Severe pneumonia in hospitalized young Nepalese children.

Studies on the efficacy of oral zinc, respiratory viruses and prognostic determinants

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Contents

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List of publications

Paper I

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Paper II

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Paper III

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Abbreviations

ALRI	acute lower respiratory tract infection
ARI	acute respiratory infection
BCG	Bacille Calmette-Guérin
CAP	community acquired pneumonia
CI	confidence interval
CRP	C-reactive protein
CXR	Chest x-ray
DPT	combined vaccine against diphtheria, pertussis and tetanus
EPI	Expanded Program on Immunization
GAPPD	Global Action Plan for the Treatment and Control of Pneumonia and Diarrhea
HR	Hazard Ratio
Hib	*Hemophilus Influenzae* type b
hMPV	human metapneumovirus
IMCI	Integrated Management of Childhood Illness
IQR	interquartile range
LCI	lower chest wall indrawing
LMICs	low-and-middle-income countries
LRI	lower respiratory tract infection
NPA	nasopharyngeal aspirate
OR	odds ratio
PCR	polymerase chain reaction
PCV	pneumococcal vaccine
PICU	pediatric intensive care unit
PIV	parainfluenza virus
RCT	randomized controlled trial
RR	risk ratio
RSV	respiratory syncytial virus
SpO_2	peripheral oxygen saturation
U5	children under five years of age
UNICEF	United Nations Children's Fund
URI	upper respiratory tract infection
WHO	World Health Organization

Summary

Pneumonia is a leading cause of death in children less than five years of age in low and middle income countries and contributes importantly to their disease burden. Zinc, essential for normal function of the immune system, is an important micronutrient for children. While routine zinc supplementation has been shown to reduce the risk of pneumonia in children, results from studies on the therapeutic effect of zinc for pneumonia are inconsistent.

We conducted a randomized double blind placebo controlled clinical trial, which formed the basis for the work described in this thesis. The aim of the trial was to estimate the extent to which administration of oral zinc reduces time till recovery and risk of treatment failure in children hospitalized with severe pneumonia defined as community acquired pneumonia with chest indrawing. We later performed a secondary analysis to identify clinical, radiological and laboratory prognostic factors for illness duration and risk of treatment failure. We also collected nasopharyngeal aspirates from the included children, to identify seven common respiratory viruses using a multiplex reverse transcription polymerase chain reaction assay.

In the trial we followed up 598 children that were 2 – 35 months of age and enrolled with severe pneumonia shortly after they were admitted to a centrally located general children's hospital in Kathmandu, Nepal. Trial participants were randomized to receive zinc sulphate (10 mg in children less than 12 months and 20 mg in older children) or placebo daily until discharge or up to a maximum of 14 days. The children were monitored by study physicians until they were discharged.

In this large cohort of children with severe pneumonia, the median time till recovery was 2 days and treatment failure was detected in one third.

We found a non-significant and clinically unimportant 10% reduction of illness duration in the children receiving zinc. Similarly, the proportion of children who had recovered within 72, 96 and 120 hours after enrolment or at risk of treatment failure was not significantly different between the zinc and placebo arms. Vomiting was more frequently observed in the children who received zinc.

Younger age, radiographic consolidation and hypoxia on admission were independent predictors of both delayed recovery and increased risk of treatment failure.

At least one of the seven respiratory viruses was detected in 30% of the children. Respiratory syncytial virus, detected in 14%, was by far the most common. During the study period of 30 months, the observed pneumonia epidemics occurred in 2 main annual peaks, but the timing of the epidemics varied.

The findings from this randomized controlled clinical trial contribute to the evidence that zinc is not an effective adjunct to standard treatment of hospitalized children with severe pneumonia in low and middle income countries. Younger age, hypoxia and radiographic consolidation on admission predicted delayed recovery and treatment failure, and can be useful tools to guide the management of severe pneumonia in hospitalized children in resource poor settings. Our work on molecular based detection of seven respiratory viruses in nasopharyngeal aspirates identified respiratory syncytial virus as an important pathogen in children hospitalized with pneumonia, which is relevant for a country where the epidemiology of pneumonia is changing and viral pneumonia is still unrecognized as a cause of hospital admissions.

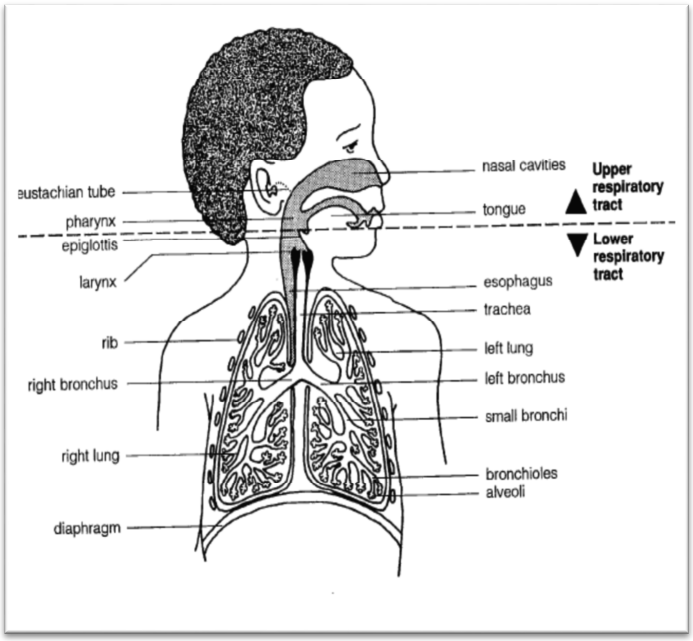
Our findings contribute to the understanding of childhood pneumonia and provide arguments for not using zinc as adjunct therapy in children hospitalized for severe pneumonia.

