EFFICACY OF ZINC IN THE TREATMENT OF PNEUMONIA IN HOSPITALISED INFANTS LESS THAN 2 YEARS OF AGE

W. ABDULLAH BROOKS
AND OTHERS

1998-025

Attachment 1.
(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 05/10/98 ~~02.09.1998~~

Principal Investigator W. Abdullah Brooks
Application No. L 98-025
Title of Study Efficacy Zinc Treatment of Pneumonia in Hospitalised Infants < 2 years old.

Trainee Investigator (if any) _____
Supporting Agency (if Non-ICDDR,B) _____
Project status:
(X) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects — Yes (No)
   (b) Non-ill subjects — (Yes) No
   (c) Minors or persons under guardianship — (Yes) No
2. Does the study involve:
   (a) Physical risks to the subjects — (Yes) No
   (b) Social Risks — Yes (No)
   (c) Psychological risks to subjects — Yes (No)
   (d) Discomfort to subjects — (Yes) No
   (e) Invasion of privacy — (Yes) No
   (f) Disclosure of information damaging to subject or others — Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) — Yes (No)
   (b) Use of fetal tissue or abortus — Yes (No)
   (c) Use of organs or body fluids — (Yes) No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study — (Yes) No
   (b) Procedures to be followed including alternatives used — (Yes) No
   (c) Physical risks — (Yes) No
   (d) Sensitive questions — (Yes) No
   (e) Benefits to be derived — (Yes) No
   (f) Right to refuse to participate or to withdraw from study — (Yes) No
   (g) Confidential handling of data — (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No
5. Will signed consent form be required:
   (a) From subjects — Yes (No)
   (b) From parent or guardian (if subjects are minors) — (Yes) No
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:
   - ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   - ✓ Protocol (Required)
   - ✓ Abstract Summary (Required)
   - ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   - ✓ Informed consent form for subjects
   - ✓ Informed consent form for parent or guardian
   - ✓ Procedure for maintaining confidentiality
   - ✓ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

agree to obtain approval of the Ethical Review Committee for any changes olving the rights and welfare of subjects before making such change.

W. Abdullah Brooks
Principal Investigator

Trainee

International Centre for Diarrhoeal Disease Research, Bangladesh

RESEARCH PROTOCOL

FOR OFFICE USE ONLY
Protocol No: Date:

RRCApproval: Yes/ No Date:
ERC Approval: Yes/No Date:

1. Title of Project (Do not exceed 60 characters including spaces and punctuations)
EFFICACY OF ZINC IN THE TREATMENT OF PNEUMONIA IN HOSPITALISED INFANTS LESS THAN 2 YEARS OF AGE

2a. Name of the Principal Investigator(s) (Last, Middle, First).	**2b. Position / Title**	**2c. Qualifications**
W. Abdullah Brooks, MD, MPH	Health & Child Survival Advisor	See CV

3. Name of the Division/ Branch / Programme of ICDDR,B under which the study will be carried out.
Clinical Sciences Division / Child Health Research / ALRI

4. Contact Address of the Principal Investigator
4a. Office Location:

4b. Fax No: 883.116
4c. E-mail: abrooks@citechco.net
4d. Phone / Ext: 2327

5. Use of Human Subjects	**5a. Use of Live Animal**	**5b. If Yes, Specify Animal Species**
Yes ☐ X	☐ **Yes**	
No ☐	☒ **No**	X

6. Dates of Proposed Period of Support *As soon as possible)*

7. Cost Required for the Budget Period
7a. Ist Year ($): **$62,365** *2nd Year ($):* *NA* *3rd Year NA*

7b. Direct Cost ($)$49,892 Total Cost ($) $62,365

8. Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Mohammed Abdus Salam — 02-09-98
Name of the Division Director — Signature — Date of Approval

9. Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

10. Signature of PI

W. Abdullah Brooks

Date: 02.09.1998

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Check here if appendix is included

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. (**TYPE TEXT WITHIN THE SPACE PROVIDED**).

Principal Investigator W. Abdullah Brooks, MD, MPH

Project Name EFFICACY OF ZINC IN THE TREATMENT OF PNEUMONIA IN HOSPITALISED INFANTS LESS THAN 2 YEARS OF AGE

Total Budget $62,365 Beginning Date Ending Date

ABSTRACT

Rationale Acute lower respiratory tract infection is the leading cause of morbidity and mortality in children below two years of age. Studies have shown that early reversal of the signs associated with severe disease; hypoxia, tachypnoea and ability to feed, is an important prognostic factor during acute illness. *Hypothesis* Zinc deficiency is widespread among children in developing countries. As it is an important regulator of cellular immunity, zinc supplementation during the acute phase of respiratory illness could work synergistically with present WHO-recommended antibiotic regimens in promoting early reversal of signs associated with adverse outcome and shorten the total duration of clinical illness. *Protocol* We propose to conduct a placebo controlled clinical trial among all infants below 2 years of age admitted to ICDDR,B's hospital in Matlab. Infants will be randomised at the time of admission to receive either 20 mg elemental zinc per day or placebo, in addition to standard WHO-recommended antibiotic therapy. All infants will be supplemented for the duration of their hospitalisation. *Data* We will compare duration of hypoxia, tachypnoea, inability to feed, reversal of lower airway obstruction and total duration of hospitalisation between treatment groups. *Goal* We seek to enhance child survival by improving the acute management of respiratory illness with a simple adjuvant that can be used in conjunction with established antibiotic regimens.

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project

1. W. Abdullah Brooks, MD, MPH Preventive Medicine/Paediatrics PI
2. Md. Yunus, MBBS, PhD Scientist Co - PI
3. ASG Faruque, MBBS, MPH Scientist Co - Investigator
4. Mathuram Santosham, MD, MPH Public Health/Paediatrics Co – Investigator
5. Robert E. Black, MD, MPH Public Health/Paediatrics Co – Investigator
6. Abdullah Baqui, MD, PhD Public Health (Epidemiology) Co – Investigator
7. MA Wahed, BSc Laboratory Specialist Co – Investigator
8. George Fuchs, MD Gastroenterology/Paediatrics Co - Investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

HYPOTHESIS

Zinc supplementation will result in clinical mitigation of those signs associated with severe pneumonia and shorter duration of illness in children with relative zinc deficiency.

Specific Aims:

OBJECTIVES

1. To determine if zinc supplementation can play a role in the acute phase of pneumonia to lessen the severity of signs associated with severe infection and shorten the overall duration of severe illness in children with zinc deficiency below 2 years of age.

SPECIFIC AIMS

1. To investigate if a 20 mg/day zinc supplement can lessen the severity of illness, measured as respiratory rate (RR), and the "danger signs": chest indrawing, cyanosis, feeding and lethargy in children under two years of age.

2. To investigate if a 20 mg/day zinc supplementation can shorten the duration of severe pneumonia characterised by the above signs.

SECONDARY AIMS

1. To measure the effect of zinc supplementation on the severity and duration of hypoxaemia.
2. To compare the severity and duration of severe signs in children with wheezing between treatment groups.

Background of the Project including Preliminary Observations

BACKGROUND

Acute lower respiratory infection (ALRI) is a leading cause of morbidity and mortality in children below five years of age(1). Pneumonia accounts for most of these deaths, and thus defines ALRI. In fact, 25-33% of the deaths among children under 5 years are attributable to pneumonia (4 million deaths/year world-wide); two thirds of which occur during infancy and over 90% of which take place in developing countries(1,2). Acute respiratory illness (ARI), overall, ranks as one of the two leading causes of hospitalisation of children in Bangladesh(3). **It has been reported that the incidence of ARI is 8-9 episodes/child/yr during the first 2 years of life, dropping to 3-4 episodes/yr in 2-4 year olds(7). Thus, the susceptibility to ARI in children under two years is more than twice that of older children.** While overall child mortality in some developing countries has declined among younger age groups, the number of deaths attributable to ALRI has remained unchanged, suggesting an increasing importance of ALRI as a cause of death among young children(8). A pooled estimate from a recent meta-analysis concluded that a case management approach to children with rapid breathing could reduce total infant mortality by 20% and mortality for all children under five years by 25%(50). It further argued that a case management approach that relies principally on early diagnosis and appropriate antibiotic therapy could reduce pneumonia case fatality by as much as 50%(50). The most recent figures for ALRI incidence in Bangladesh are from a rural setting (9). Incidence for children below age 24 months ranged 0.2 – 0.5 episodes/child/year, averaging 0.3 episodes/child/year.

Aetiology

World-wide nonbacterial ALRI is the most common paediatric pulmonary infection (10). The primary agents for nonbacterial infection are RSV, parainfluenza viruses types 1,2 and 3, and *M. pneumoniae*. Below age five years the most common cause is RSV, above that age it is M. pneumoniae(10). While nonbacterial ALRI is generally a benign and self-limited disease, children with congenital heart disease, bronchopulmonary dysplasia, prematurity and low birth weight, as well as those below two years of age are at greatest risk of severe ALRI. A study of children under 5 years old in urban Bangladesh reported that pneumonia was the most common presentation (86.5%) of viral ARI(11).

In developing countries bacterial pathogens are thought to play a greater role in severe ALRI than in developed countries(12,13,14,15,16). The two most common isolates in the 1-5 year age group are *Haemophilus influenzae* and *Streptococcus pnuemoniae*(12,15). A study of urban Bangladeshi children under age five years hospitalised with ALRI and diarrhoea reported that the overall case fatality rate was 8%. However, patients with a bacterial or a mixed viral-bacterial infection were 4 and 11 more times likely to die, respectively, than were patients with a viral infection alone (17). Therefore, in the developing world, both viral and bacterial-associated diseases are important sources of morbidity and even mortality.

Clinical Risk Factors Associated with Severe Disease and Adverse Outcome in ALRI

Respiratory Distress in the Infant and Young Child occurs primarily from either increased metabolic demand or disruption of along some point of the ventilatory pathway (18). Infants and children are especially vulnerable to respiratory distress (RD) for both structural and functional reasons. Structurally, the infant chest wall is more compliant and less stable than that of the older child(18,19). A soft sternum, the horizontal placement of the ribs and the relatively poor development of the intercostal muscles mediate this instability. The diaphragm does most of the work of breathing. The more negative the inspiratory pressure generated during the breathing cycle, the greater the likelihood of rib cage collapse during diaphragmatic descent, limiting the inflow of gases (ventilation). Moreover, the infant diaphragm is more fatigable than that of the older child (18), hence it has less functional reserve. Mechanical distortion, such as flattening from hyperaeration (e.g., from inappropriate mechanical ventilation or during an asthmatic exacerbation) or external compression (e.g., abdominal distension) will further limit ventilation.

Airway diameter is equally important to the chest wall and diaphragm in this age group (20). Infants less than four months of age are obligate nose breathers (18), which renders very young infants vulnerable to RD even during upper airway infections. At birth the diameter of the trachea is one third that of the adult and that of the bronchi is one half(18). Thus, even small increases in mucosal thickening result in more exaggerated increases in airflow resistance than in adults. Since resistance varies inversely with the fourth power of the radius of the airway, the situation is most severe when the bronchioles are involved, as is often the case during infection or reactive airways disease (19,21,22,23,24).

One additional anatomical limitation of the young infant lower respiratory tree is the absence of the pores of Kohn (the interconnections between the alveolar spaces) which increases the likelihood of wheezing and atelectasis (25). These limitations

taken together mean that the functional residual capacity (FRC) of the infant and young child airway is less than that of the older child. Therefore, even apparently small disruptions in ventilation can result in a severe decline in the partial pressure of arterial oxygen (PaO2)(18). There are key clinical signs that correlate well with compromised ventilation and are used to gauge and rate the severity of obstruction during ALRI.

