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Effectiveness and safety of the four-step versus six-step milk ladder in children with IgE-mediated cow's milk protein allergy: protocol for an open-label randomised controlled trial

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ABSTRACT

Introduction Introducing baked milk into the diet of children with cow's milk protein allergy (CMPA) has been shown to potentially accelerate the development of tolerance to non-heated milk. However, there is no standardised milk ladder (ML) protocol, and different scientific societies across countries recommend varying versions. This study aims to assess the effectiveness and safety of the four-step ML (4-ML) compared with the six-step ML (6-ML) in children with IgE-mediated CMPA.

Methods and analysis We will perform an open-label randomised trial with two parallel arms in two departments of the same academic hospital. A total of 92 children with IgE-mediated CMPA will be allocated in a 1:1 ratio to introduce cow's milk into their diet according to either 4-ML or 6-ML with a 4-week break period between subsequent steps. Oral food challenge (OFC) with tested products at each subsequent step of the ML will be conducted in hospital settings. The primary outcome will be the percentage of children with tolerance to non-heated cow's milk proteins defined as no allergic reaction to raw cow's milk (120–240 mL depending on the age of the patient) during the last OFC; measured at the end of the 12-week observation period for the 4-ML and 20-week observation period for the 6-ML. Secondary outcomes will include the percentage of children with a negative OFC to each ML step; the percentage of children with anaphylaxis (both those who were treated and those who were not treated with epinephrine); the percentage of children with exacerbation of atopic dermatitis; growth; compliance; and quality of life of the caregivers and parents' anxiety about adverse events during their child's OFC.

Ethics and dissemination The bioethics committee of the Medical University of Warsaw, Poland, approved this protocol (KB/107/2024). The findings will be published in a peer-reviewed journal and submitted to relevant conferences no later than 1 year after data collection.

Trial registration number NCT06664918.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first study with a rigorous methodology to assess the efficacy and safety of a four-step milk ladder (ML) compared with a six-step ML among children with IgE-mediated cow's milk protein allergy (CMPA).
⇒ The acquisition of tolerance to subsequent steps of the ladder will be confirmed by an oral food challenge, which is the gold standard for food allergy diagnosis. This challenge will be conducted in a hospital setting under doctor supervision.
⇒ The study will include 92 children with IgE-mediated CMPA regardless of their risk of a systemic reaction (anaphylaxis) or asthma.
⇒ This is a small, single-centre trial, including exclusively Polish children of Caucasian ethnicity, which may limit the generalisability of the results for other populations and ethnic groups.

INTRODUCTION

Cow's milk protein allergy (CMPA) is one of the most common food allergies in early childhood. Most children have an IgE-mediated pathophysiology. In the *EuroPrevall* study, the incidence of CMPA in children under 2 years of age was estimated to be less than 1–2%.[1 2] However, reliable epidemiologic data regarding food allergies in children are limited.

The first-line treatment of CMPA is the elimination of cow's milk proteins (CMPs) from the child's or diet of a breastfeeding mother. However, long-term elimination diets may be associated with risks such as delayed introduction of complementary foods, nutrient deficiencies, malnutrition, growth retardation, feeding difficulties and social exclusion.[3 4] Although the natural history of CMPA is very favourable, with the majority of children with non-IgE-CMPA developing tolerance by age 3 years,[1] an IgE-mediated CMPA may have a more protracted course and an older age of resolution.

