Zinc for severe pneumonia in very young children: double-blind placebo-controlled trial

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Summary

Background Pneumonia is a leading cause of morbidity and mortality in young children. Early reversal of severity signs—chest indrawing, hypoxia, and tachypnoea—improves outcome. We postulated that zinc, an acute phase reactant, would shorten duration of severe pneumonia and time in hospital.

Methods In a double-blind placebo-controlled clinical trial in Matlab Hospital, Bangladesh, 270 children aged 2–23 months were randomised to receive elemental zinc (20 mg per day) or placebo, plus the hospital's standard antimicrobial management, until discharge. The outcomes were time to cessation of severe pneumonia (no chest indrawing, respiratory rate 50 per min or less, oxygen saturation at least 95% on room air) and discharge from hospital. Discharge was allowed when respiratory rate was 40 per minute or less for 24 consecutive hours while patients were maintained only on oral antibiotics.

Findings The group receiving zinc had reduced duration of severe pneumonia (relative hazard [RH]=0·70, 95% CI 0·51–0·98), including duration of chest indrawing (0·80, 0·61–1·05), respiratory rate more than 50 per min (0·74, 0·57–0·98), and hypoxia (0·79, 0·61–1·04), and overall hospital duration (0·75, 0·57–0·99). The mean reduction is equivalent to 1 hospital day for both severe pneumonia and time in hospital. All effects were greater when children with wheezing were omitted from the analysis.

Interpretation Adjuvant treatment with 20 mg zinc per day accelerates recovery from severe pneumonia in children, and could help reduce antimicrobial resistance by decreasing multiple antibiotic exposures, and lessen complications and deaths where second line drugs are unavailable.

Lancet 2004; **363:** 1683–88

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Introduction

Pneumonia is a leading cause of morbidity and mortality in children less than 5 years old. About 20% of deaths in such children are attributable to pneumonia (1·9 million deaths per year).[1] Two-thirds of these deaths happen during infancy, and more than 90% are in developing countries.

Zinc is reported to prevent pneumonia,[2–5] and to prevent and treat diarrhoea.[4,6–8] It might act in the acute phase response to infection,[9,10] helping to boost the body's immune response through a defence cascade, beginning with mobilisation and sequestration of zinc to metallothionein-rich tissue, rapid upregulation of immune defence-specific protein synthesis, activation of immune defence activity such as macrophages, lymphocytes, and natural killer cells, and antibody-dependent cytotoxicity.[11] Children with good zinc status may have a more robust immune response than those with poor zinc status.[12,13] Thus, our aim was to see whether zinc, along with antibiotics, would improve the outcome of severe pneumonia.

Methods

We undertook a double blind randomised placebo-controlled clinical trial in hospitalised children who were between 2 and 23 months old at the time of admission to the Matlab Hospital, International Centre for Diarrhoeal Disease Research, Bangladesh Centre of Health and Population Research, a rural facility 50 km south of the capital Dhaka. Specifically, we investigated whether 20 mg zinc per day could shorten the duration of severe pneumonia and hospitalisation. We also measured the time to resolution of specific signs of lower airway obstruction—namely, chest indrawing, respiratory rate more than 50 per min, and hypoxia. The age-group 2–23 months was selected for its high pneumonia morbidity and restricted options to prevent community-acquired bacterial pneumonia, since vaccines to prevent *Haemophilus influenzae* and *Streptococcus pneumoniae* are not in routine use in Bangladesh. We chose a fixed dose of 20 mg of zinc per day because this is the dose being adopted for programmatic use in diarrhoea case management.[14] Additionally, we estimated that the median mg/kg exposure for this age-group would fall well below the range of acute toxicity.[15]

A study physician did a baseline physical examination, including timing of respiratory rate, assessment of breathing effort, cyanosis, mental status, and chest auscultation for crepitations or wheezing, or both. Axillary temperature was taken with a mercury thermometer. We measured baseline oxygen saturation using a pulse oximeter (Model 71000A1, BCI International Waukesha, WI, USA). Infants with cough, a respiratory rate more than 50 breaths per min, and crepitations on auscultation were diagnosed with pneumonia. Infants with pneumonia and either chest indrawing or at least one other danger