Introduction

Acute respiratory infections in children

Children under five years of age (U5) suffer from an average of three to six episodes of acute respiratory infection (ARI) in a year [1] . The level at which the vocal cords lie is used to divide the respiratory tract into upper and lower parts. The same anatomical landmark is also used to classify ARI into upper respiratory tract infections (URI) and lower respiratory tract infections (LRI) (Figure 1) [2]. The URIs includes rhinitis (common cold), otitis media, sinusitis and pharyngitis. While pneumonia and bronchiolitis are common among the LRIs [1], epiglottitis, laryngitis, laryngotracheitis (croup) and bronchitis are the other important LRIs afflicting children [3].

Figure 1. The respiratory tract



(Adapted from World Health Organization Acute respiratory infections in children: case management in small hospitals in developing countries, a manual for doctors and other senior health workers 1990)[2]

Clinical diagnosis of pneumonia and case definitions based on severity

The work described in this thesis uses community acquired pneumonia (CAP) that is defined as "the presence of signs and symptoms of pneumonia in a previously healthy child due to an infection which has been acquired outside hospital" [4]. The entity of pneumonia, an acute lower respiratory infection (ALRI), is defined by tissue pathology. The alveoli, normally filled with air in healthy individuals, are in this instance filled with pus and fluid resulting in consolidation that is either lobar or patchy in distribution. Whilst consolidation is a finding commonly associated with pneumonia of bacterial etiology, infection caused by viruses and atypical bacteria usually results in patchy inflammatory changes involving interstitial tissue and alveolar wall edema [5]. These pathological changes in the lungs form the basis for the clinical symptoms, signs and radiographic findings associated with pneumonia.

A child is usually brought to a healthcare provider by the parents/caretakers when they notice cough, fever and rapid or difficulty in breathing in the child and suspect that it might be pneumonia [6]. The presence of certain clinical signs in the child helps the health care provider confirm the parents' suspicion and assess severity in order to provide appropriate treatment and advice to the parents. Following studies in low and middle income countries (LMICs) in different regions of the world, fast breathing/tachypnea was identified as the best predictor to diagnose non-severe pneumonia in U5 children [7]. This clinical sign requiring that health workers count the respiratory rate for one full minute was adopted by the World Health Organization (WHO) in the case management algorithm of U5 children to distinguish between pneumonia and other ARI (Table 1) and is commonly used even today by clinicians and researchers alike [8]. The changes in the lungs of a child with pneumonia make it increasingly difficult to breathe and clinical signs that assess the work of breathing provide an indication of the severity. The inward movement of the lower chest

wall during the phase of inspiration, called lower chest indrawing (LCI), signifies the extra effort needed to breathe with lungs that become less compliant [9]. Similarly, nasal flaring, i.e. enlargement of the opening of the nostrils while breathing; grunting, i.e. sound made during expiration while exhaling through a partially closed glottis in an attempt to prevent alveolar collapse and raise functional residual capacity; head nodding, i.e. bobbing movement of the head occurring simultaneously with inspiration and indicating use of accessory muscles, are all signs of respiratory distress in infants and young children [10]. Central cyanosis, another indicator of severity, is a sign of impaired oxygenation by the lungs. However, it is often difficult to detect, especially in children with dark colored skin, and found late in the course of illness. Chest auscultation for the evaluation of lung sounds is an important component of the physical examination that clinicians rely on to confirm the diagnosis of pneumonia. The two most common adventitious sounds associated with pneumonia are wheezing and crepitations [11]. Wheezing, a musical, continuous sound heard during the expiratory phase signifies airway obstruction and airflow limitation [12]. Crepitations, often referred to as crackles [13], are discontinuous sounds heard mostly during inspiration and signify the presence of airway closure or secretions [11, 12]. The forceful reopening or closing of abnormally closed airways is responsible for producing this adventitious sound [14]. While the signs of breathing difficulty are easy to recognize, identifying adventitious sounds, especially in crying young children can be problematic even to the trained ear. In a review assessing accuracy of clinical features for diagnosing pneumonia in children, the inter-observer agreement for signs that could be observed, such as LCI ($\text{kappa} = 0.48$) and use of accessory muscles ($\text{kappa} = 0.59$), was better than that for identification of adventitious sounds ($\text{kappa} = 0.3$), but still inadequate [3]. Findings from a recent meta-analysis assessing the accuracy of clinical features of pneumonia were also inconclusive [15]. Among the 6 symptoms and 12 signs that were assessed in children between ages 2 months – 6 years with radiological

pneumonia as the reference standard, none were sufficient to diagnose pneumonia. The highest pooled positive likelihood ratio (LR+) of 1.9 for respiratory rate > 50 breaths per minute and lowest pooled negative likelihood ratio (LR-) of 0.3 for cough did not reach clinical significance to either rule in (LR+ of > 5.0) or rule out (LR- of < 0.2) the diagnosis of pneumonia, respectively [15]. A diagnosis of pneumonia based solely on clinical symptoms and signs may therefore be inaccurate. While the diagnosis can be verified by the presence of consolidation on a chest radiograph in high income countries (HICs), in many resource limited settings of LMICs pneumonia continues to be a clinical diagnosis. The simplified clinical definition by the WHO, which uses age-related fast breathing (Table 1) to identify young children with pneumonia, has a high sensitivity but low specificity and may result in the use of antibiotics in instances where it is not required. Children who are febrile also breathe at a faster rate and this finding was verified by a study where each one degree centigrade rise in temperature was associated with an increase in the respiratory rate that varied from 0.5 (5^{th} centile) to 2 (95^{th} centile) breaths per minute [16]. In diseases such as malaria, children with fever, fast breathing and cough may therefore easily be misclassified [17] because of the clinical overlap of symptoms and signs with pneumonia [18]. The WHO case definition for pneumonia does not differentiate pneumonia from other LRIs, such as bronchiolitis, which is the reason behind use of the term “acute lower respiratory tract infection” (ALRI) commonly encountered in studies from developing countries [19].