An assessment of tolerance acquisition to CMPs is commonly performed using the milk ladder (ML). It involves the gradual introduction of CMPs by starting with small amounts of highly heat-treated forms—commonly referred to as baked allergens—and gradually progressing to greater amounts of less processed CMP-containing products.[5] The rationale for using the ML is based on the lower allergenicity of baked CMPs combined with wheat proteins (eg, in muffins, cookies and biscuits). In a clinical trial of 100 children with CMPA (mean age, 7.5 years), most (75%) tolerated baked CMPs during an oral food challenge (OFC).[6] It is suggested that introducing baked milk into the diet of children with CMPA may also accelerate the acquisition of tolerance to non-heated milk.[7 8] Only one randomised controlled trial (RCT) assessed the effect of introducing the baked milk (a muffin for 6 months and then baked cheese for another 6 months) on the acquisition of tolerance to CMPs in children (6 months to 3 years old) with IgE-mediated CMPA, compared with strict avoidance of any milk products for 1 year.[7] In this study, a higher number of children achieved tolerance to unheated milk in the group with baked milk, compared with the control group (88.1% vs 66.7%; n=84).

Scientific evidence regarding the effectiveness and safety of the ML in children with CMPA is limited.[9] Currently, there is no standardised ML protocol, and different versions of ML are recommended by scientific societies in various countries.[10 11]

METHODS AND ANALYSIS

This study was designed following Consolidated Standards of Reporting Trials (CONSORT) 2010[12] and Standard Protocol Items: Recommendations for Interventional Trials 2013 statements.[13] Additionally, a core outcome set for IgE-mediated food allergy clinical trials and observational studies of intervention (The Core Outcome Measures for Food Allergy (COMFA)) was followed.[14]

Study objective and hypothesis

The primary objective of this study is to compare the effectiveness and safety of the four-step ML (4-ML) with the six-step ML (6-ML) in children with IgE-mediated CMPA. We hypothesise that the 4-ML will result in a higher percentage of children with IgE-mediated CMPA who can tolerate raw cow's milk (confirmed with OFC) compared with the 6-ML at the end of the observation periods (12 weeks for the 4-ML and 20 weeks for the 6-ML).

Study design

This study is an open-label, randomised superiority trial with two parallel arms and a 1:1 allocation ratio.

Study setting

Recruitment and study intervention will take place in the department of paediatrics and the department of paediatric pulmonology and allergology at the Medical University of Warsaw, Warsaw, Poland. Both departments care for a large number of children with food allergies and have extensive experience in conducting OFC. The research team is also adequately trained and experienced in conducting clinical trials.

Recruitment began in January 2025 and is expected to be completed within 48 months.

Eligibility criteria

To be considered for inclusion, the child must meet all the criteria summarised in table 1 at the time of enrolment.

Table 1 Inclusion and exclusion criteria for children with CMPA

Inclusion criteria	Exclusion criteria
► Age between 1 and 5 years. ► Diagnosis of IgE-mediated CMPA confirmed according to ESPGHAN guidelines,[24] by a positive OFC with CMPs. In high-risk children (ie, with a history of anaphylaxis),* diagnosis based on positive skin prick testing and/or elevated specific IgE to CMPs is sufficient. ► On a therapeutic elimination diet for at least 6 months or up to 12 months of age.[11] ► Eligible regardless of the risk of systemic reaction (anaphylaxis) and asthma. ► Good overall health status. ► Parents without language barriers. ► Written informed consent signed by parents. ► Good cooperation with the child's guardians.	► Uncontrolled asthma, defined as the presence of shortness of breath, chest tightness, cough and/or auscultatory changes despite treatment. ► Signs of exacerbation of a chronic disease (ie, severe atopic dermatitis). ► Signs of acute infectious disease (ie, acute runny nose, cough or fever). ► Signs of exacerbation of another allergic disease (ie, conjunctivitis, allergic rhinitis, atopic dermatitis). ► Anaphylaxis due to CMPs in the last 6 months. ► Used antihistamines within 3–10† days before the challenge (depending on the pharmacokinetics of the drug and the indication for use). ► Documented tolerance to baked CMPs or higher steps of the milk ladder. ► Use of immunosuppressive drugs or immunotherapy.

*In cases of severe anaphylactic reactions and high levels of specific IgE to CMPs, an OFC may be bypassed due to the elevated risk of anaphylaxis, which is contraindicated for OFC according to guidelines.[11]
†A time-limited contradiction (relative exclusion criterium).
CMPA, cow's milk protein allergy; CMPs, cow's milk proteins; ESPGHAN, European Society for Paediatric Gastroenterology Hepatology and Nutrition; IgE, immunoglobulin E; OFC, oral food challenge.

