Trials

STUDY PROTOCOL

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A randomized controlled non-inferiority trial of placebo versus macrolide antibiotics for *Mycoplasma pneumoniae* infection in children with community-acquired pneumonia: trial protocol for the MYTHIC Study

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Abstract

Background *Mycoplasma pneumoniae* is a major cause of community-acquired pneumonia (CAP) in school-aged children. Macrolides are the first-line treatment for this infection. However, it is unclear whether macrolides are effective in treating *M. pneumoniae* CAP, mainly due to limitations in microbiological diagnosis of previous studies. The extensive global use of macrolides has led to increasing antimicrobial resistance. The overall objective of this trial is to produce efficacy data for macrolide treatment in children with *M. pneumoniae* CAP.

Methods The MYTHIC Study is a randomized, double-blind, placebo-controlled, multicenter, non-inferiority trial in 13 Swiss pediatric centers. Previously healthy ambulatory and hospitalized children aged 3–17 years with clinically diagnosed CAP will be screened with a sensitive and commercially available *M. pneumoniae*-specific IgM lateral flow assay from capillary blood. *Mycoplasma pneumoniae* infection in screened patients will be verified retrospectively by respiratory PCR (reference test) and IgM antibody-secreting cell enzyme-linked immunospot (ELISpot) assay (confirmatory test for distinguishing between carriage and infection). Patients will be randomized 1:1 to receive a 5-day treatment of macrolides (azithromycin) or placebo. The co-primary endpoints are (1) time to normalization of all vital signs, including body temperature, respiratory rate, heart rate, and saturation of peripheral oxygen (efficacy), and (2) CAP-related change in patient care status (i.e., admission, re-admission, or intensive care unit transfer) within 28 days (safety). Secondary outcomes include adverse events (AEs), as well as antimicrobial and anti-inflammatory effects. For both co-primary endpoints, we aim to show non-inferiority of placebo compared to macrolide treatment. We

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expect no macrolide effect (hazard ratio of 1, absolute risk difference of 0) and set the corresponding non-inferiority margins to 0.7 and −7.5%. The "at least one" success criterion is used to handle multiplicity with the two co-primary endpoints. With a power of 80% to reject at least one null hypothesis at a one-sided significance level of 1.25%, 376 patients will be required.

Discussion This trial will produce efficacy data for macrolide treatment in children with *M. pneumoniae* CAP that might help to reduce the prescription of antibiotics and therefore contribute to the global efforts toward reducing antimicrobial resistance.

Trial registration ClinicalTrials.gov, NCT06325293. Registered on 24 April 2024

Keywords Anti-inflammatory, Antimicrobial, Atypical pneumonia, Azithromycin, Carriage, Colonization, Diagnosis, Resistance, Respiratory tract infection, Stewardship

Introduction

Background and rationale {6a}

Mycoplasma pneumoniae is a major cause of community-acquired pneumonia (CAP) in school-aged children [1]. Prior to the COVID-19 pandemic, *M. pneumoniae* was the most frequently detected bacterial pathogen (8%) in CAP among hospitalized U.S. children, followed by *Streptococcus pneumoniae* (4%) [2]. The proportion of *M. pneumoniae* was significantly higher in children ≥5 years of age compared with younger children (19% vs. 3%) [2]. After COVID-19 pandemic restrictions, a delayed re-emergence of *M. pneumoniae* [3] led to global CAP outbreaks [4] with detection rates of up to 64% in late 2023 (manuscript under revision).

CAP accounts for more treatment days with antibiotics in children's hospitals in the U.S. than any other condition [5]. Macrolides are the first-line treatment for a *M. pneumoniae* infection [6–8] and may have anti-inflammatory properties [9]. This class of antibiotic inhibits protein synthesis by binding to the 23S rRNA bacterial ribosome component of the large subunit (50S) [10]. Macrolides were the most commonly prescribed antibiotics during emergency departments (EDs) visits for children with CAP in the U.S., accounting for nearly half of all antibiotics given to children [11]. The widespread use of macrolides has led to a global emergence of macrolide resistance in *S. pneumoniae* [12] and *M. pneumoniae* [13]. Resistance of *M. pneumoniae* to macrolides is caused by point mutations in the 23S rRNA gene reducing the binding affinity of macrolides to the bacterial 50S ribosomal subunit [14]. The prevalence of macrolide-resistant *M. pneumoniae* (MRMP) is particularly high in Asia with >90% in some regions [13, 15–17], and it ranges from 5% to 8% in Europe and the region of the Americas, respectively [13]. Infections with MRMP strains have been previously associated with serious clinical consequences in children, leading to more severe radiological findings of pulmonary disease and even an increase in extrapulmonary manifestations (i.e., mucocutaneous and neurological disease) [18]. Overall, childhood CAP, particularly caused by *M. pneumoniae*, is an important target for antimicrobial stewardship efforts and cost-effectiveness considerations [19–21].

Macrolides are effective against *M. pneumoniae* in vitro [10], but it is still unclear whether macrolides are effective in vivo for treating *M. pneumoniae* CAP [6, 7]. A major issue in previous studies about the effectiveness of macrolides for the treatment of *M. pneumoniae* CAP is the inaccurate diagnosis of *M. pneumoniae* infection in treated children. Currently, no diagnostic test, neither polymerase chain reaction (PCR) from upper respiratory tract (URT) samples nor immunoglobulin (Ig) M serology, can reliably discriminate *M. pneumoniae* infection from carriage [22]. *Mycoplasma pneumoniae* carriage rates in the URT of healthy children vary significantly between studies from 2% up to 56% [2, 22–25]. In a previous study, we demonstrated that the detection of pathogen-specific antibody-secreting cells (ASCs) by enzyme-linked immunospot (ELISpot) assay improved the diagnosis of *M. pneumoniae* infection [23]. *Mycoplasma pneumoniae*-specific IgM ASCs were detected in children with *M. pneumoniae* CAP, but not in *M. pneumoniae* carriers with CAP caused by other pathogens or asymptomatic *M. pneumoniae* carriers [26]. The potential of the *M. pneumoniae*-specific IgM ASC ELISpot assay in diagnosing *M. pneumoniae* CAP has also later been corroborated by others [27]. Improved diagnosis with the IgM ASC ELISpot assay may contribute to evaluate the efficacy of macrolides on the outcome of CAP patients with true *M. pneumoniae* infection.

In our previous study, one-third of CAP patients with confirmed *M. pneumoniae* infection by the IgM ASC ELISpot assay were not treated with an antibiotic in vitro active against *M. pneumoniae*, but all of these children fully recovered [28]. A mild and self-limiting disease in the absence of antibiotic treatment has frequently been reported since the first descriptions of *M. pneumoniae* disease [29–32]. This observation about a self-limiting disease in a substantial proportion of *M. pneumoniae* CAP patients supports the hypothesis of an immune-mediated pathogenesis of *M. pneumoniae* infection. It has

been shown that T helper 1 cells contribute to inflammatory lesions in mycoplasma pneumonia in animal models [33–35] and that the interferon-γ response correlated with disease severity and/or radiological changes in *M. pneumoniae* CAP in children and adults [36–39].

Based on these findings, which suggest that host cell-mediated immunity is involved in the pathogenesis of *M. pneumoniae* CAP, we expect no clinically relevant effect of macrolides in children with *M. pneumoniae* CAP. The overall aim of the trial is to produce efficacy data for macrolide treatment in CAP patients with confirmed *M. pneumoniae* infection.

Objectives {7}

Co-primary objectives

The co-primary objectives of this trial are to determine, in ambulatory and hospitalized children aged 3–17 years with *M. pneumoniae* CAP, whether treatment with placebo is non-inferior to treatment with azithromycin in terms of (1) efficacy and (2) safety:

(1) Efficacy: Time to normalization of all vital signs (VS), including body temperature (T), respiratory rate (RR), heart rate (HR), and saturation of peripheral oxygen (SpO2);
(2) Safety: CAP-related change in patient care status (i.e., admission, re-admission, or intensive care unit [ICU] transfer) within 28 days after the index episode.

Secondary and additional objectives

The secondary and additional objectives include the evaluation of secondary and additional outcomes, respectively (see below).

Trial design {8}

The MYTHIC Study is an investigator-initiated, randomized, double-blind, placebo-controlled, multicenter, non-inferiority trial with two parallel groups (Fig. 1). This protocol publication follows the SPIRIT guidance [40] (additional file 1: SPIRIT checklist).

