Azithromycin therapy for prevention of chronic lung disease of prematurity (AZTEC): a multicentre, double-blind, randomised, placebo-controlled trial

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Summary

Background Systematic reviews have reported conflicting evidence on whether macrolide antibiotics reduce rates of chronic lung disease of prematurity (CLD) in at-risk preterm infants born at less than 30 weeks' gestation, including in those colonised with pulmonary *Ureaplasma* spp. Since an adequately powered trial has been lacking, we aimed to assess if the macrolide azithromycin improved survival without the development of physiologically defined moderate or severe CLD in preterm infants.

Methods AZTEC was a multicentre, double-blind, randomised, placebo-controlled trial conducted in 28 tertiary neonatal intensive care units in the UK. Infants were eligible if they were born at less than 30 weeks' gestation and had received at least 2 h of either non-invasive (continuous positive airway pressure or humidified high flow nasal cannula therapy) or invasive respiratory support (via endotracheal tube) within 72 h of birth. Eligible infants were randomly allocated in a 1:1 ratio using random permuted blocks of four to receive either intravenous azithromycin at 20 mg/kg per day for 3 days followed by 10 mg/kg for 7 days, or to placebo. Allocation was stratified by centre and gestational age at birth (<28 weeks *vs* ≥28 weeks). Azithromycin and placebo vials were encased in tamper-evident custom cardboard cartons to ensure masking for clinicians, parents, and the research team. The primary outcome was survival without development of physiologically defined moderate or severe CLD at 36 weeks' postmenstrual age. Outcomes and safety were analysed on an intention-to-treat basis (all randomly allocated infants, regardless of any post-randomisation events). The study was registered with ISRCRN (11650227) and is closed.

Findings Infants were recruited between Oct 9, 2019, and March 22, 2022. 799 (53·1%) of 1505 eligible infants underwent random allocation; three infants were withdrawn, including consent to use their data, leaving 796 infants for analysis. Survival without moderate or severe CLD occurred in 166 (42%) of 394 infants in the intervention group and 179 (45%) of 402 in the placebo group (three-level adjusted OR [aOR] 0·84, 95% CI 0·55–1·29, p=0·43). Pulmonary *Ureaplasma* spp colonisation did not influence treatment effect. Overall, seven serious adverse events were reported for the azithromycin group (five graded as severe, two as moderate), and six serious adverse events were reported in the placebo group (two severe, two moderate, and two mild), as assessed by the local principal investigators.

Interpretation Since prophylactic use of azithromycin did not improve survival without development of physiologically-defined CLD, regardless of *Ureaplasma* spp colonisation, it cannot be recommended in clinical practice.

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Introduction

Chronic lung disease of prematurity (CLD, also known as bronchopulmonary dysplasia [BPD]), is a neonatal lung disease associated with significant mortality and life-long morbidity, including potential premature development of chronic obstructive lung disease (COPD).[1,2] Its severity in the neonatal period is most commonly defined by the requirement for supplemental oxygen, with or without ongoing respiratory support, at 36 weeks' post-menstrual age (PMA).[3] Despite advances in neonatal care, rates of CLD have remained largely unchanged, and is primarily found in infants born preterm at less than 30 weeks' gestation.[4] The pathogenesis of CLD is multifactorial,[5] with several risk factors identified for its development. These include antenatal factors, such as chorioamnionitis, and postnatal factors, such as the requirement for oxygen therapy and invasive mechanical ventilation. The role of pulmonary colonisation by the mollicute *Ureaplasma* spp in the development of CLD has caused controversy,[6] despite several systematic reviews showing a significant association between pulmonary colonisation with *Ureaplasma* spp at birth and subsequent development of CLD.[6,7] A common pathway for risk factors leading to the development of CLD, including in infants colonised with pulmonary *Ureaplasma* spp, is polymorpho-nuclear neutrophil-driven pulmonary inflammation,

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Research in context

Evidence before this study
Before obtaining funding, we conducted a formal systematic review to assess the association of pulmonary colonisation with *Ureaplasma* spp and development of chronic lung disease of prematurity (CLD, also called bronchopulmonary dysplasia [BPD]). Subsequently, we searched Embase, MEDLINE, Scopus, CINHAL, the Cochrane Central Register of Controlled Trials, and Web of Science published from database inception to April 2016 before grant application, then annually to June 2022 to identify the availability of any new relevant information. We used the search terms "BPD", "bronchopulmonary dysplasia or CLD", "chronic lung disease of prematurity", "*Ureaplasma* spp", AND "preterm", "neonate", "infant", or "child", without language restrictions. We identified three meta-analyses, all of which noted an increased risk of development of CLD for preterm infants colonised with pulmonary *Ureaplasma* spp, compared with those who were not colonised. The search also identified a 2003 Cochrane review which included two studies, neither of which showed improvement in rates of CLD after erythromycin treatment. A 2014 meta-analysis suggested potential benefit of prophylactic azithromycin therapy in preterm infants targeting *Ureaplasma* spp, pulmonary inflammation, or both for the three included studies which used azithromycin. However, a subsequent meta-analysis in 2021 did not show a significant improvement in rates of CLD when two new studies were added. However, each interventional study was limited in size, with all meta-analyses and interventional studies calling for an adequately powered study to definitively assess whether the early use of macrolides in preterm-born infants improves survival without the development of moderate or severe CLD. Since the 1990s, studies have shown that pulmonary inflammation during the first 7–10 days of life is strongly associated with the subsequent development of CLD. Thus, we designed the AZTEC trial to definitively assess if the anti-infective activity against *Ureaplasma* spp and anti-inflammatory effects of azithromycin on this vulnerable population improved survival without the development of CLD.