Table 2 The intervention and control group in children with cow's milk proteins (CMPs)

Tested product	The amount of tested product	The amount of CMPs per portion and per 100 g/mL	Four-step milk ladder (intervention group)	Six-step milk ladder (control group)
Pasteurised cow milk or modified cow milk	120–240 mL	3.3 g CMPs/100 g	**Step 4** optimally pasteurised cow milk or modified cow milk, if the patient refuses cow's milk, then yoghurt	**Step 6** 4-week administration of the tolerated amount in the diet in a previously tested form
Yoghurt	1–3 years old: 1/4–1/2 of the cup (62.5–125 mL) 4–5 years old: 1/2–1 cup (125–250 mL)	1–3 years old: 3–6 g CMPs, 3–5 years old: 6–12 g CMPs 4.8 g CMPs/100 g		**Step 5**
	4-week administration of the tolerated amount in the diet in a previously tested form			
Baked hard cheese (eg, cheddar)	1–5 years old: 30 g	1–5 years old: 6.86 g CMPs 22.867 g CMPs/100 g	**Step 3**	**Step 4**
	4-week administration of the tolerated amount in the diet in a previously tested form			
Pancake	1.5 piece	1.47 g CMPs—1 pancake 2.205 g CMPs—1.5 pancake 1.889 g CMPs/100 g	**Step 2**	**Step 3**
	4-week administration of the tolerated amount in the diet in a previously tested form			
Muffin	1.5 muffin	0.875 g CMPs—1 muffin 1.3 g CMPs—1.5 muffin; 0.754 g CMPs/100 g	**Step 1**	**Step 2**
	4-week administration of the tolerated amount in the diet in a previously tested form			
Cookie	1–3 pieces	0.105 g CMPs; 0.282 g CMPs/100 g	–	**Step 1**

CMP content was estimated based on the international Milk Allergy in Primary Care[5] and the PRACTical ALLergy[15] recommendations.

Intervention

All OFCs conducted in this study will adhere to the PRACTical ALLergy (PRACTALL) guidelines.[15]

Children with IgE-mediated CMPA will be randomly assigned to either the intervention group (4-ML) or the control group (6-ML), with a 4-week interval between each step (table 2). The 4-ML is a modified version of the Canadian ladder.[16] The 6–ML was based on the international Milk Allergy in Primary Care (iMAP) ML.[5] For the purpose of this study, only one product was selected for testing at each step of the 4-ML and 6-ML. Identical products were selected for the same steps in both ladders; however, cookies and yoghurt were included as additional steps in the 6-ML. The amounts tested at each step were determined according to the iMAP[5] and PRACTALL[15] recommendations.

Supervised OFC

All OFCs will be performed openly over 1 day under the physician's supervision in a hospital setting. Following the PRACTALL[15] protocol for OFC, the tested food will be introduced in gradually increasing amounts (1/8, 1/8, 1/4 and 1/2) every 15–30 min or other specified time interval. The content of CMPs in all study products is presented in table 2. Specific recipes for cookie, muffin and pancake will be provided to caregivers before each planned OFC (Polish translation of iMAP recipes) for preparation at home. Although double-blind placebo-controlled food challenge is a gold standard for CMA diagnosis, we decided to perform open-label OFC because of its higher feasibility and acceptance of the parents.

Duration of each ladder step

The duration of each ML step, as well as the time from the first to the last step of the ML, has not been definitively established.[11] The authors of the Canadian ML suggest that the interval between steps should be at least 1–3 months.[16] Other authors propose that if a child tolerates an age-appropriate portion of a given product three times a week, they can proceed to the next step.[17] We determined that a 4-week break between subsequent steps was sufficient.