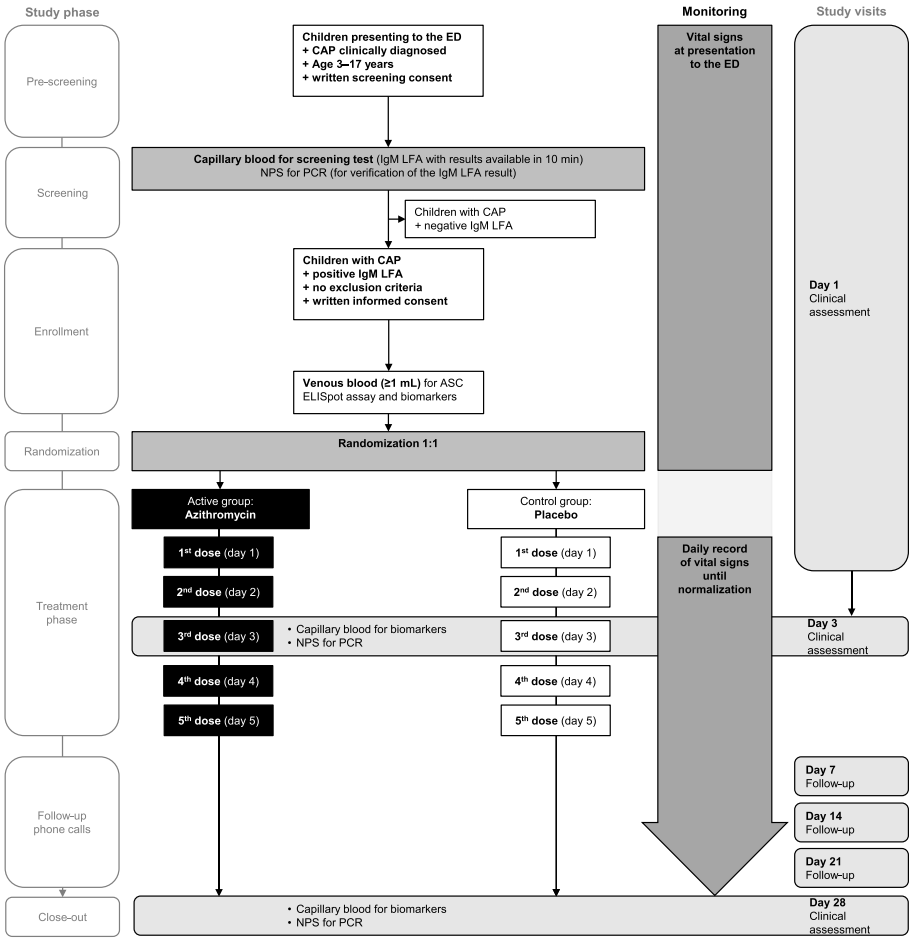

Fig. 1 Study flow. *ASC* antibody-secreting cell, *CAP* community-acquired pneumonia, *ED* emergency department, *ELISpot* enzyme-linked immunospot, *Ig* immunoglobulin, *LFA* lateral flow assay, *NPS* nasopharyngeal swab, *PCR* polymerase chain reaction

Methods: participants, interventions, and outcomes

Study setting {9}

The MYTHIC Study will recruit participants during a time period of 4 years in 13 pediatric EDs of secondary and tertiary hospitals across Switzerland (Additional file 2: table).

Eligibility criteria {10}

The ED staff will inform the local investigators about an eligible patient aged 3–17 years with clinical diagnosis of CAP. Eligibility criteria are listed in Table 1.

Pre-screening

The local investigators will initiate contact, inform the parent (and patient), and obtain a written informed consent for participation in the screening phase of the trial (Table 1: eligibility criteria, screening phase).

Screening

The patient will be subsequently screened for *M. pneumoniae* infection with an immunochromatographic point-of-care *M. pneumoniae*-specific IgM lateral flow assay (LFA; Biocard Mycoplasma pneumoniae IgM; Labsystems Diagnostics, Vantaa, Finland [41, 42]) using a capillary blood sample (Additional file 3: figure). We previously evaluated the LFA for this trial [42]: compared to *M. pneumoniae*-specific PCR from URT samples as reference test, the LFA showed a sensitivity and specificity of 86.0% and 95.1%, respectively (Additional file 3: table). All participants will also provide a nasopharyngeal swab (NPS) sample, either performed as part of the clinical routine diagnostic work-up or exclusively for the use in this study. This NPS sample will be tested with a *M. pneumoniae*-specific PCR as reference test to verify the positive IgM LFA.

Table 1 Eligibility criteria. *CAP* community-acquired pneumonia, *ED* emergency department, *FUP* follow-up, *ICU* intensive care unit, *Ig* immunoglobulin, *LFA* lateral flow assay

Study phase	Inclusion criteria (all must be fulfilled)	Exclusion criteria (excluded if any of the following are present)
Screening phase	• Children aged 3–17 years (from 3rd up to 18th birthday) presenting to the ED who will be managed ambulatory or will be admitted to general ward. • Clinical diagnosis of CAP[a]: (1) Diagnosis defined as the treating physician's documented diagnosis of CAP; AND (2) Fever ≥38.0°C (measured by any method [i.e., ear, axillary, rectal, or forehead site] in the ED or via parent report observed in the last 24h); AND (3) Tachypnea (respiratory rate above the age-specific reference value as defined in table 5 during the assessment in ED [triage or clinical examination]). • Written informed consent for participation in screening phase signed by parents or legal guardians and the patient if ≥14 years of age.	• None
Intervention phase	• Positive screening test result with the *M. pneumoniae*-specific IgM LFA (grade 2 or 3) (Additional file 3: figure). • Written informed consent for participation in intervention phase signed by parents or legal guardians and the patient if ≥14 years of age.	• Contraindication to azithromycin: documented allergy to azithromycin; cardiovascular disease, including bradycardia, arrhythmias, and/or QT-interval prolongation[b]; myasthenia gravis. • Underlying comorbidities: cystic fibrosis or other chronic lung disorders (excluding asthma), primary or secondary immunodeficiency, sickle-cell anemia, or severe cerebral palsy. • History of recurrent pneumonia (two or more episodes) or severe pneumonia (ICU admission or complications of CAP such as lung abscess, effusion, and empyema) in lifetime. • Antibiotic treatment against *M. pneumoniae* within the previous 7 days, including macrolides, tetracyclines, or fluoroquinolones. • Referral to ICU directly from the ED (e.g., development of respiratory failure). • Inability to tolerate oral medication. • Parents are unlikely to reliably complete FUP visits and questionnaires (e.g., due to language barriers or living far from the study site).

[a] This trial aims to produce translational results generalizable to a "real-world" setting. In ED settings, the diagnosis of CAP is generally based on clinical criteria [8, 9]. Therefore, inclusion criteria for this study will not be based on radiological or laboratory diagnostics. However, according to our experience from previous studies and feedback from participating centers, we expect ≥90% of children to have a chest radiograph performed as part of their diagnostic routine

[b] Co-medication with arrhythmogenic or QT-interval-prolonging drug (https://crediblemeds.org) is no exclusion criteria but will be discussed with the local investigators and/or trial management team

Enrollment

The LFA results will be available within 10 min. In case of a positive result, the local investigators will carefully check for eligibility and obtain written informed consent for participation in the intervention phase of the trial (Table 1: eligibility criteria, intervention phase). For enrolled children, a venous blood sample for the *M. pneumoniae*-specific IgM ASC ELISpot assay will be drawn.

The multimodal diagnostic approach with PCR (as reference test) on all screened patients and additional IgM ASC ELISpot assay (as confirmatory test) on randomized patients will ensure a correct diagnosis and clear guidance on study procedures and statistical analyses according to different test results (Table 2).

Sample processing

Venous blood samples from enrolled patients will be sent directly to the MYTHIC Biobank at University Children's Hospital Zurich. NPS samples and capillary blood from screened and enrolled patients will be frozen and stored locally at participating sites and transferred later in batches to the MYTHIC Biobank.

Who will take informed consent? {26a}

The local investigators (study nurse, study physician, or trained ED consultant) will obtain the written informed consent for participation in the screening phase as well as the intervention phase of the trial.