Hypoxaemia A study among Kenyan infants presenting to hospital with ALRI aged 7 days to 36 months reported that 59% of the infants were hypoxaemic, defined as arterial oxygen saturation ≤ 90% by pulseoximetry(26). All of the cyanotic patients were hypoxaemic but only 11% of the hypoxaemic patients had cyanosis. Infants with hypoxaemia were 4.3 times more likely to die than were comparable infants without hypoxaemia, and that most of these deaths occurred within 48 hours of admission if hypoxaemia was not corrected. Furthermore, hypoxaemia on admission predicted short-term hospital mortality with 90% sensitivity and 34% specificity. Finally, a multiple logistic regression model using hypoxaemia as the outcome found the greatest correlation with tachypnoea (≥ 70/min in children 3-11 months and ≥ 60/min in children > 12 months) as the clinical marker in all age groups. However, in children 3-11 months the model also included retractions and grunting.

Tachypnoea Several studies have shown a correlation between tachypnoea and ALRI. Two reported that infants under 11 months of age were almost twice as likely to have pneumonia if they had a respiratory rate (RR) ≥ 50/min (19,27,28,29). Another reported that infants < 8 weeks old were eight times as likely to have pneumonia with a respiratory rate of ≥ 60/min (19,30). Others have reported similar findings in older infants and children (19), generally indicating a positive trend between the likelihood of radiographically diagnosed pneumonia and the degree of tachypnoea. The standard criteria are RR > 50 for children under 12 months and RR > 40 for children ≥ 12 months(31), which is accepted by WHO(32).

Work of Breathing and Chest Indrawing The severity of ALRI correlates positively with an infant's work of breathing (19,20,26,31,32). Grunting, nasal flaring and the use of accessory muscles resulting in retractions or chest indrawing are determinants of respiratory effort. All of these signs are associated with severe disease (19,20,32). Nasal flaring, mediated by constriction of anterior and posterior dilators naris muscles, is simple dilation of both nares. Grunting is a repetitive short upper respiratory tract sound mediated by partial closure of the vocal cord during expiration (33). This prevents lung collapse by slowing the rate of expiratory flow, thereby increasing lung volume and alveolar pressure (24). It often signals impending respiratory failure (18,19). Abnormal chest movement includes both retractions and chest indrawing. Retractions are indrawing of the soft tissues, and can occur in the supraclavicular, intercostal and subcostal areas of the chest wall(49). While the correlation of soft tissue retractions with ALRI severity is debated, there is no dispute that chest indrawing is a sign of severe obstruction (18,19,20,24,26,31,32). As explained above, the peculiarities of infant and young child chest wall structure largely restricts chest indrawing, defined as an inward movement of the lower chest wall during inspiration, to children below 24 months of age (19). The mechanism for this paradoxical movement is the traction applied by the abnormally depressed diaphragm to the supple infant chest wall. As the diaphragm is contracts during inspiration, it descends further, drawing the chest wall (i.e., the costal margin) inward. In some infants this supplies enough pressure to the abdomen to force it outward during inspiration. This abnormal movement is variably referred to as "Hoover sign", "paradoxical movement" and "seesawing"(19).

Ability to Feed Studies have shown that many infants who are in respiratory distress (i.e., with tachypnoea, grunting, retractions or chest indrawing) have reduced food intake (20,32). Frank inability to feed helps to distinguish very severe from severe pneumonia(32) and is one of the criteria for hospitalisation in children with ALRI(20), and its correction is a precondition for discharge to home. The concomitant dehydration and acidosis it precipitates also impairs the effectiveness of antibiotics by lowering the pH of the environment. Therefore, correction of dehydration and the ability to sustain an euvolaemic state have a direct effect on patient management and recovery. Moreover, the catabolic state associated with systemic illness further diminishes the capacity for convalescence and can predispose to subsequent infection. Therefore, feeding has palliative, therapeutic and disease prevention benefits.

Reactive Airways Disease and ALRI Reactive airways disease (asthma) is now defined as a chronic inflammatory disorder of the airways involving many cell types and cellular moieties, including mast cells, eosinophils, T lymphocytes, neutrophils, and epithelial cells (20). Evidence now suggests that in susceptible individuals inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly during the night and early morning. These inflammatory episodes are commonly associated with variable airway obstruction that is often reversible (wholly or partially) either spontaneously or with treatment. It has been recognised that reactive airways disease (RAD) predisposes to pneumonia (26), and that respiratory infections, particularly viral ones, trigger exacerbations of reactive airways disease and lead to subsequent development of RAD (18,20,21). Underdiagnosis of RAD and inappropriate therapies are recognised as problems and these have a major effect on asthma morbidity and mortality (20), which may be more likely in this young population. In addition to underdiagnosis, misclassification between RAD and ALRI is a problem (20,22,34).

This new definition of RAD underscores the potential for such misclassification. Both diseases share a common pathophysiology to their clinical expression, a cellular immune response to an offending agent (infectious or non-infectious) resulting in inflammation, which in turn causes intrinsic airway narrowing (35). In ALRI this progresses to interstitial infiltrates and parenchymal (alveolar) consolidation. Not only can the diseases be mediated by the same agents, the two diseases may co-exist (20,21,26). Indeed, recent data from Matlab indicate that 54% of all infants under two years of age hospitalised for ALRI had concomitant wheezing (M Yunus, non-published data). The majority of these infants had RSV infection, which is recognised as an

important risk factor for both ALRI and RAD in this age group (21). This has important implications both for patient management and outcome, for although the two diseases share some important pathophysiology, their treatments can be substantially different, even antagonistic. The immunosuppressive effect of systemic corticosteroid therapy is routinely exploited in the management of RAD episodes (36,37), but is generally contraindicated in the presence of infection (38). Since the two diseases often co-exist in the under-two year old population, or at least may be mistaken, we need to know the effect of new treatment modalities on both.

Other Risk Factors

Age Studies have demonstrated an inverse relationship between age and the incidence and severity of ALRI (2,8). A community-based study in the Philippines, using multivariate analysis, demonstrated that age less than two years was the dominant risk factor (39).

Children under one year of age are at the greatest risk of death from ARI, accounting for 63% of all ARI-related fatalities(40,41,42). In rural Bangladesh, infants five months old and younger had the highest mortality rate attributable to ARI (136/1000)(43). Half of all fatal outcomes represented infants below six months of age. Mortality data from our hospital indicate that 57% of all deaths occur in children under one year of age, while 80% are under two years of age (Figures 1 and 2).

Figure 1.

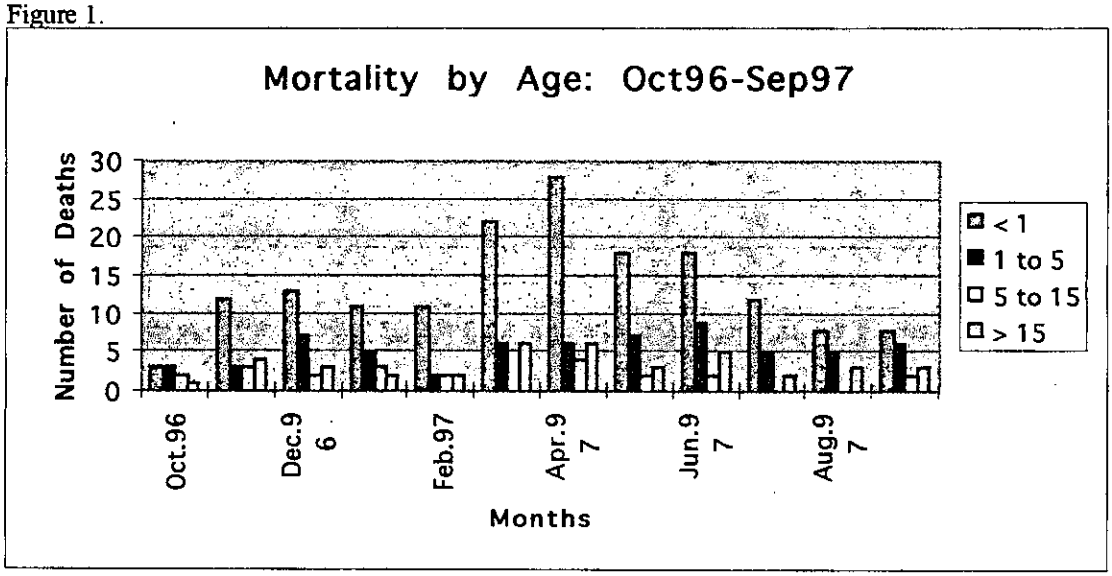
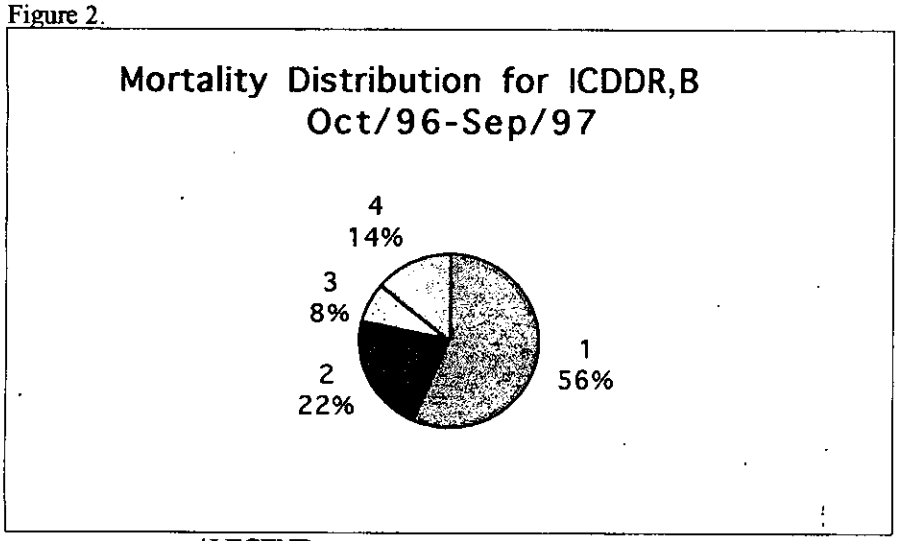


Figure 2.



*LEGEND
Group 1 = < 1 year old
Group 2 = 1 - 5 year old
Group 3 = 5 - 15 year old
Group 4 = > 15 year old

Cellular Immunity and Malnutrition

There are data on the interaction between nutritional status and immunity. In one study, 56% of the deaths among children 6 to 59 months old were attributed to malnutrition, of which 83% was of the mild-moderate form (44). Recent studies have reported a link between even sub-clinical malnutrition and impaired cellular immunity, and indicate that impaired cellular immunity is a risk factor for development of ALRI (45) and diarrhoeal disease(46,47). Several studies have demonstrated an association between malnutrition and the incidence and even the severity of pneumonia (45,48,49). Malnutrition appears to increase the risk of pneumonia-related mortality (50,51). One study reported a positive correlation between the risk of ALRI-related death and the severity of malnutrition. The likelihood of mortality for first, second and third degree malnutrition was 4.4, 11.3 and 27.0, respectively, compared to children without malnutrition (8). Other studies have reported similar increases in mortality risk for malnourished children (52,53).