The primary focus of the WHO ARI case management guidelines, initially developed in 1990 for first level health facilities in LMICs was to ensure early recognition and appropriate treatment of pneumonia based on a severity score. Cough or difficulty in breathing used as entry criteria was supplemented with additional signs to make a diagnosis, based on which treatment was provided for children between the ages of 2 months to 5 years (Table 1).The approach to sick young infants

less than 2 months of age uses a different algorithm and will not be discussed here as this thesis focuses on older children.

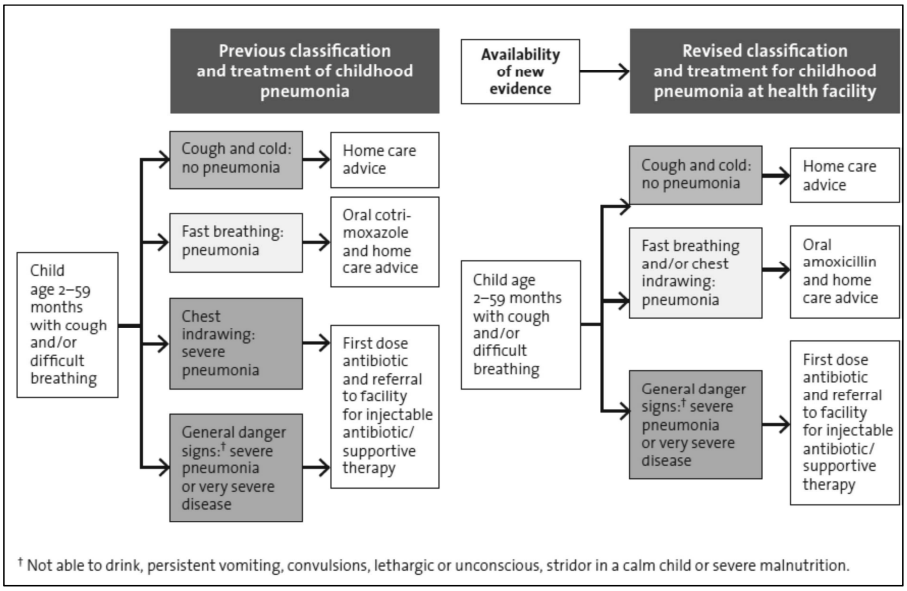
Table 1. The First World Health Organization algorithm for case management of pneumonia in small hospitals in 1990

For the child age 2 months up to 5 years with cough or difficulty in breathing:		
Clinical Signs:	**Classify as:**	**Provide Treatment:**
Central cyanosis *or* Not able to drink	Very severe pneumonia	Admit Give an antibiotic Give oxygen Give supportive care Reassess twice daily
Chest indrawing *and* No central cyanosis *and* Able to drink	Severe pneumonia	Admit Give an antibiotic Give supportive care Reassess daily
No chest indrawing *and* Fast breathing • ≥ 50 breaths per minute in a child age 2 months up to 12 months • ≥ 40 breaths per minute in a child age 12 months up to 5 years	Pneumonia	Advise mother to give home care Give an antibiotic Advise mother to return in 2 days for reassessment or earlier if child is getting worse
No chest indrawing *and* No fast breathing	No pneumonia	Advise mother to give home care

(Adapted from World Health Organization Acute respiratory infections in children: Case management in small hospitals in developing countries. A manual for doctors and other senior health workers 1990: pg 14)[2]

The WHO guidelines for ARI case management at health facilities were later incorporated into the Integrated Management of Childhood Illnesses (IMCI) in 1995 [20] and expanded to cover a wider range of conditions in 2005 [21]. A child is categorized as having very severe pneumonia if one or more of the following signs are present in children fulfilling criteria for pneumonia: central cyanosis, inability to feed or vomiting everything, convulsions, lethargy, unconsciousness, or severe respiratory distress, such as head nodding [21]. These guidelines have been regularly updated over the years [10, 21] and recently undergone a major revision with pneumonia classified into two instead of three categories based on severity (Figure 2) [22, 23].

Figure 2. Comparison of previous and revised classification and treatment of pneumonia in children 2 months to 5 years of age defined using WHO criteria at health facility level.



Adapted from Revised WHO Classification and Treatment of Pneumonia in Children at Health Facilities: Evidence Summaries. 2014: pg19 [24].

While guidelines for management of CAP in children from HICs also use clinical symptoms and signs to define a case [4, 25], a more specific diagnosis of bacterial pneumonia is sought in order to better guide treatment with antibiotics. In children with CAP that meet criteria for hospital admission, a chest radiograph, though not advised as a routine investigation, is suggested to look for consolidation [4]. Antibiotics for children less than 2 years of age with mild symptoms, especially if they have received the pneumococcal conjugate vaccine, are not recommended [4]. Likewise, antibiotics are not recommended for preschool children with CAP with clinical features compatible with a viral infection [25]. The British Thoracic Society guidelines in addition recommend that bacterial pneumonia is to be considered in "children presenting with persistent/repetitive fever > 38.5^{o}C, chest recessions and increased respiratory rate" [4]. The assessment of severity guides

decisions on whether to treat the child on an outpatient basis or in the hospital and includes identifying more signs; including low oxygen saturation identified using a pulse oximeter [25].