Table 2 Diagnostic approach for *M. pneumoniae* with test result constellations. *ASC* antibody-secreting cell, *CAP* community-acquired pneumonia, *ELISpot* enzyme-linked immunospot, *FAS* full analysis set, *FUP* follow-up, *Ig* immunoglobulin, *LFA* lateral flow assay, *NA* not available, *NPS* nasopharyngeal swab, *PCR* polymerase chain reaction, *PPS* per protocol set

Test	Method	Turn-around time	Specimen	Test result constellation[a]				
1. Screening test:	**IgM LFA**	10 min	Capillary blood	–		+		
Expected results[a]				67.2% (n = 84/125)		32.8% (n = 41/125)		
Study procedure:						Randomization		
2. Reference test:	**PCR**[b]	After close-out visit	NPS[b]	–	+	–	+	
Expected results[a]				92.9% (n = 78/84)	7.1% (n = 6/84)	9.8% (n = 4/41)	90.2% (n = 37/41)	
Interpretation of the IgM LFA result (screening test):				*Negative*	*False-negative*	*False-positive*	*Detection*	
3. Confirmatory test:	**IgM ASC ELISpot**[d]	After close-out visit	Venous blood[d,e]	NA[c]	NA[c]	–	–	+
Expected results[a]						9.8% (n = 4/41)	14.6% (n = 6/41)	75.6% (n = 31/41)
Final interpretation:				*Negative*	*False-negative*	*False-positive*	*Carriage and/or persistence*	*Infection*[d]
Study procedure:				No randomization	No randomization	FUP until final visit on day 28[b]	FUP until final visit on day 28	FUP until final visit on day 28
Statistical analysis:				Diagnostic accuracy	Diagnostic accuracy	**Intention-to-treat (FAS)**	**Per protocol (PPS)**	**Strict per protocol (strict PPS)**

[a] Expected results as proportion (number) based on results using the myCAP cohort [42] (n = 94) and KIDS–STEP cohort [43] (n = 31, unpublished results)

[b] All screened and enrolled participants will provide a NPS sample, either performed as part of the clinical routine diagnostic workup or exclusively for the use in this study. This NPS will be tested with an *M. pneumoniae*-specific PCR as reference test to verify the *M. pneumoniae*-specific IgM LFA test result

If a NPS is performed as part of clinical routine diagnostics, the sample will be frozen and stored locally so that no more than one swab will be performed on patients on day 1. The stored NPS sample will be transferred to and analyzed by *M. pneumoniae*-specific PCR at University Children's Hospital Zurich. The results of the *M. pneumoniae*-specific PCR will not be available before the close-out visit on day 28

In case a *M. pneumoniae*-specific PCR (single or multiplex) is performed for clinical reasons and indicates a false-positive *M. pneumoniae*-specific IgM LFA result, participants will be followed up until the close-out visit on day 28, but they will be excluded from per protocol analyses

[c] No venous blood sampling because not enrolled

[d] The *M. pneumoniae*-specific IgM ASC ELISpot assay may not be available in all patients (refusal to draw blood) and/or peripheral blood mononuclear cell viability can be decreased in very few instances (pre-analytical processing) and result in poor assay performance

[e] If venous blood is available also *M. pneumoniae*-specific IgM enzyme-linked immunosorbent assay (ELISA) will additionally be performed but these results will not be used to guide study procedures and statistical analyses

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Ancillary studies are being planned (e.g., *M. pneumoniae* genotyping, exhaled breath analysis, lung imaging, development of asthma, continuous VS monitoring). They will be run independently from the main trial, have their own ethics protocols and analysis plans, and need a separate written informed consent.

Intervention

Explanation for the choice of comparators {6b}

The antibiotics with the best minimum inhibitory concentration values against *M. pneumoniae* include macrolides, tetracyclines, and fluoroquinolones [10]. Macrolide antibiotics (i.e., azithromycin, clarithromycin, and erythromycin) have a more favorable side effect profile and are therefore recommended as first-line treatment for *M. pneumoniae* infections in children by the most globally recognized guidelines by the Pediatric Infectious Diseases Society (PIDS) and Infectious Diseases Society of America (IDSA) [6], British Thoracic Society (BTS) [7, 44], and National institute for Health and Care Excellence (NICE) [8]. Tetracyclines have very often adverse events (AEs) such as nausea, vomiting, photosensitive skin reactions, hypersensitivity reactions, and may also cause teeth discoloration; and fluoroquinolones have frequent AEs such as nausea, diarrhea, and may affect the developing cartilage in young children [6, 7, 45, 46]. Therefore, they are not recommended in young children below 7 years (tetracyclines) or before adolescence with skeletal maturity (fluoroquinolones) [6, 7].

Azithromycin is the most frequently used macrolide antibiotic worldwide because of its improved tolerability (over erythromycin), better taste (over clarithromycin with strong bitter intensity), and a much longer half-life that enables a 5-day treatment (compared to a 7–10-day treatment with clarithromycin) [1, 47]. In this trial, azithromycin will be used according to international guidelines once daily for 5 days, 10 mg/kg/day on day 1 and 5 mg/kg/day on days 2–5 [6, 7, 44].

Azithromycin is safe and well tolerated [48]. AEs associated with azithromycin are mainly related to gastrointestinal symptoms such as diarrhea, vomiting, abdominal pain, and nausea [49]. In adults, azithromycin can elicit arrhythmias as a potential consequence of QT-interval prolongation, particularly in patients with preexisting cardiovascular risk factors [50]. Given the low concentrations resulting from oral dosing of macrolides, the incidence of arrhythmias in adults in response to macrolides in the absence of additional risk factors is very low (<1:100,000) [51]. The risk of cardiac toxicity in children is unknown [49]. A recent randomized controlled trial (RCT) evaluating early administration of a 5-day azithromycin treatment on recurrent severe lower respiratory tract infection (LRTI) progression in preschool children reported only mild gastrointestinal symptoms in 3 of 223 (1.3%) children who received azithromycin [52]. These AEs were mild and did not lead to study discontinuation.

Intervention description {11a}

The patient will be allocated to the investigational medicinal product (IMP) at the ED. Patients will be randomized 1:1 to either azithromycin for 5 days or matching placebo for 5 days. The first dose of IMP will be administered immediately after randomization. Relevant doses will be determined according to a weight-banded dosing chart (Table 3).

Criteria for discontinuing or modifying allocated interventions {11b}

Criteria and procedures for discontinuation or modification that guarantee safety without the necessity of unblinding the IMP are listed in Table 4.

Strategies to improve adherence to interventions {11c}

Treatment adherence will be monitored during hospitalization by the local investigators on a daily basis and/or in ambulatory patients by daily documentation in the study diary and study visit on day 3 (in-hospital visit), IMP

Table 3 Dosing table for dose selection of azithromycin and placebo oral suspension

Weight band	Weight range	Day 1		Days 2–5		Total per treatment course	
	kg	mg/dose	mL/dose	mg/dose	mL/dose	mg	mL
0	≥10 to <15[a]	100	2.50	50	1.25	300	7.50
1	≥15 to <20	150	3.75	80	2.00	470	11.75
2	≥20 to ≤25	200	5.00	100	2.50	600	15.00
3	>25 to ≤35	300	7.50	150	3.75	900	22.50
4	>35 to ≤45	400	10.00	200	5.00	1200	30.00
5	>45	500	12.50	250	6.25	1500	37.50

Doses will be rounded to 0.25mL according to the oral syringe supplied with the IMP

[a] Inclusion from 3 years of age

Table 4 Criteria and procedures for discontinuation or modification of the allocated investigational medicinal product. *AE* adverse event, *FiO2* fraction of inspired oxygen, *IMP* investigational medicinal product, *ICU* intensive care unit

Discontinuation criteria	Modification criteria
• Any change in the patient's condition that justifies the discontinuation of the IMP (e.g., need for ICU transfer). • Unacceptable toxicity or AE (according to the prescribing information). • Use of a medication with a known major drug interaction with azithromycin. • Withdrawal of informed consent for IMP by patient/parent.	*Ambulatory patients:* • Need for hospital admission. *Hospitalized patients:* • Failure to maintain oxygen saturation ≥90% with FiO2 100%. • Oxygen saturation <90% for >48 h. • Clinical features of severe respiratory distress/exhaustion and/or shock/sepsis.
Procedures: • Stop IMP.	*Procedures:* • Treatment modification with antibiotics against atypical pathogens[a]; and/or • Switch to standard of care.[b]

[a] Treatment alternatives to the IMP (azithromycin as active drug) against atypical pathogens (e.g., *M. pneumoniae*, *Chlamydia pneumoniae*) must be discussed between the local investigators and the trial management team. These include clarithromycin (as another macrolide) or doxycycline in children >7 years of age (also as treatment option for infections with macrolide-resistant *M. pneumoniae* (MRMP)) [6]. In case of clinical suspicion of MRMP infection (e.g., worsening and/or non-responding symptoms), testing and (modifying) treatment for MRMP should be initiated irrespective of the study. The trial management team will also support the local team at the participating centers in managing infections with (possible) MRMP

[b] Decision about switch to standard of care (defined as treatment-as-usual, usual care, or routine care) will be made by the local team. The IMP should be continued for the total 5-day treatment duration whenever possible

return on day 28 (close-out visit) to measure remnant of suspension in bottles, and additional drug monitoring from capillary blood on days 3 and 28.