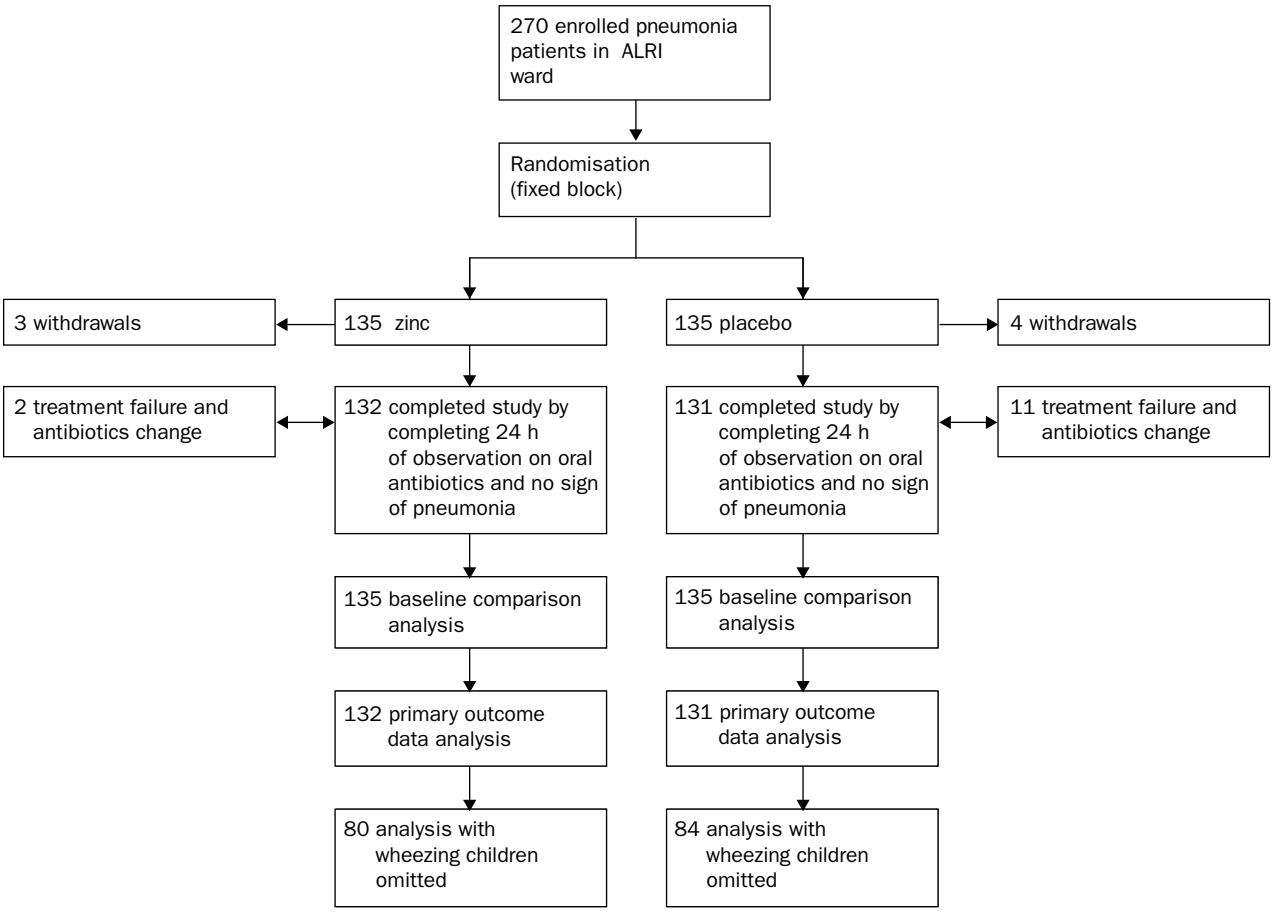

Figure 1: **Trial profile**

sign (cyanosis, inability to feed, or lethargy) were diagnosed with severe pneumonia. Children with raised respiratory rate, but who were judged not to be moving sufficient air to make breath sounds, were reassessed after nebulised albuterol administration. We included children aged 2–23 months if they had severe pneumonia, and if their parents provided informed written consent. We excluded children if they had concurrent diarrhoea (and therefore had to be managed in the diarrhoea ward), were receiving zinc supplements, or had severe malnutrition (weight for age less than 60% based on the National Center for Health Statistics reference data).[16]

Consent was obtained by a study physician who read the entire consent form to the parent, as this was an illiterate population. If parents had questions, the study physician clarified the consent form from a standardised set of key points that covered each section. Parents who indicated that they understood and agreed to the terms of the study then provided written consent. Those who could sign or draw their names did so; those who could not gave a thumb impression. The ethical review committee of the institution approved the study in accordance with the standards of the Helsinki Declaration of 1975, as revised in 1983. Analysis was per-protocol.

We then obtained 3 mL blood using trace element-free vacutainers and storage tubes for measuring serum zinc, total white blood cell with differential count, and blood culture. We also took a chest radiograph.