Pathophysiology of pneumonia and hypoxemia

Pneumonia is usually preceded by an upper respiratory tract infection, which promotes invasion of the lower respiratory tract by viruses, bacteria or other pathogens that trigger an immune response [3, 26]. The release of histamines, leukotrienes and other chemotactic factors attracts white blood cells into alveolar spaces that are thereby filled with leucocytes, fluid and cellular debris. This is associated with decreased lung compliance, increased airway resistance and obstruction of smaller airways leading to either collapse of distal air spaces or air trapping [3]. The physiological intrapulmonary shunting of de-oxygenated blood and ventilation perfusion mismatch following these pathological changes result in hypoxemia, manifested by low partial pressure of oxygen in the blood [27, 28]. A reasonably accurate assessment of arterial oxygen saturation can be obtained with a pulse oximeter. This simple and non-invasive technique, now used widely to assess and monitor peripheral oxygen saturation, especially in HICs, has been proposed as the 'fifth vital sign' for assessment of pediatric patients [29, 30]. In LMICs, the wider application of pulse oximetry is limited by the costs for acquiring the equipment, notwithstanding that required for its maintenance. When pulse oximeters are not available, the WHO guidelines recommend that clinical signs, such as cyanosis, inability to drink, severe chest indrawing, and a respiratory rate greater than 70 breaths per minute could be used for the detection of hypoxemia in children aged 2 – 59 months with pneumonia [21]. The WHO category of pneumonia based on severity [21] that is meant to guide case management has been shown to correlate with the presence of hypoxemia according to the findings of a systematic review [31]. In this review, the estimated median proportion of hypoxemia was 9.4% (IQR 7.5–18.5%) in children with severe pneumonia and 13.3%

(IQR 9.3–37.5%) in those meeting criteria for very severe pneumonia [31]. While the proportion of deaths in children with pneumonia is also likely to increase with increasing severity of hypoxemia [32, 33], a study in Papua New Guinea demonstrated that providing oxygen to hypoxemic children with pneumonia was associated with a 35% reduction in the risk of death [risk ratio (RR) 0.65; 95% CI 0.52, 0.78] [34].

Etiology of pneumonia

The WHO ARI case management algorithm recommends that all children with a diagnosis of pneumonia should be treated with antibiotics. This recommendation is based on the findings from studies conducted in the 1980s in LMICs where *Streptococcus pneumoniae* and *Hemophilus influenzae* together accounted for more than 50% of the organisms isolated in lung puncture aspirate specimens of children with severe pneumonia [35]. A review assessing the effectiveness of this case management strategy in the community estimated a 70% reduction in mortality from pneumonia in children U5, but similar estimates for hospitalized children with pneumonia could not be derived as the number of studies done in LMICs was insufficient [36].

Many bacterial and viral pathogens, alone or in combination, are known to cause CAP and the etiology varies according to the age group. Viruses are the most common cause of CAP in infants and children U5 [37]. Viruses were detected in 66% to 73% of samples from children with CAP in recent studies done in Finland [38] and the United States [39] and in 83% in children < 18 months of age in a study in Spain [40]. While respiratory syncytial virus (RSV), influenza virus, parainfluenza virus (PIV) and adenovirus have been the organisms commonly known to cause CAP, human metapneumovirus (hMPV), human bocavirus (hBoV), rhinovirus and coronavirus are more recent additions to this list [41]. *Streptococcus pneumoniae* continues to be the predominant bacterial pathogen across all age groups [38, 42] and *Mycoplasma pneumoniae* is frequently detected in the

age group of 5 – 15 years [19, 39, 43]. *Hemophilus influenzae* type B (Hib), *Staphylococcus aureus, Streptococcus pyogenes, Chlamydia pneumoniae* and *Moraxella catarrhalis* are other bacteria causing pneumonia in children [41]. Mixed infections with bacteria and virus have been documented in several studies [38, 39, 42] and in one study viral-bacterial co-infection was associated with more severe disease [42]. Bacterial pathogens causing CAP initially colonize the upper airways and then spread into the lower respiratory tract. Viral respiratory tract infections facilitate the development of bacterial pneumonia by damaging the ciliated respiratory epithelium, augmenting adhesion of bacteria to respiratory epithelial cells and promoting bacterial multiplication in the respiratory tract [44]. Viral co-infections are also common, with 2 and sometimes even 3 viral pathogens isolated in children with CAP [38, 40, 45].

The introduction of the pneumococcal and Hib conjugate vaccines in both developed and more recently in developing countries has brought about a change in the epidemiology of pneumonia. In 2010, the Hib vaccine had been introduced in 169 countries with reported coverage of 42% globally but ranging from 9% in South East Asian to 92% in the American WHO regions [46]. Pneumococcal conjugate vaccine (PCV) had been introduced to only 55 countries across the world by 2010. The data on coverage for the third dose of PCV available from only 38 countries was therefore insufficient to estimate either global or regional coverage [46]. In the most recent estimates of global pneumonia burden, which took into account both PCV and Hib vaccine use in 2010, RSV is the most common pathogen contributing to 28.8% of all episodes of childhood pneumonia with *Streptococcus pneumonia* and Hib accounting for 6.9% and 2.8% of cases, respectively [47]. While in children with severe pneumonia, the proportion with *Streptococcus pneumoniae* as the causative organism is higher (18.3%), and in those who die *Streptococcus pneumoniae* is the predominant pathogen contributing to 32.7% followed by Hib in 15.7% of cases [47].

Tests for the diagnosis of pneumonia

Identification of the causative organism in CAP has always been a challenge with many tests to choose from but none that can be considered the 'gold standard'. The best option to obtain a specimen from the site of infection is transthoracic needle aspiration, which is a procedure dating back to the late nineteenth century with 35 studies reporting this investigation in children until date [48, 49]. The organisms present in the aspirate are isolated either using conventional culture and staining of the aspirate or in more recent years by modern microbiologic methods, such as polymerase chain reaction (PCR) that detects nucleic acids of common respiratory pathogens [48, 50, 51]. The diagnostic yield from transthoracic lung aspirates has been reported to be around 50% with respect to bacterial etiology [49]. Compared to secretions from the lower airways, cultures of lung aspirates yield fewer false positives because the needle is inserted into or close to an area of consolidation identified on a chest radiograph. However, the infectious focus may still be missed or the specimen collected inadequate and culture will be affected by prior antibiotic use [49]. The most frequent complication, iatrogenic pneumothorax has been reported in 1 in 30 of more than 3000 procedures and requiring chest tube drainage in 1 in 200 instances (0.5%) according to a summary of published studies [52]. Therefore, while it can be argued that the potential benefits of transthoracic lung aspiration outweigh its risks, it should only be done when indicated and by persons that have been trained to perform, monitor and manage the complications that may occur following the procedure [50].

