

Infectious etiology modifies the treatment effect of zinc in severe pneumonia¹⁻³

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ABSTRACT

Background: Zinc is undergoing evaluation as an inexpensive therapeutic adjuvant for severe pediatric pneumonia.

Objective: We explored the effect of etiology on the treatment effect of zinc in young children hospitalized for severe pneumonia.

Design: We analyzed data from a randomized, double-blind, placebo-controlled clinical trial conducted at the Christian Medical College Hospital, a teaching hospital in Tamilnadu, India. Children aged 2–23 mo ($n = 299$) were randomly assigned to receive a 10-mg tablet of zinc sulfate or placebo twice a day during hospitalization. The primary outcomes were length of hospitalization and time to resolution of severe pneumonia stratified by etiologic classification on the basis of serum C-reactive protein (CRP) concentrations at admission.

Results: CRP concentrations were available for 295 (98.7%) of the enrolled cases. Of these 295 cases, 223 (75.6%) were classified as suspected nonbacterial pneumonias (CRP concentrations ≤ 40 mg/L). Etiology modified the treatment effect of zinc on the length of the hospital stay [hazard ratio (HR) for interaction term: 0.52; 95% CI: 0.31, 0.91; $P = 0.022$]. In the 72 suspected bacterial cases (CRP concentrations > 40 mg/L), the median length of hospitalization was ≈ 20 h longer in the zinc-supplemented group than in the placebo group (87.3 and 68.3 h, respectively; HR: 0.56; 95% CI: 0.34, 0.93; $P = 0.025$). The treatment effect was not modified in the suspected nonbacterial cases of pneumonia.

Conclusions: Our results suggest that the treatment effect of zinc for severe pediatric pneumonia may be modified by bacterial infection. Further studies are required to develop appropriate recommendations for the use of zinc in the treatment of severe pneumonia. This trial was registered at clinicaltrials.gov as NCT00198666. *Am J Clin Nutr* 2007;86:397–403.

KEY WORDS Pneumonia, zinc supplementation, treatment, etiology, inflammation

INTRODUCTION

Pneumonia remains the leading cause of morbidity and mortality in young children (1, 2). In low-income countries, undernutrition is associated with a greater severity of pneumonia, a longer duration of illness, and an increased case fatality rate (3, 4). Of the micronutrients, zinc plays a critical role in the development and maintenance of host defenses against infectious diseases (5, 6). Evidence from low-income countries indicates that detrimental effects on host immunity can occur rapidly in children with mild zinc deficiency (7). Results from preventive trials

show that zinc supplementation significantly reduces the incidence of pneumonia and chronic diarrhea in children living in areas of endemic zinc deficiency (8, 9). Significant decreases in diarrheal morbidity and mortality were also seen in zinc-supplemented children in therapeutic trials (10, 11). Whether zinc provides a similar therapeutic benefit to children with severe pneumonia is under investigation.

We recently reported that zinc supplementation was not associated with recovery in young children hospitalized for severe pediatric pneumonia in South India (12). Our results are in contrast with the results of 2 previous hospital-based trials in India and Bangladesh, which showed that zinc significantly shortened the duration of recovery in children with severe pneumonia who were receiving standard antibiotic therapy (13, 14). The disparity between the results of our study and the other 2 trials does not appear to be explained by differences in the study population or the methodology. Whereas zinc plays an important role in maintaining the integrity of the immune response, its mechanisms of action are not well understood (15). It is plausible that the therapeutic effect of zinc in severe pneumonia may be modified by pathogen-dependent immune responses. Thus, the disparity between study findings may reflect differences in the distribution of these pathogens. If the etiology of pneumonia is found to modify the effect of zinc supplementation on the recovery of severe pneumonia, an awareness of this effect modification may help us to better define a subset of patients who would most likely benefit from zinc therapy. In addition, it may lead to new hypotheses about the mechanisms underlying zinc's role in recovery from infection.

We analyzed data from our original study to explore the effect of suspected bacterial and nonbacterial etiology, classified by

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baseline serum C-reactive protein (CRP) concentrations, on the association between zinc supplementation and recovery from severe pneumonia in hospitalized children in South India who were receiving standard antibiotic therapy.