Relevant concomitant care permitted or prohibited during the trial {11d}

Decision about additional treatment with beta-lactams (such as amoxicillin) to avoid potential non-treatment of co-infecting bacterial pathogens (e.g., *S. pneumoniae*) in study patients will be made by the treating physician and will not be influenced by local investigators (Table 4).

Provisions for post-trial care {30}

No provision of post-trial care after follow-up is planned. No harm related to the trial participation is expected.

Outcomes {12}

Co-primary outcomes

(1) Time (days) to normalization of all VS, defined as T <38.0°C, RR, and HR within age-specific reference ranges, and SpO2 on room air ≥93% (Table 5), for at least 24 h (efficacy). VS will be measured before randomization (or prior to the administration of antipyretic medication at the ED). These VS measurements will be taken as the index time point for the assessment. VS will be measured after having the patient relax (without running, crying, etc. for at least 5 min) every 8 h (for hospitalized patients) or 3×/24h (for ambulatory patients) until three consecutive normal measurements of all VS (T, RR, HR, and SpO2) within 24 h are documented. Time to normalization of all VS will be aggregated as hazard and median time to event.

The resolution of all VS abnormalities has been proposed as an important primary endpoint for antibiotic trials in childhood pneumonia [53]. In contrast to adults, severe morbidity and mortality from CAP is minimal in children, particularly for *M. pneumoniae* CAP. Previous trials on macrolides for *M. pneumoniae* CAP have mainly focused on fever duration as a key endpoint [54, 55]. Lu et al. [56] observed a mean fever duration of 5 days (no statistically significant differences between patients treated with macrolides or placebo). We expect a rate of <1% of patients' VS not normalizing within a 28-day follow-up (FUP).

(2) CAP-related change in patient care status within 28 days (safety), defined as the incidence of any change in patient care status from an ambulatory to hospitalized setting (admission or re-admission), or from a hospitalized on general ward to an ICU setting (ICU transfer). CAP-related change in patient care status within 28 days will be aggregated as the proportion of patients with the event.

A recent U.S. study showed that re-admission occurred in 5% of children with CAP receiving beta-lactam monotherapy and in 2% receiving beta-lactam plus macrolide combination therapy [21]. In another U.S. CAP study, re-admission was reported in 0.5% of those who received beta-lactam monotherapy and in 0.6% of those who received beta-lactam plus macrolide combination therapy [57]. Detailed information about the proportion of patients with *M. pneumoniae* infection

Table 5 Reference values for body temperature, respiratory rate, heart rate, and saturation of peripheral oxygen on room air

Vital sign	Body temperature (T)	Respiratory rate (RR)	Heart rate (HR)	Saturation of peripheral oxygen (SpO2) on room air
Specific measurement:	Ear thermometer	Pulse oximetry	Pulse oximetry	Pulse oximetry
Unit:	°C	Breaths/min	Beats/min	%
Reference:	[2]	[58]	[58]	[7]
3 years	<38.0	21–29	86–123	≥93
4–5 years		20–27	81–117	
6–7 years		18–24	74–111	
8–11 years		16–22	67–103	
12–14 years		15–21	62–96	
15–17 years		13–19	58–92	

was not available for both studies. Based on these data, we expect a small proportion (≤5%) of patients with a CAP-related change in patient care status.

Secondary and additional outcomes

Secondary and additional outcomes, of which some were also defined as relevant endpoints for antibiotic trials in childhood CAP [53], are listed in Table 6.

Participant timeline {13}

Trial visit and contact schedules are prepared for each child at randomization and children are followed on that same schedule until the close-out visit regardless of adherence to IMP. The schedule defines visit times (with windows) necessary for data collection. An overview of trial contacts is given in Table 9.

Sample size {14}

Sample size calculations were done with regard to the per protocol set (PPS), which includes patients who are positive for *M. pneumoniae* by PCR (Table 2). To handle multiplicity with two co-primary endpoints, we apply the "at least one" success criterion [65]: we estimated the sample size for both co-primary endpoints at a one-sided significance level (α) of 1.25% (which corresponds to two-sided 97.5% confidence intervals [CIs]) and a power of 80% (β = 20%) and use the larger of the two sample sizes for the trial. This assures a minimum power of 80% to reject at least one null hypothesis.

The primary endpoint time to normalization of all VS is considered as a time-to-event endpoint, i.e., the number of days until normalization. Although we expect all patients to normalize VS during the 28-day FUP period, we used an overall event rate of 99% for the sample size calculation, in order to allow censoring. This endpoint will be compared between trial arms by a Cox proportional hazards model to estimate a hazard ratio for placebo vs. azithromycin (a hazard ratio <1 would indicate longer duration to normalization with placebo than with azithromycin). We expect no difference between treatments and thus a hazard ratio of 1. The sample size was estimated to show the non-inferiority of placebo vs. azithromycin treatment in PCR-positive patients using the method given in Chow et al. [66] (page 177), with a non-inferiority margin (δ_{HR}) of 0.7 for the hazard ratio. Assuming exponential survival times (with an overall event rate of 99% within 28 days), this non-inferiority margin of 0.7 would be equivalent to the inverse ratio of median survival times, which would be 4.8 days for placebo and 3.3 days for azithromycin (with a hazard ratio of 1 the median survival time would be 4.2 days). The maximum prolongation of the duration to VS normalization by 1.5 days may be acceptable from a clinical perspective, especially when weighed against AEs, the effect of antibiotics on microbiome, increased antibiotic resistance, and costs. These aspects are also discussed for patients with group A β-hemolytic streptococcal pharyngitis, in which modest effects of antibiotics have been observed (symptomatic improvement by only 1–2 days) [67]. Under the assumptions stated above, 302 PCR-positive patients are needed for this study (Fig. 2). Considering a drop-out rate of 14.5%, 354 patients should be enrolled. This drop-out rate is calculated from an expected drop-out of 10% due to negative PCR (false-positive screening by IgM LFA; Table 2) and an additional overall drop-out rate of 5% (due to loss to FUP or insufficient compliance), i.e., $0.1 + 0.05 \times (1 - 0.1) = 0.145$ (14.5%).

The co-primary endpoint CAP-related change in patient care status (binary) will be compared in terms of the absolute risk difference (ARD) between the two arms ($ARD = risk_{azithromycin} - risk_{placebo}$, ARD < 0 would

Table 6 Secondary and additional outcomes. *AE* adverse event, *CAP* community-acquired pneumonia, *DOOR* desirability of outcome ranking, *IMP* investigational medicinal product, *QoL* quality of life, *LFA* lateral flow assay, *RADAR* response adjusted for duration of antibiotic risk, *SAE* serious adverse event, *URT* upper respiratory tract, *VS* vital signs

Secondary outcomes:	• Overall clinical outcome based on benefits (clinical response: normalization of all VS) and harms (solicited AEs: Table 7) using desirability of outcome ranking (DOOR) and response adjusted for duration of antibiotic risk (RADAR) approach (Table 8) [59]. • Time (days) to normalization of CAP-related symptoms (i.e., cough, shortness of breath, wheeze, chest pain, sore throat, nasal congestion or runny nose, headache, muscle aches or pains, nausea or vomiting, diarrhea, reduced general condition, decreased appetite, not sleeping well, reduced activity). • QoL assessment of the patient's family until day 28 using a standardized and validated QoL questionnaire [60]. • Time (days) to return to daily routine, defined as return to childcare/school/work of patients and their families. • Development of *M. pneumoniae*-associated extrapulmonary manifestations [61, 62] within 28 days.
Additional outcomes:	• Length of hospital stay (days) in hospitalized patients after index hospitalization. • Number of medical visits (apart from the study) until day 28. • Proportion of patients (re-)treated with antibiotics for any reason until 28 days and total antibiotic exposure in days up to 28 days. • AEs/SAEs of IMP. • Microbiological indicators: proportion of patients who cleared *M. pneumoniae* in the URT within 28 days; proportion of patients in which *M. pneumoniae* became resistant to macrolides within 28 days; and proportion of patients with change in co-detecting pathogens in the URT at day 3 and 28. • Inflammatory indicators: biomarker and cytokine profiling at day 3 and 28.
Other additional outcomes (independent of study intervention):	• Degree of usefulness of informational video about the study on a five-point Likert scale.

indicate a lower risk with macrolide than placebo). We expect an absolute risk for this unfavorable event of 5% in both trial arms and thus an ARD of 0. The sample size was estimated to show the non-inferiority of placebo vs. macrolide treatment in PCR-positive patients, using the method given in Chow et al. [66] (page 90), with a non-inferiority margin (δ_{ARD}) of −7.5% for the ARD (Fig. 3). This non-inferiority would allow a maximum event rate of 12.5% in the placebo arm, which is less than 13.5%, the median acceptable failure rate in treatment of CAP identified in a survey of infectious disease physicians [68, 69]. Under the assumptions stated above, 322 PCR-positive patients are needed for this study. Considering a drop-out rate of 14.5% (as above), 376 patients should be enrolled.