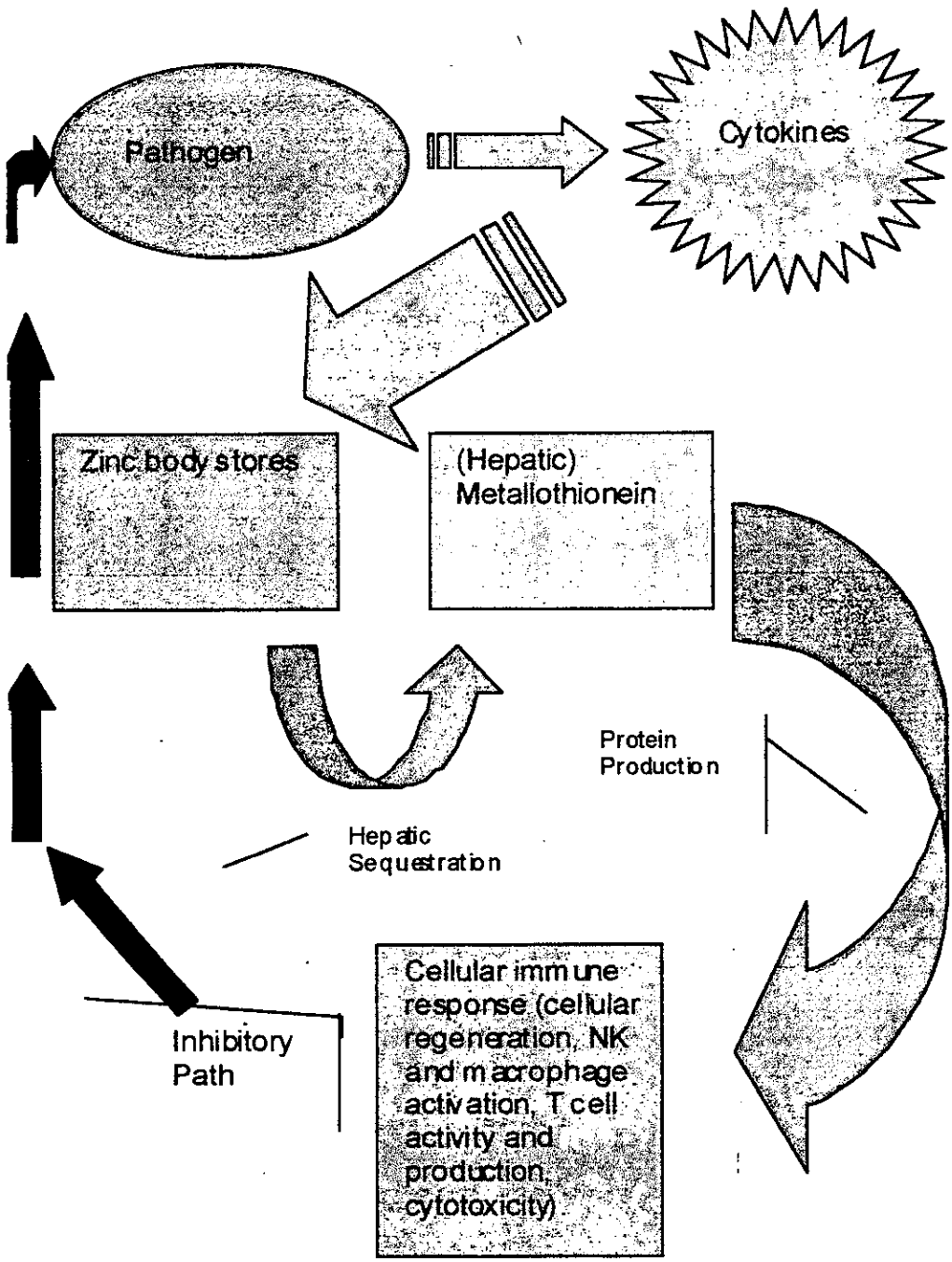
Zinc, Immunity and Morbidity

If there is a link between malnutrition and disease severity, what is the specific immunological lesion? Current evidence on the interaction between nutritional status, immunity and disease has highlighted cellular immunity (45), which appears to be compromised in malnourished children (45,49,54). This may be due, in part, to zinc deficiency since the deficit is associated with impaired cellular immunity (45,55) and correction of zinc deficiency is associated with a reduction in the incidence and prevalence of diarrhoeal disease (54,56,57,58,59,60), and a significant reduction in the incidence of respiratory infection (49,55). Indeed, there is even evidence of a correlation between the severity of malnutrition and the magnitude of the zinc effect on persistent diarrhoea (61). This effect on ALRI, albeit preventive, points to an association between ALRI and zinc. Is it reasonable to expect that zinc should have acute effects on ALRI?

Evidence suggests that zinc is part of the acute phase response to host response to stress, trauma, inflammation and infection (62,63). This response includes a significant decrease in serum zinc concentration and an internal redistribution of total body zinc stores, consisting largely of hepatic sequestration (63,64,65). There is a two-fold purpose to the acute phase response (62,63,64,65). First, it is to deprive bacteria of essential minerals for bacterial growth. Second, it is to reprioritise hepatic protein synthesis to optimise the host's ability to fight infection. Cytokines, such as TNF-α and IL-6 appear to mediate the redistribution of zinc to the intracellular zinc binding protein, metallothionein, which causes a drop in zinc serum levels and an increase of zinc in metallothionein-rich organs, such as the liver (63,65). The effect is rapid, and significant decreases in serum levels of zinc occur within 6 hours of exposure to endotoxin (lipopolysaccharide) in human volunteers (63). The cellular immune effects of zinc, mediated by its interaction with metallothionein, are inferred from immunological deficits inversely correlated with zinc stores. These deficits include: (i) impaired cell regeneration; (ii) decreased T- lymphocytes, and abnormal T-helper and/or suppressor cell function (66), (iii) impaired macrophage function (67), which depresses delayed hypersensitivity and; (vi) reduced natural killer cells and antibody dependent cytotoxicity (47). Combined, these data provide evidence for a plausible role for zinc as part of an acute phase response to infection. This response may be blunted in zinc deficient children, compromising host resistance. The data also allow us to construct a plausible model to explain why supplementation of zinc to zinc deficient infants and young children during the acute phase of disease should mitigate those clinical signs associated with severe infection. By promoting a more robust cellular immune response early on in the disease process, the extent or severity of infection may be lessened, therefore promoting a more robust cellular immune response early on in the disease process may also lessen the clinical signs associated with more severe infection. Furthermore, a more robust cellular immune response may result in a shorter duration of illness (Figure 3 next page).

Figure 3.

Acute Phase Host Response



Research Design and Methods

DESIGN

Study Design: Double blind randomised placebo controlled clinical trial.

1. To test Specific Aim 1: *Rationale*. These signs are risk factors for respiratory failure. Thus, they are markers for severe disease. *Protocol*. Baseline Assessment and Consent. Infants who are referred to ICDDR,B Matlab hospital through the ALRI surveillance programme for severe disease will be admitted into the ALRI unit of the hospital. The medical officer or one of the primary investigators will perform a physical exam detailing respiratory rate, effort, the presence of cyanosis, mental status, and findings on auscultation (crepitations, wheezing). Baseline arterial PaO2 will be measured by pulseoximeter. Infants with a RR > 50, crepitations and fever (temperature ≥ 38° C) will be diagnosed with *pneumonia*. Infants with pneumonia who have *any one* of the danger signs (chest indrawing, cyanosis, inability to feed or lethargy) will be diagnosed as having *severe*

pneumonia. Only infants with severe pneumonia will be eligible for the study, if the parents or caretakers give informed written consent. Baseline information on the history of the present illness, including duration, signs and feeding behaviour, will be obtained. Enrolled patients will have 1.0 ml of blood drawn for culture and 1.0 ml for complete blood count, as part of the surveillance programme, and 1.0 ml for baseline serum zinc for a total of 3.0 ml of blood. A chest X-ray will also be done.

Randomisation. (Table 1.) Infants will then be randomised to receive either 20 mg/day + BID (10mg elemental zinc/5 ml twice daily) of zinc as zinc gluconate or placebo (5 ml).

Management. A 20 mg (10 ml) PO zinc dose (or placebo) will be administered at the time of enrolment and within one hour of the first dose of antibiotics. Thereafter, it will be administered as a divided dose (10 mg or 5 ml) one hour before breakfast each morning and two hours after dinner each evening. Data on all of the diagnostic criteria (RR, the danger signs, and fever) as well as hypoxaemia and wheezing, will be collected thrice daily at the beginning of each eight-hourly nursing shift. Clinical management of ALRI will follow hospital routine with some modification of monitoring and oxygen administration (see Methods). Zinc supplementation and intravenous antibiotics will continue until the patient meets the criteria for recovery (see Methods). Infants will be discharged from hospital on oral antibiotics, at the discretion of the medical officer, after they have met the criteria for recovery. Blood will be drawn (1 ml) for serum zinc before discharge. Follow-up. Infants will continue to be monitored by the community surveillance system after their discharge from hospital.

Data. Our outcomes will be treatment effects. *Specific Aim 1* will generate Numerical Data: 1) Mean respiratory rate per eight-hour shift, 2) Mean PaO2 per eight-hour shift, 3) Temperature. Analysis of treatment groups can be compared by analysis of variance (ANOVA), with generation of 95% confidence intervals. Normality will be confirmed by plotting the residuals. If normality is not present, data will be analysed by non-parametric tests (Kruskal-Wallis test). Categorical Data. (See Methods for definitions.) 1. *Feeding status* (a. unable to feed, b. feeding spontaneously), 2. *Respiratory effort* (a. Chest indrawing; b. Retractions; c. Non-laboured). 3. *Appearance* (a. Cyanotic b. Non-cyanotic). 4. *Mental status* (a. Comatose/Lethargic; b. Less active; c. Alert). We will analyse these qualitatively ordered data by Chi square test for trend.

Difficulties and Alternatives. 1) Fixed vs per kg dose. A dose based on weight would ensure that every infant received the same exposure. The principal reason for selecting a fixed dose is for ease of programmatic implementation in a developing country primary care setting. This follows the practice of WHO in its intervention programmes, such as vitamin A or IMCI. Simplifying the dose regimen may enhance effectiveness. Available data suggest that the infants will only absorb a fixed proportion of ingested zinc, and that this will be determined by physiological requirement (69, 70, 71). 2) Misclassification of *abnormal breath sounds* (crepitations vs wheezing), *work of breathing* (retractions vs chest indrawing) and *feeding* categories is very likely to occur during data collection unless we can develop simple diagnostic criteria that are precise and accurate during our training and standardisation period. One solution is to ensure that project staff have experience in evaluating these clinical signs in young infants. The definitions of these clinical signs cited in *Background* will be used our training material. A paediatrician will be the project reference or "gold standard". 3) Information loss. Collection of data is periodic. If we find that patients' conditions change markedly within eight hour observation periods we can: a) shorten the intervals of observation and/or; b) transform these continuous variables into categorical ones during analysis.

Table 1.

Zinc gluconate 20 mg/day PO	Placebo
135	135

2. To test Specific Aim 2: *Rationale*. The duration of severe signs has an effect on respiratory failure. Additionally, the total duration of illness has other direct costs (hospital fees) and indirect costs (loss of income earning time, time of surrogate caretakers to care for other children left at home).