SUBJECTS AND METHODS

We measured CRP concentrations at the time of enrollment in young children who were participating in a clinical trial designed to assess the efficacy of adjunctive zinc therapy for severe pneumonia in South India. The clinical trial was conducted between September 2003 and August 2004 at the Christian Medical College (CMC) Hospital in Vellore, India. Details on subject recruitment and primary outcomes of the trial have been published (12). Briefly stated, the double-blind study was conducted in children between the ages of 2 and 23 mo who were admitted to the pediatric ward and who met the study criteria for severe pneumonia. Severe pneumonia was defined as a respiratory rate >50 breaths/min, which was accompanied by crepitations on auscultation and the presence of one or more of the following danger signs: lethargy, inability to feed, chest indrawing, or central cyanosis. The study was approved by the institutional review boards of CMC Hospital and Bloomberg School of Public Health, Johns Hopkins University.

Three hundred children were randomly assigned to receive a 10-mg dose of zinc sulfate or placebo orally twice a day throughout the duration of their hospitalization. Information on demographics, current illness, and history of respiratory illness was collected on each patient at the time of enrollment. All baseline physical findings, anthropometric data, blood test results, and CRP and plasma zinc concentrations were recorded. Enrolled children were treated according to CMC Hospital's standard protocol for the treatment of infants and children with pneumonia, which consisted of intravenous antibiotics, fluid therapy, and supportive care. During hospitalization, each child's condition was assessed by the study clinical staff at 8-h intervals, and the findings were recorded in the patient's chart. Children were given oral antibiotics when they were feeding well and when their oxygen saturation and respiratory rate were stable. Once receiving oral antibiotics, the patients were kept under observation for an additional 24 h before discharge. Children were discharged when they met all of the following 4 criteria: 1) they were entirely on oral feeds, 2) their respiratory rate was <50 breaths/min, 3) their oxygen saturation was $\geq 93\%$, and 4) the attending pediatrician had concluded that the patient's condition had resolved and further hospitalization was not required.

Measurement of CRP concentrations

Whole-blood samples were collected within 6 h of study enrollment. The sera were separated and refrigerated at -4°C for up to 4 h before CRP analysis was conducted. CRP concentrations were measured by nephelometry (Dade Behring, Marburg, Germany) in CMC Hospital's Clinical Microbiology Laboratory.

Etiologic classification of pneumonia cases

Chest radiographs and nonspecific inflammatory markers, such as CRP concentrations, are used widely for distinguishing between community-acquired bacterial and viral pneumonias because of the lack of highly accurate tests for determining the etiology of respiratory infections (16–19). Several studies have suggested high cutoffs for serum CRP concentrations, between

40 and 80 mg/L, as minimum screening limits for bacterial or pneumococcal pneumonia, noting that viral infections are rare if CRP concentrations are >40 mg/L (20–24). The results of these pediatric studies to evaluate the correlation between serum CRP concentration and infectious etiology indicated that, for this range of cutoffs, the reported sensitivity and specificity for detecting bacterial or pneumococcal pneumonia were 0.27–0.79 and 0.52–0.92, respectively. The positive likelihood ratios were from 1.45 to 8.3 and had a positive predictive value of up to 79%. Thus, the evidence suggested that the use of cutoffs in the range of CRP concentrations >40 to >80 mg/L had relatively low sensitivity but high specificity for the identification of bacterial pneumonia. The use of CRP concentrations alone as a diagnostic tool to differentiate between viral and bacterial pneumonias in clinical practice is controversial because of concerns regarding variations in sensitivity and is complicated by the lack of reference standards for microbial-specific etiologic diagnosis (24, 25). However, CRP concentrations have value as a screening tool in research for gauging respiratory disease burden by microbial etiology. Recently, researchers in South Africa used CRP concentrations ≥ 40 mg/L to define pneumococcal pneumonia to assess the vaccine efficacy of pneumococcal conjugate vaccine (26). The researchers concluded that CRP provided a better measure of vaccine efficacy than did chest radiographs. Children who had baseline CRP concentrations >40 mg/L were classified as having severe pneumonia of suspected bacterial or mixed bacterial-viral etiology. Participants who had CRP concentrations ≤ 40 mg/dL were suspected to have nonbacterial pneumonia.

