of the endpoint measures is shown in Table 4. The duration of diarrhoea since diagnosis (Me_{diff} = -8 h, 95% CI -48 to 40) as well as from intervention (Me_{diff} = -8 h, 95% CI -48 to 40), the duration of hospitalisation (M_{diff} = 0,1 days, 95% CI -1,47 to 1,67) and the duration of intravenous rehydration (Me_{diff} = 0 days, 95% CI -1 to 1) were comparable in both groups. Similarly, there were no significant differences in time until normalisation of stool consistency both since diagnosis (Me_{diff} = 8 h, 95% CI -24 to 64) as well as intervention (Me_{diff} = 8 h, 95% CI -32 to 56). No differences in time until normalisation of the numbers of stools, since diagnosis (Me_{diff} = -24 h, 95% CI -64 to 24) and intervention (Me_{diff} = -24 h, 95% CI -72 to 24) was found. Groups also did not differ in time until the improvement of the child's general condition according to the caregiver's assessment (Me_{diff} = 0 days, 95% CI -1 to 2) and severity of diarrhoea according to Vesikari Scale (M_{diff} = 0.94, 95% CI -0.94 to 2.82). 26 children did not experience adverse events (V = 0.01, p = 0.970). One patient from experimental group experienced a rash. The AE was classified as mild and managed with antihistaminic medication. The causal relationship between the study medication and the rash was assessed as possible. Chronology was compatible as the adverse event appeared 2 days after the treatment initiation. However, a viral infection could be an alternative explanation.

Discussion

Principal findings

The aim of this study was to evaluate the efficacy of pleuran in reducing the duration and severity of AGE. We demonstrated that pleuran was ineffective in shortening the duration of AGE and alleviating its symptoms in a paediatric population. A small advantage towards the experimental group in terms of duration of diarrhoea, duration of intravenous rehydration and time to normalisation of stool count was not statistically significant.

Apart from small differences, the baseline characteristics of the experimental and control groups were comparable. We excluded children who only attended an emergency department from the analysis of the endpoint "The duration of hospitalisation", because the intervention did not affect the length of hospital stay. Therefore, there was no cause-and-effect relationship. Given that the average duration of acute infectious diarrhoea is 5–7 days, we decided to exclude patients with diarrhoea lasting longer than 72 h from the study. We assumed that these children's symptoms may have resolved spontaneously regardless of treatment.

No serious adverse events were observed during the study. This observation is consistent with other studies using pleuran and other β-glucans. β-Glucans are derived from popular dietary sources and show no signs of toxicity. They are therefore considered to be safe.

To the best of our knowledge, this is the first and only RCT to evaluate the efficacy of pleuran or other β-glucans in the treatment of acute gastroenteritis in children. However, several reports have evaluated the efficacy and safety of pleuran in the prevention of recurrent respiratory tract infections (RRTIs) in children. A significant reduction in the incidence of both upper and lower respiratory tract infections was reported in RCT by Jesenak et al. (n = 175)[17]. The authors of multicentre open-label trial demonstrated a statistically significant reduction in the incidence of RTIs in children after minimum 3 months of pleuran supplementation, compared to the same group in the year before the study (n = 1030)[14]. Urbancikova et al. reported a shorter duration of HSV infection after short-term administration of pleuran, as well as shorter upper respiratory tract symptoms after long-term therapy in both children (above 6 years of age) and adults[15].

Strengths of the study

The strength of this trial is its randomised, fully blinded, placebo-controlled design. Standard Protocol Items: The Recommendations for Interventional Trials (SPIRIT) Checklist was used to prepare the study protocol, to ensure the implementation of appropriate methods[52]. In addition, the CONSORT statement was used to prepare this manuscript[43] (See Supplementary Information). Stratified randomisation with two subgroups ensured an even distribution of children with shorter and longer duration of diarrhoea at inclusion in the experimental and control groups. We decided to present the duration of diarrhoea, the time to normalisation of stool count and the time to normalisation of stool consistency as two values - from the onset of symptoms and from the start of the intervention. Although the sample size is small, this study can be used as a pilot study for further research. Our experience has allowed us to point in the direction of future studies in this area.

Limitations

The main limitation of this study is the small sample size. Before the study started, we calculated that 120 patients would be an adequate sample size. However, we have managed to recruit 27 patients, which is 23% of the expected number. During the first year of the trial, the main factor contributing to delayed recruitment was a significant number of patients meeting at least one exclusion criterion. The most common exclusion criteria were symptoms lasting more than 72 h at the time of presentation to hospital, parents administering probiotics before seeking medical advice, and patients younger than 13 months. We saw a positive trend: parents now have easy access to knowledge enabling them to confidently provide basic treatment for gastroenteritis, such as oral rehydration and probiotics, at home. As a result, they only report to the hospital after a few days if the home treatment fails. Two protocol amendments were introduced during the trial to improve the pace of recruitment (see Supplementary Information for a detailed description of these amendments). In the following months, the profile of hospitalised patients changed significantly due to the SARS-CoV-2 pandemic. Most children with acute gastroenteritis were treated as outpatients. This made it even more difficult to recruit the planned number of patients. Since the global SARS-CoV-2 pandemic subsided (January 2022), the pace of recruitment has increased significantly, but

not enough to make up for the previous two years. However, the probability of a significant effect with a larger group appears to remain low, given the considerable width of the confidence interval for the observed difference between the two groups in the endpoints analysed (at the 95% confidence level).

In this study, we focused exclusively on the clinical effect of pleuran. It would have been beneficial to test the effect of β-glucan supplementation on laboratory parameters demonstrating immune responses. In particular, mucosal immunity markers in pleuran users, e.g. salivary lysozyme or IgA levels, would provide a more comprehensive understanding of the mechanisms underlying the enhancement of the immune response. However, there is a wealth of data available on the effects of β-glucans on various laboratory markers of immune stimulation in children[17,53–56]. Another limitation of the study is that the follow-up was based on a conversation with the patient's caregivers, without a personal examination of the patient, or controlling the amount of used product. We decided to use telephone follow-up because we expected poor compliance if parents had to return to hospital with a healthy child or return the bottle with the study medication at the end of the treatment. The assessment of the patient's symptoms and condition after discharge from hospital was based on the parents' subjective assessment, which could be a source of bias.