As we consider the larger of these two sample sizes, 376 patients should be recruited for the trial. We assume that 66.7% of patients agree to screening and study participation (according to [26] and unpublished observations in the KIDS-STEP Study [43] at the participating center Zurich)

Table 7 Solicited adverse events (AEs) grading [63, 64]

Symptom	Mild (grade 1)	Moderate (grade 2)	Severe (grade 3)
Diarrhea	Looser than normal stools occurring 3–6 times/day	Looser than normal stools occurring >6 times/day	Bloody diarrhea, or diarrhea that requires medical intervention, laboratory testing, or hospitalization
Vomiting	1 episode/day	2–3 episodes/day	≥4 episodes/day
Abdominal pain	Mild or intermittent and does not interfere with daily activity	Moderate or persistent and interferes with daily activity but did not need a medical visit or absenteeism from daily routine	Prevents daily activity and resulted in medical visit or absenteeism
Allergic reaction (rash and/or pruritus)	Localized rash or pruritus without rash	Diffuse rash (maculopapular or urticarial)	Generalized rash consistent with Stevens–Johnson syndrome, erythema multiforme, or toxic epidermal necrolysis; anaphylaxis; or angioedema
Oral/pharyngeal thrush or nappy rash (Candidiasis)	Mild mucocutaneous candidiasis or diaper dermatitis, with no treatment or topical treatment only	Moderate mucocutaneous candidiasis requiring oral antimicrobial treatment	Severe mucocutaneous candidiasis; requires medical intervention, intravenous treatment, or hospitalization

Table 8 Overall clinical outcome. *AE* adverse event, *ED* emergency department, *VS* vital signs

Outcome	Adequate clinical response	Solicited AEs
1	Yes: normalization of all VS	No
2	Yes: normalization of all VS	Mild (grade 1)
3	Yes: normalization of all VS	Moderate (grade 2)
4	Yes: normalization of all VS	Severe (grade 3)
5	No: no normalization of all VS without additional ED or clinic visit or hospitalization	Any grade
6	No: no normalization of all VS with additional ED or clinic visit but without hospitalization	Any grade
7	No: no normalization of all VS with hospitalization (admission, re-admission, or ongoing hospitalization)	Any grade
8	Death (any cause)	

Adequate clinical response (defined by the co-primary outcome normalization of all VS) and harms (solicited AEs as defined in Table 7) will be documented 1×/24 h at days 3, 5 (end of treatment), 7, 14, 21, and 28. Overall clinical outcome will be aggregated as probability of better DOOR [59]. The RADAR methodology utilizes a superiority design [59]. In RADAR, all trial participants are assigned a DOOR using a two-step process: (1) categorization of all patients into an overall clinical outcome (hierarchical levels 1–8) and (2) ranking participants in the trial using two rules: (2a) when ranking the outcomes of two patients with different overall clinical outcomes, the patient with a better overall clinical outcome receives a higher rank; and (2b) when ranking the outcomes of two patients with the same overall clinical outcome, the patient treated with placebo receives a higher rank. These DOOR ranks are then compared between the treatment arms with regard to the probability of a better DOOR for a randomly selected patient with placebo vs. azithromycin (i.e., Wilcoxon–Mann–Whitney statistic [63]). The DOOR/RADAR approach is helpful for RCTs to define the optimal therapeutic strategy, since considering exclusively the primary endpoint may not allow researchers to accurately balance a proven benefit with other potential harms (i.e., impaired effectiveness or AEs)

and that 15% of screened patients are positive for *M. pneumoniae* [1, 2, 26, 70], which results in 10% of screened patients available for recruiting ($0.67 \times 0.15 = 0.10$). Thus, we expect that the number of patients to screen is 3760.

Recruitment {15}

The expected recruitment period is 4 years from January 2025 to December 2028. The estimated rate of recruitment per center is 0.5–1.5 patients per month.

Information material for participating sites includes flyers and posters placed in the waiting areas of the ED and a short informational film. A study website has been created (https://mythic-study.ch/en/) and it will include public and member-only areas. Any information material reviewed and endorsed by the ethics committee will be deposited in the publicly accessible area of the study website.

Assignment of interventions: allocation

Sequence generation {16a}

Patients will be allocated 1:1 to either azithromycin for 5 days or matching placebo for 5 days through minimization, which allows balance between treatment groups for several characteristics at all stages of the trial [71]. The following characteristics will be considered: (1) age: 3–9 years vs. 10–17 years [2, 28]; (2) patient care status: ambulatory vs. hospitalized; (3) duration of respiratory tract symptoms and/or fever before presentation to the ED: ≤6 days vs. >6 days [28, 72]; and (4) participating center: 13 centers.

The allocation of a participant to the IMP based on the aforementioned characteristics will be done using the electronic data capture (EDC) system (secuTrial; interActive Systems GmbH, Berlin, Germany). The first participant will be truly randomly allocated by the EDC system; for each subsequent participant, the treatment allocation that minimizes the imbalance on the selected characteristics between groups at that time will be identified by the EDC system. This allocation will be made with a probability of 0.8 in favor of the intervention that would minimize imbalance between treatment groups. The random element (of 0.2) ensures that allocation is not fully deterministic.

Concealment mechanism {16b}

The ZüriPharm AG at the University Hospital Zurich will assemble, blind, label, and distribute the IMP for each site to guarantee all safety regulations. Each kit (IMP, oral syringe, and measuring cup) has a unique medication ID. The medication ID and the associated treatment (active drug or placebo) are linked in a medication list, which is stored in the EDC system by the study data manager at the Clinical Trials Center (CTC) Zurich. The medication list in the EDC system includes medication IDs for medication that is available at each center (e.g., ZH123, LU123) and guarantees enough supply for each treatment arm. The medication list is concealed to all other parties.

Implementation {16c}

Prior to allocation, the local investigators must enroll the participants who fulfill all inclusion/exclusion criteria via the EDC system and enter the respective characteristics. The EDC system will then allocate the medication ID to the patient by minimization and release the medication ID to the investigator. Patients will be allocated to the IMP at the ED (for ambulatory patients) or as closely as possible to hospital admission (for hospitalized patients) within a maximum of 6 h after ED admission.