Children were then randomised to receive either 20 mg elemental zinc per day (10 mg zinc per 5 mL syrup) as acetate or placebo until they were discharged from hospital (figure 1, table 1). We preallocated group assignments by fixed randomisation using permuted blocks of variable length between two and eight. The supplement preparations were administered at the time of enrolment, within 1 h of the first dose of parenteral antibiotics on admission day, and as a divided dose 1 h before breakfast and 2 h after dinner on subsequent days. A health worker not involved with the patients' care gave

	Zinc (n=135)	Placebo (n=135)	p
Admission			
Age (months)	9·5 (6·2)	9·6 (6·0)	
Males	59·2%	71·1%	
Duration of illness (days)	2·4 (1·5)	2·6 (1·5)	
Height (cm)	66·9 (7·8)	67·1 (7·8)	
Weight (g)	6876 (1584)	6952 (1560)	
% weight-for-age	82·6 (11·7)	82·1 (11·6)	
Pulse rate/min	137 (12)	136 (10)	
Temperature (°C)	37·6 (0·9)	37·6 (0·7)	
Respiratory rate/min	66 (8)	65 (8)	
Oxygen saturation on room air	91·6% (3·4)	90·9% (7·9)	
Oxygen saturation <95%	80·0%	87·4%	
Serum zinc (μmol/L)	10·1 (1·1)	10·1 (1·0)	
Temperature >37·8°C	30·2%	26·0%	
Chest indrawing	96·3%	94·8%	
Crepitations	100%	100%	
Wheezing	38·5%	36·3%	
Dehydration	0·07%	0·07%	
Less active to lethargic	81·5%	83·7%	
Discharge			
Weight (g)	6774 (1543)	6971 (1524)	0·29
% weight-for-age	80·8 (11·7)	81·2 (11·2)	0·78
Discharge serum zinc (μmol/L)	14·8 (2·8)	11·3 (2·1)	<0·01

Data are mean (SD) unless indicated otherwise.

Table 1: **Clinical characteristics on admission and discharge by treatment group**

all doses. The zinc and placebo syrups were identical in appearance, colour, odour, and taste. The zinc and placebo syrups were prepared and labelled by ACME Laboratories (Dhamrai, Dhaka, Bangladesh).

Data for respiratory rate, chest indrawing, oxygen saturation, auscultation findings (crepitations and wheezing), fever, feeding, cyanosis, and mental status, were obtained at the beginning of every nursing shift (8 h). All enrolled patients were managed according to Matlab Hospital's standard severe pneumonia treatment guidelines. Parenteral antibiotics were ampicillin (200–400 mg/kg per day, given intravenously every 6 h) and gentamicin (6–7·5 mg/kg per day, given intravenously every 8 h). Patients who failed to improve after 48 h of antibiotics (by the eighth dose of ampicillin) or whose condition worsened, had their antibiotic changed to ceftriaxone (50 mg/kg per day intravenously). Failure to improve and worsening condition were established by respiratory rate count and severe pneumonia signs. Worsening of any one sign qualified as worsening condition and no change in any sign constituted failure to improve. Although fever was not necessary for diagnosis, it was taken as a sign of active infection, and thus had to be absent for 24 consecutive hours without the aid of antipyretics to qualify for change from severe to non-severe pneumonia.

We measured pulse oximetry using a probe on either a finger or toe. Patients were taken off oxygen by nasal cannula and continuously observed on room air for a 5-min washout period before pulse oximetry was done. We counted respirations with a timer for 60 s, after removing all clothing from the torso. We also observed the patients for chest indrawing. The counts were repeated and averaged for the actual count. If the counts differed by more than 5 breaths per min, we did a third count and the two closest readings were averaged. Children had to be awake, but not crying during all measurements.

We reclassified severe pneumonia to pneumonia (non-severe) when chest indrawing and hypoxia (oxygen saturation less than 95% on room air) were absent for 24 consecutive hours and respiratory rate was less than or equal to 50 breaths per min, at which time oral antibiotics were started. If any sign recurred, the child was reclassified as severe status until these conditions were met. The oral antibiotic was amoxicillin 40 mg/kg divided every 8 h. All children received a minimum of 5 days of antibiotics divided between parenteral and oral agents.

Children were discharged from hospital once respiratory rate fell to less than or equal to 40 per min for 24 consecutive hours, with no recurrence of respiratory distress, other danger signs, or fever (temperature >37·9°C), after drawing 1 mL of blood for measurement of post-intervention serum zinc concentration.

Serum samples (150 μL) in polypropylene tubes were diluted 1 in 12 with nitric acid and Brij35, a non-ionic detergent. We measured zinc concentration by flame atomic absorption spectrophotometry, using standard solutions prepared in the same matrix. The spectrophotometer was equipped with a nebuliser assembly (Shimadzu, Tokyo, Japan), and an acetylene and air burner. A zinc hollow-cathode lamp was used with a background correction wavelength of 213 nm. We measured zinc by aspiration of a series of working standards (at least four) and preparation of a standard curve for each lot by plotting the concentration versus absorbance using software provided by the manufacturer. Two quality control serum samples (low and high range) were then run with each lot. We dropped and repeated the result if any value of the quality control samples was greater than 2 SD from the mean.