The collection of blood for culture to identify bacteria and nasal swabs/nasopharyngeal aspirates for detection of viruses in hospitalized children is recommended in clinical practice guidelines of HIC [4, 25]. The identification of pathogens in blood culture in CAP helps to guide antimicrobial therapy and thereby decrease the problem of drug resistance. However, blood culture is not sensitive

enough for diagnosing bacterial pneumonia in children mainly because the majority of cases are not bacteremic [53]. Moreover, factors such as growth of contaminants, prior use of antibiotics, inadequate sampling and processing techniques may further limit its usefulness [25]. Such factors are likely to be even more common in developing country settings. In recent studies conducted in the United States, blood cultures were positive in only 2% to 7% of patients fulfilling diagnostic criteria for pediatric CAP [54-56], while it was substantially higher, up to 21%, in children with complicated pneumonia (pleural effusion/empyema, lung abscess or necrotizing pneumonia) [55, 56]. In another study in the United States, although pathogens were detected in 2% of children with uncomplicated pneumonia, all of them were contaminants, raising the question of whether this procedure is of any use in the evaluation of fully immunized children with CAP [57].

For the detection of viruses, the advent of PCR assays has improved the ability to identify an increasing number of organisms in respiratory specimens from children with respiratory infections [40, 58, 59]. However, isolation of viruses in healthy asymptomatic children [39, 60-63] and bacterial co-infection in 22% to 33% of children with CAP [37, 64] poses diagnostic challenges.

The finding of alveolar consolidation on a chest radiograph is frequently used for defining pneumonia in hospitalized children [65] and in studies of CAP. The inter-observer agreement between readers for radiographic findings of pneumonia in children, however, has always been a contentious issue, although there is evidence to suggest better consensus for presence of alveolar consolidation than other infiltrates [66, 67]. Radiographic findings, however, do not differentiate well between viral and bacterial etiology [68, 69]. Chest radiographs as a 'gold standard' in research and clinical settings as a proxy for diagnosing bacterial pneumonia may thus be inadequate [70].

Therefore, while establishing a diagnosis of pneumonia poses a challenge due to the lack of specific symptoms and signs and accurate diagnostic tests, the search for better diagnostic procedures

continues. There is good evidence to suggest that non-invasive imaging of the lung parenchyma in children, using ultrasonography, may be a reliable alternative to radiology [71]. Other advances in this field have been the development of tools and biomarkers for accurate classification of CAP by etiology and severity. In a study analyzing host gene expression profiles of peripheral leukocytes, the authors were able to differentiate between viral and bacterial infections with accuracy exceeding 85% [72]. Distinctive gene expressions were observed in patients with respiratory infections due to influenza A virus, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* [72]. There are also indications that application of this technology may better classify children with CAP according to disease severity, thereby enabling targeting of specific therapies that could lead to improved outcomes [64].

Determinants of severe pneumonia incidence and its outcomes

The number of children that die before reaching the age of five years has decreased from an estimated 9.9 million in 2000 to 6.3 million in 2013,which is also unacceptably high [73]. Pneumonia accounts for 15% of these mostly preventable deaths [73] concentrated in the world's poorest regions of Sub-Saharan Africa and South Asia [74], with the majority occurring in children less than 2 years of age [75]. Identifying factors that increase the risk of acquiring severe pneumonia is important because they can be used to target interventions aimed at decreasing the incidence of severe pneumonia in these vulnerable young children and the number of pneumonia related deaths. In a meta-analysis of 36 studies on severe ALRI in U5 children, 'definite' risk factors identified were low birth weight, under nutrition, lack of exclusive breast feeding, exposure to indoor air pollution, overcrowding, incomplete immunization at one year and human immunodeficiency virus (HIV) infection [76]. All the seven factors demonstrated a significant association with severe ALRI consistently across all identified studies which is why the term definite

was used. An additional seven factors; parental smoking, lack of maternal education, vitamin D deficiency, male sex, preterm births, anemia, and zinc deficiency were also found, but their association with severe ALRI was not consistent and they were defined as 'likely' risk factors [76] .

Interventions with proven efficacy exist but do not reach children at the highest risk and, in many instances, even if they do, their usage remains poor. In a review of studies from developing countries, young infants (0 – 5 months), who were not breastfed were at an increased risk of acquiring pneumonia (RR 2.07; 95% CI 0.19, 22.64), needing hospitalization (RR 4.06; 95% CI 1.48, 11.14) and death (RR 14.97; 95% CI 0.67, 332.74) compared to those who were exclusively breastfed [77]. WHO and United Nations Children's Fund (UNICEF) recommends all mothers worldwide to exclusively breastfeed their infants for the first 6 months but the proportions in which this practice is followed are only 36% and 49% for countries in South Asia and Sub-Saharan Africa, respectively [78]. Similarly, while on an average 65% of care givers seek appropriate care for children with symptoms of pneumonia in South Asia, in the Sub-Saharan African region this is practiced by only 46% of care givers [78]. The Expanded Program on Immunization (EPI), a cost effective and important public health intervention, saves millions of lives and protects against illness and disability by providing vaccines [Bacille Calmette–Guerin (BCG), three doses of Diphtheria-Pertussis-Tetanus (DPT) and Polio, Measles] to children in their first year of life [78]. However, in 2012, approximately 22.6 million (17%) children worldwide did not receive three doses of DPT vaccine, with more than half of incompletely vaccinated children living in the three LMICs of India, Nigeria and Indonesia [79].

While the interventions discussed above prevent children from acquiring pneumonia, identifying factors associated with an increased probability of survival after a child has been hospitalized for the treatment of severe pneumonia is equally important. Knowledge regarding risk factors for

adverse outcomes such as treatment failure, prolonged stay and death are needed to better utilize scarce resources in LMICs. However, there are only a few studies from LMICs reporting predictors of outcome in hospitalized children with severe pneumonia [33, 80-84]. The factors associated with an increase in case fatality are young age [33, 82], hypoxia on admission [33, 80, 81], undernutrition [80, 82] and signs of severe illness [80, 83]. Young children are also at higher risk of treatment failure [85], admission to intensive care units with mechanical ventilation and prolonged duration of hospital stay [84].

