

BMJ Open Azithromycin and high-dose vitamin D for treatment and prevention of asthma-like episodes in hospitalised preschool children: study protocol for a combined double-blind randomised controlled trial

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ABSTRACT

Introduction Previous randomised controlled trials (RCTs) suggest antibiotics for treating episodes of asthma-like symptoms in preschool children. Further, high-dose vitamin D supplementation has been shown to reduce the rate of asthma exacerbations among adults with asthma, while RCTs in preschool children are lacking. The aims of this combined RCT are to evaluate treatment effect of azithromycin on episode duration and the preventive effect of high-dose vitamin D supplementation on subsequent episodes of asthma-like symptoms among hospitalised preschoolers.

Methods and analysis Eligible participants, 1–5 years old children with a history of recurrent asthma-like symptoms hospitalised due to an acute episode, will be randomly allocated 1:1 to azithromycin (10 mg/kg/day) or placebo for 3 days (n=250). Further, independent of the azithromycin intervention participants will be randomly allocated 1:1 to high-dose vitamin D (2000 IU/day+ standard dose 400 IU/day) or standard dose (400 IU/day) for 1 year (n=320). Participants are monitored with electronic diaries for asthma-like symptoms, asthma medication, adverse events and sick-leave. The primary outcome for the azithromycin intervention is duration of asthma-like symptoms after treatment. Secondary outcomes include duration of hospitalisation and antiasthmatic treatment. The primary outcome for the vitamin D intervention is the number of exacerbations during the treatment period. Secondary outcomes include time to first exacerbation, symptom burden, asthma medication and safety.

Ethics and dissemination The RCTs are approved by the Danish local ethical committee and conducted in accordance with the guiding principles of the Declaration of Helsinki. The Danish Medicines Agency has approved the azithromycin RCT, which is monitored by the local Unit for Good Clinical Practice. The vitamin D RCT has been reviewed and is not considered a medical intervention. Results will be published in peer-reviewed journals and presented at international conferences.

Strengths and limitations of this study

- This is a large combined randomised controlled trial (RCT) consisting of two independent interventions, which evaluate if a 3-day course of azithromycin (10 mg/kg/day) compared with placebo reduces the duration of asthma-like episodes in hospitalised preschool children, and if high-dose vitamin D supplementation (2000 IU/day+ standard dose 400 IU/day) for 1 year compared with standard dose (400 IU/day) reduces the subsequent number of asthma-like episodes requiring oral corticosteroids and/or emergency department visits and/or hospitalisation.
- If the interventions are proven effective, this RCT could have a huge impact on paediatric asthma management as the findings can be directly implemented in clinical practice and thereby address an unmet clinical need for treating and preventing recurrent asthma-like episodes in young children.
- The children are carefully monitored with electronic diary recordings of asthma-like symptoms, antiasthmatic treatment, and adverse events assuring robust and clinically relevant efficacy and safety endpoints.
- Exploratory effect modifiers in both study arms include the airway immune profile, respiratory pathogens, airway microbiome assessments, blood eosinophil count, total IgE and specific IgE levels and genetic markers, which enable analysing underlying mechanisms of treatment effects and whether there are specific phenotypes or episode characteristics, where azithromycin and vitamin D treatment are more effective.
- The azithromycin intervention could raise concern regarding antibiotic resistance and microbial derangements, which will be evaluated.

Trial registration numbers NCT05028153, NCT05043116.

INTRODUCTION

Childhood asthma is often preceded by recurrent episodes of asthma-like symptoms during the first years of life.¹ Severe episodes with asthma-like symptoms cause a high rate of emergency department (ED) visits and often require hospitalisation^{2,3} with high socioeconomic demands.⁴ Typical triggers for asthma-like symptoms are respiratory tract infections (RTIs) of both viral and bacterial aetiology,^{5,6} exercise, allergens, pollutants and tobacco smoke. Exacerbations in preschoolers are most often triggered by RTIs⁷ and have a poor response to oral corticosteroids (OCS) that are one of few options for treating exacerbations in this age group.⁸ Preventive treatment of asthma in preschoolers is maintenance inhaled corticosteroids (ICS) as monotherapy and/or an oral leukotriene receptor antagonist (LTRA), which is effective for achieving daily symptom control, whereas many children still experience exacerbations requiring OCS and/or hospitalisation.⁹ Currently, there are no interventions aiming at reducing the high morbidity associated with acute exacerbations, which is a major unmet clinical need in this age group of vulnerable young children.¹⁰

Azithromycin for treatment of asthma-like episodes

Antibiotics are not recommended for treating exacerbations of asthma-like symptoms in young children, although they are commonly used for this purpose.^{6,11} As exacerbations of asthma-like symptoms can be triggered by bacterial RTIs, we previously conducted a randomised controlled trial (RCT) of azithromycin versus placebo including 158 episodes of asthma-like symptoms in 72 children from the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC₂₀₁₀) cohort aged 12–36 months with a history of recurrent asthma-like symptoms.¹² The RCT showed that 3 days azithromycin (10 mg/kg/day) reduced the episode duration by 63% compared with placebo. Furthermore, the airway microbiota and airway immune responses during the episode was shown to predict children, who benefitted from the treatment.^{13,14}

A comparable American RCT included 443 children aged 12–71 months with a history of severe episodes with asthma-like symptoms showing that 5 days azithromycin (12 mg/kg/day) reduced the risk of progression from mild to severe lower respiratory tract illness with need for OCS by 36%, but episode duration was not reported.¹⁵ A Canadian RCT included 222 children aged 12–60 months from an ED, admitted with acute asthma-like symptoms showing no effect on the duration of symptoms from 5 days azithromycin course (10 mg/kg/day).¹⁶ This study differed from the American RCT and our previous RCT as the children did not have a history of recurrent asthma-like symptoms. However, a subanalysis among children with recurrent asthma-like symptoms showed

similar results,¹⁶ but had a low number of subjects in the subgroup.