Vitamin C as comparator

In our study, we chose to use a commercially available pleuran preparation that also contains vitamin C. In order to minimise the effect of this vitamin on the results of the study, a matching placebo was manufactured, containing the same concentration of vitamin C. The used dose of vitamin C (2 mg/kg of body weight, maximum 50 mg/day) meets the EFSA recommended daily allowance for children[57]. The longest ingestion of the preparation was 13 days. To our knowledge, there are no data indicating a possible effect of this dose on the duration or severity of diarrhoea, either in children or adults. We found no studies concerning the role of vitamin C in acute gastroenteritis treatment. Although an effect of vitamin C on immune mechanisms in humans has been suggested for years, studies to date have not confirmed those hypotheses. According to Cochrane Systemic Review, chronic use of much higher doses of vitamin C led to a small and clinically insignificant reduction in the duration of the common cold of 14% and 18% with the use of 200 mg /day and 1000 mg/ day respectively[58].

Probiotics as exclusion criteria

In order to increase the reliability of the study results, we decided to exclude patients who had been given probiotics during the episode of AGE. Probiotics have been shown to reduce the length of hospital stay and to relieve the symptoms of AGE in children[3,5–7]. However, there is not enough evidence that they work to recommend them routinely. Guidelines from major medical societies are inconsistent, ranging from "should be considered"[3,5] to "are not recommended"[10]. The administration of probiotics prior to recruitment was one of the main factors contributing to the under-enrolment of the sample. Given that a significant number of patients now receive a probiotic prior to medical advice, the authors of this study believe that further research should not exclude the use of probiotics, but instead administer pleuran as an adjunctive treatment. This approach is also supported by the potential prebiotic properties of β-glucans, which could enhance the effectiveness of probiotics. It is important to note that all study participants should receive the same strain of probiotic bacteria. Moreover, a β-glucan with proven prebiotic properties for particular probiotic strain should be used. For example, studies have shown the stimulatory effect of pleuran on the growth of *Lacticaseibacillus* strains, so this β-glucan-probiotic pair could be one of potential choices for further trials[59].

Conclusions

Despite promising results in children with respiratory infections, our experimental study shows that the use of pleuran does not reduce the duration and symptoms of acute gastroenteritis in children. Therefore, there is no evidence to support its use in infectious diarrhoea. However, given pleuran's ability to stimulate intestinal innate immunity, prebiotic properties and good tolerability, larger studies are needed to further investigate its potential as a nutraceutical in the paediatric population. The authors of this article hypothesise that the combination of pleuran with probiotic *Lacticaseibacillus* strains, particularly those demonstrated to alleviate the symptoms of gastroenteritis in children (e.g. *Lacticaseibacillus rhamnosus* GG or *Limosilactobacillus reuteri* DSM 17938), may represent a promising avenue for further investigation. This study can be used as a pilot study for further research.

Data availability

The study protocol is publicly available via the ClinicalTrials platform (NCT03988257) and published in BMJ. The data that support the findings of this study are available upon reasonable request from corresponding author. Data requestors would need to sign a data access agreement to gain access.

Received: 25 September 2024; Accepted: 17 March 2025
Published online: 15 May 2025

References

1. Szajewska, H. et al. Use of probiotics for management of acute gastroenteritis. *J. Pediatr. Gastroenterol. Nutr.* **58** (4), 531–539 (2014).
2. WHO. (2024). https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease. WHO Fact sheets: Diarhhoeal Disease.
3. Guarino, A. et al. European society for pediatric gastroenterology, hepatology, and nutrition/european society for pediatric infectious diseases Evidence-Based guidelines for the management of acute gastroenteritis in children in Europe. *J. Pediatr. Gastroenterol. Nutr.* **59** (1), 132–152 (2014).
4. PROTECT. The paediatric burden of rotavirus disease in Europe. *Epidemiol. Infect.* **134** (5), 908–916 (2006).