Added value of this study
To our knowledge, the AZTEC trial is the first adequately powered study to address this key knowledge gap, which has existed for several decades, around the effectiveness of macrolides in preventing the development of CLD in preterm infants at high risk of developing CLD. By conducting a robust, adequately powered, double blind, multi-centre, randomised controlled trial, we have demonstrated that azithromycin does not improve survival without development of physiologically defined moderate or severe CLD. Although the use of azithromycin was reassuring with few adverse events noted, none of the secondary outcomes including components of the primary outcome of death, severity of CLD, or treatment interaction with pulmonary *Ureaplasma* spp colonisation, invasive and non-invasive respiratory support, corticosteroid use, treatment for nosocomial infections, treated patent ductus arteriosus, severe intraventricular haemorrhage, and necrotising enterocolitis, were modified by treatment. We did find a significant reduction in rates of treated retinopathy of prematurity in survivors, although not when death was included in the outcome.

Implications of all the available evidence
The AZTEC study has definitively shown that the use of early azithromycin in preterm-born infants at high risk of developing CLD does not improve survival without the development of physiologically defined moderate or severe CLD. This is concordant with the conclusions of a 2021 systematic review, which also did not suggest efficacy of azithromycin in decreasing rates for the combined outcome of CLD and death. Since increased use of antibiotics in preterm infants is associated with increased morbidity (eg, due to necrotising enterocolitis), increased mortality, and increased antibiotic microbial resistance, caution is required to prophylactically use azithromycin in this vulnerable population to prevent the development of CLD.

which results in injury to the lung parenchyma with the subsequent tissue remodelling leading to dysregulated future lung growth and development. Trials of corticosteroids targeting pulmonary inflammation are extensive, but given the safety profile (eg, concerns over long-term neurodevelopmental outcomes), these treatments are often reserved for infants who are dependent on ventilators, in whom the potential benefits could outweigh the risks.[8] Therefore, there has been a search for new alternative therapies to specifically target pulmonary inflammation to ameliorate rates of CLD.

An ideal treatment would target both the infective and pulmonary inflammatory pathways which contribute to the development of CLD. Macrolide antibiotics such as azithromycin have potent anti-infective and anti-inflammatory activities, and are frequently used in several respiratory conditions including COPD[9] and cystic fibrosis.[10] Besides its antibiotic properties, azithromycin has several anti-inflammatory activities against neutrophil-driven inflammation, including: decreasing pro-inflammatory cytokine production; decreasing neutrophil chemotaxis and recruitment into the alveolar spaces while also enhancing bacterial clearance; and restricting biofilm formation.[11] Importantly, azithromycin has potent activity in the lungs as it is concentrated in resident pulmonary cells; its concentration in the lungs is a hundred times greater than in plasma.[12] Azithromycin has thus emerged as a potential treatment for preterm infants at risk of developing CLD due to its activity against *Ureaplasma* spp,[13] and its powerful anti-inflammatory properties against neutrophil activity in the lung.[11]

Azithromycin treatment started early in life in preterm infants at risk of developing CLD has the potential to target both pulmonary *Ureaplasma* spp colonisation and neutrophil-driven pulmonary inflammation, which consistently peaks at days 7–10 after birth in infants who subsequently develop CLD.[14–16]

Systematic reviews of previous trials of early macrolide treatment have been conflicting, citing the lack of an adequately powered study to confirm the place of macrolides in the treatment of CLD in preterm infants.[17,18] Therefore, we conducted the adequately powered Azithromycin Therapy for Chronic Lung Disease (AZTEC) trial, which aimed to assess whether early treatment with 10 days of intravenous azithromycin improved rates of survival without the development of physiologically-defined moderate or severe CLD in preterm infants born at less than 30 weeks' gestation. We also assessed the impact of any respiratory *Ureaplasma* spp colonisation on survival without development of CLD.

Methods

Study design

AZTEC was a double-blind, randomised, placebo-controlled trial conducted in 28 tertiary neonatal intensive care units in the UK according to the previously published protocol.[19] The trial was approved by the Medicines and Healthcare Products Regulatory Agency, the Wales 2 Research Ethics Committee (reference 18/WA/0199), and the Health Research Authority. It was overseen by the trial management group, which reviewed trial data monthly according to a pre-defined monitoring plan, and an independent data and safety monitoring committee (appendix p 3) which acted according to a specified internal charter. An independent trial steering committee (appendix p 3) provided executive oversight according to established terms of reference. The study was registered with ISRCRN (11650227).

Participants

Infants were eligible if they were born at less than 30 weeks' gestation and had received at least 2 h of either non-invasive (continuous positive airway pressure [CPAP] or humidified high flow nasal cannula [HHFNC] therapy) or invasive respiratory support (via endotracheal tube) within 72 h of birth. Eligible infants also required: a high probability of an indwelling intravenous line for drug administration for ten days of study treatment while an inpatient at the recruiting centre; follow-up to primary outcome to be feasible, and for their parent or guardian to be able to provide written informed consent for their infant to take part in the study. Infants were excluded if a serious congenital anomaly was known at birth, if they had previous exposure to a macrolide antibiotic (not maternal), or if the local investigator's opinion was that survival beyond 72 h was unlikely.

Randomisation and masking

The randomisation list was prepared by an independent statistician; treatment was randomly allocated in a 1:1 ratio using random permuted blocks of four by a computer program (Sortition, Oxford Innovation, Oxford, UK), stratified by centre and gestational age at birth (<28 weeks *vs* ≥28 weeks). Each infant from multiple births was randomised separately. Parents, clinical, and research teams were masked to the intervention, as the active and placebo investigational medicinal product (IMP) were packaged in identical 10 ml vials that were independently masked by Saint Mary's Pharmaceutical Unit (Cardiff, UK) by encasing the vials in a tamper-evident custom cardboard carton ensuring that contents were not visible during reconstitution.[19,20] Reconstituted IMP was colourless.