Table 9 Participant timeline. *AE* adverse events, *ASC* antibody-secreting cell, *C* clinic, *ED* emergency department, *ELISA* enzyme-linked immunosorbent assay, *ELISpot* enzyme-linked immunospot, *H* home, *HR* heart rate, *IMP* investigational medicinal product, *LFA* lateral flow assay, *NA* not available, *NPS* nasopharyngeal swab, *PCR* polymerase chain reaction, *P* phone, *QoL* quality of life, *RR* respiratory rate, *SpO2* saturation of peripheral oxygen, *T* body temperature

Procedure	**Pre-screening**	**Screening**	**Enrollment (within 6 h after admission to ED)**	**Randomization (within 10 h after admission to ED)**	**Treatment phase**					**Follow-up**				**Close-out**
Time point	t_{-1}	t_{-1}	t_{-1}	t_0	t_1	t_2	t_{3+}	t_4	t_5	t_{6+}	t_{7+}	t_{8+}	t_{9+}	t_{final}
Days in trial	1	1	1	1	1	2	3	4	5	6–28 (until normalization)	7	14	21	28
Time window (range) if patient at home (days in trial)							3–5				±1 (6–8)	±2 (12–16)	±3 (18–24)	±4 (24–32)
Clinic (C), phone (P), home (H)	C	C	C	C	C	C/H	C	C/H	C/H	C/H	P	P	P	C
Trial participation														
Screening phase:														
Eligibility check (screening)	X													
Written informed consent (screening)	X													
Capillary blood: LFA		X												
NPS: multiplex PCR		X					X							X
Intervention phase:														
Eligibility check (intervention)			X											
Written informed consent (intervention)			X											
Venous blood: ASC ELISpot			X											
IMP allocation (minimization)				X										
IMP administration					X	X	X	X	X					
Clinical assessment														
Medical history				X										
Physical examination				X			X							X
Vital signs (T, RR, HR, SpO2)	X					X	X	X	X	X	(X)	(X)	(X)	X
Symptom review, including AEs				X		X	X	X	X	X	X	X	X	X
Concomitant care review				X		X	X	X	X	X	X	X	X	X
QoL assessment							X				X	X	X	X
Laboratory and radiological assessment														
Capillary blood: biomarkers			X				X							X
Blood: ELISA			(X)											
Chest radiograph			(X)											
Other assessment														
Study film perception														X

Information about eligible patients that will undergo screening will be collected in a screening log at the local sites by the treating physician or local investigators. Each eligible patient receives a patient ID (documented in the screening log). Patients who will be included and allocated to IMP (medication ID) will be documented in the designation log and entered into the electronic case report form (eCRF) in the EDC system by the local investigators. Patient ID, medication ID, and date of allocation will be added to the eCRF accessible from the local site. The designation log will be held at each site and in copy at the CTC Zurich.

Assignment of interventions: blinding

Who will be blinded {17a}

Blinding will be ensured using placebo, which is provided by the ZüriPharm AG and indistinguishable from the active treatment in any way but the active ingredient. ZüriPharm AG and the study data manager (who links medication ID to active drug and placebo in the EDC system) are unblinded. All caregivers (including nurses, treating physicians), the parent (and patient), the investigators, and outcome assessors will be blinded to the allocated treatment. The trial statistician will be blinded when performing the blinded sample size review but will be unblinded for the final analysis.

Procedure for unblinding if needed {17b}

In the MYTHIC Study, no situations needing emergency unblinding are foreseen. AEs caused by drug toxicity and needing discontinuation of the drug are expected to be extremely rare when administering azithromycin in regular doses and for 5 days. The acute toxicity of a one-off azithromycin overdose is very limited. There is no specific antidote and management is symptomatic. Allergic reactions to any of the ingredients of the formulation can occur. However, these are extremely rare. In situations where an allergic reaction due to IMP is suspected, and further regimen doses are due, IMP is to be discontinued (Table 4).

In case of the need for emergency unblinding due to unforeseen circumstances in a study participant, the trial management team must be contacted. Unblinding will occur through the EDC system by a person with an appropriate right. The medication ID released by the EDC system will be entered into a screening log. This screening log will be stored locally in every study center and can be used as backup for unblinding procedures in case the EDC system would not be available for any technical circumstances.

Data collection and management

Plans for assessment and collection of outcomes {18a}

The VS measurement method depends on the patient care status. In hospitalized patients, VS will be measured by routine clinical monitoring using locally available equipment and procedures. VS will be documented in the clinical information system and the EDC system. In ambulatory patients, T will be measured by digital ear thermometer and RR, HR, and SpO2 by Masimo SafetyNet Radius PPG along with access to the Masimo SafetyNet mobile application (Masimo, Irvine, CA, USA). The parent will receive a trial box including the IMP kit, digital ear thermometer, Masimo SafetyNet Radius PPG, study diary, and instruction sheet (e.g., for use of patient self-documentation with the EDC system, QR code to the study website). The trial box will be returned at the close-out visit including the IMP bottles.

Plans to promote participant retention and complete follow-up {18b}

The parent (and patient) will be thoroughly instructed by the local investigators before discharge about the further study schedule, including in-hospital visits (days 3 and 28) and FUP phone calls (days 7, 14, and 21) to ensure data collection.

Data management {19}

Clinical trial data will be collected in the EDC system (secuTrial), which runs on a server maintained by the IT-department of the University Hospital Zurich. The eCRF will be implemented (set up and adjusted) by the data management group at the CTC Zurich.

Confidentiality {27}

All data collected during the course of the study will be kept strictly confidential and only accessed by trial management team members, statistician, local investigators, and designated staff of the CTC Zurich for EDC system administration and monitoring and of the ethics committee and/or competent authority for audits and inspections. Clinical data collected as part of this study are coded by the patient ID. No personal data are stored apart from year of birth, age at inclusion, and sex. Data will be stored in the EDC system, which is accessible via a standard browser on a WWW-connected device. Password protection ensures that only authorized persons can enter the system to view, add, or edit data collected during the course of the study according to their permissions.

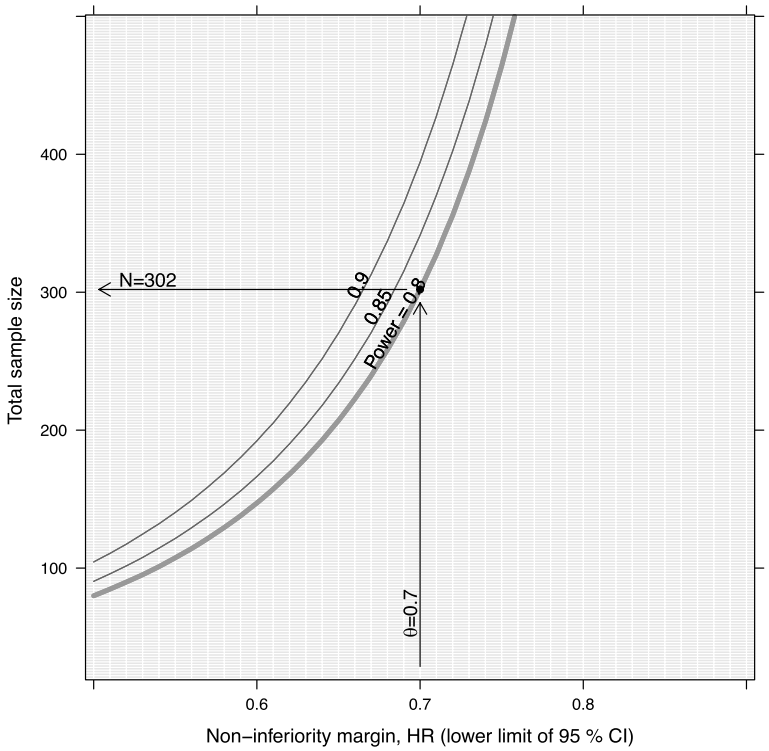

Fig. 2 Sensitivity of the sample size for the co-primary endpoint duration to normalization of vital signs with regard to the non-inferiority margin, δ_{HR}, expecting no difference between treatments (hazard ratio [HR] = 1)

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

All unused samples (blood, NPS) will be stored in the MYTHIC Biobank. Biobank storage is only allowed with written informed consent independent from the MYTHIC Study.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

To assess the non-inferiority of placebo vs. azithromycin regarding the co-primary endpoint time to normalization of VS, we will estimate the hazard ratio of placebo vs. azithromycin with a two-sided 97.5% CI using a Cox proportional hazards model on the PPS (Table 2; SPIRIT item #20c [40]). Non-inferiority of placebo will be declared if the lower limit of the CI will be larger than the non-inferiority margin (i.e., the whole CI lies entirely above the margin).

To assess the non-inferiority of placebo vs. macrolide regarding the co-primary endpoint CAP-related change in patient care status, we will estimate the absolute risk difference (ARD = $\text{risk}_{\text{azithromycin}} - \text{risk}_{\text{placebo}}$) with a two-sided 97.5% CI on the PPS. Non-inferiority of placebo will be declared if the lower limit of the CI will be larger than the non-inferiority margin (i.e., the whole CI lies entirely above the margin).