Another important factor that determines survival is the quality of care provided to children with severe pneumonia admitted to a health facility. The IMCI strategy, developed in 1995 to identify and treat major childhood illnesses, strengthen counseling of care takers, provide preventive services and speed up the referral of sick children, focused only on outpatient management [20]. Following evidence of significant deficiencies in the recognition and care of sick children brought to the hospital, the WHO IMCI emergency triage assessment and treatment guidelines to improve pediatric referral level care in developing countries were established in 2000 [10]. Since then, the IMCI strategy has been adopted by more than 100 developing countries. Evaluation of the program indicates that it is cost-effective [86] and the performance and quality of care provided by health workers with IMCI training is better than those who are not trained [87]. However, despite reports of successful implementation of IMCI in more than 75% of districts by many countries, the actual coverage remains poor. Some of the measures recommended to scale up coverage are to encourage community health workers and the private sector to adopt this IMCI approach for common childhood illnesses [88].

Another initiative of WHO/UNICEF, the Integrated Global Action Plan for the Treatment and Control of Pneumonia and Diarrhea (GAPPD), aims to substantially reduce preventable deaths due to these

two diseases in U5 children [74]. The discussion that follows is related to issues mostly relevant to pneumonia. The goals for tackling pneumonia are to reduce the incidence of severe pneumonia by 75% compared to 2010 levels and deaths from pneumonia to fewer than 3 per 1000 live births by 2025 and the proposed strategy is to deliver interventions that work using the "protect, promote and treat" framework [74]. The suggested interventions for reducing pneumonia morbidity and mortality are promotion of breast feeding to protect young children, providing measles, pertussis, Hib and PCV vaccines to prevent children from getting pneumonia, reducing household indoor air pollution, and treat appropriately those diagnosed with pneumonia using standard case management in health facilities and the community [74]. The proposed targets can only be achieved if interventions reach every child at risk. Therefore, coverage targets proposed by GAPPD, to be maintained or reached by the end of 2025 in each country, are 90% full-dose coverage of each relevant vaccine (80% coverage in every district); 90% access to appropriate pneumonia case management (80% coverage in every district); that at least 50% of infants are exclusively breastfed during the first 6 months of life, as well as the virtual elimination of pediatric human immunodeficiency virus (HIV) infection [74].

Zinc – function and metabolism

Zinc was discovered as an important element for human health in the 1960s and although it has been widely studied, many questions regarding its mechanism of action and utility still remain unanswered [89]. It is the second most abundant essential trace element in the human body [90] and is required for cellular division, differentiation and growth, which make its demand high in individuals with a rapid growth rate, such as children. Organs that are dependent on continuous cell division for proper function, such as the immune system and the gut epithelium, are particularly sensitive to zinc deficiency [91, 92]. In the airway epithelium, zinc has been demonstrated to exert

anti-oxidant and anti-apoptotic properties [90]. Zinc deficiency affects the functions of both the innate and adaptive immune systems. The activity of natural killer cells and phagocytosis by macrophages and neutrophils is impaired by decreased zinc levels [93]. The generation of reactive oxygen species, such as superoxide anion that destroys pathogens inside neutrophils following phagocytosis, is also affected by zinc deficiency [94]. In the adaptive immune system, zinc deficiency affects the development and function of the T cells much more than the B cells. The alterations in the cell mediated immune system range from thymic atrophy with subsequent decrease in lymphocyte numbers to imbalance in the T-cell subsets. Zinc deficiency is associated with diminished production of T_H1 cytokines (IFN-γ, IL-2 and TNF-α) while levels of those associated with the T_H2 response (IL-4, IL-6 and IL-10) are less affected [95]. Although less affected, zinc deficiency also leads to a reduction in B cells and antibody production [92]

A regular intake of zinc is required as there are no stores in the body from which it can be easily mobilized. Recommended daily allowances for zinc are 2 mg/day for infants below 6 months, 3 mg daily for young children up to 3 years and 5 mg for older children until the age of 8 years [96]. How well-nourished the body is with respect to zinc is dependent on the quantity and bioavailability of this element in the food [97]. Although meat and fish are the most readily bioavailable sources of zinc, cereals and pulses continue to be the main sources of zinc in the diet globally (5). Bioavailability of zinc in foods is influenced by plant ligands, such as phytate, dietary fibers and lignin as well as calcium. Phytates make insoluble complexes with zinc in the gut and presence of calcium enhances this effect [98].

Assessing zinc deficiency in populations versus individuals

Inadequate intake of absorbable zinc is the most common cause of zinc deficiency [99]. Based on data derived from national food balance sheets, 17.3% of the global population is at risk of

inadequate zinc intake with values ranging from 7.5% in high income regions to 30% in South Asia [100]. The reason for this disparity lies in the dietary source of zinc. In high income regions, approximately 40% of zinc is derived from meat and 20% from cereals whereas in South Asia about 70% of zinc is derived from cereals and 5% from meat. However, these data on zinc are more representative of food intake by adults rather than children, who are also more vulnerable to zinc deficiency [100]. The proportion of U5 children with stunting (length/height for age < -2 Z) is probably a better indicator of zinc status [101]. Countries classified as high risk of zinc deficiency are those with estimated prevalence of inadequate zinc intake of > 25% and stunting of > 20%. Nepal with 41% stunted U5 children [102] and estimated inadequate intake of zinc among 24% of the population is classified as being at medium risk of zinc deficiency [100].