Procedures

Active therapy was ten days of intravenous azithromycin (Zedbac, Aspire Pharma, Petersfield, UK), given once daily at 20 mg/kg for the first 3 days followed by 10 mg/kg for a further 7 days by slow intravenous infusion via a peripheral or central intravenous line (appendix p 4). Placebo therapy consisted of sterile water for injection. The dose regimen was based on previous evidence of eradication of pulmonary *Ureaplasma* spp colonisation using a dose of 20 kg/mg for 3 days[21] and the further treatment at a 10 mg/kg dose for days 4–10 targeted the pulmonary inflammation which has been shown to peak at 7–10 days after birth.[14] An endotracheal aspirate (if receiving endotracheally intubated mechanical ventilation) or a nasopharyngeal aspirate sample (if not endotracheally intubated; appendix p 4) was collected to assess baseline pulmonary *Ureaplasma* spp colonisation before administration of the first IMP dose.

A daily log was completed, separated into weekly case report forms for 3 weeks, to prospectively record study data. A further data collection was undertaken at 36 weeks' PMA to obtain primary outcome data and to supplement secondary outcome data collection.

Outcomes

The primary outcome was a composite outcome of survival without development of physiologically defined moderate or severe CLD at 36 weeks' PMA.[3,22] The diagnosis and severity categorisation were based on previous recommendations and studies.[3,22,23] CLD was initially diagnosed if the infant was receiving respiratory support, supplementary oxygen, or both for the first 28 days of age. Assessment of CLD severity at 36 weeks' PMA was categorised as: mild (if the infant was breathing room air); moderate (if the infant required >21% and <30% supplemental oxygen [or low-flow oxygen at 0·01–1·0 L/min]) but was not receiving any respiratory support); or severe (if the infant was still receiving any respiratory support—invasive via by endotracheal tube, CPAP, or HHFNC—regardless of

degree of supplemental oxygen requirement or was receiving ≥30% oxygen [or low-flow ≥1·1 L/min], with or without any respiratory support). Supplemental oxygen

Figure 1: Trial profile
PMA=postmenstrual age. *Some parents gave multiple reasons. †One eligible infant was screened once during enrolment but was inadvertently randomly allocated twice. ‡Three infants were fully withdrawn from the study, including the use of any data collected previously.

requirement at 36 weeks' PMA was formally assessed for the moderate CLD group with an oxygen reduction test (appendix p 5) to confirm an absolute requirement for supplemental oxygen.[24]

Secondary outcomes at 36 weeks' PMA, which were composite outcomes incorporating death, comprised mortality, severity of CLD, effect of baseline pulmonary *Ureaplasma* spp colonisation, the number of days of supplemental oxygen, number of days of invasive or non-invasive ventilation, treatment for patent ductus arteriosus (PDA), treatment of nosocomial infections (positive blood culture, cerebrospinal fluid culture, or both, or antibiotic treatment for ≥5 days), severe intraventricular haem-orrhage (IVH, grade III or IV); necrotising enterocolitis ([NEC] Bell stage ≥II), treatment for retinopathy of prematurity (ROP), the highest liver and renal function measures, and serious adverse events or reactions.[24]

Pre-specified subgroup analyses on the primary outcome comprised of presence or absence of *Ureaplasma* spp colonisation at baseline, infant being inborn or outborn, gestational age, and recruiting centre. Post-hoc analyses included estimation of the effect of the postnatal corticosteroid use and estimation of secondary outcomes in survivors only.