High-dose vitamin D for prevention of asthma-like episodes

In children with asthma, vitamin D insufficiency is prevalent year-round and may increase the risk of exacerbations.^{17,18} A recent systematic review and meta-analysis, which included several RCTs of vitamin D supplementation in children and adults showed that vitamin D significantly reduced exacerbations requiring OCS by 36% (n=658 adults; 22 preschoolers) and ED visits or hospitalisations by 61% (664 adults; 277 school-aged children; 22 preschoolers) compared with supplementation with 400 IU/day.¹⁹ The systematic review underlines the shortage of RCTs in children, especially in preschoolers, where the largest preventive effect is to be expected as vitamin D has been shown to reduce the risk of RTIs,²⁰ which are the main trigger of exacerbations in young children. Moreover, an RCT in adults (n=8) has suggested that high-dose vitamin D (2000 IU/day) vs standard dose (400 IU/day) influences the expression of several genes in white blood cells affecting a wide variety of biologic functions including genes involved in immune function.²¹

Currently, there are eight published paediatric vitamin D RCTs with respiratory outcomes,²² but only two includes preschoolers. The first is a safety pilot study of the DIVA RCT (NCT03365687) randomising 22 preschool-aged children to an oral bolus of 100,000 IU plus 400 IU/day for 6 months or 400 IU/day. This was safe and raised serum 25-hydroxyvitamin D (25(OH)D) ≥75 nmol/L, but was underpowered to show differences in unplanned healthcare attendances due to acute wheeze.²³ The second is an American RCT investigating 12 months maintenance vitamin D repletion (oral 400 IU/day) vs rapid repletion (oral 400 IU/day+intramuscular injection of either 300,000 IU (<5 years of age) or 600,000 IU (>5 years of age)) to children 2–14 years of age (mean age 6 years, 231 completers) with moderate-to-severe asthma exacerbations and 25(OH)D <25 ng/mL to prevent subsequent exacerbations.²⁴ Rapid compared with maintenance repletion significantly reduced unplanned visits for asthma exacerbations among children with baseline levels of 3–11 ng/mL during the initial 3 months of treatment, but not thereafter.²⁴

Apart from the safety pilot study of the DIVA RCT and the study by Alansari *et al* there are no other published RCTs of vitamin D supplementation to preschoolers with asthma-like symptoms. Though, the DIVA study on ‘vitamin D in prevention of exacerbations of asthma in preschoolers’ are currently recruiting participants, and is aiming to recruit 865 children.²³ Therefore, RCTs in preschoolers are needed to establish whether high-dose vitamin D supplementation can prevent exacerbations with asthma-like symptoms requiring OCS and/or ED visits and/or hospitalisation.²⁵

It is important to investigate potential mechanisms of both azithromycin and high-dose vitamin D to be able to

pinpoint specific phenotypes and episode characteristics, where treatment may be most effective.

Objectives

The main objective of the azithromycin arm of the study is to evaluate if 3 days azithromycin treatment (10 mg/kg/day) compared with placebo reduces the current episode duration.

The main objective of the vitamin D arm is to investigate whether high-dose supplementation (2000 IU/day+standard dose (400 IU/day) compared with standard dose (400 IU/day) reduces the number of subsequent episodes requiring OCS and/or ED visits and/or hospitalisation. This will be studied among hospitalised children aged 1–5 years with a history of recurrent asthma-like symptoms.

METHODS

This study is a combined double-blind RCT with two independent interventions. Children, who meet the inclusion criteria for both interventions, will be asked to participate in one treatment arm consisting of azithromycin versus placebo and a second arm of high dose vitamin D (2000 IU/day+standard dose 400 IU/day) versus standard dose (400 IU/day) (see [figure 1](#)). The study is coordinated by researchers at the COPSAC research unit at Herlev-Gentofte Hospital. Planned start to recruit participants is April 2022, and we estimate a 3-year timespan for recruiting all participants (ie, April 2024).

Participants, intervention and outcomes

Study setting

The study will be conducted at three paediatric departments in Zealand, Denmark: Herlev-Gentofte University Hospital, Hvidovre University Hospital and Slagelse Hospital. Additional paediatric departments will be included if needed.

Eligibility criteria: inclusion/exclusion

The inclusion criteria for participation in both study arms are: (1) hospitalisation due to an episode of asthma-like symptoms, (2) age 12–71 months, (3) a medical history of episode(s) with asthma-like symptoms and treatment with short-acting beta-agonists (SABA) as monotherapy, or in combination with ICS and/or LTRA. Further, parents must have fluent Danish skills. The specific inclusion and exclusion criteria for participation in the two study arms are listed in [table 1](#).

The exclusion criteria aim to minimise the risk of allergic reactions and of including children, who suffer from pneumonia or other bacterial infections, rickets, moderate to severe vitamin D deficiency ($25(\text{OH})\text{D} < 25 \text{ nmol/L}$), as the Danish Paediatric Society's guidelines regarding treatment of vitamin D deficiency recommend 800 IU/day when $25(\text{OH})\text{D} < 25 \text{ nmol/L}$,²⁶ and risk of vitamin D intoxication ($25(\text{OH})\text{D} > 150 \text{ nmol/L}$).