We calculated the sample size on the basis of mean duration of severe pneumonia while in hospital of 6 days (SD 2·5, data from 365 days of hospital chart review), and an effect size of 20%, with a 5% chance of a type I error and a 10% chance of a type II error. This calculation resulted in a sample size of 132 children per group, or 264 in total. Because there were no available data on duration of the signs of severe pneumonia, we looked at the proportion of patients who had these signs at the end of 48 h hospitalisation (by review of 365 days of hospital records). 94% had chest indrawing, 98% tachypnoea (respiratory rate more than 50 per min), and 70% inability to feed. We then calculated the sample size needed to detect a 20% difference for each sign, except for inability to feed, which we believed would reverse substantially and thus assumed a 30% effect. These calculations yielded sample sizes of 86, 65, and 123 children per group, respectively. Thus, the maximum number of children needed for the study was 264. Although we did not expect high rates of attrition, we adjusted the final sample size to 270 to allow for possible cases of withdrawal.

We constructed Kaplan-Meier plots of duration of each outcome for the zinc and placebo groups. We set admission as time=0, and we censored event assessment if the outcome of interest did not happen during the follow-up period. Outcomes for duration were chest indrawing, respiratory rate more than 50 per min, oxygen saturation, severe pneumonia, respiratory rate more than 40 per min, and hospitalisation. We used a Cox proportional hazards model to compare the relative hazard (RH) of the outcome in the zinc group with that in the placebo group and to explore associations between baseline covariates and outcome. Baseline covariates with a significant association with the outcome were included in the model along with treatment group. We treated tied scores in duration outcomes using the exact partial (conditional logistic) calculation, as time was measured in discrete 8-hourly shifts. We also used the Cox proportional hazards model to check for potential interactions between all covariates and treatment group on the outcomes of interest.

We tested the proportional hazards assumptions using tests of specification (re-estimation, analysis and plotting of Schoenfeld residuals) and goodness of fit. We compared mean pre-supplementation and post-supplementation serum zinc concentrations within groups by paired *t* tests, whereas two-sample *t* tests were done between groups. We analysed categorical variables using χ^2 to calculate relative odds and 95% CI. We calculated p values for comparisons of dichotomous variables between groups using Fisher's exact test (two-tailed). All data were analysed using the Stata/SE version 8.0 statistical analysis package.

Role of the funding source

The funding sources had no role in study design, data collection, data analysis, data interpretation, or the decision to publish.

Results

In total, 270 children were enrolled between Aug 23, 1999, and Aug 19, 2001. Of these, six (three from each group) left hospital against medical advice, and one (from the placebo group) withdrew from the study, but completed treatment in hospital (figure 1). Most (172 [64%]) patients were younger than 12 months, and most (177 [65%]) were male, which results from local health-seeking practices.

	Zinc (n=117)	Placebo (n=115)
Normal	10 (8·5%)	7 (6·1%)
Alveolar infiltrates	69 (59·0%)	69 (60·0%)
Interstitial infiltrates	15 (12·8%)	16 (13·9%)
Consolidation	14 (11·9%)	12 (10·4%)
Mixed consolidation/infiltrate	7 (6·0%)	8 (7·0%)
Peribronchial cuffing	2 (1·7%)	3 (2·6%)

Table 2: **Chest radiograph findings on admission**

The mean duration of illness before hospitalisation was 2·5 days (SD 1·5), with no difference between groups. By caretakers' reports, 64% of study children had at least one previous pneumonia episode, with a mean time interval of 6 weeks (SD 6).

A paediatrician not involved in patient care read all chest radiographs. Of 270 patients, 232 (86%) had an admission chest radiograph, whereas 38 did not because the machine was absent for repairs. Of those who had a radiograph, 210 (90·5%) had findings consistent with pneumonia (17 [7·3%] were normal, and 5 [2·2%] had only peribronchial cuffing), which did not differ by group (table 2). Radiographic findings were not a selection criterion, however, and all patients had clinically severe pneumonia. Moreover, radiographic findings had no effect on duration of illness or hospitalisation in the analysis.

Baseline serum zinc concentrations were 10·1 μmol/L (SD 1·1) and 10·0 μmol/L (1·0) for zinc and placebo groups, respectively. At the end of the study (table 1), both groups had higher serum zinc concentrations than at baseline: 14·5 μmol/L ($p<0·0001$) for zinc and 11·2 μmol/L for placebo group ($p<0·0001$). The difference between the zinc and placebo groups' discharge zinc concentrations was also significant ($p<0·0001$). Although children in the zinc group weighed less than those in the placebo group both at admission and on discharge, these differences were not significant, nor were their weight-for-age comparisons (table 1).