The most commonly used biomarkers of zinc status, i.e. plasma or serum zinc concentrations, may not correctly identify zinc deficiency in individuals but may be useful as indicators of zinc status at the population level [99]. However, this indicators, in the absence of a better biomarker, are still used in many studies to assess zinc status of individuals [103]. Moreover, the interpretation of the commonly reported baseline plasma/serum zinc levels in children with pneumonia need consideration because zinc is redistributed from the plasma to the liver in response to an acute infection or inflammation [99].

Zinc for the prevention and treatment of common childhood infections

According to prevalence estimates from the United Nations, zinc deficiency is responsible for 116.000 (1.7%) deaths in children 12 – 59 months of age globally [104]. In a systematic review assessing the impact of zinc supplementation in randomized clinical trials in developing countries, there was a 9% (RR 0.91; 95% CI 0.82, 1.01) reduction in all - cause mortality , reduction in diarrheal deaths by 18% (RR 0.82; 95% CI 0.64, 1.05) and pneumonia deaths by 15% (RR 0.85; 95% CI 0.65,

1.11)[105]. The review also estimated impact on disease occurrence and found that administration of zinc at a median dose and duration of 10 mg/day for 6 months resulted in reductions in the incidence of pneumonia by 19% (RR 0.81; 95% CI 0.73, 0.90) and diarrhea by 13% (RR 0.87; 95% CI 0.81, 0.94), respectively [105].

Oral zinc for the treatment of acute diarrhea was recommended by the WHO and UNICEF in 2004 and many countries have adopted this recommendation. Several reviews and meta-analyses support the utility of this inexpensive intervention [106-108]. In addition, effectiveness studies have suggested that oral zinc given for acute diarrhea reduces all - cause mortality by 46% and hospital admission rates by 23% [109]. However, in the studies that measured this effect it was difficult to disentangle the effect of oral rehydration solution (ORS) from the effect of zinc as ORS was included in the intervention [110, 111].

Many studies have been done to explore the therapeutic effect of zinc on acute respiratory infections. The common cold is a leading cause of doctor visits and absenteeism from school and work and is associated with complications, such as otitis media, sinusitis and exacerbations of reactive airway diseases. Pre-school children may have 6 to 10 episodes of common cold per year [112]. While there is no proven effective treatment for this illness, two recent meta-analyses [113, 114] concluded that oral zinc given within 3 days of the onset of illness significantly reduced illness duration. However, most of the studies were in adults and there was substantial heterogeneity between the trials. Until date, 14 clinical trials on the efficacy of zinc in addition to standard treatment given during childhood pneumonia have been published [115-129]. The trials that have measured the effect of zinc have done so in diverse populations and used different definitions of pneumonia and recovery. The results are conflicting, indicating that the therapeutic effect of zinc for pneumonia/ALRI, if any, is limited.

Global burden of pneumonia

It is estimated that 6.3 million children U5 die every year worldwide; 99% of these deaths occur in developing countries. Pneumonia is the leading cause and accounts for almost one million of these deaths [73]. Four out of five deaths occur in children less than 2 years of age [75]. The incidence of pneumonia in U5 children is ten-fold higher in LMICs with an estimated 0.22 episodes per child-year, as opposed to 0.024 in HICs [47]. However, the same does not hold true for severe pneumonia because for LMICs the estimated proportion of episodes that are severe is 11.5% compared to 26.7% for HICs [47]. The estimates for severe episodes in HICs are based on data generated from hospitalized children but for LMICs these are derived from community based studies [47] with severe pneumonia defined by the presence of LCI, thus representing a need for hospitalization[8]. In a systematic analysis of data from hospital based studies, severe ALRI resulted in 12 million hospital admissions in children U5 in 2010 [130]. The estimated incidence of hospital admissions for severe ALRI, which is 10 episodes per 1000 in HICs, is half the overall rate of 20 episodes per 1000 U5 children in LMICs, which is a more plausible estimate of the disease burden in these settings [130]. This review also highlights the inequities that exist in access to health care in LMICs, with more than one-third of children with severe ALRI not reaching hospitals and four- fifths of ALRI related deaths occurring outside of hospital setting [130].

Pneumonia burden in Nepal

U5 children account for 10% of the total population of 26.5 million in Nepal [131]. The community based – Integrated Management of Childhood Illness (CB-IMCI) program, initially implemented in 1999, has since 2009 covered all 75 districts of the country. In Nepal, ARI is classified and managed according to WHO guidelines [132] in government health institutions. A true estimate of the incidence of pneumonia in U5 children of Nepal does not exist because there is no system for active

surveillance in the community. Integrated Health Management Information System (HMIS) implemented since 1994 manages information on all health services mostly delivered through the government's health facilities. In the Nepalese fiscal year 2069/70 B.S. (16 July 2012 - 15 July 2013), among children visiting health facilities, 243/1000 U5 were diagnosed with pneumonia of which 0.4% were classified as having severe pneumonia [133]. According to the Demographic and Health Survey for the period 2006 – 2010, among the 5% of U5 children with symptoms of ARI in the two weeks preceding the survey, only 50% were taken for advice or treatment to a health care provider [102]. Therefore, while the incidence of pneumonia reported by the government is similar to the projected country level estimate of 0.24 episodes per child year by the Child Health Epidemiology Reference Group [47], it does not take into account the many sick children that do not actually reach health facilities. It also does not include data from the sick children that visit private physicians, especially in urban areas of the country. Over the last 10 years the incidence of childhood pneumonia and the proportion of children who have severe disease seem to have declined steadily in Nepal [134]. The country introduced the pentavalent DTP-HepB-Hib vaccine into its Expanded Program on Immunization in 2009 [135] and started introducing the pneumococcal conjugate vaccine in January 2015 [136] . The coverage for the pentavalent DTP-HepB-Hib3 vaccine is > 80% in 91% of the 75 districts in Nepal [137]. These initiatives are likely to further reduce the occurrence of pneumonia of bacterial etiology in children.