5. Shane, A. L. et al. 2017 infectious diseases society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. *Clin. Infect. Dis.* **65** (12), e45–80 (2017).
6. Dinleyici, E. C., Eren, M., Ozen, M., Yargic, Z. A. & Vandenplas, Y. Effectiveness and safety of *Saccharomyces boulardii* for acute infectious diarrhea. *Expert Opin. Biol. Ther.* **12** (4), 395–410 (2012).
7. Freedman, S. B., Ali, S., Oleszczuk, M., Gouin, S. & Hartling, L. Treatment of acute gastroenteritis in children: an overview of systematic reviews of interventions commonly used in developed countries. *Evid. Based Child. Health.* **8** (4), 1123–1137 (2013).
8. van den Berg, J. & Berger, M. Y. Guidelines on acute gastroenteritis in children: a critical appraisal of their quality and applicability in primary care. *BMC Fam Pract.* **12** (1), 134 (2011).
9. Lo Vecchio, A. et al. Comparison of recommendations in clinical practice guidelines for acute gastroenteritis in children. *J. Pediatr. Gastroenterol. Nutr.* **63** (2), 226–235 (2016).
10. NICE. Diarrhoea and vomiting caused by gastroenteritis in under 5s: diagnosis and management. *NICE guideline* (2009). https://www.nice.org.uk/guidance/cg84/resources/diarrhoea-and-vomiting-caused-by-gastroenteritis-in-under-5s-diagnosis-and-management-pdf-975688889029
11. MacGillivray, S., Fahey, T. & McGuire, W. Lactose avoidance for young children with acute diarrhoea. *Cochrane Database Syst. Reviews*; 10:CD005433 (2013).
12. Giavasis, I. Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Curr. Opin. Biotechnol.* **26**, 162–173 (2014).
13. Khan, A. A., Gani, A., Khanday, F. A. & Masoodi, F. A. Biological and pharmaceutical activities of mushroom β-glucan discussed as a potential functional food ingredient. *Bioactive Carbohydr. Diet. Fibre.* **16**, 1–13 (2018).
14. Rennerova, Z. et al. Beta-(1,3/1,6)-D-glucan from pleurotus ostreatus in the prevention of recurrent respiratory tract infections: an international, multicentre, open-label, prospective study. *Front. Pediatr.* **10:**999701 (2022).
15. Urbancikova, I. et al. Efficacy of pleuran (β -Glucan from *Pleurotus ostreatus*) in the management of herpes simplex virus type 1 infection. *Evidence-Based Complement. Altern. Med.* **2020**, 1–8 (2020).
16. Paśnik, J., Ślemp, A., Cywińska-Bernaś, A., Zeman, K. & Jesenak, M. Preventive effect of pleuran (β-glucan from pleurotus ostreatus) in children with recurrent respiratory tract infections - Open-label prospective study. *Curr. Pediatr. Res.* **21** (1), 99–104 (2017).
17. Jesenak, M. et al. Immunomodulatory effect of pleuran (β-glucan from pleurotus ostreatus) in children with recurrent respiratory tract infections. *Int. Immunopharmacol.* **15** (2), 395–399 (2013).
18. Meng, Y., Lyu, F., Xu, X. & Zhang, L. Recent advances in chain conformation and bioactivities of Triple-Helix polysaccharides. *Biomacromolecules* **21** (5), 1653–1677 (2020).
19. Wzorek-Łyczko, K., Woźniak, W., Piwowarczyk, A. & Kuchar, E. The anti-infective effect of β-glucans in children. *Int. J. Vitam. Nutr. Res.* **94** (3-4), 96-307 (2024).
20. Grau, J. S., Sirvent, L. P., Morera Ingles, M. & Urgell, M. R. Beta-glucans from pleurotus ostreatus for prevention of recurrent respiratory tract infections. *Acta Pediatr. Esp.* **73**, 186–193 (2015).
21. Auinger, A., Riede, L., Bothe, G., Busch, R. & Gruenwald, J. Yeast (1,3)-(1,6)-beta-glucan helps to maintain the body's defence against pathogens: a double-blind, randomized, placebo-controlled, multicentric study in healthy subjects. *Eur. J. Nutr.* **52** (8), 1913–1918 (2013).
22. Fuller, R. et al. Yeast-derived β-1,3/1,6 glucan, upper respiratory tract infection and innate immunity in older adults. *Nutrition* **39–40**, 30–35 (2017).
23. Bergendiova, K., Tibenska, E. & Majtan, J. Pleuran (β-glucan from pleurotus ostreatus) supplementation, cellular immune response and respiratory tract infections in athletes. *Eur. J. Appl. Physiol.* **111** (9), 2033–2040 (2011).
24. Talbott, S. M. & Talbott, J. A. Baker's yeast Beta-Glucan supplement reduces upper respiratory symptoms and improves mood state in stressed women. *J. Am. Coll. Nutr.* **31** (4), 295–300 (2012).
25. Rao, K. S. et al. Role of immune dysregulation in increased mortality among a specific subset of COVID-19 patients and immune-Enhancement strategies for combatting through nutritional supplements. *Front. Immunol.* **11:**1548. (2020).
26. Geller, A. & Yan, J. Could the induction of trained immunity by β-Glucan serve as a defense against COVID-19? *Front. Immunol.* **11:**1782. (2020).
27. Liang, J. et al. Edible fungal polysaccharides, the gut microbiota, and host health. *Carbohydr. Polym.* **273**, 118558 (2021).
28. Delatte, S. J. et al. Effectiveness of beta-glucan collagen for treatment of partial-thickness burns in children. *J. Pediatr. Surg.* **36** (1), 113–118 (2001).
29. Zeković, D. B., Kwiatkowski, S., Vrvić, M. M., Jakovljević, D. & Moran, C. A. Natural and modified (1→3)-β-D-Glucans in health promotion and disease alleviation. *Crit. Rev. Biotechnol.* **25** (4), 205–230 (2005).
30. El Enshasy, H. A. & Hatti-Kaul, R. Mushroom immunomodulators: unique molecules with unlimited applications. *Trends Biotechnol.* **31** (12), 668–677 (2013).
31. Lee, D. H. & Kim, H. W. Innate immunity induced by fungal β-Glucans via Dectin-1 signaling pathway. *Int. J. Med. Mushrooms.* **16** (1), 1–16 (2014).
32. Vetvicka, V., Vannucci, L., Sima, P. & Richter, J. Beta glucan: supplement or drug?? From laboratory to clinical trials. *Molecules* **24** (7), 1251 (2019).
33. Garrett, W. S., Gordon, J. I. & Glimcher, L. H. Homeostasis and inflammation in the intestine. *Cell* **140** (6), 859–870 (2010).
34. Leentjens, J. et al. The effects of orally administered Beta-Glucan on innate immune responses in humans, a randomized Open-Label intervention Pilot-Study. *PLoS One.* **9** (9), e108794 (2014).
35. Tsukada, C. et al. Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of β-glucan. *Cell. Immunol.* **221** (1), 1–5 (2003).
36. Netea, M. G. et al. Trained immunity: A program of innate immune memory in health and disease. Science 2016;352(6284). (1979).
37. Lin, Y. L., Liang, Y. C., Lee, S. S. & Chiang, B. L. Polysaccharide purified from *Ganoderma lucidum* induced activation and maturation of human monocyte-derived dendritic cells by the NF-κB and p38 mitogen-activated protein kinase pathways. *J. Leukoc. Biol.* **78** (2), 533–543 (2005).
38. Karacsonyl, S. & Kuniak, L. Polysaccharides of pleurotus ostreatus: isolation and structure of pleuran, an alkali-insoluble β-D-glucan. *Carbohydr. Polym.* **24** (2), 107–111 (1994).
39. EL-Deeb, N. M. et al. ŸW, et al. Modulation of NKG2D, KIR2DL and cytokine production by pleurotus ostreatus glucan enhances natural killer cell cytotoxicity toward cancer cells. *Front. Cell. Dev. Biol.* **13** (7), 165 (2019).
40. Abrams, D. I. et al. Antihyperlipidemic effects of pleurotus ostreatus (oyster mushrooms) in HIV-infected individuals taking antiretroviral therapy. *BMC Complement. Altern. Med.* **11** (1), 60 (2011).
41. Schneider, I. et al. Lipid Lowering effects of oyster mushroom (Pleurotus ostreatus) in humans. *J. Funct. Foods.* **3** (1), 17–24 (2011).
42. Jayasuriya, W. J. A. B. N., Wanigatunge, C. A., Fernando, G. H., Abeytunga, D. T. U. & Suresh, T. S. Hypoglycaemic activity of culinary *Pleurotus ostreatus* and *P. cystidiosus* mushrooms in healthy volunteers and type 2 diabetic patients on diet control and the possible mechanisms of action. *Phytother. Res.* **29** (2), 303–309 (2015).
43. Schulz, K. F., Altman, D. G. & Moher, D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMC Med.* **8** (1), 18 (2010).
44. Wzorek-Łyczko, K., Piwowarczyk, A. & Kuchar, E. Protocol of the study: the effectiveness of pleuran in the treatment of acute gastroenteritis in children—a randomised, placebo-controlled, double-blind trial (EPTAGE). *BMJ Open.* **11** (3), e042370 (2021).