Children aged 12 months or older resolved their respiratory illness earlier than younger infants for all measured outcomes. The RH of hospital duration for children aged 12 months or older compared with children younger than 12 months was 0·44 (95% CI 0·34–0·57). Other duration outcomes were similarly associated with age. Since 64% of the children in the study were younger than 12 months, all hazard calculated modelling controlled for age as a continuous variable. No excess vomiting, serious adverse events, or deaths happened in either group. None of the children relapsed from non-severe to severe pneumonia after status change, probably because of the stringent requirements for status change—ie, that respiratory rate, chest indrawing, and low oxygen saturation are all resolved for a consecutive 24 h. No evidence of interaction was apparent between covariates.

After age was controlled for, each severe pneumonia indicator improved in zinc-supplemented children compared with those receiving placebo (table 3). There were shorter durations of chest indrawing, respiratory rate more than 50 per min, and hypoxia, leading to shorter overall duration of severe pneumonia (figure 2), as well as total hospital stay (tables 3 and 4, figure 2). This resulted in a mean difference of 4 (95% CI 4·2–4·9) versus 5 (4·5–5·5) days of severe pneumonia, and 5 (4·8–5·5) versus 6 (5·1–6·1) days of hospitalisation for the zinc and placebo groups, respectively. Subgroup analysis by quartiles for admission weight-for-age and admission serum zinc concentration showed no consistent pattern for the effect of zinc on total hospital duration (data not shown).

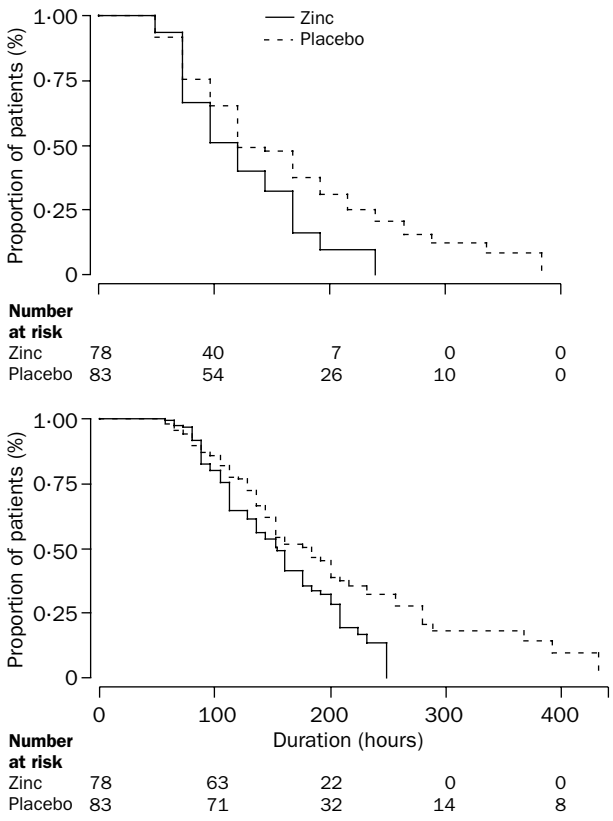

Figure 2: **Kaplan-Meier survival curves when wheezing patients omitted**

Upper=severe pneumonia duration. Lower=hospital duration.

Since our objective was to examine the effect of zinc on severe bacterial pneumonia as an adjuvant to standard antimicrobial management, and wheezing is probably due to bronchiolitis (a viral infection) or reactive airways disease (asthma), we repeated the analysis with wheezing children omitted. Differences between the zinc and placebo groups were greater for all clinical signs and

	Median duration (h, 95% CI)		Outcome relative hazard (95% CI)
	Zinc (n=132)	Placebo (n=131)	
Outcomes			
Chest indrawing	40 (39–48)	48 (40–56)	0·80 (0·61–1·05)
Respiratory rate >50 per min	48 (40–56)	56 (40–64)	0·74 (0·57–0·98)
Hypoxia	80 (72–96)	88 (80–104)	0·79 (0·61–1·04)
Severe pneumonia resolution	72 (72–96)	96 (72–96)	0·70 (0·51–0·98)
Respiratory rate >40 per min	104 (88–112)	112 (104–128)	0·75 (0·57–0·98)
Hospital (days)	112 (104–112)	112 (111–129)	0·75 (0·57–0·99)

Table 3: **Age-adjusted hazard ratios by group and outcome**

	Median duration (h, 95% CI)		Outcome relative hazard (95% CI)
	Zinc (n=80)	Placebo (n=84)	
Outcome			
Chest indrawing	48 (40–64)	48 (33–71)	0·68 (0·48–0·96)
Respiratory rate >50 per min	48 (40–65)	56 (40–87)	0·65 (0·46–0·92)
Hypoxia	88 (72–104)	96 (80–108)	0·74 (0·53–1·04)
Severe pneumonia resolution	84 (72–96)	96 (72–120)	0·61 (0·40–0·92)
Respiratory rate >40 per min	104 (88–128)	128 (104–136)	0·66 (0·47–0·94)
Hospital (days)	112 (104–129)	128 (112–144)	0·67 (0·47–0·94)

Table 4: **Age-adjusted hazard ratios by group and outcome when wheezing children omitted**

overall duration of severe pneumonia and time in hospital when these children were omitted (table 4). Zinc supplementation did not affect either duration of severe pneumonia or overall length of hospital stay for wheezing children (RH=1·04, 95% CI 0·77–1·40 and 1·02, 0·072–1·44, respectively).