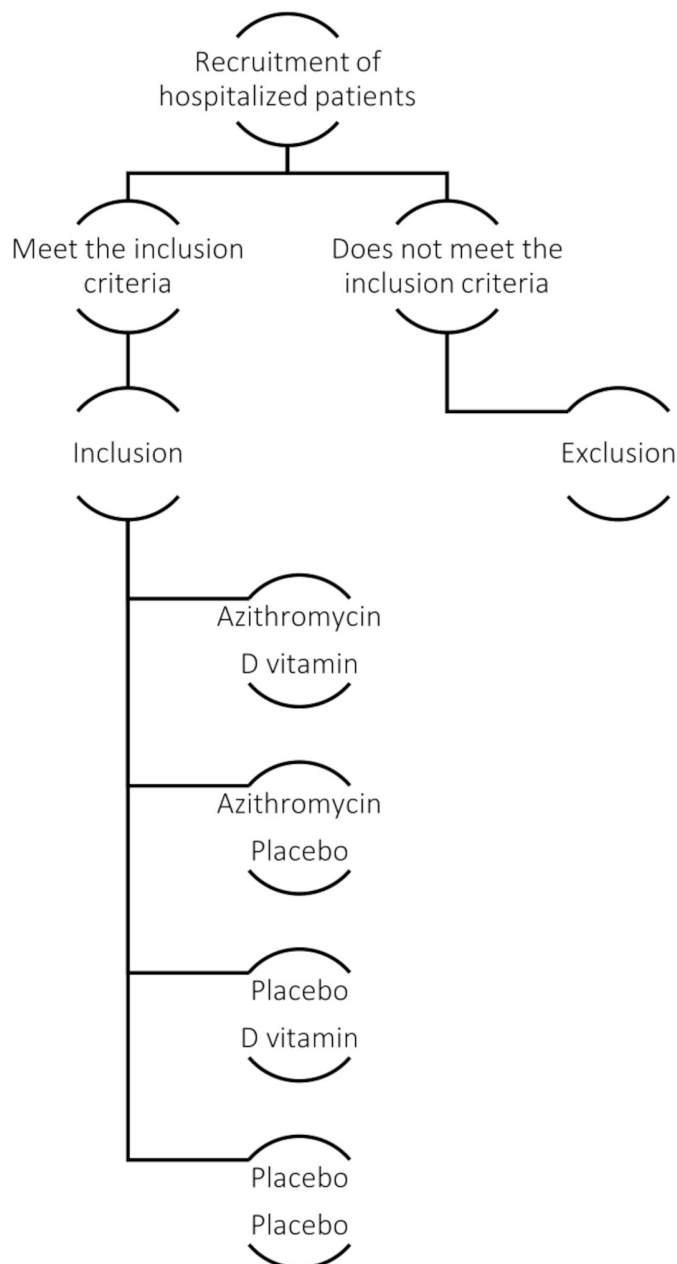


Figure 1 Flow diagram of enrolment of patients. Patients who meet the inclusion criteria in both study arms (ie, vitamin D and azithromycin) can participate in both studies independently of each other.

Interventions

In the azithromycin arm, the children are randomised to a 3-day course of azithromycin (10 mg/kg/day) or placebo. The interventions consist of powder for oral suspension 40 mg/mL azithromycin or placebo in identical looking 22.5 mL packings. The parents will report their child's asthma-like symptoms defined as wheeze or whistling sounds, breathlessness, or troublesome cough severely affecting the well-being of the child, use of asthma medication, adverse events (AEs) and sick leave via a link sent per text messaging once daily for 21 days via the secure system Research Electronic Data Capture (REDCap).

Table 1 Overview of the inclusion and exclusion criteria for azithromycin and vitamin D

Inclusion criteria	
Azithromycin and vitamin D study	<ul style="list-style-type: none"> ▶ Hospitalisation due to an episode of asthma-like symptoms ▶ Age 12–71 months ▶ Medical history of episode(s) with asthma-like symptoms and treatment with SABA as monotherapy, or in combination with ICS and/or LTRA
Exclusion criteria	
Azithromycin and vitamin D study	<ul style="list-style-type: none"> ▶ Clinical signs of pneumonia (tachypnoea: respiratory rate >50 and/or fever: temperature >39°C and/or C reactive protein >50) ▶ Chronic lung disease (other than asthma/wheeze) ▶ Impaired liver or kidney function ▶ Neurological or psychiatric disorders ▶ Congenital or acquired prolonged QT interval ▶ Clinically relevant bradycardia, cardiac arrhythmia, or severe heart failure.
Azithromycin study	▶ Macrolide allergy
Vitamin D study	<ul style="list-style-type: none"> ▶ Daily intake of vitamin D supplementation or receives a combination of vitamin and dietary supplements containing vitamin D >400 IU/day (~10 µg/day) ▶ Moderate to severe vitamin D insufficiency (ie, 25(OH)D < 25 nmol/L) ▶ Malnourished (ie, children >2 years—age-specific BMI less than the third percentile and children <2 years—weight or height in relation to age are less than the third percentile) ▶ Newly arrived refugee/immigrant from regions with high incidence of rickets ▶ Receives medication that alters calcium or vitamin D absorption/metabolism ▶ Familial occurrence of hypercalciuria and/or kidney stones ▶ High level of vitamin D (i.e., 25(OH)D >150 nmol/L)

BMI, body mass index; ICS, inhaled corticosteroids; LTRA, leukotriene receptor antagonist; 25(OH)D, 25-hydroxyvitamin D; SABA, short-acting beta-agonists.