45. Schnadower, D. et al. Validation of the modified Vesikari score in children with gastroenteritis in 5 US emergency departments. *J. Pediatr. Gastroenterol. Nutr.* **57** (4), 514–519 (2013).
46. WHO. The Treatment of diarrhoea: a manual for physicians and other senior health workers 4th rev. (2005).
47. Friedman, J. N., Goldman, R. D., Srivastava, R. & Parkin, P. C. Development of a clinical dehydration scale for use in children between 1 and 36 months of age. *J. Pediatr.* **145** (2), 201–207 (2004).
48. Wojtyniak, K., Szajewska, H. & Dziechciarz, P. Translation to Polish, cross-cultural adaptation, and validation of the Bristol stool form scale among healthcare professionals and patients. *Gastroenterol. Rev.* **13** (1), 35–39 (2018).
49. Kassambara, A. https://CRAN.R-project.org/package=rstatix. 2023. rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.7.2.
50. Wickham, H. et al. Welcome to the tidyverse. *J. Open. Source Softw.* **4** (43), 1686 (2019).
51. Gohel, D. https://CRAN.R-project.org/package=flextabl. 2021. flextable: Functions for Tabular Reporting. R package version 0.6.10.
52. Chan, A. W. et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann. Intern. Med.* **158** (3), 200 (2013).
53. Li, F., Jin, X., Liu, B., Zhuang, W. & Scalabrin, D. Follow-up formula consumption in 3- to 4-Year-Olds and respiratory infections: an RCT. *Pediatrics* **133** (6), e1533–e1540 (2014).
54. Vetvicka, V., Richter, J., Svozil, V., Rajnohová Dobiášová, L. & Král, V. Placebo-driven clinical trials of yeast-derived β-(1–3) glucan in children with chronic respiratory problems. *Ann. Transl Med.* **1** (3), 26 (2013).
55. Richter, J. et al. Clinical trials of yeast-derived β-(1,3) glucan in children: effects on innate immunity. *Ann. Transl Med.* **2** (2), 15 (2014).
56. Sarinho, E. et al. Production of interleukin-10 in asthmatic children after Beta-1-3-glucan. *Allergol. Immunopathol. (Madr).* **37** (4), 188–192 (2009).
57. Scientific Opinion on Dietary Reference Values for vitamin C. *EFSA Journal* **11** (11), 3418 (2013).
58. Hemilä, H. & Chalker, E. Vitamin C for preventing and treating the common cold. *Cochrane Database Syst. Reviews.* **2013**(1):CD000980 (2013).
59. Synytsya, A. et al. Glucans from fruit bodies of cultivated mushrooms pleurotus ostreatus and pleurotus eryngii: structure and potential prebiotic activity. *Carbohydr. Polym.* **76** (4), 548–556 (2009).

Acknowledgements
We would like to thank Dr Zbigniew Lewandowski for helping us to design the study and for providing statistical expertise.

Author contributions
All authors meet The ICMJE Guidelines for Authorship. Anna Piwowarczyk (AP) initially conceptualized the study. Katarzyna Wzorek-Łyczko (KWŁ), Ernest Kuchar (EK), AP, and Henryk Szymański (HSz) initiated the study design and helped with its implementation. KWŁ and WW (Weronika Woźniak) conducted the study. KWŁ and AP provided statistical expertise and analysed the data with blinded statistician supervision. EK was a grant holder. KWŁ and AP, wrote the first draft of the manuscript. All authors have contributed to and approved the final version.

Funding
The study was funded by The Nutricia Foundation's research grant No. RG3-2018, covering costs of recruitment, equipment, and administrative costs. The statutory resources of The Department of Pediatrics with Clinical Assessment Unit at The Medical University of Warsaw were acquired to finance the voluntary liability insurance of the researcher. The active product and placebo were manufactured, weighed, packaged, and donated to the study by PLEURAN s.r.o., Slovakia. Both The Nutricia Foundation and PLEURAN s.r.o. had no role in the design of this study, its execution, analyses, interpretation of the data, or decision to submit results. An agreement was signed between the Medical University of Warsaw (WUM) and the PLEURAN s.r.o. substantially limiting the right of PLEURAN s.r.o., in particular, no financial obligations between the parties and no rights to documentation created as a result, in the course of or in connection with the Study for PLEURAN. WUM committed to sharing with PLEURAN unprocessed data (not including the personal data of patients) obtained during the study, provided that in case PLEURAN concluded separate results using this database, and at the same time, PLEURAN would like to refer to the study, these results should be agreed with WUM.