Statistical analysis
Based on prevailing data at the time of the study design,[24,25] we estimated our sample size on the assumption we would achieve a 12% improvement in survival without the development of physiologically defined moderate or severe CLD (from 50% to 62%), with an estimated mortality rate of 25%. With a two-sided α-level of 5% and 90% power, we estimated that 716 infants would be required to adequately power the study. Assuming an estimated 10% drop out rate, we calculated that 796 infants should be recruited. An a priori statistical analysis plan, approved by the trial management group, data and safety monitoring committee, and independent trial steering committee has been published previously,[26] (see appendix pp 6–7). Outcomes and safety were analysed on an intention-to-treat basis (all randomly allocated infants, regardless of any post-randomisation events). The primary outcome was analysed using three-level logistic regression to account for clustering of multiple births and participants within centres, and adjusted for gestational age as a fixed effect. Results are reported as unadjusted, two level (gestational age and centre), and three level (addition of multiple birth) adjusted odds ratios (aORs) and adjusted absolute risk differences (aARDs) with 95% CIs and p values. Eligible infants who missed their oxygen reduction test were assigned to the moderate CLD group. The following predefined sensitivity analyses of the primary outcome were conducted: first, imputing missed oxygen reduction tests; second, excluding infants who had missed the oxygen reduction test from the analyses; and third, removing these infants for complete case analysis. The effect of baseline *Ureaplasma* spp colonisation is
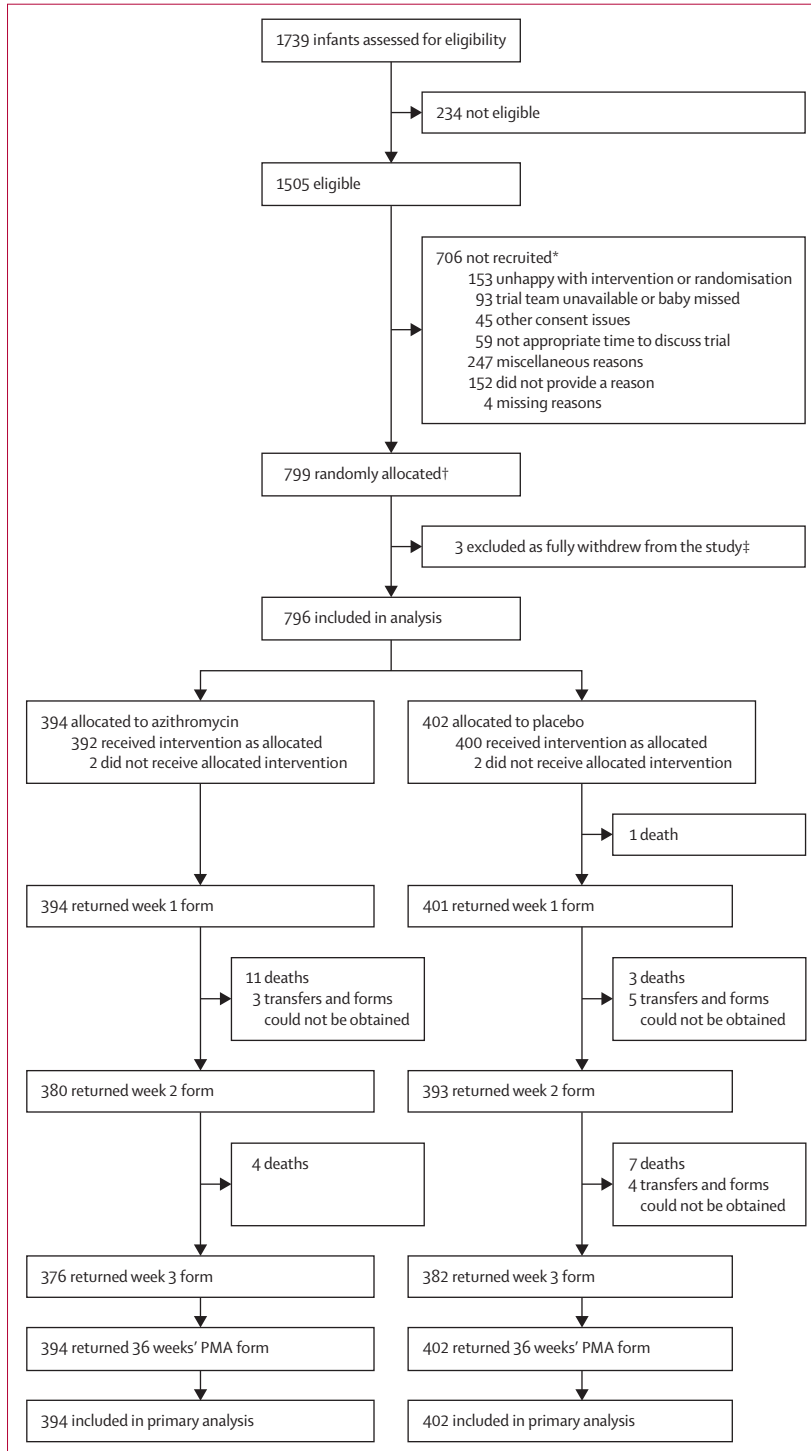
reported by extending the primary analysis to include an interaction term of *Ureaplasma* spp colonisation by treatment group. Differential treatment effects were also explored by gestational age, centre, and whether an infant was born at the recruiting centre (inborn) or transferred to the recruiting centre (outborn). Complier average causal effect analysis was used to investigate the effect of time-to-initiation of IMP and proportion of IMP taken.

Secondary outcomes were analysed using logistic regression, if dichotomous, using the three-level modelling approach described previously in survivors only, and in a composite analysis incorporating death. Days on supplemental oxygen and respiratory support (invasive ventilation via endotracheal tube or non-invasive respiratory support) were analysed as counts, with the in-survivors analysis including count data up until the point of death (if death occurred) and the composite-incorporating-death analysis defined as days alive without respiratory support between random allocation and the 36 weeks' PMA timepoint. These outcomes were modelled using a multilevel Poisson regression with robust standard errors to account for any heteroskedasticity (eg, minor deviations from the underlying assumption in the Poisson model that the mean equals the variance).[27] Multilevel linear regression models with Box-Cox transformations were used for maximum liver and renal function values (including in those dying). Secondary outcomes were not adjusted for multiple comparisons and there were no interim data analyses.

To analyse *Ureaplasma* spp, respiratory samples (endotracheal and nasopharyngeal aspirates) were transferred to the central laboratory (Central Biotechnology Services, Cardiff University, UK). Following initial processing, manual DNA extraction was performed to maximise yield from samples expected to contain limited biomass with final elution volumes of 50 µL. DNA quantification was subsequently performed. A published multiplex real-time quantitative PCR was performed to identify the presence of *Ureaplasma parvum*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* using primers shown in the appendix (p 8).[28,29] A standard curve of plasmid DNA containing concatemers of primers and probe targets separated by intervening sequences of 30 bp was generated for each run. A cut-off value of 10 copies per µL was used as a threshold for detecting presence of *Ureaplasma* spp in these clinical respiratory samples (see appendix p 7).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Infants were recruited between Oct 9, 2019, and March 22, 2022, with a complete suspension to recruitment between Jan 26 and July 10, 2020, and a partial suspension by several recruiting centres (between Jan 15 and April 31, 2021) due to the COVID-19 pandemic. 28 tertiary level neonatal intensive care units throughout the UK participated (median recruitment per centre 18, recruitment range 3–117 infants). 1739 infants born at less than 30 weeks' gestation were screened, and of those,