In the vitamin D arm, the children are randomised to one daily oral dose of vitamin D supplementation of 2000 IU (~50 µg) or an identical lookalike placebo for 1 year. We recommend all parents to continue supplementing their child with 400 IU/day (~10 µg) as this is recommended by Danish Health Authority.²⁷ Therefore, the intervention group will receive a total intake of 2400 IU/

Month = January (e.g.)	Date	1	2	3	4	5	6	...
Yes=x, No=0	Weekday	Mon	Tue	Wen	Thu	Fri	Sat	...
Healthy (i.e., no lung symptoms, no asthma medication). If yes, you do not have to fill in more.								
Lung symptoms	Cough							
	Shortness of breath							
	Wheeze							
Treatment of lung symptoms - Acute	Blue spray*							
	Oral steroid course**							
Treatment of lung symptoms - Preventive	Brown spray***							
	Asthma pill****							
Child's sick leave from daycare								
Possible adverse events								
If yes describe*****								

Figure 2 Diary the parents fill out on information on asthma-like symptoms, asthma medication use, AEs and sick leave. Further, a checkbox for administration of azithromycin is added to diary during the first 3 days, and a checkbox for how many days the current week D-pearls were given. *SABA. **OCS. ***ICS. ****LTRA. *****Possible to write text. AE, adverse event; ICS, inhaled corticosteroids; LTRA, leukotriene receptor antagonist; OCS, oral corticosteroids; SABA, short-acting beta-agonists.

day (~60 µg/day). The intervention consist of D-Pearls (ie, small and soft ox gelatin capsules 1000 IU/D-Pearl) or placebo in identical looking D-Pearls produced by Pharma Nord. To avoid seasonal variations in 25(OH)D levels at baseline and the seasonal variation in RTIs, the intervention is given for 12 months. The parents will report their child's asthma-like symptoms, use of asthma medication, AEs and sick leave via a weekly REDCap link, where they can fill out daily information from the prior week (see [figure 2](#)).

Outcomes

The primary outcome in the azithromycin study arm is the duration after randomisation of the episode with doctor-diagnosed asthma-like symptoms, so severe that the symptoms have led to hospitalisation.

Asthma-like symptoms are defined as wheeze or whistling sounds, breathlessness, or troublesome cough severely affecting the well-being of the child, which is a validated method to monitor asthma-like symptoms,^{28–30} and used several times in our clinical observational studies of young children,^{28 29 31–33} and in two RCTs including our previous azithromycin RCT.^{12 34} Secondary outcomes are: (1) symptom burden; (2) length of hospitalisation; (3) intensity of episode treatment; (4) leave from daycare; (5) health economic benefits; (6) gut microbiome and resistance profiles (assessed by metagenomic sequencing for composition, diversity and abundance of bacterial

taxa and antibiotic resistance genes³⁵ at baseline, 3 weeks, 3 months and 1 year after treatment with azithromycin. Evaluation of effect modification by (7) airway microbiome (virus detection by PCR and bacterial 16S rRNA sequencing), and (8) airway immune profile. Further, safety by (9) AEs for 3 weeks.

The primary outcome in the vitamin D study arm is the number of episodes with asthma-like symptoms requiring OCS and/or ED visit and/or hospitalisation over a 12-month period after randomisation. Secondary outcomes are: (1) time to first exacerbation; (2) number of episodes with asthma-like symptoms; (3) length of hospitalisation; (4) symptom burden between exacerbations; (5) treatment during exacerbations; (6) step-down asthma maintenance therapy; (7) health economic benefits. Evaluation of effect modification by (8) baseline 25(OH)D level; (9) genetic variation and expression in the vitamin D pathway, for example, vitamin D receptor and vitamin D binding protein; (10) genetic variation and expression in known childhood asthma loci, for example, 17q21; (11) airway microbiome; (12) airway immune profile and (13) atopic status, that is, blood eosinophil count, total immunoglobulin E (IgE) and specific-IgE levels towards aeroallergens; (14) number of asthma-like episodes the previous year; (15) requiring versus not requiring OCS the last year. Further, safety profile by clinically significant; (16) hypercalciuria; (17) hypercalcaemia; (18) 25(OH)D>150nmol/L; and (19) AEs

for 1 year. Furthermore, (20) prevention of and possible reduction of symptom burden from COVID-19.

Participant timeline

Recruitment of participants will start April 2022 and is expected to last 3 years. The study will be completed when at least 250 participants are recruited for the azithromycin study arm and 320 participants for the D vitamin study arm. Study duration per participant for the azithromycin arm is 21 days, the first 3 days with treatment, and faecal samples are further collected at inclusion, 21 days, 3 months and 1 year after intervention. Study duration per participant for the vitamin D arm is 1 year (see figure 3). The estimated completion of the study is April 2025.

Recruitment

A doctor and a nurse from the research team will contact the paediatric wards every morning on weekdays to screen for newly admitted patients eligible to include in the study. If this is the case, layperson summaries, including participant information and the informed consent form, which describes the experiment and its procedures, will be handed to the parents. If the parents after reading the layperson summaries are interested in participating, the research team will make an appointment with the family on the same day or latest within 3 days. Inclusion is only possible while the child is hospitalised. We will inform