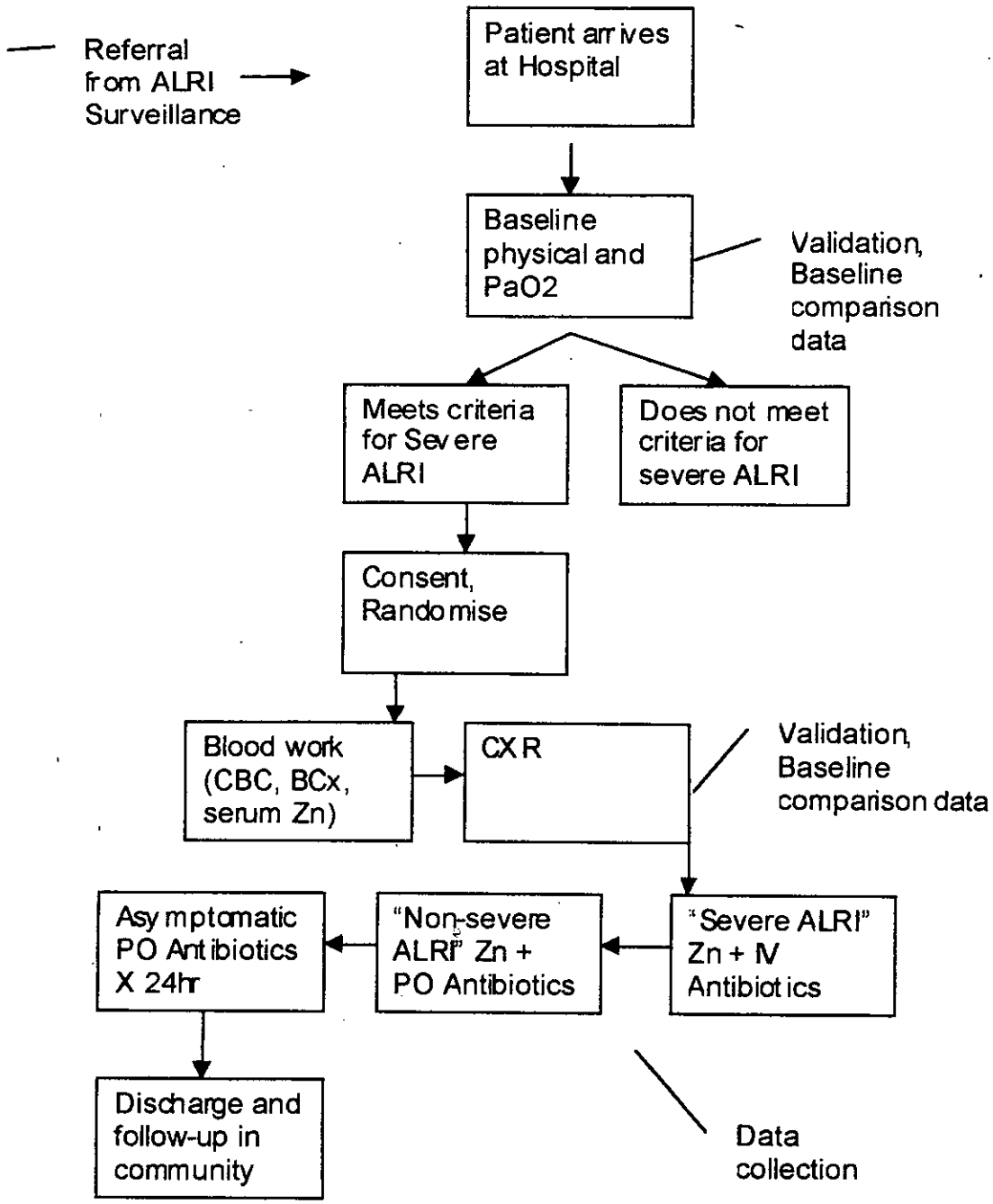
Protocol. Recruitment, patient management, data collection and decision to discharge will be as outlined above. The duration of each of the severe signs will be measured thrice daily, at the beginning of each eight-hourly nursing shift. The duration of each severe sign will be from the time of enrolment to the last 8-hour shift, inclusively, for which the patient is noted to have that sign or recorded value. The duration of severe pneumonia will be from the time of enrolment to the last 8-hour period, inclusively, for which the patient meets the criteria for severe pneumonia.

Data. Specific Aim 2 will generate Continuous Numerical Data: 1) Duration of tachypnoea (RR > 50) measured in hours; 2) duration of hypoxia measured in hours; 3) duration of each of the signs of severity, measured in hours. Analysis: Kaplan-Meier survival curve. Treatment and placebo group data will be compared by logrank test, to test the null hypothesis that the groups are the same with respect to duration of illness and hospital stay. Hazard ratio will be performed to compare the relative duration of illness times and time in hospital between groups. Modelling will be performed by Cox Regression. The interval between the enrolment and recovery times will be compared between treatment groups using ANOVA.

Difficulties and Alternatives. Two caretaker behaviours will adversely affect this outcome; "absconding" (leaving against medical advice) and failing to leave when discharged, usually for logistical reasons. In the first case, data from patients who "abscond" will be treated as censored in continuous data (Kaplan-Meier) analysis. Other data will also be retained in the analysis and the total number of absconding patients will be reported as withdrawals and will be compared by treatment group using ANOVA. An attempt will be made to follow up these patients through the network of community health workers in Matlab and categorise their reasons for withdrawing from the study. We will compare these as categorical variables by treatment group. In the case of the latter, the date and time of the decision to discharge will be entered into the analysis.

3. To test Secondary Aim 1: *Rationale.* Hypoxaemia is a risk factor for respiratory failure and death. Interventions that could lessen its severity or duration should improve outcomes in the management of severe pneumonia. *Protocol.* Hypoxaemia will be surveilled at the time of enrolment, as described above. Patients will have their arterial PaO2 measured by trained staff using pulseoximetry. Thereafter, PaO2 will be checked at the beginning of each eight-hourly nursing shift. *Severe hypoxaemia* will be defined as a PaO2 < 90% on room air, *Moderate* 92% - 90% (inclusively). *Data.* The severity and duration will be analysed as outlined above for continuous data. *Difficulties and Alternatives.* 1) Missing data. As with the other periodic measurements, if the intervals of assessment are too great, we can collect data at more frequent intervals. 2) Inappropriate definition. We will collect data on several children who not have pneumonia and compare our definition of hypoxaemia with the local standard, and make any necessary adjustments.

4. Design summary. Figure 4.



TIMELINE

- The study is projected to last for 12 months. The ALRI unit averages about 50 patients per month, in the required age group. Assuming a refusal rate of 10%, it would take no more than 10 months to complete the data collection, and could take less. Laboratory processing of samples would proceed in parallel. Analysis would begin no later than month 11. Write-up would be completed by the end of month 12.

Table 2.

Task	Time	In	Months				
Training	X						
Data Collection		X	X	X	X		
Data entry		X	X	X	X		
Laboratory Analysis			X	X	X		
Analysis						X	
Write-up						X	X
	1	2-3	4-5	6-7	8-9	10-11	12

METHODS

List of Contents

1. Definitions (Diagnoses)
2. Inclusion, exclusion criteria
3. Sample size calculation
4. Population and study site
5. Randomisation
6. Clinical management
7. Laboratory
8. Quality assurance
9. Consent procedures

Definitions

Severe pneumonia (Signs). Four criteria: **1)** Tachypnoea (respiratory rate > 50/min); **2)** *Any one* of the "danger signs": chest indrawing, central cyanosis, inability to feed, lethargy; **3)** Fever (temperature ≥ 38° C), **4)** Crepitations on auscultation. Infants with concomitant wheezing will fit the definition. Wheezing without the other abnormal auscultatory findings will not fit the definition.

Recovery Three criteria for **24 consecutive hours**: **1)** Respiratory rate ≤ 40/min; **2)** No danger signs (i.e. signs of sever pneumonia); **3)** No fever (temperature < 38° C).

Severe hypoxaemia PaO2 < 90% on room air.

Moderate hypoxaemia PaO2 90 – 92%, inclusively.

Auscultation (abnormal)

Crepitations **Short** crackling noises on **inspiration** (from the Latin *crepo* "to rattle") resembling the sound of rubbing hairs between the fingers. They may also be heard on expiration in more severe cases.

Wheezing. **Long** high-pitched whistling or musical sound on **expiration** (also on inspiration with severe RAD) (23).

Neurological signs

Alert Spontaneous age-appropriate behaviour.

Less Active Lack of spontaneity, but age appropriate behaviour.

Lethargic Responds to verbal stimuli with age-specific appropriate or inappropriate behaviour.

Time frames

Duration of illness The time and date of enrolment to the time and date of the last 8 hour shift for which the patient criteria for severe pneumonia.

Feeding

Unable to Feed will be recorded for any infant who requires any portion of daily maintenance feeds to be supplemented by IV or nasogastric tube in order to maintain adequate hydration.

Spontaneous Feeding will be recorded for any infant who receives all daily maintenance feeds by mouth.

Inclusion Criteria

Infants 2 – 23 months old, referred by the Matlab ALRI surveillance system to the Matlab hospital who meet our definition of severe ALRI, whose caretakers provide written consent.

Exclusion criteria

Infants who have a history of chronic heart or renal disease, active measles, severe malnutrition requiring separate medical attention, signs of systemic illness (sepsis, meningitis), those presently receiving zinc supplements, or whose caretakers withhold consent.

Sample size calculations

All calculations assume the probability of a Type I error of 5% and a Type II error of 10% (i.e., Power of 80%). The most recent data from Matlab Hospital (Yunus, non-published data) indicate that the following percentages of infants aged 14.6 months, SD 12.7, had the following symptoms for two days: chest indrawing 94%; tachypnoea 98%; inability to feed 70%; wheezing 54%. The duration of illness was 5.7 days, SD 5. These findings were based on 639 illnesses observed in 526 children.

In order to detect a 30% decrease in the duration of:

- Chest indrawing, we need 38 infants per group.
- Tachypnoea, 31 per group.
- Feeding, 94 per group.
- Duration, 135 per group.
- Wheezing, 160 per group.

Wheezing will not be a main outcome. Therefore, we need to recruit 135 infants in each group for a total study population of 270 infants.

Population and study site

Matlab is located 55 km south-east of Dhaka in the Ganges-Meghna delta. In order to evaluate new intervention strategies an integrated health services and research programme has been created that provides clinical services through four health sub-centres, three community treatment centres and a primary care hospital. These provide services to a target population of 100,000. The hospital is a 70-bed inpatient facility with a 10-bed ALRI unit. In 1996 the unit treated 427 paediatric patients for severe ALRI. For 1997 the figures declined slightly to 403. Recent data indicate that the admissions have increased for this year.

Randomisation

We will use *fixed randomisation*. Allocation of study subjects will be uniform, as the assignment probabilities for test treatments and control groups are equal, according to the formula:

$$P1 = P2 = \ldots = Pi = \ldots = Pt+1$$

Where Pi, I = 1, ..., t, denote assignment probabilities for the t test treatment and Pt+1 denotes the assignment probability for the control group, and where ${}^{(t+1)}\sum_{(i-1)}$ Pi = 1. Randomisation will be achieved by permuted blocks of variable length, where the smallest block size will be equal to the sum of integers of the allocation ratio, according to the formula ${}^{(t+1)}\sum_{(i-1)} ri = B$, where B is the minimum block size. As there are two treatment groups, B = 2. Thus, the range of block sizes will be 2 to 8. The larger the block the greater the likelihood of departure from the allocation ratio, so blocks greater than 4 times the number of treatment cells will be avoided (68). The block sizes used for the construction of the allocation schedule will not be divulged to the project personnel until after the trial to avoid unmasking.

Preparation of Zinc Syrup

Design of treatment groups

Infants will be randomised to one of two groups (TABLE 1). Group A will receive 10 mg of elemental zinc twice daily for a total of 20 mg/day and Group B will receive placebo twice daily. The samples will be mixed and stored in bottles, for a total of 270 bottles.

Requirements for zinc and placebo

1. Zinc gluconate for Group A (20 mg ÷ BID):

2. Concentration 2 mg/ml. Dose is 10 mg/5 ml. Total = 20 doses maximum per infant. Volume = 100 ml.

3. Placebo for Group B 5 ml per dose for total of 20 doses. Volume = 100 ml.

4. Both groups will receive one 100 ml bottle that will be kept by the infant's bedside.

Special instructions

1. Supplement and placebo have to be identical in taste, appearance, and smell. The taste of the zinc needs to be masked as well (being given to young infants 2 –24 months).

2. To dissolve the zinc gluconate, any base substance normally used for vitamin supplements can be used, but must be vitamin-free.