The HealthNuts cohort study among children with IgE-mediated egg allergy showed an increased likelihood of raw egg tolerance (p=0.009) associated with frequent administration of baked eggs (5 times per month) compared with less frequent administration (0–4 times

per month).[18] However, the optimal frequency of allergen administration has not been determined.

In our study, similarly to the study by Nowak-Wegrzyn *et al*,[6] we will recommend children consume one serving containing a tolerable amount of CMPs at least three times a week, in the same form as tested during the last negative OFC. Initial dose of consumed CMP-containing product should be the same as tested during OFC. If the child demonstrates good tolerance of the specified amount of CMPs during the first 2 weeks, the daily portion may be increased by up to 2–3 times according to the current step of the ML. Parents will be encouraged to keep a minimum interval of 2 hours between servings. If the allergen is not administered for more than a week, the interval between subsequent OFCs should be extended proportionally to the length of the break.

Children will be recommended to consume CMPs only in the form and amount used during the OFC. For example, if tolerance to baked milk is confirmed, only a muffin or cake prepared according to the provided recipe and in a tolerated amount could be administered at home. Additionally, parents will be discouraged from serving any commercial foods due to the unknown CMP content. For participants at a higher level of ML, consumption at home will include both the amount of CMPs tolerated during the last OFC and 1–2 additional portions of CMPs from a lower step of the ML. However, the child should not consume more than three products containing CMPs per day

Participants will be discouraged from consuming CMP-containing products from higher steps of the ML at home without previously confirmed tolerance through an OFC in a hospital setting.

Children with positive OFC

In the case of positive OFC with baked milk (first step of the ladder), children will continue the strict elimination diet at home. In the case of a positive OFC with products from higher steps of the ML, children will not progress to the next step of the ML. At home, they will continue to consume CMPs in the previously tolerated form, in the amount and frequency specified by the research team. An additional OFC with raw milk (the final step of the ladder) will be conducted at the end of the trial. After completing the ML (reaching the final step), parents will be encouraged to regularly include milk and CMP-containing products in their child's diet.

Study procedure

Children aged 1–5 years diagnosed with IgE-mediated CMPA will be invited to participate in the study. The recruiting physician, familiar with the study protocol, will perform an eligibility screening based on the patient's medical records, interview and physical examination. During a face-to-face meeting with the patient's caregivers, the physician will collect any missing information about the inclusion and exclusion criteria, explain the study procedures, risks and benefits and provide a leaflet describing the study. Following this, two copies of written informed consent will be obtained from the caregivers.

After enrolment, each child will be randomly assigned to either the intervention group (the 4-ML) or the control group (the 6-ML) (table 2), with a 4-week break period between subsequent steps. Children will start introducing CMPs into their diet using the appropriate ML (see Intervention).

Education of caregivers of children on the ML

Parents will be informed that during the 4-week time interval between subsequent OFC, their child should continue consuming the tolerated amount of CMPs at home (in the same form and dose as during the negative OFC). During the break period, products introduced at home should optimally be administered after the child returns from nursery or kindergarten, in the early afternoon, to allow for observation of the child's reaction after consuming the entire dose of the tested meal.

The tested product should not be administered on an empty stomach. Children should avoid strenuous physical exercise, exposure to significant airborne allergens (dust, pollen, if the child is sensitised) and hot baths for 2–4 hours after consumption. These factors, known as cofactors, may change a child's tolerance to consumed CMPs. During the administration of the study food product, the child should continue a complete milk-free diet, which includes avoiding milk and products of other ungulates (eg, goat's milk, sheep's milk).

Parents will be informed both verbally and in writing about the possibility of adverse effects during the administration of the allergen at home. They will receive instructions on how to handle such effects, including the treatment of anaphylaxis (online supplemental additional file 1). Before introducing the study food at home, the study personnel will ensure that all medications listed in the adverse event instructions are available at home and parents/caregivers are trained how to recognise and treat allergic reactions. All parents will be encouraged to contact the research team if they have any problems with introducing subsequent steps of the ML into their child's diet.