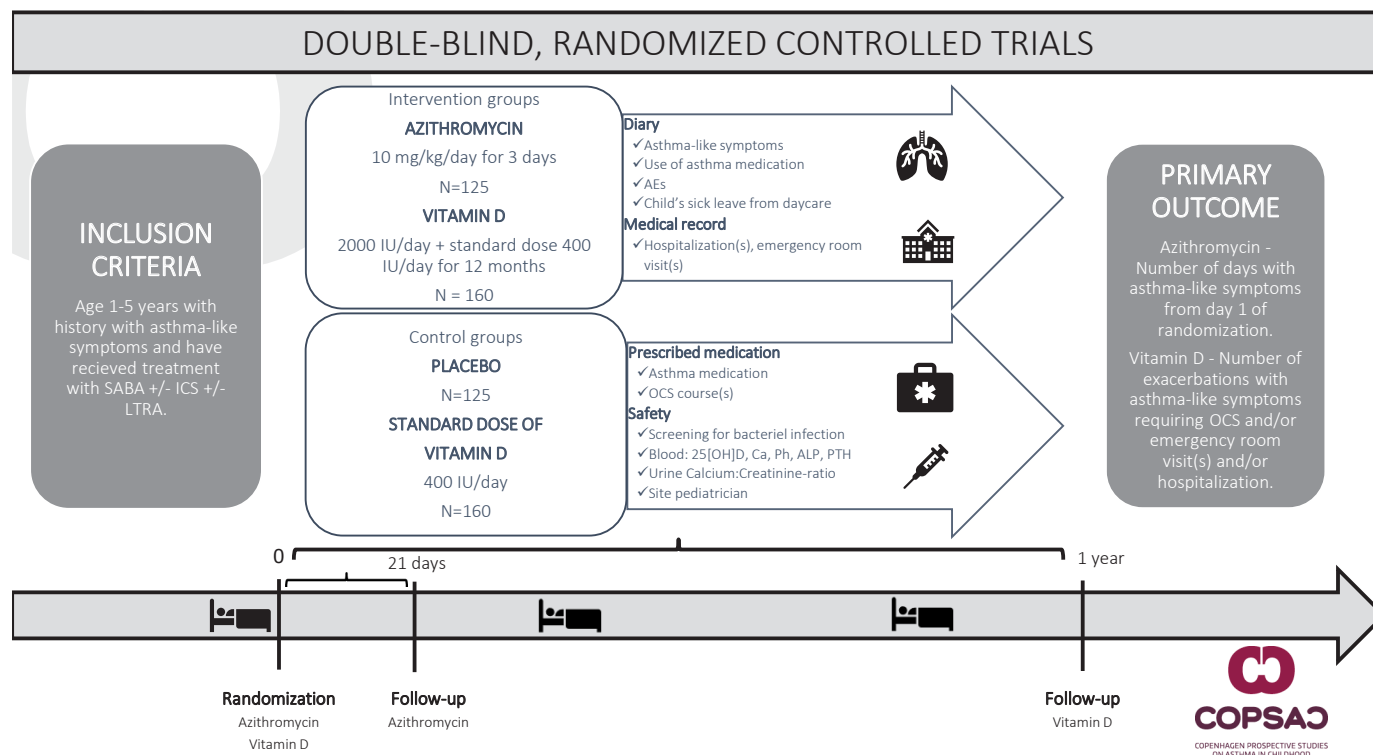


Figure 3 Overview of the study timeline for both study arms (ie, 21 days in the azithromycin arm and 1 year in the vitamin D arm). AEs, adverse events; ALP, alkaline phosphatase; Ca, calcium; ICS, inhaled corticosteroids; LTRA, leukotriene receptor antagonist; OCS, oral corticosteroids; Ph, phosphorus; PTH, parathormone; SABA, short-acting beta-agonists; 25(OH)D, 25-hydroxyvitamin D.

orally about the studies purposes, content, risks, form etc. Here, the parents will have the possibility to ask questions before finally deciding to participate.

Adverse events

Azithromycin is a commonly used macrolide in children and is shown safe in several RCTs including the RCT performed in the COPSAC₂₀₁₀ cohort.^{12 36 37} Of known AEs the most frequent are gastrointestinal (ie, diarrhoea, nausea, vomiting), whereas other AEs are rare (eg, allergic reaction).

AEs due to vitamin D treatment are rare. The European Food Safety Authority has provided tolerable upper intake levels (ULs) of vitamin D for children of all ages, concluding that for 1–10 years, UL is 2000 IU/day.²² Recently, the TARGet Kids! Collaboration conducted an RCT randomising 703 healthy 1–5 years old children to 2000 IU/day or 400 IU/day for a minimum of 4 months to reduce RTIs, where no vitamin D related AEs were reported.³⁸ Based on above-mentioned studies, the intervention dose chosen in this RCT is 2000 IU/day+ standard dose 400 IU/day. Potential AEs of high-dose vitamin D treatment include (1) hypocalcaemia and (2) hypercalcaemia/calciuria. These AEs are rare and are assessed at inclusion, after 3 months, and at a 1-year follow-up visit by measuring 25(OH)D; calcium (Ca), both ionized Ca and serum Ca; phosphorus (Ph); alkaline phosphatase (ALP); parathormone (PTH); and urine calcium:creatinine ratio, which will be evaluated by an independent paediatrician.

If a child unexpectedly develops symptoms of severe AEs, such as intoxication or allergic reaction, treatment will be stopped immediately, randomisation unblinded, and the participant will be referred for assessment and treatment. Further, if a participant is diagnosed with moderate to severe vitamin D deficiency (25(OH)D < 25 nmol/L) the child will be excluded from the study and referred to their general practitioner for a higher supplementation dose according to Danish Paediatric Society's recommendations.²⁶

Sample size

The main outcome in the azithromycin study arm is the duration of the episode with asthma-like symptoms in days; that is non-normally distributed. Due to some observations of zero days duration, we will add a pseudocount of 1 day duration for transformation of all durations: $\log(\text{duration}+1) = \beta_0 + R * \beta_1 + \text{error}$, where R is the intervention. Based on the COPSAC₂₀₁₀ RCT duration of symptoms,¹² we expect a SD of $\log(\text{duration}+1) = 1.04$. With a power of 80% and a two-tailed alpha (α) of 5%, a delta (λ) in geometric mean ratio of 1.5, corresponding to a 50% reduction in symptoms duration, we need a sample size of $n = 230$ children randomised in two groups on a 1:1 and expecting a drop out of 10%. To increase power, we aim to recruit 250 children (125/arm).