The two non-inferiority tests will be performed and interpreted independently (of each other) and the trial considered successful if non-inferiority can be shown for at least one of the primary outcomes. Should the resulting CIs exclude the reference value for no difference (1 for hazard ratio, 0 for ARD), the result can be interpreted as superiority of either treatment [73]. To complement the main analyses above, the following sensitivity and additional analyses are planned:

- We consider the PPS as the main set for showing non-inferiority and the sample size calculation was also done with regard to the PPS. In addition, we will also test non-inferiority in the strict PPS and the full analysis set (FAS). Since the three analysis sets (FAS, PPS, and strict PPS) differ considerably in size and composition (Table 2; SPIRIT item #20c [40]), we will consider the PPS as the relevant set for concluding non-inferiority, but we will discuss differences in the conclusions based on the different sets. For a robust interpretation of the non-inferiority test, these analyses should lead to similar conclusions as the main analysis [69].
- For CAP-related change in patient care status, we will present the 2×2 table of events per treatment arm

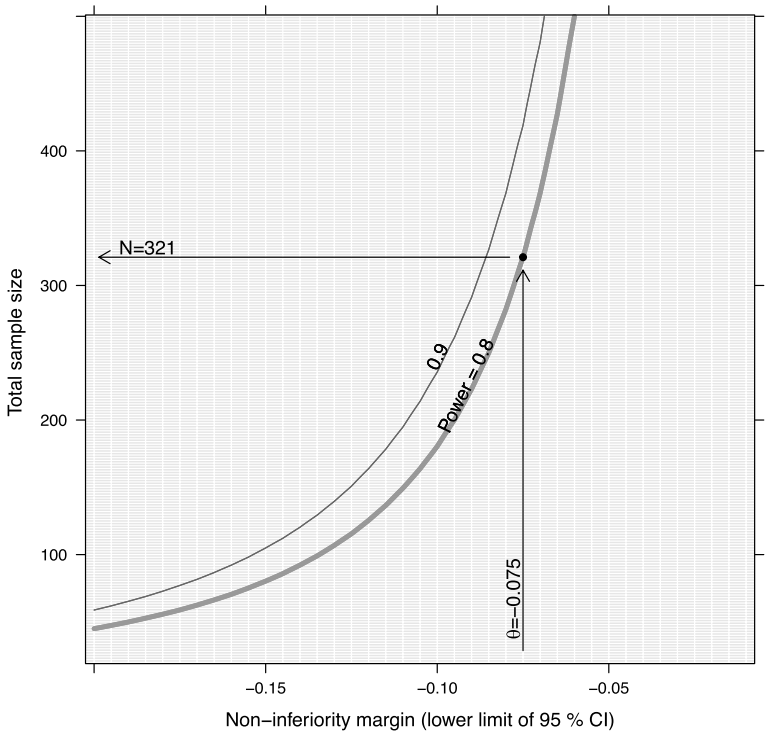

Fig. 3 Sensitivity of the sample size for the co-primary endpoint CAP-related change in patient care status with regard to the non-inferiority margin, δ_{ARD}, expecting no difference between placebo and azithromycin (absolute risk difference = 0), assuming a probability for a change in patient care status of 0.05

and an unadjusted odds ratio estimate for all three analysis sets.

- For time to VS normalization, we will plot Kaplan–Meier curves and estimate median time-to-event by trial arm.
- Covariate-adjusted analyses will be performed for both endpoints and all analysis sets, considering all variables used in the minimization for treatment allocation. For time to VS normalization, a mixed-effects Cox proportional hazards model with a random intercept per center will be used (coxme package in R). For change in patient care status, a generalized linear mixed-effects model will be used. Explanatory variables in both models are treatment (placebo vs. azithromycin), age (continuous), patient care status (ambulatory vs. hospitalized), and prodromal symptom duration (continuous).

More detailed methodology for summaries and statistical analyses, also for all secondary and additional objectives, is documented in a separate statistical analysis plan, which will be finalized before database closure and published later according to "Prospective reporting of statistical analysis plans" [74].

Interim analyses {21b}

An interim analysis for safety will be conducted after 1/3 and 2/3 of the patients have completed the 28-day FUP. The independent data monitoring committee (IDMC) will oversee and discuss the results. Access to interim data and interim analysis results will be limited to the IDMC and the statistician. We will do a blinded sample size review, using an internal pilot study design. The sample size review will be done for the binary co-primary endpoint, since the sample size estimation for this endpoint depends on the overall risk of a change in patient care status as a nuisance parameter [75]. The overall event rate for the time-to-event co-primary endpoint (proportion of patients with VS normalization within 28 days) is less uncertain and larger (less important nuisance parameter). We will estimate the overall risk of a change in patient care status ad interim, after 250 patients have the primary outcome measurement (2/3 of the planned sample size, $\widehat{N}_{init}$), as the proportion of patients who had the event, ignoring treatment groups. This estimate of the overall risk will then be used to recalculate the sample size, $\widehat{N}_{recalc}$, as described above. The final sample size

will be the larger of the original sample size and the recalculated sample size, $\widehat{N} = \max\left(\widehat{N}_{\text{init}}; \widehat{N}_{\text{recalc}}\right)$.

Formal statistical stopping rules will not be used in the MYTHIC Study although the IDMC charter will specify guidelines for when the IDMC will alert the trial management team to the need to discontinue the trial. These guidelines will be conservative to guard against premature discontinuation of the trial from early inspection of the data.

Methods for additional analyses (e.g., subgroup analyses) {20b}

Exploratory subgroup analyses are planned for the following baseline characteristics regarding the two co-primary outcomes: age (3–9 vs. 10–17 years and continuous in years); patient care status (ambulatory vs. hospitalized); prodromal symptom duration (≤6 days vs. >6 days and continuous in days); confirmation of *M. pneumoniae* infection by both PCR and IgM ASC ELISpot assay (binary yes vs. no); radiologically confirmed CAP (binary yes vs. no); and sex (binary male vs. female). For each subgroup variable, a mixed-effects Cox proportional hazards model will be fitted to the time to normalization and a generalized linear mixed-effects model to the CAP-related change in patient care status. Treatment, the subgroup variable, and the interaction between the subgroup variable and treatment will be included as explanatory variables. A statistically significant interaction between one of the subgroup variables and treatment would indicate a different treatment effect in the corresponding subgroups (or along age gradient). We will also compute group-specific treatment effects (with 95% CI), fitting a separate model for the corresponding subgroups, which will be reported together with the interaction *p*-value.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

The FAS will include all patients who gave written informed consent and were enrolled. Patients in the FAS will be analyzed according to the randomly assigned treatment, adhering to the intention-to-treat principle. The PPS will include all patients from the FAS who are PCR-positive (Table 2) and who are sufficiently compliant to treatment (≥80% of the medication used). The strict PPS will include all patients from the PPS who additionally have confirmed *M. pneumoniae* infection by IgM ASC ELISpot assay (Table 2). Patients in the PPS and strict PPS will be analyzed according to the received treatment (in case there are any deviations from the randomized treatment). In order to analyze all patients in the FAS (and the other sets), missing outcome and important covariate data will be multiply imputed using chained equations, as implemented in the R package mice [76], using $m = 100$ imputations per missing value.

Plans to give access to the full protocol, participant level-data, and statistical code {31c}

The full protocol is added as Additional file 4 and has been made available at ClinicalTrials.gov (NCT06325293). An anonymized subset of most important participant-level data and statistical code for data analysis will be made available per the funder policy upon completion of the trial.

Oversight and monitoring

Composition of the coordinating center and trial steering committee {5d}

The coordinating center is composed of the trial management team, members of the CTC Zurich, patient and public involvement (PPI) contributors, and the trial steering committee (TSC). The CTC Zurich is responsible for data management including the EDC system administration and monitoring. PPI contributors (parents) were identified through a survey among previous pneumonia study participants. They were involved in the development and set-up of the study and will be represented by PPI facilitators in the trial management team. The TSC is composed of the principal investigator, sponsor, co-investigators, trial manager and statistician and will oversee the trial implementation and conduct at the study sites. The trial will follow national and international standards for Good Clinical Practice (GCP) and comply with regulatory and ethical requirements.

Composition of the data monitoring committee, its role, and reporting structure {21a}

The IDMC includes experts in the field of pediatrics, pediatric infectious diseases, and/or clinical trials, the trial statistician, and another independent statistician. The IDMC will be independent from the sponsor and competing interests. Its responsibility will be to safeguard the interests of trial participants, assess the safety of the interventions during the trial, and contribute to monitoring the overall conduct of the clinical trial. Additional roles for the IDMC include consideration of implications of arising external evidence for safety and trial continuation, as well as advising on protocol modifications proposed by the investigators. The IDMC members will sign a IDMC charter.

Adverse event reporting and harms {22}

Solicited AEs and serious adverse events (SAEs) are collected in the EDC system. SAEs will be additionally reported to the sponsor by email to the study center within 24 h of the local investigator becoming aware of the event and assessed by the local investigator indicating

seriousness, severity (Table 7), expectedness, and causality. This initial report must be followed by the completed and signed SAE form in the eCRF within 7 days. The sponsor will promptly re-evaluate the seriousness, severity, expectedness, and causality of the SAE and report it to the relevant regulatory authorities and the IDMC, as appropriate.