3. Taste may be improved by non-nutritious substances only (flavours, sucrose).

4. Any preservative normally used in vitamin syrups may be used to maintain shelf-life up to 12 months.

5. Bottles must have a measurement scale attached to show amount for each dose.

Clinical Management

All enrolled patients will be treated with ampicillin (200-400 mg/kg/day ÷ Q6° IV) and gentamicin (6-7.5 mg/kg/day ÷ Q8° IV) for their ALRI, which is the standard starting regimen at this hospital. Serum gentamicin levels will be monitored according to hospital routine. Supplemental humidified 40% oxygen will be given by nasal prongs for PaO2 < 95% on room air. Supplemental oxygen will be increased to 100% by mask if the PaO2 remains < 92% on nasal prongs or if the infant is centrally cyanotic. Clinical parameters of respiratory rate, effort, feeding and wheezing, as well as appearance (cyanosis) and mental status (lethargy) will be recorded every six hours. Patients who fail to improve by the end of 48 hours of antibiotics (by the eighth dose of ampicillin) or whose condition worsens will have their antibiotic changed to a third generation cephalosporin (ceftriaxone). When signs of severity have been absent for 24 hours, the IV antibiotics will be changed to oral (PO). Infants with no sign of respiratory distress after 24 hours of PO antibiotics will be discharged to home.

Laboratory Procedures

ICDDR,B Hospital, Dhaka, has a modern medical laboratory and routinely performs standard haematological tests, such as haemoglobin, haematocrit, white blood cell count and differential count according to internationally established methods. Samples will be collected by butterfly needle into polypropylene syringes, then injected into trace element-free polypropylene tubes using 18 – 22 gauge needles to minimise zinc contamination.

Zinc Assay. Samples (150 µl) in polypropylene tubes are diluted 1:12 with HNO3 and Briiz-35. Zinc is determined by flame atomic absorption spectrophotometry (AAS) using standard solutions prepared in the same matrix. AAS is equipped with nebulizer assembly (Shimadzu) and C2H2/air burner. Zinc hollow-cathode lamp is operated with background correction wavelength of 213 nm. Determination of zinc is done by aspiration of a series of working standards (at least 4) and a standard curve is prepared for each lot by plotting the concentration (mg/l) versus absorbance using software provided by the manufacturer. Two quality control sera (low and high range) are run with each lot and if any value of these quality control samples is not within the acceptable range, the result is ignored and the whole lot is repeated.

Quality Assurance

Periodic review and testing of staff on the study definitions and diagnostic criteria will be done and the degree of inter-observer variation will be quantified. A manual with the definitions and protocol will be kept in the ALRI unit of the hospital. The percentage of agreement and kappa statistic will be calculated to assess inter-observer variation. The principal investigators will make spot checks in the unit three times per week.

Consent Procedures

Consent will be obtained using a standardised form prior to enrolment in the study. Parents will be advised that their children's participation in this experimental study is voluntary and that they may withdraw their children from the study at any time, and that as new information becomes available that might affect this study, it will be made available to them. (SEE APPENDIX.)

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. (TYPE WITHIN THE PROVIDED SPACE).

SEE METHODS (POPULATION AND STUDY SITE).

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. **(TYPE WITHIN THE PROVIDED SPACE).**

SEE DESIGN. DETAILS OF DATA ANALYSIS ARE PROVIDED WITH FOR ITEMISED STUDY AIM.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

GOALS

Previous studies have demonstrated a reduction in the incidence of ALRI with zinc supplements (48,49), thus establishing a link between zinc and ALRI. Our study differs in two respects. First, we seek to investigate an acute treatment effect on the major risk factors associated with adverse outcome, which is a follow-up of the finding that case management improves outcome (50). Additionally, if there is an acute treatment effect, we seek to determine if it is additive or synergistic when combined with early diagnosis and antibiotic therapy. It will be left to future studies to determine whether or not this intervention has any effect on case fatality or total child mortality. Second, we wish to investigate the acute effect of zinc on pneumonia specifically, as opposed to ALRI, which includes laryngitis, bronchitis and bronchiolitis. Pneumonia carries a much higher risk of mortality than these other diseases. It is important to quantify its specific effects on pneumonia, not only because pneumonia accounts for a high burden in morbidity and mortality, but also because present therapies are not always successful. This is because there is no therapy for most viral infections, and bacterial infections are prone to antimicrobial resistance. Moreover, the specific pathogen and its susceptibilities are generally not known, which can further delay appropriate therapy and compromise case management.

Details of Consent and privacy are provided in the attached consent form (Appendices).

~~We do not believe that there are any added risks to the study subjects (see attached response to reviewers (Reviewer 1, response~~ 2; Reviewer 2, responses 5, 7). The antibiotic treatment and management will be the same between groups, and will reflect the best practices of the standard of care for severe pneumonia. The potential benefits are a reduction in the severity and duration of pneumonia, as described in the protocol.

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

NA.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

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Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

The results will be published in a major peer-reviewed journal for global consumption by the international scientific and professional health provider communities.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. **(DO NOT EXCEED ONE PAGE)**

N/A.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	**Position**	**Date of Birth**

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	**Degree**	**Year**	**Field of Study**

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

Bibliography

SEE ATTACHED CV.

APPENDIX

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

See attached consent form

Signature of Investigator/ or agents
Date:

Signature of Subject/ Guardian
Date:

Continuation Sheet (Number each sheet consecutively)

PI: W. Abdullah Brooks, MD, MPH: Efficacy Zinc Acute Management ALRI < 2 y/o

Budget for 1 year	Name	Pay Level	No. of Staff	% Effort	Mo'ly Rate	Man/ month	US $ Subtotal
Personnel							
3122	Dr. Md Yunus	Co-PI	1	0.20	1,688.00	12	4,051
3121	Research Medical Officer	No-A	1	1.00	632.00	12	7,584
3121	Nurse	GS5	1	1.00	347.00	12	4,164
3121	Data analysis		1	1.00	832.00	2	1,664
3121	Secretarial		1	0.20	510.00	12	1,224
3121	Data Entry Technician	GS3	1	1.00	224.00	12	2,688
	Subtotal						**$21,376**
Travel							
	Domestic						2080
	Subtotal						**$2,080**
Supplies and Materials							
3701	Drugs						1,000
3704	Office supplies						500
	Subtotal						**$1,500**
Lab. Test and Other							
4809	Serum Zinc						1,608
4810	Chest X-ray						582
4813	Hospitalisation costs						19,296
4817	Internet						200
4821	Library Service Charge						250
4806	Photocopying						500
	Subtotal						**$22,436**
Others							
3900	Fax, DHL, Phone						300
4000	Training						200
4300	Print & Publication						500
4500	Misc						1500
	Subtotoal						**$2,500**
	Total Direct						**$49,892**
	Total Indirect (25%)						**$12,473**
	Total Costs						**$62,365**

S. Moin
18/8/98

Shamima Moin
Controller, Budget & Costing

Detailed Budget for New Proposal

~~SEE ATTACHED SIGNED BUDGET.~~

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

Budget Justification

1. **Dr. Yunus** runs the Matlab Hospital and has experience in ALRI-related surveillance and studies. He will assist with project supervision and personnel training. He will contribute the equivalent of 1 day per 5-day workweek, or 20% time. His annual salary is US $20,256. Thus, 0.2 * 20,256 = US $4,051.
2. **Research Medical Officer** is needed fulltime. This person will be dedicated to the project to recruit patients, guarantee fulfilment of study criteria, obtain consent, conduct physical exams and take a history, draw blood, monitor patient progress and make clinical judgements about recovery, while working independently. Therefore, the person must be at least at the NO-A level. The NO-A monthly salary is US $ 632. For one full year the salary is US $7,584.
3. **Nurse** is needed fulltime to oversee and ensure appropriate level care is given to study subjects, blood work and supplemental care is provided in systematic and timely fashion to avoid significant inter-patient differences in quality of care, to train non-dedicated nurses in basic pneumonia supportive management such as pulseoximetry, and to monitor patient progress with the medical officer. The nurse must be capable of working independently and supervising other nurses. Therefore, the nurse must be at least at the GS5 level. The monthly salary is US $347. For one full year the salary is US $4,164.
4. **Data analysis** is needed for two weeks at the beginning of the study and for six weeks at the end. The initial period is necessary in order to validate our questionnaire and to ensure the adequacy of our data management. The second period is to conduct the data analysis. The monthly salary of a programmer is US $832, which for two months is US $1,664.
5. **Secretarial** support is a requirement of the Clinical Sciences Division.
6. **Travel expenses** are based on transportation and lodging costs between Dhaka and Matlab for the PI. The PI will spend 2 weeks (14 days) at Matlab at the start of the trial to train the staff and oversee the initiation of the study. During the first two months of the trial, the PI will visit the site once weekly, with one overnight stay (8 days). During the remaining 10 months, the PI will visit once monthly (10 days). The cost is US $65 per day for a total of 32 days, equalling US $2,080.
7. **Supplies** of drugs are based on estimates of the cost of zinc from ACME and Opsonin, and syrup-related expenses such as measuring cups and teaspoons, and trace element-free serum collection tubes (approximately US $0.25 each * 2 per patient * 270 patients = US $135). Office supplies are estimated at about US $40 per month for a total of US $500 in 12 months.
8. **Serum Zinc** is based on the actual cost of the spectrophotometric test at US $3.00 per test for 2 test per 270 patients for a total of US $1,620.
9. **Chest X-rays** cost US $2.15 per 270 patients for a total of US $582.
10. **Hospitalisation costs** are set at US $12 per 270 patients averaging 6 days of stay for a total of US $19,440.
11. **Internet** costs related to the project are predicted to run about US $16 per month for a total of US $200.
12. **Library service charges** for retrieval and photocopying will average US $40 per month. Most use will be at the time of manuscript preparation.
13. **Photocopying charges** will cover the costs of printing questionnaires and training materials at US $0.10/page. The questionnaires will be six pages, plus an average of three pages per patient for daily records for a total of US $243. Training materials will cost about US $50 for developing and copying. The remaining US $200 will be applied to miscellaneous project-related copying and printing expenses averaging US $16 per month.
14. **Fax, DHL, Phone charges** are estimates assuming US $12.50 per month for project-related faxes and phone calls, plus 3 DHL packages at US $50 each for the 12-month period.

15. **Training** costs are estimates of costs for training videos (US $70) and VCR rentals for teaching personnel pneumonia assessment.
16. **Print and publication** includes the cost for manuscript production and publication, as well as project-related printing costs such as inkjet printer cartridges (approximately US $30 each).
17. **Miscellaneous** expenses include costs for ALRI timers, paediatric probes for pulseoximetry (US $115 per box), thermometer covers for electronic thermometers (US $49 per box), and unforeseen project-related expenses.
18. **Indirect costs** are at 25% since the funding source is USAID via Johns Hopkins.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

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1. Protocol 98-006 – Received. Duration 1 year (September 1998 – September 1999).
   1.1. Johns Hopkins University (USAID). US $13,355.
2. Protocol 98-020 – Under consideration. Duration 2 years (1998 – 2000)
   2.1. Swiss Development Corporation. US $50,000
   2.2. CHR (Targeted Research) US $65,000
   2.3. Johns Hopkins University. US $115,120 Project Total US $230,120

Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included ☐
2. Approval of the Division Director on Face Sheet ☐
3. Certification and Signature of PI on Face Sheet, #9 and #10 ☐
4. Table on Contents ☐
5. Project Summary ☐
6. Literature Cited ☐
7. Biography of Investigators ☐
8. Ethical Assurance ☐
9. Consent Forms ☐
10. Detailed Budget ☐

EFFICACY OF ZINC IN THE TREATMENT OF PNEUMONIA IN HOSPITALISED INFANTS LESS THAN 2 YEARS OF AGE – Abstract

Summary

Rationale Acute lower respiratory tract infection is the leading cause of morbidity and mortality in children below two years of age. Studies have shown that early reversal of the signs associated with severe disease; hypoxia, tachypnoea and ability to feed, is an important prognostic factor during acute illness. *Hypothesis* Zinc deficiency is widespread among children in developing countries. As it is an important regulator of cellular immunity, zinc supplementation during the acute phase of respiratory illness could work synergistically with present WHO-recommended antibiotic regimens in promoting early reversal of signs associated with adverse outcome and shorten the total duration of clinical illness. *Protocol* We propose to conduct a placebo controlled clinical trial among all infants below 2 years of age admitted to ICDDR,B's hospital in Matlab. Infants will be randomised at the time of admission to receive either 20 mg elemental zinc per day or placebo, in addition to standard WHO-recommended antibiotic therapy. All infants will be supplemented for the duration of their hospitalisation. *Data* We will compare duration of hypoxia, tachypnoea, inability to feed, reversal of lower airway obstruction and total duration of hospitalisation between treatment groups. *Goal* We seek to enhance child survival by improving the acute management of respiratory illness with a simple adjuvant that can be used in conjunction with established antibiotic regimens.