At any stage of the study, parents may withdraw consent for further participation in the study, and this will not affect the child's further care and treatment.

Assessments

The child's anthropometric measurements (height and weight) will be obtained at the time of inclusion in the study and at the end of the observation period (12 weeks or 20 weeks, depending on the study arm). The weight (kg) and standing height (cm) will be measured following standard methods. Body mass index (BMI) will be calculated using the standard equation. BMI-for-age and BMI-for-length/height z-scores will be computed using the WHO AnthroPlus software V.1.0.4.

Caregivers will be assessed for their subjective opinion on the quality of life before the first OFC and after

completing the ML (after 12 or 20 weeks, depending on the study arm). This will be done using a dedicated and validated scale for evaluating the quality of life in allergy patients: The Food Allergy Questionnaire of Life—Parent Form (FAQLQ-PF).[19] FAQLQ-PF was developed and validated to examine parental perception of health-related quality of life in children with food allergies aged 0–12 years. The questionnaire is divided into three domains: emotional impact, food anxiety and social and dietary limitations. The total score is calculated as the mean of three subscales. The minimum score is 0 and the maximum score is 6. The core questionnaire includes 14, 26 and 30 questions in the versions developed for children younger than 4 years of age, aged 4–6 and 7–12 years old, respectively.

Caregivers will also assess their OFC-related anxiety level before each OFC using the Subjective Units of Distress Scale (SUDS),[20] a commonly used method for measuring anxiety levels during exposure. Parents will rate their level of anxiety on a scale from 1 to 100, where 0 represents no anxiety or complete relaxation and 100 represents extreme anxiety, the worst ever experienced. This scale aims to help caregivers and doctors track improvements throughout the treatment.

Additionally, in children with atopic dermatitis, skin lesions will be assessed using the variant of the Severity Scoring of Atopic Dermatitis (SCORAD): the objective SCORAD Index (oSCORAD)[21] before the first OFC and during the examination following each observation period. The SCORAD Index is essential for determining the severity of atopic dermatitis and evaluating disease improvement during and after therapy. In the SCORAD Scale, the severity of atopic dermatitis is assessed based on objective symptoms—extent and severity of lesions—and subjective symptoms, such as itching and sleep disturbances, experienced by the patient over the last 72 hours. The oSCORAD is a version of the SCORAD Scale that omits the assessment of subjective symptoms by the patient. The maximum score on this scale is 83 points; however, in the most severe cases, an additional 10 points can be added for disfiguring or function-limiting changes. There are three degrees of severity of AD following the oSCORAD Scale: mild (<15 points), moderate (15–40 points) and severe (>40 points).

Adherence

To assess compliance and the incidence of anaphylaxis, during the 4-week break between OFCs, parents will monitor the number of portions and the frequency of allergen administration at home and report this information in the Milk Ladder Monitoring Diary developed by the study authors (online supplemental additional file 2).

Follow-up

1 month after completing the ML, patients will be followed up by the research team to assess their ongoing tolerance and continued inclusion of milk in their diet.

Box 1 Criteria of a positive oral food challenge (OFC) according to the PRACTical ALLergy guidelines[15]

The OFC should be stopped if any of the following symptoms occur during the OFC:

Skin
- ⇒ Three or more urticarial lesions
- ⇒ Angioedema
- ⇒ Confluent erythematous, pruritic rash

Respiratory
- ⇒ Wheezing
- ⇒ Repetitive cough
- ⇒ Difficulty breathing/increased work of breathing
- ⇒ Stridor
- ⇒ Dysphonia
- ⇒ Aphonia

Gastrointestinal
- ⇒ Vomiting alone not associated with gag reflex
- ⇒ Severe abdominal pain (such as abnormal stillness, inconsolable crying or drawing legs up to abdomen) that persists for ≥3 min

Cardiovascular
- ⇒ Hypotension for age not associated with vasovagal episode

If two or more of the following symptoms are present, the OFC should be stopped:

Skin
- ⇒ Persistent scratching for ≥3 min

Respiratory
- ⇒ Persistent rubbing of the nose or eyes for ≥3 min
- ⇒ Persistent rhinorrhoea for ≥3 min

Gastrointestinal
- ⇒ Diarrhoea

Outcomes

Primary outcome

The primary outcome will be the percentage of children who acquired tolerance to non-heated CMPs defined as unreactive patients during OFC with non-heated cow's milk (the last step of ML; a pasteurised cow milk or modified cow milk, the amount of 120–240 mL depending on the age of the patient, max. 7.95 g of CMPs) after the end of the observation period in accordance with the study protocol (depending on the study arm: 12 and 20 weeks).

A positive OFC is defined in accordance with the PRACTALL guidelines (box 1).[15]

Secondary outcomes

- Percentage of children with negative OFCs to each cow's milk form (cookie, muffin, pancake, cheese, yoghurt, milk, if applicable) according to the ML protocol at the end of the observation period (12 or 20 weeks, depending on the study arm). This will be reported as the percentage of children with negative OFCs after receiving the full planned amount for each OFC.
- Percentage of children with negative OFCs to each cow's milk form (cookie, muffin, pancake, cheese, yoghurt, milk, if applicable) according to the ML protocol after the end of the observation period (12 or 20 weeks, depending on the study arm). This will

be reported as the percentage of children with negative OFCs after receiving any tolerated dose during the trial.

- Percentage of children who experienced anaphylaxis, defined by the clinical criteria of anaphylaxis according to World Allergy Organization 2020,[22] during the observation period (12 or 20 weeks, depending on the study arm) according to the study protocol.
- In a subgroup of children with anaphylaxis, a percentage of children who required epinephrine administration during the observation period (12 or 20 weeks, depending on the study arm) according to the study protocol.
- Change in BMI-for-age z-score and length/height-for-age z-score at the end of the observation period (12 or 20 weeks, depending on the study arm) compared with baseline (before the first OFC).
- Change in total score of the FAQLQ-PF[19] at the end of the observation period (12 or 20 weeks, depending on the study arm), compared with baseline (before the first OFC).
- Assessment of parents' anxiety about adverse events during their child's OFC before each OFC, using the SUDS.[20]
- In a subgroup of children with atopic dermatitis, the percentage of children with mild, moderate and severe atopic dermatitis, assessed using the oSCORAD[21] Scale before each OFC (ie, during the examination following each observation period) and after each OFC.
- Percentage of children with full compliance to the intervention protocol, defined as performing all OFCs in the hospital setting and then regular introduction of the milk-containing products according to the individual tolerance following the study protocol, at the end of the observation period (12 or 20 weeks, depending on the study arm).
- Percentage of children with full compliance to the intervention protocol, defined as performing all OFCs in the hospital setting and then regular introduction of the milk-containing products according to the individual tolerance following the study protocol (see Adherence) at the end of the observation period (12 or 20 weeks, depending on the study arm).

Concomitant care

If a child is suspected of having a food allergy other than CMPA, it will be appropriately diagnosed and managed. However, any procedures cannot interfere with the course of this study; for example, potential immunotherapy will be contraindicated.

Parents will be encouraged to contact the study physician if they observe any health issues in their child.

Criteria for discontinuing the study

Participation in the study may be discontinued in the event of withdrawal of consent by one or both parents, any risk to the child's health, non-compliance with recommendations, loss of the opportunity to observe the child, or termination of the study.

In the event of the recruited child's premature withdrawal from the study, the investigator will ensure that all evaluable endpoints are entered into the clinical trial documentation (case report form (CRF)), unless parents/guardians decline further participation in the study. If discontinuation of the intervention will be due to the occurrence of a definite and/or potential adverse event, the child's follow-up will continue and will be recorded in both the medical record and the CRF.