The main outcome in the vitamin D study arm is the number of episodes with asthma-like symptoms requiring OCS and/or ED visit and/or hospitalisation over a 12-month period (λ), which is estimated at 0.94/child in the placebo arm based on previous RCTs with ICS.³⁹ A sample size of 145 children per arm will provide 80% power with a two-tailed α of 5% to detect a 35% relative reduction in the mean number of events (ie, $\lambda = 0.94$ in the placebo vs $\lambda = 0.611$ in the intervention group), based on a previous observed rate ratio of 0.64 for exacerbations requiring OCS in a meta-analysis.¹⁹ We expect a 10% drop-out and therefore aim to recruit 320 children (160/arm).

Blinding and randomisation

The participants will be randomly allocated 1:1 in both study arms, independently. The randomisation in both study arms will be done at Glostrup Pharmacy by a computer-generated list of random numbers in blocks of varying size (ie, 4, 6 or 8). Copies of the randomisation code will be kept in sealed envelopes at the Glostrup Pharmacy.

Treatment compliance in the azithromycin study arm is ascertained by parents checking a REDCap link checkbox after administration during the first 3 days.

Treatment compliance in the vitamin D study arm is ascertained by parents weekly via a REDCap link, where there is a checkbox for how many days the current week D-pearls were given and by counting the returned blister packs (both used and unused) at the 1-year follow-up visit.

All participants, parents and members of the research team are blinded to group allocation. Efficacy and safety analyses will be performed under allocation concealment. Both study arms will remain double-blinded until all children have been recruited and throughout the data validation and primary outcome analysis phases.

Data collection, management and analyses

Data collection methods

Through an interview with the child and parent(s) data on baseline health including, data on former episode(s) with asthma-like symptoms, current and former asthma medication, atopic status and data on environment and sociodemographic will be collected and directly entered in the REDCap trial database (see [table 2](#) for details recorded). Moreover, we will extract data on prescribed medication and information from the child's medical records from birth and 4 years ahead from date of inclusion. We will monitor asthma-like symptoms, defined as wheeze or whistling sounds, breathlessness or troublesome cough severely affecting the well-being of the child, via diary data registered online via the REDCap system. The parents will report daily for 21 days their child's asthma-like symptoms, use of medication, possible AEs and sick leave. A link for this registration is sent once daily via REDCap. If the child also participates in the vitamin D study arm, the parents continue to fill out the diary once weekly for 1 year. The participants included in the

Table 2 Overview of data we will access at the time of inclusion and over study procedures

Participant	Baseline	21 days	3 months	1 year	Description	References
Azithromycin study arm						
Faecal sample	X	X	X	X	Measurement of gut microbiota using 16S rRNA sequencing and antibiotic resistance genes assessed by metagenomic sequencing	35 40
Diary	X	X			Diary on asthma-like symptoms, medication, sick leave and AEs is registered daily by parents via text messaging through REDCap	12 28
Hypopharyngeal aspirate	X				Measurement of airway microbiota and viruses	41
Filter-paper sampling	X				Evaluation of immune mediator profiles in the upper airway epithelial lining fluid	42
Blood sample	X				Inflammatory markers (ie, C-reactive protein, white blood cell count)	
Anthropometrics	X				Height, weight	
Physical examination	X				Assessment of fever, tachypnoea, chest recessions, wheezing and lung and heart auscultation, and examination of the skin, ears, nose and throat	
Interview	X				Baseline characteristic	Age, sex, ethnicity, address
					Growth data	Weight, height, BMI z-score
					Birth data	Gestational age, birth weight and length, delivery mode, siblings at birth.
					Atopic status	Food allergies, allergic rhinoconjunctivitis, atopic dermatitis
					Asthma medication	SABA, ICS, OCS, LTRA (current and former)
					Former episode(s) with asthma-like symptoms	Time, numbers, treatment (incl. medication and emergency room visit(s), hospitalisation(s), visit to general practitioner or treated at home)
					Other relevant data	Daycare status (if yes - also start date), smoking in the home, animal in the home
					Parents and sibling(s) atopic status	Asthma diagnosis, food allergies, allergic rhinoconjunctivitis, atopic dermatitis
					Socioeconomic status	Parent's education level, parents age, parents' income.

Continued

Table 2 Continued

Participant	Baseline	21 days	3 months	1 year	Description	References
Vitamin D study arm						
Diary	X			X	Diary on asthma-like symptoms, medication, sick leave and AEs is registered daily the first 21 days, afterwards weekly, by the parents, through text messaging via REDCap	12 28
Hypopharyngeal aspirate	X			X	*	41
Filter-paper sampling	X			X	*	42
Nasal epithelial scrape	X			X	Gene expression	43
Urine sample	X		X	X	Calcium:creatinine ratio	
Anthropometrics	X				*	
Physical examination	X				*	
Blood sample	X		X	X	At inclusion and at the 1-year follow-up visit: 25(OH)D, Ca, Ph, ALP, PTH, C reactive protein, white cell count. Gene expression and methylation in vitamin D pathways and childhood asthma risk loci. At 3 months after inclusion 25(OH)D, Ca, Ph, ALP, PTH. Total-IgE and specific-IgE towards inhalant allergens will be examined after 1 year.	
Interview regarding exhibitions	X				*	

If the child is participating in both study arms the overlapping procedures will only be taken once.