1. Rationale for conducting the study in children less than 2 years of age is, as described above, because this comprises the most vulnerable age group to pneumonia morbidity and mortality. We need to study children with pneumonia to study the effects of zinc in the acute stage of the disease.

2. The potential risks include:
   2.1. The potential of infection from phlebotomy if sterile technique is not observed.
   2.2. Mild gastrointestinal distress from zinc (rare).

3. Minimising risks
   3.1. Infection likelihood will be minimised by using sterile technique in phlebotomy.
   3.2. Mild gastrointestinal distress will be monitored with daily routine physical exams, as well as observation of food intake.

4. Confining access of all study records to project staff will protect confidentiality. We will keep all study records in a locked file cabinet. Personal information will not be shared with anyone outside of the study staff, nor will personal identifiers be used or reported in the analysis or distribution of the data.

5. We will obtain signed informed consent at the time of randomisation after completion of a physical exam and ascertaining whether or not the patient has severe pneumonia and meets the study criteria (see Figure 4 p 15 of protocol and enclosed consent form). This will take place in the intake ward of the hospital. Parents will be informed by the study personnel that participation is voluntary and will not prevent the child from receiving the standard therapy provided for severe pneumonia.

6. The interview will be conducted in the ALRI unit of the hospital, which is a ten-bed facility. The duration of the interview will be approximately 10 minutes, not including the physical exam. It will focus on the history of the illness, previous illnesses, risk factors for severe disease, immunisation history and sociodemographic variables.

7. The potential benefits to the patient and family include:
   - 7.1. A shorter duration of illness
   - 7.2. Less severe illness, as defined in the protocol
   - 7.3. The potential benefits to society and the health community include:
     - 7.3.1. Adjuvants that can improve the early clinical management of severe pneumonia, in conjunction with appropriate antibiotic therapies.
     - 7.3.2. A reduction in the prevalence of severe pneumonia via a reduction in the duration of illness (secondary prevention).
     - 7.3.3. A better understanding of the specific factors associated with adverse outcome in severe pneumonia, e.g. hypoxaemia.
   - 7.4. That these benefits far outweigh the risks of bacteraemia from poor blood drawing technique or mild gastrointestinal distress needs no expatiation.

8. The study will require no records, organs, tissues or foetal material, but will require body fluids (blood) in order to assay serum zinc at both the beginning and end of the study for each patient.

Responses to Reviewers

Reviewer1

1. *Make the point of positive impact Zn in prevention trials*
   1.1. A statement has been added that the preventive effect demonstrates an association between zinc and ALRI in two places:
      1.1.1. Zinc immunity and morbidity p 8.
      1.1.2. Goals, p 10.

2. *Justify dose of 20 mg/day vs mg/kg (fixed dose).*
   2.1. In the Difficulties and Alternatives section on page 12, the protocol now states, "A dose based on weight would ensure that every infant received the same exposure. The principal reason for selecting a fixed dose is for ease of programmatic implementation in a developing country primary care setting. This follows the practice of WHO in its intervention programmes, such as vitamin A or IMCI. Simplifying the dose regimen may enhance effectiveness. Available data suggest that the infants will only absorb a fixed proportion of ingested zinc, and that this will be determined by physiological requirement." References are provided.

3. *Management 20mg/day needs to be clarified.*
   3.1. Page 12, Methods now states, "A 20 mg (10 ml) PO zinc dose (or placebo) will be administered at the time of enrolment and within one hour of the first dose of antibiotics. Thereafter, it will be administered as a divided dose (10 mg or 5 ml) one hour before breakfast each morning and two hours after dinner each evening".

Reviewer2

1. *Assumption sufficient Zn deficient children, not included are severe deficiency – clarify.*
   1.1. Two articles are cited that provide clinical and epidemiological evidence of high prevalence of zinc deficiency specifically among Bangladeshi children, references 45 and 46:
      1.1.1. Zaman K, Baqui AH, Yunus M, Sack RB, Chowdhury HR, Black RE. Malnutrition, cell-mediated immune deficiency and acute upper respiratory infections in rural Bangladeshi children. Acta Pædiatr 1997; 86:923-7.
      1.1.2. Baqui AH, Black RE, Sack RB, Chowdhury HR, Yunus M, Siddique AK. Malnutrition, cell-mediated immune deficiency and diarrhoea: A community-based longitudinal study in rural Bangladeshi children. Am J Epidemiol 1993;137:355-365.
   1.2. Zinc deficiency is not an exclusion criterion (see Exclusion Criteria p 16). Children will only be excluded if they have such severe malnutrition that they should be hospitalised for their nutritional status.

1.3. Therefore we believe that the majority of enrolled patients will be relatively zinc deficient and that we will be able to test our hypothesis.

2. *ALRI 8 – 9 episodes/child/yr (should be ARI). Clarify.*
   2.1. The reference to ALRI incidence is a mistake. The statement on page 3 Background has been corrected to read, "*It has been reported that the incidence of **ARI** is 8-9 episodes/child/yr during the first 2 years of life, dropping to 3-4 episodes/yr in 2-4 year olds (7). Thus, the susceptibility to ARI in children under two years is more than twice that of older children.*" ALRI incidence in Bangladesh is as stated in the protocol.

3. *Two citations, Sazawal et al 1998, and Sazawal and Black 1992. Explain what those studies found and how they are different.*
   3.1. We have added a discussion under the heading "Goals" (last paragraph p10) to address this question. It identifies two goals, and in so doing, underscores the essential differences between this and previous studies. *"Previous studies have demonstrated a reduction in the incidence of ALRI with zinc supplements (48,49), thus establishing a link between zinc and ALRI. Our study differs in two respects. First, we seek to investigate an acute treatment effect on the major risk factors associated with adverse outcome, which is a follow-up of the finding that case management improves outcome (50). Additionally, if there is an acute treatment effect, we seek to determine if it is additive or synergistic when combined with early diagnosis and antibiotic therapy. It will be left to future studies to determine whether or not this intervention has any effect on case fatality or total child mortality. Second, we wish to investigate the acute effect of zinc on pneumonia specifically, as opposed to ALRI, which includes laryngitis, bronchitis and bronchiolitis. Pneumonia carries a much higher risk of mortality than these other diseases. It is important to quantify its specific effects on pneumonia, not only because pneumonia accounts for a high burden in morbidity and mortality, but also because present therapies are not always successful. This is because there is no therapy for most viral infections, and bacterial infections are prone to antimicrobial resistance. Moreover, the specific pathogen and its susceptibilities are generally not known, which can further delay appropriate therapy and compromise case management."*

4. *Management section, 20 mg/day BID.*
   4.1. We have addressed this in Reviewer 1, 3.1.

5. *Decision not to collect interim serum Zn samples – explain.*
   5.1. The decision to assay serum Zn levels at baseline and at the end of the study can be easily justified. The *baseline sample* will do three things.
      5.1.1. Validate the hypothesis that there is a relative Zn deficiency in the population. These values can be compared with published values on other infants and children.

5.1.2. Assure internal validity, i.e. that the two groups are the same apart from the Zn supplementation.

5.1.3. Enlarge the database, and give us a better understanding, of the serum Zn levels (as a rough estimate of Zn stores) of sick children correlated with their nutritional status. These data will facilitate decisions on programmatic intervention, i.e. on whom and when to intervene.

5.2. The *terminal sample* will do two things.

5.2.1. Document that the Zn treatment group actually received the supplement, based on a rise in serum levels, which we can expect, based on the experience of previous studies.

5.2.2. Allow for an objective correlation of findings with treatment status.

5.3. We have explained that Zn is part of the acute phase response. As such it is taken up rapidly by metallothionein-rich organs under the influence of cytokines. During infection, then, serum zinc levels can remain low, as they do during other stresses, trauma and exercise. This phenomenon has been demonstrated (63, 64, 65). It is not clear how we could interpret serum Zn values during active infection. Since interim samples may be of questionable value, it is unlikely that the Ethical Review Committee of the Centre would sanction their use in ill children with marginal nutritional status. The important comparison will be between outcomes as defined in the protocol and verifiable treatment status.

6. *Laboratory procedures unclear.*

6.1. Samples collected in polypropylene tubes; but vacutainers first – clarify.

6.1.1. Blood will be collected by butterfly needle into polypropylene syringes, then injected into trace element-free tubes. This has been added to the methods section of the protocol (p 19).

6.2. Dilution of samples too extreme not recognised by spectrophotometers. Usually 1:3 or 1:4.

6.2.1. We agree that it is more common to use dilutions of 1:4, but we do not agree that higher dilutions exceed the sensitivity of the spectrophotometer. The sensitivity to optical density on the spectrophotometer can be optimised (adjusted) to accommodate higher dilutions such as those used by our laboratory at ICDDR,B. Our laboratory follows a modified version of the guideline established by the Association of Official Analytical Chemists (AOAC). These guidelines do state that 400 μL of serum is diluted 1:4. However, it has been the practice for several years at the Centre to use smaller aliquots of serum, since they are being drawn from children with marginal or inadequate nutritional status. Our laboratory uses serum aliquots of 150 – 200 μL in dilutions of 1:12. The effective working range of serum concentration is 0.4 mg/L – 1.6 mg/L, which is an acceptable range. Using this same matrix we can produce a

standardisation curve which is comparable to that achieved with larger samples and lower dilutions. Finally, studies using comparable dilutions to those used at our Centre have been published.[1]

7. *Will provision Zn at time of infection increase bacterial load?*
   7.1. There are no data with which to authoritatively answer this question concerning pneumonia. There are three observations that we can make.
      7.1.1. The first is from our protocol p 8, concerning the acute phase response, of which Zn is a part. "There is a two-fold purpose to the acute phase response (62,63,64,65). First, it is to deprive bacteria of essential minerals for bacterial growth. Second, it is to reprioritise hepatic protein synthesis to optimise the host's ability to fight infection. Cytokines, such as TNF-α and IL-6 appear to mediate the redistribution of zinc to the intracellular zinc binding protein, metallothionein, which causes a drop in zinc serum levels and an increase of zinc in metallothionein-rich organs, such as the liver (63,65)."
      7.1.2. In prevention studies, serum Zn has been shown to be higher in supplemented groups than in non-supplemented groups (48, 49, and Bates et al.[2] Despite the greater availability of Zn in the serum of these children, their incidence of disease is less. This suggests that the immune response does not allow invading pathogens to benefit from the higher levels of Zn, indeed it seems to be protective. This may only be true if the Zn is supplied prior to infection. But there is a third observation.
      7.1.3. When Zn is used to treat acute or persistent diarrhoeal illness, the duration and severity of infection is less in Zn supplemented groups than in non-supplemented groups. Granted, we do not know the mechanism by which this occurs, i.e. if it is due to effects on cellular immunity, water and electrolyte absorption, upregulation of enterocytes or brush border enzymes, etc, but the extra Zn in the gut does not confer an advantage to the enteric pathogens. Again, it appears to be protective.
   7.2. Thus, the likelihood that transient increases in serum and gut luminal Zn will benefit pathogens in the respiratory tract is low. We anticipate that adding Zn to the competing tendencies between the acute phase response and pathogen replication, in the presence of an intact immune system, will favour the host.