Definition and measurement of primary outcomes

The 2 primary outcomes of the present study were the length of hospitalization and the time to resolution of severe pneumonia. In our original study, we used 3 different definitions of severe pneumonia to evaluate recovery: 1) respiratory rate >50 breaths/min and oxygen saturation $<93\%$; 2) inability to feed, respiratory rate >50 breaths/min, and oxygen saturation $<93\%$; and 3) chest indrawing, respiratory rate >50 breaths/min, and oxygen saturation $<93\%$.

Our initial analysis indicated that all 3 definitions provided similar results. Therefore, we used the following combination of findings to evaluate recovery status in the current analysis: inability to feed, respiratory rate >50 breaths/min, and oxygen saturation $<93\%$. The duration of hospitalization was defined as the number of hours that elapsed between enrollment and discharge. We also explored the time to resolution of tachypnea (ie, respiratory rate >50 breaths/min), inability to feed orally, fever (ie, axillary temperature $>37.5^{\circ}\text{C}$), and lethargy.

Statistical analysis

The effects of zinc supplementation on the outcomes of interest stratified by etiology of infection were analyzed on an intention-to-treat basis. Data were analyzed with STATA software (version 8.0; Stata Corp, College Station, TX). The *t* test for continuous variables and 2-tailed chi-square analysis or Fisher's exact test for contingency data were used, as appropriate, to assess treatment group differences at baseline. Kaplan-Meier survival functions were used to measure the median duration of outcomes and to assess the effect of treatment on the study outcomes. Cox proportional hazards regression models were constructed to adjust the treatment effects for potential confounding



TABLE 1

Selected baseline characteristics (as categorical variables) of patients with severe pneumonia assigned zinc ($n = 149$) or placebo ($n = 146$)[†]

Characteristic	Zinc		Placebo	
	n (%)			
Suspected nonbacterial pneumonia (CRP ≤ 40 mg/L)				
Male	83/106 (73.5)		81/117 (69.2)	
Age < 12 mo	71/106 (67.0)		84/117 (71.8)	
Underweight (weight-for-age z score ≤ -2)	45/106 (42.5)		46/117 (39.3)	
Hyperpyrexia (axillary temperature >38.4 °C)	11/105 (10.5)		21/117 (18.0)	
Suspected bacterial pneumonia (CRP > 40 mg/L)				
Male	23/43 (53.5)		17/29 (58.6)	
Age < 12 mo	28/43 (65.1)		23/29 (79.3)	
Underweight (weight-for-age z score ≤ -2)	17/43 (39.5)		9/29 (31.0)	
Hyperpyrexia (axillary temperature > 38.4 °C)	11/43 (25.6)		6/29 (20.7)	

[†] Treatment groups were stratified by etiologic classification (nonbacterial and bacterial). CRP, C-reactive protein. None of the differences between groups were statistically significant (ie, $P < 0.05$); chi-square test was used to compare proportions.

factors and to evaluate effect modification. The hazard ratios (HRs) compared the recovery rates from the indicators in the zinc-supplemented group with those in the placebo group. HRs > 1 are associated with the probability that the duration of the outcome will be shorter in the zinc group than in the placebo group. The analyses were based on clinical outcomes, and the time to recovery was defined as the end of the last consecutive 16-h period in which the participant did not meet the definition of severe pneumonia. Associations between the intervention and outcomes of interest in the regression models were considered to be statistically significant at $P \leq 0.05$. Interaction terms were statistically significant at $P \leq 0.1$.