*As in the Azithromycin study arm.

AEs, adverse events; ALP, alkaline phosphatase; BMI, body mass index; Ca, calcium; ICS, inhaled corticosteroids; IgE, immunoglobulin E; LTRA, leukotriene receptor antagonist; OCS, oral corticosteroids; 25(OH)D, 25-hydroxyvitamin D; Ph, phosphorus; PTH, parathormone; REDCap, Research Electronic Data Capture; SABA, short-acting beta-agonists.

azithromycin study will deliver a blood sample at baseline, measuring C reactive protein and white blood cell count. Faecal samples will be collected at baseline, 3 weeks, 3 months and 1 year after inclusion. Both studies include biosamples for measuring airway microbiota, pathogenic bacteria, vira and the airway immune profile. The vitamin D study arm also includes samples from the nose for gene expression of the upper airway. Further, in the vitamin D study arm at inclusion and after 3 months (ie, at their local hospital) a blood sample and a urine sample will be taken for measuring 25(OH)D, Ca, Ph, ALP, PTH and urine Calcium:Creatinine ratio, which will be evaluated by an independent paediatrician for signs of vitamin D intoxication and vitamin D insufficiency.

Further, they will attend a clinical follow-up visit at the COPSAC research clinic 1 year after enrolment for collection of a variety of bio samples, screening for AEs and interviewing the parent(s) regarding the recent years' exacerbations, medication use and step-up/step-down in preventive asthma medication.

The planned study procedures are listed in [table 2](#). Further, a more in-depth description of the methods for

sampling and assessments of the biosamples is given in online supplemental material.

Statistical methods

An intention-to-treat analysis will be carried out with all randomised children by treatment group.

Azithromycin study

We will analyse the duration of an episode of asthma-like symptoms and SABA use after treatment with Quasi-Poisson regression models. This type of regression is ideal for modelling counts, such as duration because it captures both skewness and variance heterogeneity and provides an easy-to-interpret quantification of effects as relative change in mean counts. Further, we will analyse time to resolution of asthma-like symptoms using survival analysis, for example, Cox regression or accelerated failure time method, though specific model chosen will be dependent on model fit. We will evaluate potential effects of factors such as recruiting centre and age of the child by inclusion of these in the models.

We will analyse baseline factors potentially modifying the treatment effect such as airway immune profile; airway microbial colonisation; and clinical appearance, including current use of antiasthmatic treatment as interaction terms in the models. The modifying effect of airway immune profile and airway microbial colonisation are investigated to confirm earlier findings.

This is planned, as we previously showed that lower levels of TNF-alpha and CCL22 and higher IL-10 in the upper airway predicted better treatment response to azithromycin.¹⁴ Furthermore, we showed that the composition of the airway microbiota during an acute episode modified the effect of azithromycin treatment with high richness or high relative abundance of *Veillonella* and *Neisseria* being associated with a better treatment effect of azithromycin.¹³

We will apply unsupervised compositional methods such as principal component analysis and alpha-diversity and beta-diversity measures for the analysis of inflammatory markers and microbiota as well as univariate single variable testing and additionally supervised methods such as partial least squares-discriminant analysis (PLS-DA)/sparse PLS-DA to evaluate jointly contributing variables.

Safety analyses will include all children who receive the intervention, including those, if any, without a primary outcome measure.

Vitamin D study

We will analyse the number of exacerbations requiring OCS and/or ED visit and/or hospitalisation per child during the 12-month follow-up using a Quasi-Poisson regression model to compute the incidence risk ratio.

Six subgroup analyses are planned: 25(OH)D (<75 vs ≥75 nmol/L and first vs fourth quartile), sex (male/female), atopy (sIgE≤0.35 or >0.35 kUa/L), elevated blood eosinophil count (≤0.3 or >0.3×10⁹ cells/mL), body mass index (Z-score≤2 vs >2 SD) and ethnicity (Caucasian vs non-Caucasian). Specific ethnicities will be registered, and if possible, we will stratify analysis on ethnicity.

Effect modification analyses are planned for baseline airway immune mediator levels, respiratory pathogens (virus and bacteria), airway microbiome and for genetic variations in the vitamin D pathway/metabolism and childhood asthma risk loci.

The time to first exacerbation requiring OCS and/or ED visit and/or hospitalisation will be compared between groups using a cox regression model. Symptom/hospitalisation duration during an exacerbation will be compared between groups using a Quasi-Poisson or general linear regression model. Similar analyses will be carried out for intensity of SABA and OCS use. Daily symptom burden between exacerbations will also be compared using a Poisson or general linear regression model.

The χ^2 test or Mantel-Haenszel method will serve to compare categorical outcomes, including the number of children with ≥1 episode of clinically significant hypercalciuria, hypercalcaemia, elevated serum 25(OH)D and AEs.

No adjustment for multiple outcomes is planned in any of the study arms.

Data management and monitoring

Acquired data are stored in the secure REDCap system provided by the Capital Region of Copenhagen. The data are directly entered electronically, both by researchers and the parents. The azithromycin study arms will be monitored by the unit of Good Clinical Practice (GCP), Bispebjerg Hospital. GCP will oversee randomisation, data management, progress monitoring and all analyses. A combination of remote monitoring activities and routine monitoring visits are conducted to ensure that each site adheres to the study protocol, GCP guidelines and data collection completeness.