	Azithromycin (N=394)	Placebo (N=402)
Neonatal		
Median gestational age, weeks (IQR)	27·0 (25·4–28·4)	27·1 (25·3–28·7)
Median birthweight, g (IQR)	891 (700–1120)	900 (705–1140)
Male	212 (54%)	230 (57%)
Female	182 (46%)	172 (43%)
Intrauterine growth restriction*	61 (16%)	62 (15%)
Heart rate over 100 bpm at 5 min	353/391 (90%)	351/399 (88%)
Mode of ventilation prior to randomisation†		
Invasive	319/376 (85%)	323/391 (83%)
Non-invasive	57/376 (15%)	68/391 (17%)
Mother		
Race or ethnic group‡		
UK or Irish White	308 (78%)	305/401 (76%)
Indian, Pakistani, or Bangladeshi	25 (6%)	23/401 (6%)
Chinese or Asian	12 (3%)	10/401 (2%)
Black, African, or Caribbean	25 (6%)	29/401 (7%)
Any other White background	19 (5%)	19/401 (5%)
Any other ethnic group	5 (1%)	15/401 (4%)
Index of Multiple Deprivation 2022§		
1 (most deprived)	112/378 (30%)	112/383 (29%)
2	78/378 (21%)	72/383 (19%)
3	61/378 (16%)	82/383 (21%)
4	66/378 (17%)	53/383 (14%)
5 (least deprived)	61/378 (16%)	64/383 (17%)
Mode of delivery		
Vaginal delivery	167 (42%)	162 (40%)
Caesarean section	227 (58%)	240 (60%)
Born at recruiting site (inborn)	354 (90%)	354 (88%)
Main cause of preterm birth		
pPROM	107 (27%)	117 (29%)
Preterm labour (without pPROM)	113 (29%)	114 (28%)
Antepartum haemorrhage	28 (7%)	44 (11%)
Pregnancy induced hypertension	26 (7%)	33 (8%)
Obstetric intervention for fetal reasons (eg, chorioamnionitis)	87 (22%)	65 (16%)
Other maternal illness	33 (8%)	29 (7%)
Multiple pregnancies	88 (22%)	106 (26%)
Antenatal maternal corticosteroids administration	369 (94%)	379 (94%)
Antenatal maternal antibiotic treatment within 5 days of delivery‡	141/389 (36%)	168/399 (42%)
Antenatal maternal magnesium sulphate‡	342/393 (87%)	356/400 (89%)

Data are n (%) unless otherwise stated. bpm=beats per min. pPROM=preterm premature rupture of membranes. *Intrauterine growth restriction defined as birth weight below the 10th percentile for gestational age. †29 randomly allocated infants had received supportive ventilation prior to randomisation but mode of ventilation was not available. ‡Missing baseline data were due to the data not being entered by local centres. §Index of Multiple Deprivation quintile was generated from the mother's postcode; 35 mothers provided an invalid postcode.

Table 1: Neonatal and maternal characteristics at baseline

	Primary outcome		Death component of primary outcome	
	Survival without moderate or severe CLD	Died or had moderate or severe CLD	Died	Survived
Placebo (n=402)	179 (45%)	223 (55%)	31 (8%)	371 (92%)
Azithromycin (n=394)	166 (42%)	228 (58%)	34 (9%)	360 (91%)
Unadjusted				
OR (95% CI), p value	0·91 (0·69 to 1·29), 0·50	..	1·13 (0·68 to 1·88), 0·64	..
ARD (95% CI)	–0·02 (–0·09 to 0·05)	..	0·01 (–0·03 to 0·05)	..
Two-level model*				
aOR* (95% CI), p value	0·91 (0·67 to 1·23), 0·55	..	1·12 (0·66 to 1·89), 0·67	..
aARD* (95% CI)	–0·02 (–0·08 to 0·04)	..	0·01 (–0·03 to 0·05)	..
Three-level model†				
aOR† (95% CI), p value	0·84 (0·55 to 1·29), 0·43	..	1·28 (0·39 to 4·18), 0·69	..
aARD† (95% CI)	–0·03 (–0·09 to 0·04)	..	0·00 (–0·00 to 0·01)	..

Odds ratios (95% CI) and ARDs (95% CI) show the comparison between azithromycin and placebo (reference group). OR=odds ratio. ARD=absolute risk difference. aOR=adjusted odds ratio. aARD=adjusted absolute risk difference. CLD=chronic lung disease of prematurity. *Adjusted for gestational age and clustering within 28 recruitment centres. †Adjusted for gestational age and clustering within multiple births (ie, 707 mothers delivered 796 infants) within 28 recruitment centres.

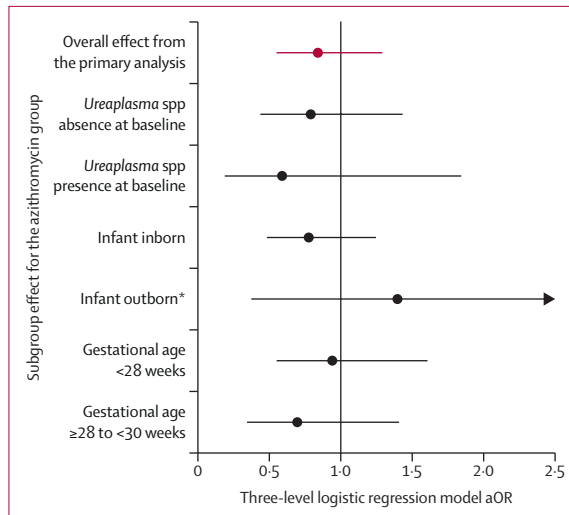*Table 2:* **Primary composite outcome and death**

Figure 2: **Forest plot of the overall effect and subgroup-specific effects for primary outcome (three-level model)**
aOR >1 favours azithromycin. aOR=adjusted odds ratio. *Born outside recruiting site. Upper bound=5·22.

were withdrawn, including consent to use their data, leaving 796 infants for analysis (n=394 for azithromycin and n=402 for placebo). An additional nine were withdrawn after random allocation (azithromycin 6 and placebo 3). One infant was inadvertently randomised twice (the initial allocation was implemented). Participant demographics are shown in table 1. No discernible differences were noted between the intervention and placebo groups besides greater antenatal maternal antibiotic administration in the placebo group (42% *vs* 36%, respectively). Overall, the median gestational age at birth was 27·0 weeks (IQR 25·3–28·6). Of the total 796 infants, 502 (63%) were born at less than 28 weeks' gestation and 294 (37%) were born at 28 to less than 30 weeks' gestation (appendix p 15). Median birthweight for the whole population was 895 g (IQR 703–1130), 442 (56%) infants were male and 354 (44%) infants were female, 467 (59%) were born by caesarean section, and the majority (708 [89%]), were born at their recruiting hospital (table 1).