13 children had their antimicrobial therapy changed because of treatment failure, ten in the placebo group and three in the zinc group (relative odds 3·4, 0·86–19·9, p=0·08). Removal of wheezing children from the analysis reduced the numbers to nine and two (relative odds 4·6, 0·90–45·1, p=0·06). In the less than 12-months-old age range, nine children in the placebo group failed treatment versus two in the zinc group (relative odds 4·6, 0·90–45·1, p=0·06). Removal of wheezing children from this group resulted in eight versus one requiring antibiotic change (relative odds 9·1, 1·1–411·2, p=0·03)

Discussion

We have shown clinically and statistically significant reductions in recovery time from severe pneumonia and overall hospital stay in children less than 2 years old given zinc with standard antimicrobial therapy. This improvement seems to result from substantial reductions in the resolution times of each of the severe pneumonia indicators, including chest indrawing, severely raised respiratory rate, and hypoxia, indicating a consistency between these specific signs and the diagnosis of severe pneumonia. The zinc supplement was safe and well tolerated in children as young as 2 months old.

The reductions in the duration of severe pneumonia and its components and overall hospitalisation might be mediated by the role of zinc in the acute phase response described previously,[17–20] mediated by cytokines during acute infection.[21–23] Since this response has been characterised,[24–27] we did not attempt to measure it directly. Thus, by maximising tissue bioavailability of zinc in these children, they may have had a more robust immune response and recovered more quickly.

Another possible effect of zinc is on the extent of inflammation and its resolution rate surrounding infection. Zinc supplementation might protect the lung from inflammatory states, whereas zinc deficiency might enhance airway inflammation and cellular damage.[28] In the presence of zinc, animals had decreased inflammation of other organ systems and increased bacterial inhibition and cellular regeneration.[29–31] Thus, zinc may reduce inflammation, and lower airway obstruction, in supplemented children and contribute to faster inflammation resolution time, manifested by shorter duration of chest indrawing, high respiratory rate, and hypoxia. The benefit from zinc seems to increase after 100 h of illness. This lag period in effect onset has been reported elsewhere,[32] and might be inherent in the mechanism of the zinc effect.

Children with wheezing had no adverse effects from zinc, nor did they receive a benefit in this study. This study was not designed to assess the effects of zinc on lower respiratory disease associated with wheezing, thus absence of observed effect should not be taken as absence of effect. Future studies could examine the effect of zinc on acute respiratory illness with wheezing.

The effects on treatment failure are striking, have significant implications for reduction of antimicrobial resistance by decreasing multiple antibiotic exposures, and could help reduce complications and death in situations where second line drugs are not available. Acute phase reactants enhance β-lactam effectiveness[33–35] and early bacterial clearing,[36] which might explain this reduction in duration of illness and treatment failure. Whether zinc has an effect on these specific reactants is an area for future study.

Although this study was not designed to measure the potential direct and indirect cost savings associated with a reduction in treatment failure, these savings are no less substantial than the epidemiological effect. The 20 mg zinc tablets now in production, which can be used instead of the syrup, would cost a modest US$0·15 for a treatment course, resulting in 1 fewer hospital day at a rate of US$25 per day (Matlab Hospital). In view of the high number of children in this age-group hospitalised with severe pneumonia, the cost savings could be substantial.

That zinc-deficient children would mount a more robust immune response when given zinc in the acute illness phase is intuitively appealing, but a pharmacological effect, rather than correction of zinc deficiency, could provide an alternative explanation for our findings. The population from which these children came has a high prevalence of undernutrition and zinc deficiency;[6,37–40] however, the patients' low admission serum zinc concentrations are difficult to interpret because serum zinc decreases during the acute phase response to illness, and consistent with this reduction, even the placebo-supplemented group had a significant increase in serum zinc at recovery. The effects of zinc were not associated with patients' initial anthropometric status or serum zinc concentration, although the numbers in the subgroup analyses may have been too small to detect differences. Zinc might boost the acute phase and protective immune responses irrespective of a child's zinc status, in which case even zinc-replete children would derive a benefit. Zinc supplementation has benefited tissue repair in at least one zinc-replete animal model.[30]

This study needs to be replicated in other populations, including those with and without a high prevalence of zinc deficiency and, as with zinc studies of diarrhoeal disease, should include a follow-up period to look at effects on subsequent illness. Additionally, both animal and human studies should also be undertaken to describe the precise mechanism by which zinc interacts with the acute phase response, including more detail on its effects on immunity. Such studies would allow the global public-health significance of these findings to be assessed, and the results best applied to improve child health and survival.