Declarations

Competing interests
All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript. An agreement between the Medical University of Warsaw (WUM) and the PLEURAN s.r.o. was described above.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-94893-3.

Correspondence and requests for materials should be addressed to K.W.-Ł.

7. Podsumowanie i wnioski

Wnioski:

1. Brak potwierdzenia skuteczności klinicznej pleuranu w leczeniu AGE.

Na podstawie przeprowadzonego, podwójnie zaślepionego badania klinicznego z randomizacją należy stwierdzić, że pleuran nie wykazuje istotnej klinicznie skuteczności w leczeniu ostrych biegunek infekcyjnych u dzieci.

2. Potwierdzenie bezpieczeństwa pleuranu w populacji dziecięcej.

Preparat zawierający pleuran charakteryzuje się doskonałym profilem bezpieczeństwa w populacji dzieci, bez występowania poważnych działań niepożądanych podczas 14- dniowej obserwacji, co pozwala na bezpieczne stosowanie preparatu w pozostałych wskazaniach, jak też w przyszłych badaniach klinicznych.

3. Ograniczona przydatność pleuranu w praktyce klinicznej AGE.

W oparciu o uzyskane wyniki nie ma podstaw naukowych do rekomendowania pleuranu jako terapii wspomagającej w leczeniu ostrych zakażeń przewodu pokarmowego u dzieci.

4. Potencjał zastosowania pleuranu w innych wskazaniach.

Wykazane mechanizmy immunomodulującego działania oraz doskonały profil bezpieczeństwa sugerują potencjał zastosowania pleuranu w innych wskazaniach, szczególnie w profilaktyce zakażeń układu oddechowego oraz wspieraniu odporności u dzieci z niedoborami odporności. Dalsze badania powinny koncentrować się na tych obszarach zastosowań.

5. Konieczność ukierunkowanych dalszych badań wieloośrodkowych.

Ze względu na heterogeniczność wyników dostępnych w piśmiennictwie oraz ograniczenia metodologiczne pojedynczych badań, konieczne są dalsze randomizowane próby kliniczne o większej mocy statystycznej (n > 500), prowadzone w wielu ośrodkach medycznych. Przyszłe badania powinny uwzględniać stratyfikację według wieku, etiologii infekcji oraz statusu żywieniowego pacjentów. Dalsze badania powinny koncentrować się na identyfikacji optymalnych populacji pacjentów oraz wskazań klinicznych, w których immunomodulujące właściwości β-glukanów mogą przynieść największe korzyści terapeutyczne. Szczególnie obiecujące wydają się zastosowania w profilaktyce infekcji u dzieci z niedoborami odporności oraz w terapii wspomagającej chorób autoimmunizacyjnych. Biorąc pod uwagę zdolność pleuranu do stymulacji odporności wrodzonej w obrębie jelitowego układu chłonnego, jego właściwości prebiotyczne oraz dobrą tolerancję, konieczne są szerzej zakrojone badania nad jego zastosowaniem w zakażeniach przewodu pokarmowego. Na podstawie dotychczasowej literatury oraz naszych doświadczeń wysunęliśmy hipotezę, że połączenie pleuranu z probiotykami z rodzaju *Lacticaseibacillus*, zwłaszcza szczepami skutecznymi w łagodzeniu objawów biegunki dzieci (np. *L. rhamnosus* GG lub *L. reuteri* DSM 17938), może stanowić obiecujący kierunek dalszych badań – a niniejsze badanie może pełnić rolę pilotażową. Przedstawiona metodyka badawcza może służyć jako fundament dla przyszłych projektów oceniających skuteczność innych substancji bioaktywnych pochodzenia naturalnego, przyczyniając się do rozwoju medycyny opartej na dowodach w obszarze nutraceutyków pediatrycznych.

Podsumowanie

Niniejsza rozprawa doktorska stanowi pionierską próbę oceny skuteczności pleuranu w leczeniu ostrej biegunki infekcyjnej (AGE) u dzieci. W ramach cyklu trzech publikacji przeprowadziliśmy kompleksową analizę: od przeglądu literatury, przez opracowanie protokołu badania klinicznego, po realizację randomizowanej, podwójnie zaślepionej próby kontrolowanej placebo. Mimo, że badanie nie potwierdziło skuteczności pleuranu w skracaniu czasu trwania biegunki ani łagodzeniu jej objawów, wykazano bardzo dobry

profil bezpieczeństwa tej substancji w populacji dziecięcej. Wyniki te pozwalają na wyciągnięcie ważnych wniosków praktycznych i metodologicznych oraz wskazują potencjalne kierunki dalszych badań – zwłaszcza w zakresie zastosowania pleuranu w profilaktyce infekcji dróg oddechowych czy jako składnika terapii wspomagającej u dzieci z zaburzeniami odporności. Rozprawa wnosi istotny, oryginalny wkład do rozwoju badań nad bioaktywnymi polisacharydami w medycynie wieku rozwojowego.