767 (96%) of 796 infants received the first dose of IMP within 72 h of age, being similar between the intervention group (median 49·4 h [IQR 36·2–63·8]) and placebo group (51·1 h [36·9–63·3], appendix pp 9,15). Four infants did not receive IMP, with three dying shortly after random allocation, and one owing to a local clinical decision. Infants who received at least ten, nine, eight, and seven doses were 567 (71%), 636 (80%), 678 (85%) and 710 (89%) of 796, respectively (appendix p 9). Protocol deviations are reported in the appendix (pp 10–11). Baseline respiratory samples were available from 667 infants (84%). There were 376 endotracheal samples (192 and 184 from the azithromycin and placebo groups, respectively), and 291 nasopharyngeal aspirate samples (144 and 147, respectively).

741 (93%) of 796 infants had their primary outcome physiologically assessed. 55 (7%) infants receiving low-flow supplemental oxygen at 36 weeks' PMA did not undergo an oxygen reduction test, so were classified as having moderate CLD (34 [9%] of 394 and 21 [5%] of 402 in the intervention and placebo groups, respectively). Survival without moderate or severe CLD occurred in 166 (42%) of 394 infants in the intervention group and 179 (45%) of 402 in the placebo group (three-level adjusted OR [aOR] 0·84, 95% CI 0·55–1·29, p=0·43; table 2). These findings remained unchanged after all sensitivity analyses (appendix p 12). For the imputation analysis, the three-level aOR was 0·92 (0·59–1·45), with identical results for the complete case analysis. Intraclass correlation coefficients for all models are presented in the appendix (p 13).

Detailed subgroup analyses for the primary outcome are shown in figure 2 and the appendix (pp 13–14). By 36 weeks' PMA, 34 infants (9%) in the azithromycin group and 31 infants (8%) in the placebo group had died. No between-group differences were noted for rates of death (three-level aOR 1·28 [95% CI 0·39 to 4·18],

1505 were deemed eligible (appendix p 8). Of the 234 not eligible, the common reasons for failures to participate for the screened infants were first, that the local investigator did not anticipate survival beyond 72 h after birth (n=76), second, follow-up to 36 weeks' PMA was considered unlikely (n=37), and third, completion of ten days of intravenous treatment was thought unlikely (n=32). 799 (53·1%) of 1505 eligible infants underwent random allocation (figure 1). Three infants

p=0·69). By 36 weeks' PMA, 194 (54%) of 360 infants in the intervention group and 192 (51%) of 371 in the placebo group developed moderate or severe CLD (table 3). No differences were noted for rates of moderate or severe CLD between the two treatment groups (three-level aOR 0·85 [95% CI 0·55 to 1·32], p=0·48). No between-group differences were noted for postnatal corticosteroid use, treated PDA, treated nosocomial infection, IVH, or severe NEC (table 4, appendix p 16). Treated ROP in survivors was decreased in the intervention group (12 [4%] of 340) when compared with the placebo group (26 [7%] of 350; aOR 0·42 [95% CI 0·18 to 0·95]), but not when those who died were incorporated into the outcome definition (45 [12%] of 373 in the intervention group when compared with 55 [15%] of 379 in the placebo group; table 4). As explained in the appendix (pp 6–7), the quality

	No or mild CLD, n (%)	Moderate or severe CLD, n (%)	Unadjusted		Two-level model		Three-level model	
			OR (95% CI), p-value	ARD (95% CI)	aOR* (95% CI), p value	aARD* (95% CI)	aOR† (95% CI), p value	aARD† (95% CI)
Placebo (n=371)								
Total	179 (48%)	192 (51%)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
No	60 (16%)	NA	..	..	..	..	..	..
Mild	119 (32%)	NA	..	..	..	..	..	..
Moderate	NA	70 (19%)	..	..	..	..	..	..
Severe	NA	122 (33%)	..	..	..	..	..	..
Azithromycin (n=360)								
Total	166 (46%)	194 (54%)	0·92 (0·69 to 1·23), 0·56	–0·02 (–0·09 to 0·05)	0·92 (0·68 to 1·26), 0·61	–0·02 (–0·09 to 0·05)	0·85 (0·55 to 1·32), 0·48	0·02 (–0·09 to 0·04)
No	50 (14%)	NA	..	..	..	..	..	..
Mild	116 (32%)	NA	..	..	..	..	..	..
Moderate	NA	87 (24%)	..	..	..	..	..	..
Severe	NA	107 (30%)	..	..	..	..	..	..
Totals (n=731)								
Total	345 (47%)	386 (53%)	NA	NA	NA	NA	NA	NA
No	110 (15%)	NA	NA	NA	NA	NA	NA	NA
Mild	235 (32%)	NA	NA	NA	NA	NA	NA	NA
Moderate	NA	157 (21%)	NA	NA	NA	NA	NA	NA
Severe	NA	229 (31%)	NA	NA	NA	NA	NA	NA

CLD=chronic lung disease of prematurity. OR=odds ratio. aOR=adjusted odds ratio. ARD=absolute risk difference. aARD=adjusted absolute risk difference. NA=not applicable. *Adjusted for gestational age and clustering within 28 recruitment centres. †Adjusted for gestational age and clustering within multiple births (ie, 707 mothers delivered 796 infants) within 28 recruitment centres.