Contributors

W Abdullah Brooks was the principal investigator and was responsible for the study conception, design, implementation, principal data analysis, and manuscript preparation. M Yunus was the co-principal investigator, and helped to do the study, staff supervision, training, and data review. M A Wahed was involved in staff training, supervision, and did all the spectrophotometry for zinc. K Nahar and S Yeasmin provided day-to-day running of the study, principal patient recruitment and staff supervision, and data review. M Santosham and R E Black contributed to study design, interim discussions of the project's progress, data analysis, and manuscript preparation.

Conflict of interest statement

None declared.

Acknowledgments

This study was funded by the Johns Hopkins Family Health and Child Survival Cooperative Agreement with the US Agency for International Development, by a cooperative agreement from US Agency for International Development (HRN-A-00–96–90005–00), and by core donors to the International Centre for Diarrhoeal Disease Research, Bangladesh Centre for Health and Population Research. We thank Prof Marie Diener-West in the Department of Biostatistics at the Bloomberg School of Public Health, Johns Hopkins University, for her advice about data analysis and manuscript preparation; and ACME Laboratories (Dhaka, Bangladesh) for preparing the zinc and placebo syrups.

References

1 Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. Estimates of world-wide distribution of child deaths from acute respiratory infections. *Lancet Infect Dis* 2002; **2:** 25–32.

2 Sazawal, S, Black RE, Jalla S, Mazumdar S, Sinha A, Bhan MK. Zinc supplementation reduces the incidence of acute lower respiratory infections in infants and preschool children: a double-blind, controlled trial. *Pediatrics* 1998; **102:** 1–5.

3 Ninh NX, Thissen JP, Collette L, Gerard G, Khoi HH, Ketelslegers JM. Zinc supplementation increases growth and circulating insulin-like growth factor I (IGF-I) in growth-retarded Vietnamese children. *Am J Clin Nutr* 1996; **63:** 514–19.

4 Bhutta ZA, Black RE, Borwn KH, et al. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Zinc Investigators' Collaborative Group. *J Pediatr* 1999; **135:** 689–97.

5 Bhandari N, Bahl S, Taneja S, et al. Effect of routine zinc supplementation on pneumonia in children aged 6 months to 3 years: randomised controlled trial in an urban slum. *BMJ* 2002; **324:** 1358.

6 Roy SK, Tomkins AM, Akramuzzaman SM, et al. Randomised controlled trial of zinc supplementation in malnourished Bangladeshi children with acute diarrhoea. *Arch Dis Child* 1997; **77:** 196–200.

7 Bhutta ZA, Bird SM, Black RE, et al. Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials. *Am J Clin Nutr* 2000; **72:** 1516–22.

8 Baqui AH, Black RE, Arifeen S, et al. Effect of zinc supplementation started during diarrhoea on morbidity and mortality in Bangladeshi children: community randomised trial. *BMJ* 2002; **325:** 1059.

9 Ling PR, Schwartz JH, Jeevanandam M, Gauldie J, Bistrian BR. Metabolic changes in rats during a continuous infusion of recombinant interleukin-1. *Am J Physiol* 1996; **270:** E305–12.

10 Gaetke LM, McClain CJ, Talwalkar RT, Shedlofsky SI. Effects of endotoxin on zinc metabolism in human volunteers. *Am J Physiol* 1997; **272:** E952–56.

11 Cousins RJ, Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. *Physiol Rev* 1985; **65:** 238–309.

12 Sazawal S, Jalla S, Mazumder S, Sinha A, Black RE, Bhan MK. Effect of zinc supplementation on cell-mediated immunity and lymphocyte subsets in preschool children. *Indian Pediatr* 1997; **34:** 589–97.

13 Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr* 1998; **68:** 447S–63.

14 Fontaine O. Effect of zinc supplementation on clinical course of acute diarrhoea. *J Health Popul Nutr* 2001; **19:** 339–46.

15 Fosmire GJ. Zinc toxicity. *Am J Clin Nutr* 1990; **51:** 225–27.

16 Hamil PV, Drizd TA, Johnson CL. Physical growth: National Center for Health Statistic Percentages. *Am J Clin Nutr* 1979; **32:** 607–29.

17 Jiang SL, Samols D, Sipe J, Kushner I. The acute phase response: overview and evidence of roles for both transcriptional and post-transcriptional mechanisms. *Folia Histochem Cytobiol* 1992; **30:** 133–35.

18 Peters M. Actions of cytokines on the immune response and viral interactions: an overview. *Hepatology* 1996; **23:** 909–16.

19 Stratowa C, M Audette. Transcriptional regulation of the human intercellular adhesion molecule-1 gene: a short overview. *Immunobiology* 1995; **193:** 293–304.