Study modifications

If any adverse events occur during OFCs, the participation of the child in the trial will be interrupted, and appropriate treatment will be administered based on the clinical situation, following the department's standard procedures. The interval before progressing to the next step of the ladder will be individually planned. If any adverse reaction occurs after consuming the tested food, the child should be examined by the attending physician.

Harms

Patients participating in the study may not receive additional benefits beyond standard care. The proposed OFC procedures do not differ from the standard procedures. The only difference is the allocation of patients to either a 12-week or 20-week observation time. Outside of the study protocol, the length of observation would typically be decided only by the child's doctor.

An additional 15 min is required to complete a survey dedicated to assessing caregivers' quality of life and anxiety levels. At home, parents will keep the Milk Ladder Monitoring Diary developed by the study authors.

All adverse events will be reported in the CRF, classified according to the International Conference on Harmonisation Good Clinical Practice guidelines[23] and evaluated for their duration, intensity and causal relationship to the intervention. Participants will benefit from telephone and email contact with the primary investigator, ensuring that any potential adverse events are promptly reported and consulted by a physician.

As indicated in the CONSORT[12] extension on harms document, all those symptoms will be reported for all randomised participants, including those who withdraw from the study. The data on adverse events will be presented for each study arm and each type of adverse event separately, with an exact count of each event and a distinction between patients with single and multiple events.

Sample size

There are limited reports regarding the standardisation of baked allergens in an OFC with CMP, making it challenging to calculate the sample size based on existing data. In the only randomised trial that assessed the effect of introducing baked CMP in children with IgE-mediated

CMPA on the time required to acquire tolerance to CMP, a 20% difference between the groups was observed.[7] Based on this, it was estimated that 42 patients per group would be needed to achieve 80% power with a two-sided significance level of 5%. Considering an anticipated 10% dropout rate, it was calculated that 46 patients per group (92 in total) would be necessary. The sample size was determined using the Sample Size Calculator (https://clincalc.com/stats/samplesize.aspx).

Time of the study with justification

Based on study site capacity, a period of 48 months is considered sufficient to complete recruitment and conduct interventions in both groups.

Sequence generation and allocation concealment

Children will be randomised to either the intervention or control group immediately after enrolment. An independent investigator with no clinical involvement in the trial will generate random assignments using a computer-generated random number table. The random number will be generated in blocked randomisation (using the block of 2) with a 1:1 allocation ratio. The randomisation will be created using StatsDirect statistical software (StatsDirect statistical software; http://www.statsdirect.com; England: StatsDirect, 2024).

Allocation concealment will be ensured through the use of opaque, sealed and numbered envelopes. The data regarding intervention assignments will be stored in a sealed envelope in a secure location within the administrative part of the department. An independent person will open the next consecutively numbered envelope and provide the allocation information to the study physician. Since this is an open-label trial, both the participants and the study team will be aware of the treatment allocation on randomisation. In the event of any medical issues, the assigned treatment information will already be accessible to the study physicians and principal researcher, eliminating the need for code-breaking procedures.

Blinding

Due to the nature of the study, it is an open-label trial without blinding at any stage.

Data collection and management

All study participants will be assigned a study identification number. CRFs will be completed on paper forms. Data will then be entered and stored in a password-protected electronic database. The original paper copies of CRFs and all study data will be stored in a locker within the study site, accessible to the involved researchers only.

Statistical analysis

Descriptive statistics will be used to summarise baseline characteristics. For continuous variables, comparison between groups will be done using the Student's t-test or Mann-Whitney U test, depending on whether or not the variables are distributed normally. The appropriate statistical test will be employed to compare percentages, either the χ^2 test or Fisher's exact test.

For continuous outcomes, the differences in means or medians (depending on the distribution of data), and for dichotomous outcomes, the relative risk (RR) and number needed to treat, all accompanied by a 95% CI, will be calculated. The difference between study groups will be considered statistically significant if the p value is less than 0.05, if the 95% CI for RR does not include 1.0, or if the 95% CI for mean difference does not include 0. All statistical tests will be two-tailed and performed at the 5% level of significance.