8. Opinia Komisji Bioetycznej

Komisja Bioetyczna
przy Warszawskim Uniwersytecie Medycznym

Tel.: 022/ 57 - 20 -303
Fax: 022/ 57 - 20 -165

ul. Żwirki i Wigury nr 61
02-091 Warszawa

e-mail: komisja.bioetyczna@wum.edu.pl
www.komisja-bioetyczna.wum.edu.pl

KB/45../2018

Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym
w dniu 18 kwietnia 2018 r. po zapoznaniu się z wnioskiem:

Lek. Katarzyna Wzorek-Łyczko,
Klinika Pediatrii z Oddziałem Obserwacyjnym,
ul. Żwirki i Wigury 63A, 02-091 Warszawa,

dotyczącym: wyrażenia opinii w sprawie badania pt :„Skuteczność pleuranu w leczeniu ostrej biegunki infekcyjnej u dzieci-badanie z randomizacją, kontrolowane placebo, prowadzone metodą podwójnie ślepej próby."

wyraża następującą
o p i n i ę

- stwierdza, że jest ono dopuszczalne i zgodne z zasadami naukowo-etycznymi*.
- ~~stwierdza, że jest ono niedopuszczalne i niezgodne z zasadami naukowo-etycznymi.*~~

Uwagi Komisji – *verte*

Komisja działa na podstawie art.29 ustawy z dnia 5.12.1996r. o zawodzie lekarza /Dz.U.nr 28/97 poz.152 wraz z późn.zm./, zarządzenia MZiOS z dn.11.05.1999r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych /Dz.U.nr 47 poz.480/, Ustawy prawo farmaceutyczne z dnia 6 września 2001r. (Dz.U.Nr 126, poz. 1381 z późn. zm.) oraz Zarządzenie nr 56/2007 z dnia 15 października 2007r. w sprawie działania Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym /Regulamin Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym/.
Komisja działa zgodnie z zasadami GCP .
W załączeniu: skład komisji oraz lista obecności

Przewodnicząca Komisji Bioetycznej
Dr hab. n. med. Magdalena Kuźma-Kozakiewicz

*niepotrzebne skreślić

Komisja wyraża pozytywną opinię w sprawie przeprowadzenia wnioskowanych badań- na warunkach określonych we wniosku oraz dodatkowo zastrzegając:
1/ obowiązek przedstawienia Komisji:

- wszystkich zmian w protokole mających wpływ na przebieg oraz ocenę badania,
- wszystkich przypadków zdarzeń niepożądanych,
- zawiadomienia o przyczynach przedwczesnego zakończenia badania,
- sprawozdania w toku przeprowadzonych badań-za sześć miesięcy,
- raportu końcowego.

strona podpisowa do uchwały Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym nr KB/.....45..... z dnia 18 kwietnia 2018r.

1. Dr hab. n.med. Magdalena Kuźma –Kozakiewicz .....
2. Dr hab. n. med. Tomasz Grzela .....
3. Dr hab. n. med. Andrea Horvath-Stolarczyk .....
4. Prof. dr hab. n. med. Paweł Piątkiewicz .....
5. Dr hab. n. med. Marek Postuła .....
6. Dr hab. n. med. Marcin Ufnal .....
7. Dr n. med. Katarzyna Kosińska-Kaczyńska .....
8. Dr hab. n. farm. Sylwia Flis .....
9. Dr n. med. Agnieszka Serafin .....
10. Ks. Paweł Śmierzchalski .....
11. Prof. dr hab. Maria Boratyńska .....

Lista obecności
na posiedzeniu Komisji Bioetycznej
w dniu 18 kwietnia 2018

1. Dr hab. n.med. Magdalena Kuźma –Kozakiewicz .....
2. Dr hab. n. med. Tomasz Grzela .....
3. Dr hab. n. med. Andrea Horvath-Stolarczyk .....
4. Prof. dr hab. n. med. Paweł Piątkiewicz .....
5. Dr hab. n. med. Marek Postuła .....
6. Dr hab. n. med. Marcin Ufnal .....
7. Dr n. med. Katarzyna Kosińska-Kaczyńska .....
8. Dr hab. n. farm. Sylwia Flis .....
9. Dr n. med. Agnieszka Serafin .....
10. Ks. Paweł Śmierzchalski .....
11. Prof. dr hab. Maria Boratyńska .....

9. Oświadczenia wszystkich współautorów publikacji

Warszawa 04.06.2025
(miejscowość, data)

Prof. dr hab. n. med. Ernest Kuchar

OŚWIADCZENIE

Jako współautor pracy pt. „The anti-infective effect of β-glucans in children" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowił: nadzór nad przygotowaniem przeglądu literatury, interpretacją wyników, nadzór nad przygotowaniem publikacji.
Mój udział procentowy w przygotowaniu publikacji wynosił: 10%

Wkład Katarzyny Wzorek-Łyczko w powstawanie publikacji określam jako: 65%
Obejmował on sformułowanie problemu badawczego, przeprowadzenie przeglądu literatury, interpretacja wyników analizy, przygotowanie manuskryptu, prowadzenie korespondencji z recenzentami. (merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Katarzyny Wzorek-Łyczko.
(imię i nazwisko kandydata do stopnia)

KIEROWNIK
Klinika Pediatrii z Oddziałem Obserwacyjnym
prof. dr hab. n. med. Ernest Kuchar
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 04.06.2025
(miejscowość, data)

Dr n. med. Anna Piwowarczyk

OŚWIADCZENIE

Jako współautor pracy pt. „The anti-infective effect of β-glucans in children" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowił: sformułowanie problemu badawczego, nadzór nad metodyką przygotowania przeglądu literatury, interpretacją wyników, przygotowanie publikacji.
Mój udział procentowy w przygotowaniu publikacji wynosił:20%

Wkład Katarzyny Wzorek-Łyczko w powstawanie publikacji określam jako: 60%
Obejmował on sformułowanie problemu badawczego, przeprowadzenie przeglądu literatury, interpretacja wyników analizy, przygotowanie manuskryptu, prowadzenie korespondencji z recenzentami. (merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Katarzyny Wzorek-Łyczko.
(imię i nazwisko kandydata do stopnia)

Anna Piwowarczyk
(podpis oświadczającego)

***w szczególności** udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 04.06.2025
(miejscowość, data)

Lek. Weronika Woźniak

OŚWIADCZENIE

Jako współautor pracy pt. „The anti-infective effect of β-glucans in children" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowił przeprowadzenie przeglądu literatury, przygotowanie manuskryptu. Mój udział procentowy w przygotowaniu publikacji wynosił: 5%