20 Roberts RM, Mathialagan N, Duffy JY, Smith GW. Regulation and regulatory role of proteinase inhibitors. *Crit Rev Eukaryot Gene Expr* 1995; **5:** 385–436.

21 Ramadori G, Christ B. Cytokines and the hepatic acute-phase response. *Semin Liver Dis* 1999; **19:** 141–55.

22 Ramadori G, Van Damme J, Rieder H, Meyer zum Buschenfelde KH. Interleukin 6, the third mediator of acute-phase reaction, modulates hepatic protein synthesis in human and mouse. Comparison with interleukin 1 beta and tumor necrosis factor-alpha. *Eur J Immunol* 1988; **18:** 1259–64.

23 Ramadori G, Sipe JD, Dinarello CA, Mizel SB, Colten HR. Pretranslational modulation of acute phase hepatic protein synthesis by murine recombinant interleukin 1 (IL-1) and purified human IL-1. *J Exp Med* 1985; **162:** 930–42.

24 Braunschweig CL, Sowers M, Kovacevich DS, Hill GM, August DA. Parenteral zinc supplementation in adult humans during the acute phase response increases the febrile response. *J Nutr* 1997; **127:** 70–74.

25 Goldblum SE, Cohen DA, Jay M, McClain CJ. Interleukin 1-induced depression of iron and zinc: role of granulocytes and lactoferrin. *Am J Physiol* 1987; **252:** E27–32.

26 Iwata M, Takebayashi T, Ohta H, Alcalde RE, Itano Y, Matsumura T. Zinc accumulation and metallothionein gene expression in the proliferating epidermis during wound healing in mouse skin. *Histochem Cell Biol* 1999; **112:** 283–90.

27 Thurnham DI. Micronutrients and immune function: some recent developments. *J Clin Pathol* 1997; **50:** 887–91.

28 Truong-Tran AQ, Ruffin RE, Foster PS, et al, Altered zinc homeostasis and caspase-3 activity in murine allergic airway inflammation. *Am J Respir Cell Mol Biol* 2002; **27:** 286–96.

29 Cho YH, Lee SJ, Lee JY. Antibacterial effect of intraprostatic zinc injection in a rat model of chronic bacterial prostatitis. *Int J Antimicrob Agents* 2002; **19:** 576–82.

30 Duff M, Ettarh RR. Crypt cell production rate in the small intestine of the zinc-supplemented mouse. *Cells Tissues Organs* 2002; **172:** 21–28.

31 Luk HH, Ko JK, Fung HS, Cho CH. Delineation of the protective action of zinc sulfate on ulcerative colitis in rats. *Eur J Pharmacol* 2002; **443:** 197–204.

32 Sazawal S, Black RE, Bhan MK, Bhandari N, Sinha A, Jalla S. Zinc supplementation in young children with acute diarrhea in India. *N Engl J Med* 1995; **333:** 839–44.

33 Casal J, Gimenez MJ, Aguilar L, et al. Beta-lactam activity against resistant pneumococcal strains is enhanced by the immune system. *J Antimicrob Chemother* 2002; **50** (suppl S2)**:** 83–86.

34 Groeneveld AB, Bossink AW, van Mierlo GJ, Hack CE. Circulating inflammatory mediators in patients with fever: predicting bloodstream infection. *Clin Diagn Lab Immunol* 2001; **8:** 1189–95.

35 Szalai AJ, van Ginkel FW, Wang Y, McGhee JR, Volanakis JE. Complement-dependent acute-phase expression of C-reactive protein and serum amyloid P-component. *J Immunol* 2000; **165:** 1030–35.

36 Noursadeghi M, Bickerstaff MC, Herbert J, Moyes D, Cohen J, Pepys MB. Production of granulocyte colony-stimulating factor in the nonspecific acute phase response enhances host resistance to bacterial infection. *J Immunol* 2002; **169:** 913–19.

37 Simmer K, Khanum S, Carlsson L, Thompson RP. Nutritional rehabilitation in Bangladesh—the importance of zinc. *Am J Clin Nutr* 1988; **47:** 1036–40.

38 Rahman MM, Wahed MA, Fuchs GJ, Baqui AH, Alvarez JO. Synergistic effect of zinc and vitamin A on the biochemical indexes of vitamin A nutrition in children. *Am J Clin Nutr* 2002; **75:** 92–98.

39 Osendarp SJ, van Raaij JM, Darmstadt GL, Baqui AH, Hautvast JG, Fuchs GJ. Zinc supplementation during pregnancy and effects on growth and morbidity in low birthweight infants: a randomised placebo controlled trial. *Lancet* 2001; **357:** 1080–85.

40 Osendarp SJ, Santosham M, Black RE, Wahed MA, van Raaij JM, Fuchs GJ. Effect of zinc supplementation between 1 and 6 mo of life on growth and morbidity of Bangladeshi infants in urban slums. *Am J Clin Nutr* 2002; **76:** 1401–18.