An intention-to-treat model that includes data from all randomised participants will be used in the analysis, including those with low compliance or those who drop out or withdraw their consent will be applied. A per-protocol analysis that includes all participants who finish the study according to the protocol will also be performed.

Monitoring

The study will be carried out in accordance with the protocol, as it will be registered. No changes in the study protocol are expected to be made after the study starts. However, in case of any unexpected circumstances requiring alterations of the protocol, changes will be immediately applied to the protocol registry site at clinicaltrials.gov, and, if relevant enough, reported to the Bioethics committee. An independent Data and Safety Monitoring Board (DSMB) will be created before the start of the study. The DSMB will review data after recruitment from 25%, 50% and 75% of participants to assess the study progress (including rate of recruitment, completeness of data and their appropriate collection) and all the adverse events. The number of recruited patients will be monitored and kept up to date; appropriate changes (ie, training of the recruiting physicians, study leaflets, addition of new recruitment centres) will be applied to the study procedure and protocol if the pace of recruitment is not high enough to finish the study within the established time, which is 4 years.

ETHICS AND DISSEMINATION

Research ethics approval

The bioethical committee of The Medical University of Warsaw issued approval for the study before recruitment commenced (approval number: KB/107/2024). Verbal and written information regarding informed consent will be presented to the caregivers. Any modifications to the protocol that may affect the course of the study will be presented to the bioethical committee.

Protocol amendments

This is a first version of study protocol dated on 21 June 2024. Any protocol modification should be the object of an amendment, which will be dated and signed by all the parties involved in the development of the initial

protocol. The amendment will be submitted to the appropriate ethics committees, either for approval or for information, depending on the nature and the importance of the changes to the study conditions and according to the specific regulations.

Informed consent process

Written informed consent will be obtained from all participants before enrolment (online supplemental additional file 3). The research team will discuss the study with the child and caregiver and answer any questions. Two copies of the written informed consent will be obtained from the child's caregivers.

Confidentiality

All information collected during the study will be considered confidential and will not be disclosed. The identity of study participants under no circumstances will be communicated to the sponsor or any official bodies.

Access to data

Only the principal investigator and study coordinator will be given access to the de-identified electronic dataset. All data sets will be password protected. To ensure confidentiality, data will be blinded for any identifying participant information. The datasets used and/or generated during this study will be made available from a given author on reasonable request, after the publication of results, no later than 3 years from the completion of data analysis.

Ancillary and post-trial care

The compensation of harms will be covered by study insurance policies. For post-trial care, see the Follow-up section.

Dissemination policy

The study protocol will be made freely available to the public through open-access registration and publication. The results of this RCT, whether positive or negative, will be published in a peer-reviewed journal. Abstracts will also be submitted to relevant national and international conferences. Adherence to the CONSORT[12] guidelines will be ensured in this RCT to guarantee compliance with best practices for reporting clinical trials.

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Competing interests DW has nothing to declare. AH has participated as a clinical investigator, advisory board member and speaker for several companies, including BioGaia, Danone, Dicofarm, HIPP, Nestlé, NNI, Nutricia and Mead Johnson. AS has participated as a clinical investigator and speaker for Nestlé and receives research support from Nutricia. AN-W receives research support from DBV Technologies and Siolta speaking fees from Nestle, Danone and Thermo Fisher; royalties from UpToDate; she serves as an Associate Editor for the *Annals of Allergy, Asthma & Immunology*, chair of the ABAI Board of Directors, director of the AAAAI Board, and the chair of the Medical Advisory Board of the International FPIES. KG has participated as a speaker for Mead Johnson. HS serves as a board member of the International Scientific Association for Probiotics and Prebiotics, a role which is unpaid and voluntary. She has participated as a clinical investigator, advisory board member, consultant and speaker for several companies, including Arla, BioGaia, Biocodex, Danone, Dicofarm, Nestlé, NNI, Nutricia, Mead Johnson and Novalac.

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