RESULTS

Three hundred children were enrolled in the trial, and one-half were randomly allocated to each treatment group. One of the participants withdrew from the study against medical advice shortly after enrollment, and that child was omitted from the

analysis. CRP concentrations were available for 295 (98.7%) of the 299 severe pneumonia cases. Of this number, 223 (75.6%) were classified as suspected nonbacterial pneumonias (CRP concentrations ≤ 40 mg/L), and 72 were classified as suspected bacterial pneumonias (CRP concentrations > 40 mg/L). Overall, $\approx 70\%$ of the participants were boys. The mean (\pm SD) age was 9.6 ± 5.9 mo, and 70% of the children were < 12 mo of age. Approximately 39% of the children were underweight (weight-for-age z score ≤ -2.0), and 22% of the children had plasma zinc concentrations < 9.18 μ mol/L at the time of enrollment. Baseline characteristics did not differ significantly by treatment group when stratified by etiology of infection (**Table 1** and **Table 2**). The proportion of suspected bacterial pneumonias was greater in the zinc group than in the placebo group (29% compared with 20%); however, the difference was not statistically significant.

In the present analysis, we found evidence of significant effect modification by etiology on the length of hospital stay (HR for interaction term: 0.52; 95% CI: 0.31, 0.91; $P = 0.022$) and on the duration of recovery from severe illness (HR for interaction term: 0.56; 95% CI: 0.29, 1.05; $P = 0.074$). When we stratified by etiology, a significant negative treatment effect was observed for suspected bacterial infections (CRP > 40 mg/L), and no treatment effect was observed for suspected nonbacterial infections (CRP ≤ 40 mg/L; **Table 3**). In children with suspected bacterial infections, the median length of hospitalization was ≈ 20 h longer in the zinc-supplemented group than in the placebo group (87.3 and 68.3 h, respectively; HR: 0.56; $P = 0.025$; **Figure 1**). The median duration of severe pneumonia tended to be longer in the zinc group than in the placebo group; however, the association was not statistically significant (92.8 and 82.8 h, respectively; HR: 0.61, $P = 0.100$; **Figure 2**). None of the 3-factor interaction models, which included baseline zinc status and underweight status, were statistically significant.

We also explored the effect of high fever (axillary temperature ≥ 38.4 °C) on the treatment effect of zinc within 72 h of admission. As with elevated CRP concentrations, high fever has been shown to be significantly associated with bacterial pneumonia (27). Our analysis showed that the effect of high fever on the treatment effect of zinc was congruent with our findings in which etiologic classification was defined on the basis of CRP concentrations (**Table 4**).

After the CRP and fever analyses were completed, we reviewed the bacterial culture data we had collected. As expected, the proportion of culture-confirmed cases was low, $\approx 9.4\%$

TABLE 2

Selected baseline characteristics (as continuous variable) of patients with severe pneumonia assigned zinc ($n = 149$) or placebo ($n = 146$)[†]

Characteristic	Zinc			Placebo		
	n	Median	95% CI	n	Median	95% CI
Suspected nonbacterial pneumonia (CRP ≤ 40 mg/L)						
White blood cell count ($10^9/L$)	106	13.9	12.3, 15.7	115	14.4	12.4, 15.8
Serum CRP concentration (mg/L)	106	6.8	3.3, 10.1	117	5.3	3.3, 9.1
Zinc concentration (μ mol/L)	105	10.7	10.3, 11.5	116	10.6	10.1, 11.17
Suspected bacterial pneumonia (CRP > 40 mg/L)						
White blood cell count ($10^9/L$)	43	15.3	13.2, 17.9	29	20.1	15.3, 24.6
Serum CRP concentration (mg/L)	43	70.7	56.8, 93.9	29	100	66.8, 115.1
Zinc concentration (μ mol/L)	43	10.6	9.6, 11.5	29	10.2	9.6, 12.2

[†] Treatment groups were stratified by etiologic classification (nonbacterial and bacterial). CRP, C-reactive protein. None of the differences between groups were statistically significant (ie, $P < 0.05$); Mann-Whitney rank-sum test was used to compare medians.