Wkład Katarzyny Wzorek-Łyczko w powstawanie publikacji określam jako: 65%
Obejmował on sformułowanie problemu badawczego, przeprowadzenie przeglądu literatury, interpretacja wyników analizy, przygotowanie manuskryptu, prowadzenie korespondencji z recenzentami. (merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Katarzyny Wzorek-Łyczko.
(imię i nazwisko kandydata do stopnia)

Weronika Woźniak
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 04.06.2025
(miejscowość, data)

Prof. dr hab. n. med. Ernest Kuchar

OŚWIADCZENIE

Jako współautor pracy pt. „Protocol of the study: the effectiveness of pleuran in the treatment of acute gastroenteritis in children- a randomised, placebo controlled, double- blind trial (EPTAGE)" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowił: kierownictwo i nadzór nad grantem badawczym, nadzór nad przygotowaniem projektu badania, nadzór nad przygotowaniem publikacji.
Mój udział procentowy w przygotowaniu publikacji wynosił:10%

Wkład Katarzyny Wzorek-Łyczko w powstawanie publikacji określam jako: 60%
Obejmował on sformułowanie problemu badawczego, zaprojektowanie badania, przygotowanie dokumentacji koniecznej do prowadzenia badania, uzyskania zgody Komisji Bioetycznej, ubezpieczenia badania, rozliczenia grantu badawczego, przygotowanie manuskryptu, prowadzenie korespondencji z recenzentami. (merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Katarzyny Wzorek-Łyczko.

(imię i nazwisko kandydata do stopnia)

KIEROWNIK
Kliniki Pediatrii z Oddziałem Obserwacyjnym
prof. dr hab. n. med. Ernest Kuchar
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 04.06.2025
(miejscowość, data)

Dr n. med. Anna Piwowarczyk

OŚWIADCZENIE

Jako współautor pracy pt. „Protocol of the study: the effectiveness of pleuran in the treatment of acute gastroenteritis in children- a randomised, placebo controlled, double- blind trial (EPTAGE)" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowił: sformułowanie problemu badawczego, autorstwo koncepcji i projektu badania, nadzór nad przygotowaniem projektu, przygotowanie manuskryptu.
Mój udział procentowy w przygotowaniu publikacji wynosił: 30%

Wkład Katarzyny Wzorek-Łyczko w powstawanie publikacji określam jako: 60%
Obejmował on sformułowanie problemu badawczego, zaprojektowanie badania, przygotowanie dokumentacji koniecznej do prowadzenia badania, uzyskania zgody Komisji Bioetycznej, ubezpieczenia badania, rozliczenia grantu badawczego, przygotowanie manuskryptu, prowadzenie korespondencji z recenzentami. (merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Katarzyny Wzorek-Łyczko.
(imię i nazwisko kandydata do stopnia)

Anna Piwowarczyk
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 04.06.2025
(miejscowość, data)

Prof. dr hab. n. med. Ernest Kuchar

OŚWIADCZENIE

Jako współautor pracy pt. „A randomised trial of pleuran in peadiatric acute gastroenteritis" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowił: kierownictwo i nadzór nad grantem badawczym, nadzór nad przeprowadzeniem badania, nadzór nad przygotowywaniem publikacji.
Mój udział procentowy w przygotowaniu publikacji wynosił: 10%

Wkład Katarzyny Wzorek-Łyczko w powstawanie publikacji określam jako: 60%
Obejmował on sformułowanie problemu badawczego, przeprowadzenie rekrutacji do badania, prowadzenie dokumentacji koniecznej do rozpoczęcia i prowadzenia badania interwencyjnego, zgromadzenie danych, synteza i interpretacja wyników badania, przygotowanie manuskryptu, prowadzenie korespondencji z recenzentami. (merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Katarzyny Wzorek-Łyczko.
(imię i nazwisko kandydata do stopnia)

KIEROWNIK
Kliniki Pediatrii z Oddziałem Obserwacyjnym
prof. dr hab. n. med. Ernest Kuchar
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 04.06.2025
(miejscowość, data)

Dr n. med. Anna Piwowarczyk

OŚWIADCZENIE

Jako współautor pracy pt. „A randomised trial of pleuran in peadiatric acute gastroenteritis" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowił: sformułowanie problemu badawczego, nadzór nad poprawnym przeprowadzeniem badania, nadzór nad poprawnością prowadzonej dokumentacji, rekrutacja pacjentów do badania, nadzór nad interpretacją wyników, przygotowanie publikacji.
Mój udział procentowy w przygotowaniu publikacji wynosił: 20%

Wkład Katarzyny Wzorek-Łyczko w powstawanie publikacji określam jako: 60%
Obejmował on sformułowanie problemu badawczego, przeprowadzenie rekrutacji do badania, prowadzenie dokumentacji koniecznej do rozpoczęcia i prowadzenia badania interwencyjnego, zgromadzenie danych, synteza i interpretacja wyników badania, przygotowanie manuskryptu, prowadzenie korespondencji z recenzentami. (merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Katarzyny Wzorek-Łyczko.
(imię i nazwisko kandydata do stopnia)

Anna Piwowarczyk
.....
(podpis oświadczającego)

***w szczególności** udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa 04.06.2025
(miejscowość, data)

Lek Weronika Woźniak

OŚWIADCZENIE

Jako współautor pracy pt. „A randomised trial of pleuran in peadiatric acute gastroenteritis" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowił: rekrutacja pacjentów do badania.
Mój udział procentowy w przygotowaniu publikacji wynosił: 5%

Wkład Katarzyny Wzorek-Łyczko w powstawanie publikacji określam jako: 60%
Obejmował on sformułowanie problemu badawczego, przeprowadzenie rekrutacji do badania, prowadzenie dokumentacji koniecznej do rozpoczęcia i prowadzenia badania interwencyjnego, zgromadzenie danych, synteza i interpretacja wyników badania, przygotowanie manuskryptu, prowadzenie korespondencji z recenzentami. (merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Katarzyny Wzorek-Łyczko.
(imię i nazwisko kandydata do stopnia)