Table 3: Severity of CLD (component of the primary outcome)

	Composite incorporating death analysis†				Analyses of survivors only‡§			
	Azithromycin (n=394)	Placebo (n=402)	IRR (95% CI)	OR (95% CI)	Azithromycin (n=394)	Placebo (n=402)	IRR (95% CI)	OR (95% CI)
Invasive ventilation	49 (41–57)¶	48 (42–56)¶	0·99 (0·95–1·04)	NA	6 (2–21)¶	6 (2–26)¶	1·05 (0·90–1·23)	NA
Non-invasive ventilation	23 (8–37)¶	23 (10–36)¶	0·90 (0·73–1·10)	NA	39 (18–55)¶	37 (17–54)¶	1·08 (0·92–1·27)	NA
Postnatal corticosteroids§	168/394 (43%)	174/402 (43%)	NA	0·91 (0·47–1·77)	158/384 (41%)	164/392 (42%)	NA	0·86 (0·43–1·59)
Treated patent ductus arteriosus	144/388 (37%)	159/395 (40%)	NA	0·79 (0·47–1·33)	123/367 (34%)	136/372 (37%)	NA	0·78 (0·46–1·31)
Nosocomial infection	256/394 (65%)	247/401 (62%)	NA	1·20 (0·86–1·68)	246/384 (64%)	239/393 (61%)	NA	1·20 (0·87–1·67)
Severe intraventricular haemorrhage	45/313 (14%)	42/318 (13%)	NA	1·11 (0·63–1·95)	38/306 (12%)	30/306 (10%)	NA	1·30 (0·71–2·36)
Bell stage ≥II necrotising enterocolitis	57/394 (14%)	60/401 (15%)	NA	0·97 (0·50–1·88)	29/366 (8%)	42/383 (11%)	NA	0·65 (0·33–1·31)
Treatment for retinopathy of prematurity	45/373 (12%)	55/379 (15%)	NA	0·76 (0·36–1·60)	12/340 (4%)	26/350 (7%)	NA	0·42 (0·18–0·95)

Data are n/N (%) median (IQR), or OR (95% CI) unless otherwise stated. IRR=incidence rate ratio. OR=odds ratio. *Three-level analysis adjusted for gestational age, multiple births, and multiple centres. †Composite incorporating death analyses defined as days alive without outcome. ‡Survivor analyses based on number of days of respiratory support before death. §This analysis was not prespecified in the statistical analysis plan. ¶Data are median (IQR).

Table 4: Secondary outcome measures (three-level analysis)*

of data collection for daily supplemental oxygen and invasive or non-invasive respiratory support was insufficient for reliable analysis. Therefore, daily records of participating infants receiving invasive or non-invasive respiratory support were obtained from the National Neonatal Data Analyses Unit, which collates neonatal data submitted by almost all UK neonatal units. No differences were noted between the intervention and placebo groups for either form of respiratory support (table 4). Recruiting centres used different liver enzymes, but the highest serum creatinine, alkaline phosphate, and liver enzymes were similar between the groups (appendix p 16), except for marginally lower aspartate aminotransferase in the azithromycin intervention group versus placebo group (median 43 international units per L [IQR 23 to 65] *vs* 43 [27 to 80], three-level model Box-Cox transformed coefficient –0·12 [95% CI –0·22 to –0·01], p=0·034; data available for 312 [39%] of 796 infants).

Ureaplasma parvum, *Ureaplasma urealyticum*, or both species were identified in 148 (22%) of 667 pretreatment baseline respiratory samples, including 14 infants colonised with both serovars. Pulmonary colonisation with *Ureaplasma* spp was 66 (45%) of 148 in the intervention group, and 82 (55%) of 148 in the placebo group. Since mycoplasma detection was uncommon, and was not part of the prespecified aims, we have not reported these data. No subgroup treatment effect was noted on the primary outcome by respiratory *Ureaplasma* spp. colonisation (interaction aOR 0·59 [95% CI 0·19–1·84]), nor by the other predefined subgroups of those born at less than 28 weeks' gestation, whether an infant was born at the recruiting centre, or by recruitment centre (appendix p 14).

Of the 13 serious adverse events reported (appendix p 16), local investigators thought five were possibly related to azithromycin: two in the placebo group (one case of atrial ectopics [unmasked by the local investigator] and one supraventricular tachycardia); and three in the intervention group (one cardiac arrest, one occurence of acute kidney injury, and one case of congenital pyloric stenosis). Independent ECG review for cardiac events did not show any cases of prolonged corrected QT interval. Overall, seven serious adverse events were reported for the azithromycin group (five graded as severe, two as moderate), and six serious adverse events were reported in the placebo group (two severe, two moderate, and two mild; appendix p 16).

Discussion

Pulmonary inflammation and *Ureaplasma* spp colonisation have been postulated to be on the causal pathway for development of CLD, which is characterised by recruitment of neutrophils into the lung with subsequent increases in reactive oxygen species and proteases (eg, neutrophil elastase), resulting in lung tissue damage. Therefore, these mechanisms are reasonable targets for intervention to ameliorate lung disease in preterm infants. Azithromycin is an attractive therapeutic option due to its action against *Ureaplasma* spp and in reducing neutrophilic inflammation in the lungs.[11] Previous studies using macrolides to prevent development of CLD have produced conflicting results, including the systematic reviews by Nair and colleagues[18] and Razak and colleagues.[17] We conducted an adequately powered study, and to our knowledge the largest single trial to date, which exceeded our power calculation due to lower than anticipated death rates and a very low drop-out rate. We did not observe improved survival without the development of physiologically-defined moderate or severe CLD with azithromycin treatment, nor did *Ureaplasma* spp colonisation affect the primary outcome. Azithromycin treatment did not affect the important secondary outcomes, including rates of death, CLD, corticosteroid use, the need for invasive or non-invasive ventilation, treated nosocomial infection, or treated PDA, NEC, or IVH. Rates of treated ROP were lower in surviving infants in the intervention group, but not when death was included in the outcome. The safety profile of azithromycin was reassuring: the single occurrence of pyloric stenosis is within the expected UK rates of 2–3 per 1000 newborn infants.[30,31] The rates of reported adverse cardiac events were similar between the two groups.[32]