TABLE 3Effect of treatment on median number of hours to recovery by clinical indicator stratified by etiology¹

Outcome	Zinc		Placebo		Hazard ratio ² (95% CI)	P
	n	Median (95% CI)	n	Median (95% CI)		
Duration of hospitalization						
Suspected nonbacterial pneumonia	106	69.5 (65.2, 74.5)	117	73.2 (69.0, 84.3)	1.15 (0.9, 1.50)	0.302
Suspected bacterial pneumonia	43	87.3 (68.9, 111.3)	29	68.5 (60.7, 90.0)	0.56 (0.34, 0.93)	0.025
Severe illness ³						
Suspected nonbacterial pneumonia	106	78.6 (66.8, 92.2)	117	76.2 (72.3, 90.8)	1.13 (0.84, 1.52)	0.412
Suspected bacterial pneumonia	43	92.8 (70.7, 121.2)	29	82.8 (67.2, 97.3)	0.61 (0.34, 1.10)	0.100
Tachypnea ⁴						
Suspected nonbacterial pneumonia	106	70.2 (64.8, 87.3)	117	73.2 (69.0, 84.3)	1.17 (0.89, 1.53)	0.257
Suspected bacterial pneumonia	43	87.0 (68.9, 97.9)	29	71.8 (60.7, 90.0)	0.59 (0.35, 0.99)	0.047
Fever ⁵						
Suspected nonbacterial pneumonia	106	68.5 (64.2, 74.5)	117	73.2 (69.0, 84.3)	1.15 (0.88, 1.49)	0.310
Suspected bacterial pneumonia	43	87.3 (68.9, 111.3)	29	68.5 (60.7, 90.0)	0.56 (0.34, 0.93)	0.025
Lethargy						
Suspected nonbacterial pneumonia	106	68.5 (64.2, 73.3)	117	73.2 (69.0, 84.3)	1.15 (0.88, 1.50)	0.306
Suspected bacterial pneumonia	43	87.3 (68.9, 111.3)	29	68.5 (60.7, 90.0)	0.56 (0.34, 0.93)	0.025

¹ Recovery criteria were met for 16 consecutive hours after the last report of a clinical indicator.

² Data were analyzed by using Cox proportional hazards regression models. Interaction between zinc treatment and etiology of infection: hospitalization ($P = 0.022$), severe pneumonia ($P = 0.074$), tachypnea ($P = 0.034$), fever ($P = 0.022$), and lethargy ($P = 0.024$).

³ Severe illness: inability to feed, O₂ saturation <93%, and respiratory rate >50 breaths/min.

⁴ Respiratory rate >50 breaths/min.

⁵ Fever >37.5 °C.

(28/298), given the practice of self-medication with antibiotics before hospitalization in this setting. Whereas the pathogens were not identified specifically, 89% (25/28) were gram-positive bacteria. We then explored the treatment effect of zinc in culture-confirmed bacterial pneumonia cases (**Table 5**). The results showed that the median lengths of hospitalization and recovery from severe pneumonia were greater in the zinc-supplemented children with bacterial pneumonia than in the children in the placebo group. In the confirmed bacterial cases, the magnitude and direction of the HRs for the effect of zinc on the 2 outcomes of interest were compatible with the results of the analysis in which etiologic classification was based on serum CRP status at enrollment. CRP data were available for 27 of the 28 culture-positive cases. Of these, 7 of 27 (25.9%) had CRP concentrations >40 mg/L. However, the mean (\pm SD) CRP concentration in the culture-confirmed cases tended to be higher than in the culture-negative cases (43.0 ± 20.7 and 30.3 ± 44.0 mg/L, respectively, $P = 0.175$).

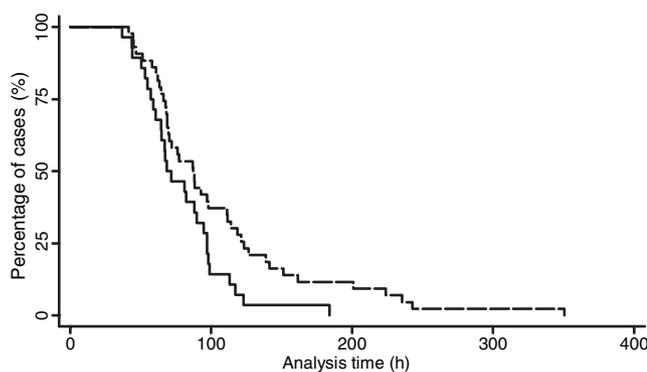


FIGURE 1. Kaplan-Meier survival curves of the median duration of hospitalization in the zinc treatment group (dashed line; $n = 43$) and in the placebo group (solid line; $n = 29$) in suspected bacterial pneumonia cases.