Reviewer3

1. *Hypothesis as stated cannot be tested. No immunological parameters.*

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[1] Reimold E, Besch D. Detection and elimination of contaminants interfering with the determination of zinc in plasma. Clin Chem 1978;24:675-680.

[2] Bates et al. A trial of zinc supplementation in young rural Gambian children. Br J Nutr 1993 Jan;69:243-55.

1.1. The hypothesis (p 11) has been changed to read, "Zinc supplementation will result in clinical mitigation of those signs associated with severe pneumonia and shorter duration of illness in children with relative zinc deficiency". The references to cellular immunity have been dropped from the hypothesis and are part of the background justification for the proposed intervention.

2. *No evidence for rapid effects of zinc on immune responsiveness. Rapid resolution likely due antibiotics not Zn. No estimate for the time course of the acute phase response. Effects of supplementation must be measured within time frame hospitalisation. Serum Zn measurements will not provide evidence of immunological effects.*
   2.1. In fact, there is evidence from both animal models and human volunteers (p 9) for an acute phase response under immunological regulation (62 – 65). That the response helps to improve immunity is provided from data on immunological lesions in Zn deficit states (47, 66, 67). The reviewer is correct that there are no direct data on rapid effects of Zn on immune responsiveness. This is what we seek to collect evidence for, indirectly, by investigating clinical responsiveness.
   2.2. In severe pneumonia antibiotic response is seldom "rapid". Fever and tachypnoea can persist up to 48 hours even with appropriate therapy. However, by giving both groups identical antibiotic therapy, we can control for the influence of antibiotic effect in the analysis and measure the contribution from Zn.
   2.3. The sources we cite (p 9) state that the acute phase response occurs within 6 hours (63).
   2.4. We agree that serum Zn will not provide evidence of immune responsiveness. We are not measuring immune responsiveness, but the Zn effect on specific indices of severe infection. Our outcomes are all clinical effects. We can speculate that these are mediated by cellular immunity, but there are other effects mediated by Zn such as cellular membrane stability, which may be important here.

3. *Induction/regulation immune effector may not be rapid. More accurate outcome may be reduction ALRI episodes/child/yr. Thus follow up essential. No description of follow up.*
   3.1. We have addressed the issue of immune responsiveness and cellular immunity. Again, this is background to justify why Zn in the acute phase of pneumonia may be beneficial, but there may be other effects of equal or greater importance. We will measure only clinical outcomes.
   3.2. Other studies have looked at the effects on resistance to new infection (incidence). We have provided a rationale as to why we want to look at treatment effects (Reviewer 2, 3.3.1).
   3.3. The study is not designed as a follow-up study, per se. What we intended is that the patients enrolled in the study are referred from an existing community surveillance study. As such, there is an extant follow-

up structure to monitor discharged infants when they are sent back into the community. They are not lost to follow-up.

4. *Inappropriate to hypothesise that Zn has effects on cellular immunity when design cannot test this.*
   4.1. We have addressed this in Reviewer 3, 2.1 – 2.4 and 3.1.

5. *Volume of blood to be drawn at baseline not stated.*
   5.1. The volume of blood drawn at enrolment is 4 ml. This has been added to the protocol (To test specific aim 1, p 12).

6. *Specific documentation of Zn deficiency and impaired cellular immunity/Zn supplementation and improved immune responsiveness is lacking.*
   6.1. We will measure baseline serum Zn as an indicator of Zn status, as described above (Reviewer 2, 5). The issue cellular immunity has been addressed above.

7. *No indication of individual investigator expertise or responsibilities.*
   7.1. The principal investigator's CV was enclosed, and four of the co-investigators are widely published in acute respiratory infections.

U.S.Agency for International Development
Child Health Research Project
Bureau for Global Programs, Field Support and Research
Center for Population, Health and Nutrition
Office of Health and Nutrition
Child Survival Division

f a c s i m i l e

Date: 8/12/98

To: DR. ABDULLAH BROOKS
ICDDR,B/CSD
Fax: 880-2-883116

From: Andrew Clements
Fax: 202-216-3702
Tel: 202-712-1083

Re: PROPOSAL REVIEWS

Pages: 8 (including this one)

U.S. Agency for International Development
Child Health Research Project

Bureau for Global Programs, Field Support and Research
Center for Population, Health and Nutrition
Office of Health and Nutrition, Child Survival Division
Rm 3.07-080B RRB, Washington, DC 20523-3700
Tel: 202-712-1083
Fax: 202-216-3702
Email aclements@usaid.gov

August 12, 1998

Dr. W. Abdullah Brooks
Clinical Sciences Division, ICDDR,B
GPO Box 128
Dhaka 1000, Bangladesh

Dear Dr. Brooks,

We are forwarding to you the three reviews of the proposal entitled "Efficacy of zinc in the treatment of acute lower respiratory tract infection in hospitalised infants less than 2 years of age."

We request that the investigators address the concerns of the External Peer Reviewers and amend the proposal accordingly. Please return the revised proposal along with the responses to the reviewers' comments to us by the 2nd of September. We will review the amended proposal and, if acceptable, let you know of its approval shortly thereafter.

Please contact me if you have any questions or concerns.

Sincerely,

Andrew Clements, Ph.D.
Child Health Research Project

cc : Ruth Frischer, Ph.D.
Robert Black, MD, MPH

ICD 2

EVALUATION OF CHILD HEALTH RESEARCH PROPOSAL

Reviewer's name:

Name of proposal: Efficacy of zinc in the treatment of acute lower respiratory tract infection in hospitalised infants less than 2 years of age

Name of proposed investigator:
Brooks, W.A. et al.

Date of review: July 1998

The USAID Child Health Research Project requires external reviews of all proposals considered for funding. This review serves to ensure high quality of the research supported by USAID, as well as to provide specific suggestions to investigators to assist in revising research proposals. Reviewers comment will be used by USAID personnel in determination of project funding recommendations, as well as by the investigators themselves, who will receive them anonymously.

This is a well written and structured proposal and it contains detailed information about how the project is to be implemented, which outcomes will be studied, and the methodology that will be used. The proposed design (prospective treatment/placebo control) is appropriate to address the objectives of the study and sample size calculations were adequately done. The proposed research is also well justified and its policy relevance is clearly identified. The analytical plan is also adequate. I have only very minor suggestions.

1) In the background section, under 'zinc, immunity and morbidity', the authors should make the point that a few community zinc supplementation trials have documented a positive impact of zinc supplementation on reducing incidence and/or severity of acute respiratory infections. Although the purpose of these trials was different in that they were preventive, as opposed to therapeutic as is the present proposal, they provide evidence that an association between zinc supplemention and acute respiratory infections in young children has been demonstrated before.

2) The authors should justify the dose of 20 mg/day that they proposed to provide to the subjects. Why a fixed dosing as opposed to a dose per kg of body weight, specially since the age range of participating children is wide (2-24 months)?

3) A detail: Under "Management", p. 12: the text says that a dose of 20 mg of zinc will be administered one hour before breakfast each morning and two hours after dinner each evening. This would mean 40 mg/day. Is the 20 mg amount separated in two doses, one given in the morning and one at night? Please clarify.

EVALUATION OF CHILD HEALTH RESEARCH PROPOSAL

Reviewer's name:

Name of proposal: Efficacy of zinc in the treatment of acute lower respiratory tract infection in hospitalised infants less than 2 years of age

Name of proposed investigator: W. A. Brooks, M. Yunus, M. Santosham, R. Black, A. Baqui, M. Wahed, G. Fuchs

Date of review 8/7/98

The USAID Child Health Research Project requires external reviews of all proposals considered for funding. The review serves to ensure high quality of the research supported by USAID, as well as to provide specific suggestions to investigators to assist in revising research protocols. Reviewers comments will be used by USAID personnel in determination of project funding recommendations, as well as by the investigators themselves, who will receive them anonymously.

The proposal requests funds to conduct a placebo-controlled clinical trial to test whether provision of a 20 mg zinc supplement/day in conjunction with WHO-recommended antibiotic therapy will improve outcomes among infants below two years of age with acute lower respiratory tract infections. The objectives of the study will be to determine if zinc supplementation can lessen the severity of signs associated with severe infection and shorten the overall duration of severe illness in children with zinc deficiency.

The study population is to be drawn from Metlab in Bangladesh. This clinical services unit draws from a population stated to be 100,000 people. Individual patients will be recruited from the ALRI unit which admits about 50 infants in the correct age range for the study each month. An assumption is made that there will be sufficient zinc deficient infants among the test group to adequately address the proposed hypothesis, although this is not a known parameter. Children will not be included in the study only if they are zinc deficient and this will make the real testing of the hypothesis more difficult. If all the children were of sufficient zinc status, there would be no way to test the hypothesis. This is unlikely to be the case, but no evidence of the prevalence of zinc status in the population is presented in the proposal, although reference is made to the likelihood of suboptimal zinc status in populations in the developing countries.

(1)

The proposal states that the incidence of acute lower respiratory infection is 8 to 9 episodes/year during the first year of life, but incidence in Bangladesh ranges from 0.2 to 0.5 episodes/year. Reasons for this disparity should have been discussed. It raises the questions of whether this study should be done in a location in which prevalence of ALRI is greater.

(2) ARI

The background for the study is generally well done. There is much more discussion of the presentation of acute lower respiratory disease than there is the interaction between

this disease and zinc; however the linkage is established through citation of appropriate references.

This study is apparently a continuation or a variation on studies which have been done linking zinc status to respiratory disease. There are two citations of work (Sazawal, et al, submitted for publication; Sazawal and Black, 1992) which relate to the present proposed research. There should have been a clearer explanation of what those studies found and how the present work links to those studies. It is important to understand how the proposed work differs from the work that has already been done by these investigators.

In the study design, there is an inconsistency which needs to be addressed. The design calls for providing a supplement of 20 mg zinc/ day to each of the infants in the test group (and a placebo for the control group), yet the design says that the infants will be provided with a zinc supplement (20 mg) given in the morning and in the evening. This would be twice the dose proposed in the hypothesis and it would be helpful to know which is to be used.

Another design question would relate to the decision to not collect samples for zinc determination from the children during the course of the study. Plasma zinc would be determined on entry into the study thus allowing for estimation of the zinc status of the infant, but the decision to not determine zinc at intervals during the study should be explained. Such information might be helpful in understanding the response to the supplement in the infants.

The laboratory procedures to be followed is unclear. For the zinc determination, it states that samples will be collected in polypropylene tubes. This seems unlikely unless the children are to be bled directly into the tubes. It seems more reasonable that the samples are to be collected, either with vacationers or hypodermic syringes and the samples (of plasma) stored in polypropylene tubes, but this involves some inference on the part of the reviewer that is perhaps not correct. Diluting the samples by the among listed in the procedures seems extreme. Most atomic absorption spectrophotometers could not recognize a sufficient signal with a dilution of this magnitude. A most usual dilution is a 1:3 or 1:4 dilution and some clarity of the procedure to be followed for analysis would be helpful.

The consent procedure form to be used is stated as being in the appendix. No appendix was provided to this reviewer and hence could not be assessed for appropriateness. The budget was also not included and therefore could not be addressed.

An overarching questions that should be addressed by the investigators comes from the premise used for the work. The authors state that a normal response to infection is the sequestration of zinc in order to prevent bacterial growth, probably mediated through TNF alpha and IL 6. While there is no question that the robustness of the response to infection is lessened in a zinc deficient individual, will the provision of added zinc at the time of infection (and presumably the elevation of plasma zinc values) actually

increase the bacterial load in an infected individual. It is clear that provision of zinc before an individual becomes ill would be beneficial; it is less clear that provision of zinc during illness will improve the outcome.