Our results are consistent with other smaller studies which do not support the routine prophylactic use of macrolides to decrease rates of CLD, despite convincing evidence of eradicating pulmonary *Ureaplasma* spp with the azithromycin dose used in our study.[13] The meta-analysis available during the design of the AZTEC study noted a reduction in the combined outcome of CLD or death after prophylactic azithromycin use regardless of pulmonary *Ureaplasma* spp colonisation.[18] However, a 2021 systematic review, published during our recruitment phase, which included two new studies (n=80 and n=121) did not suggest azithromycin is effective in decreasing rates for the combined outcome of CLD and death, but there was a trend towards reduction of these outcomes in those colonised with *Ureaplasma* spp, although the evidence was considered low-quality.[17] We aimed to start treatment as early as possible after birth, and continued for ten days to both eradicate pulmonary *Ureaplasma* spp colonisation and to target the neutrophilic pulmonary inflammation associated with the development of CLD.[14] Our *Ureaplasma* spp colonisation rate was 22%, which is marginally lower than rates reported in the literature.[33] This lower rate is probably due to assessment of pulmonary *Ureaplasma* spp colonisation soon after birth, with testing only at baseline within 72 h of birth before administration of the first dose of IMP. Given the differing patterns of *Ureaplasma* spp colonisation over time,[34] left untreated many more infants would have been shown to have pulmonary *Ureaplasma* spp colonisation. Work is underway to assess the true rate of *Ureaplasma* spp in the placebo group, and to assess

whether intervention with azithromycin eradicated pulmonary *Ureaplasma* spp colonisation. This is anticipated, as *Ureaplasma* spp has low resistance rates to macrolides in the UK.[35] We remain confident that the treatment regime was appropriate, as it was based on a previously established dose and schedule.[20]

In addition to targeting the *Ureaplasma* spp, our hypothesis was also based around proof-of-concept in addressing pulmonary inflammation through the effect of azithromycin on reducing neutrophil activity and proinflammatory cytokine production.[36] Additional work is underway to determine if azithromycin treatment modulated proinflammatory cytokine concentrations, including whether this is more prevalent in the *Ureaplasma* spp colonised group or non-colonised group. Azithromycin having an effect on cytokine production seems probable, given its widespread efficacy in other neutrophilic associated respiratory diseases and its purported mechanisms of action.[37]

If azithromycin did indeed eradicate pulmonary *Ureaplasma* spp colonisation and decrease pulmonary inflammation, it would be unclear why rates of CLD were not affected, especially in the group with *Ureaplasma* spp colonisation. While the study was neither designed nor powered to assess whether eradication of *Ureaplasma* spp affected survival without development of CLD, there was no indication that rates of CLD were affected at all by azithromycin, nor by presence of respiratory *Ureaplasma* spp colonisation. Alternative respiratory pathways (eg, oxidant injury or surfactant dysfunction) or non-respiratory pathways (eg, the presence of significant PDA or systemic infection) could continue to substantially contribute to lung injury, resulting in unaltered rates of CLD.[38,39] Alternatively, our choice of primary outcome of survival without development of physiologically defined moderate or severe CLD at 36 weeks' PMA might not be optimal, and assessing respiratory status at a later timepoint could show a benefit, as evidenced by a study showing decreased wheezing at age 1 year after using superoxide dismutase compared with placebo, despite no effect on BPD (CLD) at 36 weeks' PMA.[38] Assessment of respiratory status at a corrected age of 1 or 2 years is currently underway to assess if the intervention modulates respiratory morbidity and, neurodevelopment in the medium term.[40]

A potentially important finding was the decrease in treated ROP in the azithromycin group. While this is unlikely to be due to a direct effect of azithromycin on the eye, azithromycin treatment could result in a decreased need for higher concentrations of supplementary oxygen, or could decrease systemic proinflammatory cytokines, which might have a secondary effect on the development of ROP. Whether this observation is real or spurious, given multiple secondary outcomes and no pre-planned adjustment for multiple testing, this warrants further investigation, as ROP remains a significant outcome for preterm infants.

The key strengths of our study are adequate power, robustly followed protocol and double-blinding, timely recruitment within our 2·5 year target despite the two pauses, a very low dropout rate, a priori statistical analyses plan, the inclusion of a large representative UK population, and timely early treatment. By only including infants requiring respiratory intervention at birth, we recruited a population who is at high-risk for CLD and most likely to benefit from the intervention, borne out by the high rates of CLD and greater male prevalence in our cohort. The trial had a high enrolment rate (53% of eligible infants were consented and randomly allocated). Important limitations include the missed oxygen reduction tests, inadequate collection of respiratory support data, and lower than anticipated baseline sampling. The higher rates of infection were due to using a broader definition of clinician-treated infection, positive cultures, or a combination of both rather than identifying infection purely on grounds of positive culture results only. However, none of these would have affected the overall results, so we are confident the results are robust.

In conclusion, the outcome of this trial coupled with other recently published interventions to reduce rates of CLD[23,41] indicate the challenges of treating this multifactorial disease.[42] Taken together, these results suggest that a single intervention during the neonatal period might not be effective; novel strategic combinations of multiple interventional strategies and trial designs are required to address this conundrum. Finally, it will be important to assess the longer-term effect of azithromycin on respiratory outcomes and neurodevelopment, especially to assess longer-term effectiveness and safety.[40]

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