DISCUSSION

We observed that etiology of infection, as classified by serum CRP concentration, modified the effect of zinc supplementation on the recovery of children aged <2 y hospitalized for severe pneumonia who were receiving standard care. Specifically, zinc-treated children had a significantly longer hospital stay and a slower recovery from severe illness than did the children who were receiving a placebo if their illness was classified to be of bacterial etiology on the basis of CRP concentrations at baseline (CRP > 40 mg/dL). The length of hospitalization and the period of recovery from severe pneumonia were comparable between treatment groups in children with nonbacterial pneumonias. Our finding of a negative treatment effect of zinc on bacterial pneumonias is supported by data on culture-confirmed cases of bacterial pneumonia collected in the present study. Whereas CRP concentrations

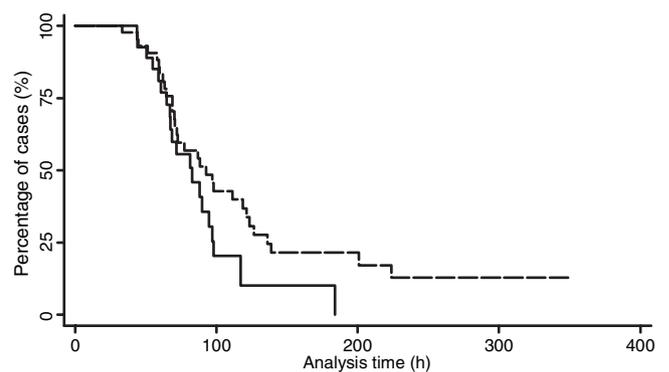


FIGURE 2. Kaplan-Meier survival curves of the median duration of recovery from severe pneumonia in the zinc treatment group (dashed line; $n = 43$) and in the placebo group (solid line; $n = 29$) in suspected bacterial pneumonia cases. Severe illness was defined as the inability to feed, a respiratory rate <50 breaths/min, and oxygen saturation <93%.



TABLE 4

Effect of treatment on median number of hours to recovery by clinical indicator in children by high fever status at enrollment¹

Outcome	Zinc		Placebo		Hazard ratio ² (95% CI)	P
	n	Median (95% CI)	n	Median (95% CI)		
Duration of hospitalization						
Axillary temperature <38.4 °C	127	70.2 (66.8, 87.2)	122	75.0 (69.3, 86.1)	0.99 (0.77, 1.28)	0.773
Axillary temperature ≥38.4 °C	22	87.3 (68.9, 111.6)	27	67.5 (55.0, 76.9)	0.71 (0.40, 1.27)	0.250
Severe illness ³						
Axillary temperature <38.4 °C	127	87.0 (68.9, 95.2)	122	84.3 (73.2, 97.2)	1.07 (0.80, 1.43)	0.633
Axillary temperature ≥38.4 °C	22	92.2 (69.5, 121.2)	27	62.7 (55.0, 76.9)	0.51 (0.27, 0.97)	0.042

¹ Recovery criteria were met for 16 consecutive hours after the last report of a clinical indicator.² Data were analyzed by using Cox proportional hazards regression models. Interaction between zinc treatment and etiology of infection: hospitalization ($P = 0.213$) and severe pneumonia ($P = 0.019$).³ Severe illness: inability to feed, O₂ saturation <93%, and respiratory rate >50 breaths/min.

alone have relatively low sensitivity for detecting bacterial pneumonia, elevated CRP concentrations are associated with pneumonias of bacterial etiology. It is conceivable that CRP concentrations may depend on the specific bacterial pathogen responsible for infection. Additionally, peak CRP concentrations may not have been measured because nearly 67% of the cases reported being ill for ≥3 d before seeking care.