Frequency and plans for auditing trial conduct {23}

Monitoring activities will be conducted by the CTC Zurich independent from investigators and the sponsor. The extent and nature of monitoring activities will be defined and described in a study specific monitoring plan. Audits and inspections may be performed by designated staff of the ethics committee and/or competent authority. Access to source documents will be granted for these purposes. However, all involved parties will keep personal data of participants strictly confidential.

Plans for communicating important protocol amendments to relevant parties (e.g., trial participants, ethical committees) {25}

Protocol amendments are only implemented after approval by the regulatory authorities and communicated to all relevant parties. Substantial amendments that impact trial specific procedures require additional on-site or web-based training provided to all investigators.

Dissemination plans {31a}

The data from all centers will be analyzed together and published as soon as possible in peer-reviewed journals, as well as being presented at national and/or international conferences. The results will be submitted for Open Access publication in high impact peer-review journals likely to be read by health professionals in the management of CAP in children around the globe. The work will be presented at key medical conferences. To maximize the impact of the trial its findings will be disseminated more widely through abstracts for oral and poster presentations submitted to the main relevant national and international conferences.

Once the trial has been published, all families who participated will be notified of the results. The study website will provide information for collaborators, participants, and the public, with the results of the trial eventually posted there. The social media presence of the organizations involved will also be used to highlight news about the trial.

For the main results of the trial a press release will be produced, in collaboration with the press office of the journal publishing the results, which will be distributed to Swiss and global media, to encourage press coverage. This will enable a wider audience to be reached.

Discussion

The MYTHIC Study is a multicenter, randomized, double-blind, placebo-controlled, non-inferiority trial of placebo vs. macrolide antibiotics that aims to provide conclusive evidence on the effectiveness of macrolide treatment for *M. pneumoniae* infection in ambulatory and hospitalized children with CAP.

Numerous previous studies have already tried to evaluate the effect of macrolides for *M. pneumoniae* CAP in children but were unable to draw any conclusions. A Cochrane review [55] evaluated seven RCTs on the effectiveness of antibiotic treatment for *M. pneumoniae* LRTI in children. However, the diagnostic criteria, the type and duration of treatment, inclusion criteria, and outcome measures differed significantly, making it impossible to draw any specific conclusions. A systematic review [54] including 17 randomized and non-randomized studies corroborated these results by showing insufficient evidence to support or refute the use of macrolides for *M. pneumoniae* LRTI. Also this review reported significant limitations of included studies such as substantial bias and subjective outcomes, inability to correct for timing of intervention, and most importantly, difficulty interpreting testing modalities [54]. Even so, another observational CAP study did not demonstrate benefits of empirical macrolide therapy in those children with *M. pneumoniae* infection [21]. However, it is important to note that lack of evidence is not evidence of inefficacy of macrolides for *M. pneumoniae* CAP when considering the limitations of the previous studies.

When prescribing macrolides in clinical practice, clarithromycin should be preferred over azithromycin [8, 77]. Azithromycin strongly promotes the development of antimicrobial resistance due to its very long half-life (48 to 108 h) and the associated long-lasting plasma levels (measurable plasma levels >1 μg/L up to 30 days following 3-day treatment) [77]. The development of macrolide resistance in *M. pneumoniae* during a course of treatment with azithromycin has already been demonstrated in children with *M. pneumoniae* CAP [78]. Nevertheless, azithromycin was selected as the investigational drug because it only needs to be given once a day, is available in a child-friendly formulation as a suspension, and most importantly, because it has a much better taste compared to the strong bitter intensity of clarithromycin, which is essential for an IMP in terms of compliance. However, as azithromycin promotes the development of antimicrobial resistance in *M. pneumoniae*, the MYTHIC Study will not only investigate for MRMP at randomization but also assess the effect of azithromycin on macrolide resistance development and bacterial clearance during the course of treatment (antimicrobial effects), in addition to the inflammation and immune response (anti-inflammatory effects).

The MYTHIC Study will also allow to evaluate the efficacy of macrolides in *M. pneumoniae* CAP patients with varying degrees of disease severity (ambulatory vs. hospitalized). By using the patient care status as a characteristic in the allocation through minimization the balance between treatment groups for both hospitalized and ambulatory children is guaranteed and treatment effects can be evaluated on subgroup levels. This could be essential because possible treatment effects of macrolides could vary depending on the severity of the disease.

As previous studies failed to demonstrate an effect of macrolides in *M. pneumoniae* CAP, mainly due to insufficient diagnostic testing modalities, the MYTHIC Study gives a unique opportunity to overcome these limitations using the novel IgM ASC ELISpot assay which is the only test that reliably diagnoses *M. pneumoniae* infection. The multimodal diagnostic approach with on-site point of care IgM LFA as screening test, PCR as reference test, and IgM ASC ELISpot assay as confirmatory test will be the major advantage compared to previous studies evaluating the effect of macrolides in children with *M. pneumoniae* CAP.

In conclusion, the MYTHIC Study has the potential to produce the first efficacy data for macrolides in *M. pneumoniae* CAP that might help to reduce the prescription of antibiotics and therefore contribute to the global efforts toward reducing antimicrobial resistance.

Trial status

Recruitment is planned from the start in January 2025, using trial protocol version 1.3 (24 April 2024), until December 2028.

Abbreviations

AE	Adverse event
ARD	Absolute risk difference
ASC	Antibody-secreting cell
BTS	British Thoracic Society
CAP	Community-acquired pneumonia
CI	Confidence interval
CTC	Clinical Trials Center
DOOR	Desirability of outcome ranking
eCRF	Electronic case report form
ED	Emergency department
EDC	Electronic data capture
ELISpot	Enzyme-linked immunospot
FAS	Full analysis set
FUP	Follow-up
GCP	Good Clinical Practice
HR	Heart rate
ICU	Intensive care unit
IDSA	Infectious Diseases Society of America
IDMC	Independent data monitoring committee
Ig	Immunoglobulin
IMP	Investigational medicinal product
LFA	Lateral flow assay
LRTI	Lower respiratory tract infection
MRMP	Macrolide-resistant *Mycoplasma pneumoniae*
NICE	National institute for Health and Care Excellence
NPS	Nasopharyngeal swab
PCR	Polymerase chain reaction
PIDS	Pediatric Infectious Diseases Society
PPI	Patient and public involvement
PPS	Per protocol set
QoL	Quality of life
RADAR	Response adjusted for duration of antibiotic risk
RCT	Randomized controlled trial
RR	Respiratory rate
SAE	Serious adverse event
SpO2	Saturation of peripheral oxygen
T	Body temperature
TSC	Trial steering committee
URT	Upper respiratory tract
VS	Vital signs

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13063-024-08438-6.

Additional file 1. SPIRIT checklist.

Additional file 2. Participating sites.

Additional file 3. Screening test (lateral flow assay).

Additional file 4. Trial protocol (version 1.3, 24 April 2024).

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Authors' contributions

PMMS is the principal investigator and CB the sponsor of the MYTHIC Study who devised the trial concept, with input from SvF, MS, CA, UH, RL, and KMPB. PMMS, CB, CA, RL, and KMPB secured the trial grant. PMMS, CB, SvF, RT, EO, and MvW designed the clinical trial. SS is responsible for the sample procedures and the MYTHIC Biobank. SvF is the trial statistician and developed the statistical sections of the trial grant and trial protocol. RT, EO, and MvW provided trial management. PMMS wrote the first draft of the manuscript. All authors contributed to subsequent drafts and approved the final version. All authors adhere to the authorship guidelines and have agreed to publication.

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Availability of data and materials

Not applicable. An anonymized subset of most important participant-level data and statistical code for data analysis will be made available per the funder policy upon completion of the trial.

Declarations

Ethics approval and consent to participate

Approval by ethics committees in Switzerland (BASEC ID: 2023-01295) on 14 May 2024. Parents or legal guardians and the patient if ≥14 years of age provide informed consent prior to study procedures commencing.

Consent for publication

Not applicable. No identifying images or other personal or clinical details of participants are presented here or will be presented in reports of the trial

results. The participant information materials and informed consent forms are available from the corresponding author on request and also deposited in the publicly accessible area of the study website.

Competing interests
The authors declare that they have no competing interests.

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