Ethics and dissemination

The studies are conducted in accordance with the guiding principles of the Declaration of Helsinki and are approved by the Danish ethical committee (H-20065249, H-20066827). The Danish Medicines Agency has approved the azithromycin study arm (EudraCT number: 2020-004420-42). The vitamin D intervention has been reviewed as well and is not considered to be a medical intervention. All families will receive written information about the study prior to participation and both parents will give verbal and written informed consent before enrolment. Acquired data are stored in the secure REDCap system. No provision is given to the participants, and they have the possibility to withdraw from the study at any time.

No participant identifiers will be used in the dissemination of this research. Results will be disseminated to the medical community via national/international conferences and publications in peer-reviewed journals, to lay people via social and public media, and to families of preschoolers with asthma by involving patient organisations and networks. All results, negative as well as positive, will be published. Further, the results will be available on our websites: www.copsac.com and www.dbac.dk.

Public and patient involvement

No patients were involved in setting the research question or the outcome measure, nor are they involved in developing plans for recruitment, design or implementation of the study. No patients will be asked to advise on interpretation or writing up of results. We will disseminate the results of the research to study participants and the public.

DISCUSSION

This paper describes the design and methodology of a combined RCT consisting of two independent interventions with the overall aim to improve both secondary prevention (high-dose vitamin D) and tertiary prevention (azithromycin) of asthma-like symptoms in preschoolers. This has the potential to fill in a huge medical need as

the prevalence of childhood asthma has increased markedly in the last decades,² and there are limited treatment possibilities for both secondary and tertiary prevention of exacerbations.

We hypothesise that azithromycin treatment shortens episode duration of asthma-like symptoms so severe that the symptoms have led to hospitalisation of preschool children. If successful, the project will provide evidence of azithromycin as a potential standard treatment for recurrent acute asthma-like episodes requiring hospitalisation. A secondary aim is to assess the individual responses to azithromycin treatment based on the airway immune profile and the airway microbiome. Previously, we investigated levels of 18 cytokines and chemokines *in vivo* in the upper airway to assess the value of these mediators for predicting treatment response to azithromycin. Levels of TNF- α , IL-10 and CCL22 were found to predict treatment response.¹⁴ Further, in the same study, we also found that the composition of the airway microbiota during the acute episode modified the treatment effect of azithromycin. A high bacterial richness was associated with a better treatment effect and high relative abundance of several individual bacterial taxa similarly increased the treatment effects.¹³ If replicated, these results may pave a path for a personalised treatment strategy from immunological and/or microbial profiles, so only children, who will benefit from azithromycin will be treated. Thereby, we aim to pinpoint the children, which will have the best treatment effect to lower the number of children treated with azithromycin.

The azithromycin study can raise concerns regarding antibiotic resistance. However, the intervention only targets children with recurrent asthma-like episodes, who currently receive or have received antiasthmatic medication, which narrows the group, where the treatment will be relevant. A focus on personalised treatment in the future will further narrow down the group, where azithromycin is indicated. Finally, we have previously reported that azithromycin treatment only leads to short-term deranged gut microbiota (ie, 21 days after intervention), which was normalised later in childhood (ie, 1–3 years after intervention).⁴⁰

We hypothesise that high-dose vitamin D treatment will lead to more effective secondary prevention of episodes with asthma-like symptoms in preschoolers currently receiving or having received anti-asthmatic medication. We will investigate potential mechanisms of action to elucidate the protective role of vitamin D, which includes effect modification analyses of airway immune mediator levels, the airway microbiome, and genetic variations and gene expression in the vitamin D pathway/metabolism, and known childhood asthma risk loci. This strategy has the potential to highlight specific phenotypes of asthma and episode characteristics, where vitamin D supplementation is most effective. Importantly, vitamin D supplementation has been found to reduce the risk of RTI in a subgroup analysis within a meta-analysis of children aged 1–16 years by 40% (n=513 children).²⁰ This suggests that

vitamin D may be particularly beneficial for prevention of episodes in preschoolers, where RTI is the main trigger of exacerbations. This effect on RTIs further suggests that vitamin D has its impact via the immune system. Hence, exploring the role of baseline vitamin D levels and the impact of vitamin D on airway immune mediators would enhance our understanding of mechanisms of action.

This combined placebo-controlled, double-blind, RCT with two independent interventions has several strengths, which include (1) enrolment of hospitalised preschoolers with asthma-like symptoms irrespective of atopy, phenotype and baseline 25(OH)D (vitamin D arm); (2) a short-term (ie, 3 days for azithromycin), safe and easy single daily dosing without bolus treatment for vitamin D arm; (3) a 12-month intervention for vitamin D ensuring that the study period continues throughout winter and covers the peak incidence of exacerbations and RTIs and the seasonal decline in 25(OH)D; (4) primary outcomes that address unmet clinical and societal needs; (5) exploration of potential mechanisms of action to direct personalised treatment; (6) safety profile documentation and (7) interventions that can easily be implemented in clinical practice. RCTs are necessary as a basis for evidence-based guidelines before being able to (1) implement azithromycin as part of the tertiary prevention of asthma in preschool children and (2) implement vitamin D supplementation as a part of secondary prevention of asthma in preschool children.

The project will be performed by the research team at COPSAC in collaboration with paediatric dept. at Herlev-Gentofte University Hospital, Hvidovre University Hospital and Slagelse Hospital, Denmark.

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Contributors BC, JS, HB, KB, LMH and JNK designed the studies. JNK was responsible for writing the first draft of the manuscript. UR were responsible for the data system REDCap and contact to the pharmacy and the GCP unit. JNK, BC, JS, UR, NF, TM, LMH, A-MMS, KB and HB read and critically revised the manuscript.

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