Our results are compatible with the results of a recent therapeutic trial that showed that adjuvant zinc therapy in indigenous Australian children with severe pneumonia was significantly associated with increased morbidity. The risk of readmission within 120 d in the zinc group was more than double the risk of the placebo group (28). More than 90% of the participants had radiographic evidence of lobar pneumonia, a finding thought to be associated with *Streptococcus pneumoniae* infection (16, 17). Whereas there was no difference in the duration of hospital stay between groups, the median time to normalization of the respiratory rate was ≈10 h longer in the zinc-supplemented group than in the placebo group; however, this difference was not statistically significant.

The acute phase response (APR) is mediated by a number of proinflammatory cytokines and is characterized by the increased synthesis of acute phase plasma proteins, including CRP, and by decreases in plasma zinc concentrations (29). For most infections, these controlled cytokine-mediated changes are beneficial to the host by optimizing T cell function, thereby facilitating recovery. However, the pathology associated with sepsis and severe pneumonia is thought to result from exaggerated host

inflammatory responses to pathogen products (29, 30). In an exaggerated APR, excess inflammation reduces phagocytosis and prolongs the severity of symptoms and the period of infection. Bacterial pathogens, which include gram-negative bacterial endotoxin, pneumococcal peptidoglycan and pneumolysin, and *Staphylococcus aureus* enterotoxin, are a frequent cause of an exaggerated APR. In contrast, APRs are often reduced in acute viral infections (31).

Zinc has been shown to up-regulate the production of several proinflammatory cytokines engaged in the APR. Evidence from in vitro models indicates that zinc stimulates monocytes in a dose-dependent fashion to release the acute phase proinflammatory cytokines interleukin-2, interleukin-6, and tumor necrosis factor- α (32). In animal studies, zinc administered 24 h before the experimental induction of endotoxemia was found to stimulate the release of proinflammatory cytokines and to increase the expression of heat shock proteins, which, in turn, were correlated with decreased cellular damage in vitro and improved pulmonary function in vivo (33, 34). This evidence is supported by data from prophylaxis trials that showed increases in the incidence of fever, tachypnea, and rhinorrhea in zinc-supplemented children (35, 36). Results from several trials conducted in populations that have endemic zinc deficiency indicate that zinc prophylaxis reduces the incidence of severe pneumonia (35, 37), which may suggest that the up-regulation of proinflammatory cytokines may serve to potentiate the immune system against future severe infection in zinc-deficient children.

TABLE 5

Effect of treatment on median number of hours to recovery by clinical indicator in children with pneumonia etiology confirmed by bacterial culture¹

Outcome	Zinc		Placebo		Hazard ratio ² (95% CI)	P
	n	Median (95% CI)	n	Median (95% CI)		
Duration of hospitalization						
Nonbacterial pneumonia	138	70.2 (67.0, 77.2)	132	71.2 (67.5, 76.9)	0.99 (0.78, 1.26)	0.781
Bacterial pneumonia	11	126.5 (53.7, 200.9)	17	90.0 (64.8, 111.0)	0.49 (0.21, 1.11)	0.085
Severe illness ³						
Nonbacterial pneumonia	138	78.6 (70.2, 92.2)	132	75.9 (71.2, 84.3)	0.99 (0.76, 1.31)	0.994
Bacterial pneumonia	11	126.4 (53.7, 214.5)	17	89.9 (62.8, 111.0)	0.38 (0.15, 0.97)	0.045

¹ Recovery criteria were met for 16 consecutive hours after the last report of a clinical indicator.² Data were analyzed by using Cox proportional hazards regression models. Interaction between zinc treatment and etiology of infection: hospitalization ($P = 0.137$) and severe pneumonia ($P = 0.120$).³ Severe illness: inability to feed, O₂ saturation <93%, and respiratory rate >50 breaths/min.