The reviewer would recommend approval for this study if the questions raised can be addressed adequately.

EVALUATION OF CHILD HEALTH RESEARCH PROPOSAL

Reviewer's name:

Name of proposal: Efficacy of Zinc in the Treatment of Acute Lower Respiratory Tract Infection in Hospitalised Infants less than 2 Years of Age

Name of proposed investigator: Brooks, W. Abdullah

Date of review July 30, 1998

The USAID Child Health Research Project requires external reviews of all proposals considered for funding. The review serves to ensure high quality of the research supported by USAID, as well as to provide specific suggestions to investigators to assist in revising research protocols. Reviewers comments will be used by USAID personnel in determination of project funding recommendations, as well as by the investigators themselves, who will receive them anonymously.

1) Goals: The primary goal of this proposal is to assess the efficacy of zinc supplementation in the clinical management of acute lower respiratory tract infections (ALRI) in hospitalized infants less than 2 years of age. The investigators hypothesize that supplementation will be associated with increased immunity, resulting in reversal of clinical signs (hypoxia/hypoxaemia, tachypnoea, inability to feed, etc.) and a shorter duration of illness. The hypothesis is based on the assumptions that "zinc deficiency is widespread" and that "zinc is an important regulator of cellular immunity".

2) Design: The study is designed as a double-blind randomized, placebo-controlled clinical trial. Analysis will be based on the assessment of clinical signs using an algorithm for severe ALRI. However, the hypothesis as formulated, that zinc supplementation enhances cellular immune responsiveness with amelioration of clinical disease, cannot be tested by the proposed research plan. No immunological parameters will be analyzed to document the hypothetical immunomodulatory effects of zinc supplementation.

There is no evidence for the rapid effects of zinc supplementation on immune responsiveness during the acute phase response outlined in Figure 3. Such rapid resolution of clinical signs will more than likely be due to antibiotic treatment. The investigators do not provide an estimate of the time course for the acute phase response (Fig. 3). Preliminary data detailed in the Sample Size Calculations section indicate the average duration of ALRI illness in the target population is 5.7 (S.D. 5) days. Effects of supplementation must be measurable within that time frame. Nevertheless, measurement of serum zinc levels (baseline and at recovery) as proposed will not provide evidence of immunologic effects.

Efficacy of zinc in the treatment of ALRI...
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Induction and regulation of immune effector function may not be a rapid process. Enhancement of immune recognition and response may evolve gradually to allow increases in cell number and function. In addition, immunologic resolution of clinical signs may require natural boosting (repeated exposure to pathogen). Given a longer time course for zinc effectiveness, one could envision that a more accurate outcome of supplementation would be a reduction in the number of ALRI episodes/child/year. Thus, follow-up would be essential. The investigators propose to follow infants within the community surveillance system but neither this system nor the plan for follow-up is described.

3) Appropriateness: Research designed to assess the efficacy of low-cost, safe micronutrient supplementation is worthwhile, especially if improved nutrition can be correlated with a reduction in illness. However, it is inappropriate to hypothesize that zinc has effects on cellular immunity when the proposed research is not designed to test this assumption.

4) Budget: No budgetary information has been included, so it is impossible to comment on its appropriateness.

5) Ethics: The volume of blood to be drawn from patients at the baseline collection should be indicated [1 ml is to be drawn for the zinc assay at recovery].

6) Background: In general, the review of pertinent literature is good. Specific documentation of zinc deficiency and impaired cellular immunity/zinc supplementation and improved immune responsiveness is lacking.

7) Other: There is no indication of individual investigator expertise or responsibilities within the context of the proposed research.

EFFICACY OF ZINC IN THE TREATMENT OF PNEUMONIA IN HOSPITALISED INFANTS LESS THAN 2 YEARS OF AGE

Date (dd/mm/yy): __/__/__

Patient Name: _____

Address: _____

Hospital Record No: _____

Identification No:_____

Date of Birth (mm/yy): __/__

Period of birth month (circle): Beginning / Middle / End

Age (months): _____

Date of illness: __/__/__

Time of onset of illness (24 hr): _____hr

(Number of hours of illness): _____

Has patient had cough?
For how many days?

Has patient had chest indrawing?
For how many days?

Has patient been feeding:
Same More Less Unable to feed

Has patient had fever?
For how many days?

Was infant given ORS at home? Y/N Homemade or Packet (circle)
If packet, how many packets_____

Has patient received any drugs (medicine) since illness started? Y/N
Number of days of antibiotic use_____

Birth History

Full-term infant: Y/N

If not full term, approximate gestational age (in weeks): _____

Home delivery or Hospital delivery (circle)
Did the baby cry immediately? Y/N

Was the infant hospitalised after birth? Y/N

Did the infant have any severe illness during the first two months of life? Y/N
If yes, was the infant treated with any medicine?

Past Medical History

Has infant ever been hospitalised? Y/N

Have you ever had to seek medical care for the infant? Y/N

Vaccinations to date (number of):

BCG ___	DPT___	OPV ___
(Birth)	(6, 10, 14 weeks)	(6, 10, 14 weeks)

Vaccination status: Up to date? Yes ___ No ___

Socioeconomic Status

Family income (monthly): TK _____

What form of transportation do you usually use? (Circle)
Foot Bicycle Rickshaw Taxi Bus Auto Other

Do you own or rent your present home?
If you rent, how much do you pay per month? _____

Maternal Education (number of years): _____ years

Paternal Education: (number of years): _____ years

Number of persons in household: _____

Number of children under 5y/o in household: _____

Type of latrine available: Open field___ Hanging___ Sanitary___

Is there any open sewerage near your home? Yes___ No___

Water source: None___ Open___ Tube well___ WASA___ Well___

Water storage: Open container___ Covered container___

History & Physical Examination

Name: _____

Patient Record No: _____

Hospital Record No: _____

Today's Date (dd/mm/yy): __/__/__

Recorder: _____

Height: _____cm

Admission Weight _____g

Vital signs:

Pulse Temp RR BP

Respiratory exam

Effort: Non-laboured Retractions Chest-indrawing

Nasal flaring Grunting

Auscultation: Clear Crepitations Decreased air entry

Bronchial breath sounds Wheezing

Hydrational status

No sign Some Severe

Mental status

Alert Less active Lethargic Comatose

1st Blood sample taken: Serum Zinc 1 _____ Date __/__/__

2nd Blood sample taken Serum Zinc 2 _____ Date __/__/__

CURRICULUM VITAE

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Education

1994-Present	Johns Hopkins School of Hygiene and Public Health, Baltimore, MD Preventive Medicine Residency
1994-1995	Johns Hopkins School of Hygiene and Public Health, Baltimore, MD Master of Public Health (MPH)
1991-1994	The New York Hospital/Cornell Medical Ctr. New York, NY Residency - Pediatrics
1985-1991	Stanford University School of Medicine, Stanford CA Medical Doctorate (MD)
1983-85	American River College Sacramento, CA Post-baccalaureate sciences
1976-1978	University of California, Los Angeles Bachelor of Arts (BA) International Relations
1974-1976	University of California, San Diego Political Science

Research Activities

July-October 1995	International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) Dhaka, Bangladesh Multicentre Study on Lower Osmolar oral rehydration solution (ORS). Clinical trial to assess the efficacy of lower osmolarity ORS relative to standard ORS (WHO formula) in adults with cholera and infants under two years of age with acute watery diarrhoea.
July-October 1995	ICDDR,B Dhaka, Bangladesh Analysis of surveillance data on risk factors for Amoebic dysentery among patients hospitalised and clinic patients at the Diarrhoea Hospital.

July-October 1995	ICDDR,B Dhaka, Bangladesh Analysis of surveillance data on risk factors for Cryptosporidiosis among hospitalised and clinic patients at the Diarrhoea Hospital.
July-October 1992	University Hospital, Salvador Bahia, Brazil. Field study on risk factors for invasive diarrhoeal disease among slum children in Salvador. Included collection of epidemiological data as well as isolation of pathogens and testing with DNA probes.
Oct-December 1990	The Center for Disease Control and Prevention Atlanta, GA Epidemiology Intelligence Service Clerkship for Medical Students - Enteric Branch. Included outbreak investigation of E. coli 0157-H7 in Helena MT among children at an elementary school, and surveillance system analysis for Salmonella typhi carriers among state health departments in all 50 states and territories.
July-Sept 1986	The National Institutes of Health (NIH) Bethesda, MD Division of Parasitology. Isolation of Leishmania major attachment proteins to the macrophage, as a possible means of prevention of parasitic infection.
1985-1986	Stanford University School of Medicine Stanford, CA Identification of heat-shock proteins in Fasciola hepatica morphogenesis, as a model for morphological regulation in Schistosoma species.

Professional

1996-1997	Chief Resident, Johns Hopkins Preventive Medicine Pgm Baltimore, MD
1996	Consultant for PAHO, EPI/SVI Georgetown, Guyana Planned and implemented mass vaccination campaign against measles for all children aged 12 - 59 months
1996	Consultant for Consultants in Epidemiology and Occupational Health Washington, DC Principal investigator in risk factor analysis for Non-Hodgkins Lymphoma

1995+	St. Joseph's Hospital Pediatric Express Baltimore, MD Emergency room physician for pediatric patients
1995+	Sinai Hospital Emergency Room Baltimore, MD Emergency room physician for pediatric patients
1995+	Baltimore County Health Department Baltimore, MD Physician for School-based clinics in Baltimore County School District
1994+	Kennedy Krieger Institute Baltimore, MD Pediatric physician for inpatients

Grants

1996	Small study on serological-based evaluation of silicate exposure in patients with scleroderma: NIH; Lawless D, Brooks W, Lamm S, Teed K. Not Funded.

Presentations

April 1993	Department of Pediatrics Grand Rounds: Original research results on risk factors for invasive diarrhoeal disease among slum children in Salvador, Bahia, Brazil The New York Hospital/Cornell Medical Ctr, NY, NY
Oct 1990	Genetic evidence of the common origin and essential oneness of the human race: Association for Bahá'í Studies, Atlanta, GA

Awards and Honours

Jul-Oct 1995	International Fellow, The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) Clinical Sciences Division Dhaka, Bangladesh
1994-1995	Health and Child Survival Fellows Scholarship for Master of Public Health degree Johns Hopkins School of Hygiene and Public Health, Baltimore, MD
1985-1986	Medsholars Award for independent research Stanford University School of Medicine, Stanford, CA

1986	Ciba-Geigy Summer Internship Award for medical student research National Institutes of Health, Bethesda MD
1974-1975	Pacific Palisades Chamber of Commerce Scholarship for Academic Achievement and Student Leadership

Teaching Experiences

1995	Johns Hopkins University Intersession Course on Public Health Career Options
1993	Methodist Community Hospital, Brooklyn NY Chief Resident: Clinical training of pediatric residents as part of that hospital's accreditation process

Memberships and Activities

1994+	American Public Health Association
1993+	American Academy of Pediatrics
1995+	Association of Preventive Medicine Residents
1992+	American Academy for the Advancement of Science
1990+	Bahá'í International Health Agency
1977+	Member of Bahá'í Faith, Bahá'í Community of the United States of America

Professional Licensure and Certification

Board Certified, New York Board of Medical Examiners

Board Certified, Maryland Board of Medical Examiners

Certified, American Heart Association
Basic Cardiac Life Support (BCLS)
Advanced Cardiac Life Support (ACLS)
Pediatric Advanced Life Support (PALS)

Languages

French - Speaking, reading, writing proficiency

Portuguese - Speaking, reading, writing proficiency

Arabic - Reading, writing comprehension (with difficulty)

Swahili - Conversant (with difficulty)