Weronika Woźniak
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

TRZEBNICA 04/06/2025
(miejscowość, data)

Dr hab. n. med. Henryk Szymański

OŚWIADCZENIE

Jako współautor pracy pt. „A randomised trial of pleuran in peadiatric acute gastroenteritis" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowił: nadzór nad rekrutacją pacjentów do badania, nadzór nad interpretacją wyników i przygotowaniem publikacji.
Mój udział procentowy w przygotowaniu publikacji wynosił: 5%

Wkład Katarzyny Wzorek-Łyczko w powstawanie publikacji określam jako: 60%
Obejmował on sformułowanie problemu badawczego, przeprowadzenie rekrutacji do badania, prowadzenie dokumentacji koniecznej do rozpoczęcia i prowadzenia badania interwencyjnego, zgromadzenie danych, synteza i interpretacja wyników badania, przygotowanie manuskryptu, prowadzenie korespondencji z recenzentami. (merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Katarzyny Wzorek-Łyczko.
(imię i nazwisko kandydata do stopnia)

dr hab. n. med.
Henryk Szymański
1774388
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

10. Bibliografia

1. Fitzwater Sean, Chandran A, Kosek M, Santosham M. Infectious Diarrhoea. In: Water and Sanitation Related Diseases and the Environment: Chalenges, Interventions and Preventive Measures. Wiley- Blackwell; 2011. p. 47–70.

2. World Health Organization. The Global Burden of Disease: 2004 Update. 2008.

3. Czarkowski Mirosław, Wielgosz Urszula. Choroby zakaźne i zatrucia w Polsce w 2023 roku. Warszawa; 2024.

4. PROTECT. The paediatric burden of rotavirus disease in Europe. Epidemiol Infect. 2006;134(5):908–16.

5. Guarino A, Ashkenazi S, Gendrel D, Lo Vecchio A, Shamir R, Szajewska H. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases Evidence-Based Guidelines for the Management of Acute Gastroenteritis in Children in Europe. J Pediatr Gastroenterol Nutr. 2014 Jul;59(1):132–52.

6. Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Shamir R, et al. Use of Probiotics for Management of Acute Gastroenteritis. J Pediatr Gastroenterol Nutr. 2014 Apr;58(4):531–9.

7. Lo Vecchio A, Dias JA, Berkley JA, Boey C, Cohen MB, Cruchet S, et al. Comparison of Recommendations in Clinical Practice Guidelines for Acute Gastroenteritis in Children. J Pediatr Gastroenterol Nutr. 2016 Aug;63(2):226–35.

8. Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, et al. 2017 Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea. Clinical Infectious Diseases. 2017 Nov 29;65(12):e45–80.

9. NICE. https://www.nice.org.uk/guidance/cg84/resources/diarrhoea-and-vomiting-caused-by-gastroenteritis-in-under-5s-diagnosis-and-management-pdf-975688889029. 2009. Diarrhoea and vomiting caused by gastroenteritis in under 5s: diagnosis and management.

10. MacGillivray S, Fahey T, McGuire W. Lactose avoidance for young children with acute diarrhoea. Cochrane Database of Systematic Reviews. 2013 Oct 31;

11. van den Berg J, Berger MY. Guidelines on acute gastroenteritis in children: a critical appraisal of their quality and applicability in primary care. BMC Fam Pract. 2011 Dec 2;12(1):134.

12. Di Luzio NR. Update on the immunomodulating activities of glucans. Springer Semin Immunopathol. 1985 Dec;8(4):387–400.

13. Chan GCF, Chan WK, Sze DMY. The effects of β-glucan on human immune and cancer cells. J Hematol Oncol. 2009 Dec 10;2(1):25.

14. Jesenak M, Majtan J, Rennerova Z, Kyselovic J, Banovcin P, Hrubisko M. Immunomodulatory effect of pleuran (β-glucan from Pleurotus ostreatus) in children with recurrent respiratory tract infections. Int Immunopharmacol. 2013 Feb;15(2):395–9.

15. Rennerova Z, Picó Sirvent L, Carvajal Roca E, Paśnik J, Logar M, Milošević K, et al. Beta-(1,3/1,6)-D-glucan from Pleurotus ostreatus in the prevention of recurrent respiratory tract infections: An international, multicentre, open-label, prospective study. Front Pediatr. 2022 Oct 14;10.

16. Urbancikova I, Hudackova D, Majtan J, Rennerova Z, Banovcin P, Jesenak M. Efficacy of Pleuran (*β* -Glucan from *Pleurotus ostreatus*) in the Management of Herpes Simplex Virus Type 1 Infection. Evidence-Based Complementary and Alternative Medicine. 2020 Apr 13;2020:1–8.

17. Jesenak M, Urbancek S, Majtan J, Banovcin P, Hercogova J. **β** -Glucan-based cream (containing pleuran isolated from *pleurotus ostreatus*) in supportive treatment of mild-to-moderate atopic dermatitis. Journal of Dermatological Treatment. 2016 Jul 3;27(4):351–4.

18. Bobovčák M, Kuniaková R, Gabriž J, Majtán J. Effect of Pleuran (β-glucan from *Pleurotus ostreatus*) supplementation on cellular immune response after intensive exercise in elite athletes. Applied Physiology, Nutrition, and Metabolism. 2010 Dec;35(6):755–62.

19. Bergendiova K, Tibenska E, Majtan J. Pleuran (β-glucan from Pleurotus ostreatus) supplementation, cellular immune response and respiratory tract infections in athletes. Eur J Appl Physiol. 2011 Sep 20;111(9):2033–40.

20. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. Ann Intern Med. 2018 Oct 2;169(7):467–73.

21. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, et al. SPIRIT 2013 Statement: Defining Standard Protocol Items for Clinical Trials. Ann Intern Med. 2013 Feb 5;158(3):200.

22. Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMC Med. 2010 Dec 24;8(1):18.