Limited evidence suggests that, for the pathogens implicated in lower respiratory and systemic infections, the therapeutic benefits of zinc may vary by etiology. Zinc did not appear to have an effect in children with measles-related pneumonia who received standard antibiotics along with vitamin A therapy (38). However, data from in vitro studies have shown that zinc, even in low concentrations, acts synergistically with lipopolysaccharide on monocytes to enhance the induction of proinflammatory cytokines (39, 40). Consistent with this finding is evidence that shows that treatment with parenteral zinc immediately before challenge with *Salmonella typhimurium* significantly increased the case fatality rates in mice (41). When zinc was administered to pigs within 1 h of lipopolysaccharide-induced endotoxemia, the resulting proinflammatory effects were associated with deterioration of pulmonary function and higher lethality (41, 42). In a supplementation trial in adult patients with gram-negative catheter sepsis and pancreatitis, zinc supplementation was shown to be associated with an exaggerated APR (43). Data from human studies of gram-positive bacteria are lacking; however, evidence from one animal study has shown that zinc supplementation improved the survival in mice infected with *S. pneumoniae* and *Francisella tularensis* (41). Collectively, the findings support the idea that the therapeutic effect of zinc on the recovery from pneumonia may be contingent on the interaction between zinc and pathogen-dependent host inflammatory responses.

At the onset of infection, plasma zinc concentrations decline rapidly by 10–69%, and a corresponding increase in the production of zinc proteins in the liver modulates the immune response (44). It has been suggested that elevated plasma zinc concentrations in acute inflammatory states may inhibit T cell activity and impair phagocytosis (45) and that the observed decrease in plasma may serve an antiinflammatory function (43, 46). If hepatic zinc sequestration is a mechanism for regulating cytokine production, preventing the decline in plasma zinc through supplementation may potentially exacerbate infection if the APR is severe. Yet, low zinc stores are known to impair T cell function, reduce the production of proinflammatory cytokines, and decrease phagocytosis (45). Therefore, supplementation in zinc-deficient children may improve host responses in acute infection. Thus, prior zinc status and the timing of supplementation may be important factors in the recovery from infection.

Alternatively, our findings and those of Chang et al (28) suggest that zinc may possibly enhance bacterial survival or inhibit clearance of the infection. Experimental evidence supports the idea that zinc may increase microbial function, proliferation, and virulence (41, 47).

A potential limitation of the present study was that the prevalence of zinc deficiency in our study population was not directly assessed. However, dietary insufficiency, limited bioavailability from local diets, and the high incidence of infection in this population were findings that were consistent with a pattern of endemic zinc deficiency. In addition, bacterial pathogens were not identified, other than by Gram stain; therefore, the effect of specific bacterial pathogens on the treatment effect of zinc could not be explored. Misclassification of etiology is also a potential limitation given the poor sensitivity of classification criteria. The effect of misclassification was likely to have been nondifferential and therefore may have diluted the strength of the associations observed.

The results of the present analysis suggest that adjuvant zinc therapy may prolong the recovery from bacterial pneumonias

and other infections associated with acute inflammation. Our findings are compatible with previous evidence that the therapeutic effect of zinc may be contingent on the interaction between zinc and pathogen-dependent host inflammatory responses. The results are intended to generate new hypotheses for clarifying zinc's role in the treatment of childhood pneumonia. Integrating the data on etiology and multiple inflammatory markers, including cytokines, in therapeutic trials is likely to provide a more accurate picture of the interrelation between zinc, infections, and immune responses and to help target the subpopulations most likely to benefit from zinc supplementation. The effects of zinc on host inflammatory responses in pneumonia of different etiologies need to be better understood before zinc supplementation can be recommended as an adjuvant therapy for all hospitalized pneumonias.

The authors' responsibilities were as follows—CLC, AB, and MS: helped to design the study; AB, CLC PDM, LM, IA, and TM: supervised the conduct of the study; CLC, AB, and MS: analyzed and interpreted the data; PDM, LM, IA, and TM: interpreted the data; CLC and AB: designed the data management system; CLC: acted as a guarantor for the study, had full access to all the data in the study, and had final responsibility for the decision to submit for publication; and all authors: contributed to the writing or editing of the manuscript, or both, and approved of the manuscript. None of the authors had any conflicts of interest or had any advisory board affiliations with any financial or personal interests in the US Agency for International Development at the time the research was done.

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