Focus of thesis

While the main aim of this PhD work was to examine the extent to which administration of oral zinc reduces the duration of illness and risk of treatment failure, it also describes the detection of viruses from the nasopharynx and determines predictors of outcome of an episode of severe pneumonia in young children admitted to a general hospital in Nepal. When this study was

conceptualized in 2001/2002, several studies had demonstrated that zinc reduced the duration of diarrhea but there were no published studies on the effect of zinc during pneumonia. The identification of risk factors that predict important outcomes, such as duration of illness and treatment failure, in children hospitalized with severe pneumonia is relevant because the focus of pneumonia case management in resource poor countries has largely been on identifying factors, that when modified, could prevent deaths [138]. The work on the etiology of severe pneumonia in hospitalized children is pertinent for a country where the epidemiology of pneumonia is changing and viral pneumonia is still unrecognized as a cause for hospital admissions. Our description of molecular based detection of seven respiratory viruses in nasopharyngeal aspirates of children hospitalized with severe pneumonia is the first of its kind in Nepal.

Study Objectives

General objective

To measure the efficacy of zinc as adjunct to antibiotic treatment, identify common viral pathogens and predictors of outcome in children in the age range of 2 – 35 months hospitalized with severe community acquired pneumonia.

Specific objectives

I: To assess the efficacy of oral zinc administered daily, for a maximum of 14 days, to infants and young children hospitalized with severe community acquired pneumonia in reducing the time to cessation and the risk of treatment failure.

II: To identify common viral pathogens from nasopharyngeal aspirates of infants and young children hospitalized with severe community acquired pneumonia using a multiplex PCR assay.

III: To measure the strength of the association between clinical, radiological and laboratory predictors and the duration and risk of treatment failure in infants and young children hospitalized with severe community acquired pneumonia.

Study Methods and Subjects

Study setting

Nepal - geography, economy and health status of children

Nepal is a landlocked country in South Asia with India to the east, south and west and China bordering the north. It is 885 kilometers long and 193 kilometers wide with a total land area of almost 150,000 square kilometers [131]. The total population of Nepal according to the census in 2011 was 26.5 million with an annual population growth of 1.35% and average population density of 181 per square kilometer [131]. For administrative purposes, Nepal is divided into five development regions, 14 zones and 75 districts (Figure 3). The country is also divided into three ecological zones, i.e. mountain, hill and plain (Terai) that run from east to west (Figure 3). The hill zone occupies 42% of the land area with altitudes ranging from 610 meters to 4876 meters above sea level. While 43% of the population resides in the hills, the distribution is not uniform. Most people live in the valleys with lower numbers living at 2000 meters and above [102]. The climatic conditions in the hills vary from tropical (<1000 meters), sub-tropical (1000 – 2000 meters), temperate (2000 – 3000 meters), sub-alpine (3000 – 4000 meters) and alpine (4000 – 5000 meters) [139]. The Kathmandu valley, at an altitude of approximately 1400 meters above sea level is the most fertile and most urbanized. The capital of the country, Kathmandu, is situated in this valley and has the highest population density of 4.408 per square kilometer [102].

The Kathmandu Valley climate is influenced by the Indian monsoon with four distinct seasons. The pre-monsoon from the months of March to May is windy, hot and humid followed by a well-defined monsoon from July to September. Most of the rain falls during this monsoon season, accounting for 82% of the average 1400 mm annual rainfall. The post-monsoon from October to November and

winter season from December to February is usually dry with occasional showers. Temperatures can drop below 0°C during the winter and can rise up to 35°C during the hot months [140].

Figure 3. Administrative map of Nepal

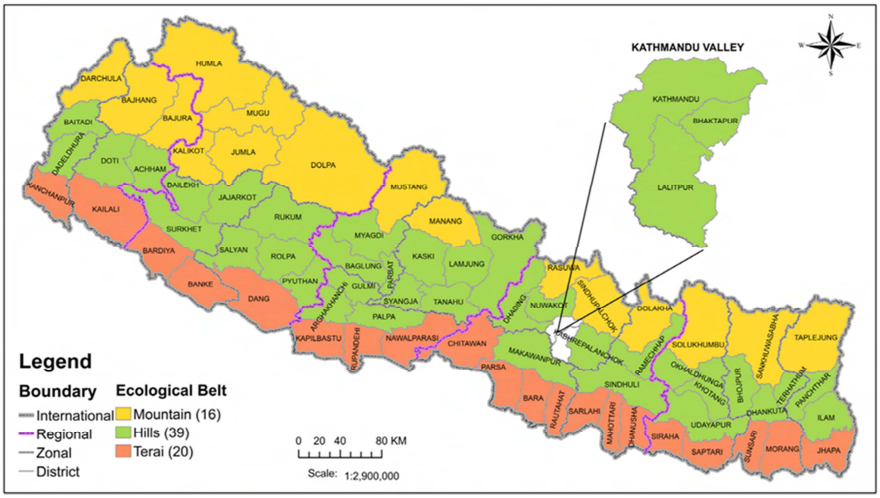


Figure adapted from Annual Report Department of Health Services (2012 - 2013)[133]

Nepal is classified as a low income country by the World Bank, a developing country by the United Nations Development Program and as a least developed country by the United Nations Conference on Trade and Development [141]. Compared to Norway, which ranks first with a very high human development index (HDI) of 0.944, Nepal ranks 145th with a low HDI of 0.541 [142]. The country's gross national income per capita in 2012 was US $ 700 [141]. The life expectancy at birth is 68.8 years with the mean number of years of schooling at 3.9 years [142].

Children less than 5 years of age comprise almost one-tenth of the total population with a male to female ratio of 1.1:1 [131]. Over the past 15 years there has been a steady decline in infant and U5 mortality. In the five year period 2006 – 2010, the U5 mortality was 54 and infant mortality 46 deaths per 1000 live births [102]. While nutritional status is also improving, among U5 children 41% are still stunted, 11% wasted and 29% are underweight. Seventy percent of infants less than 6

months of age are exclusively breastfed and breast feeding continues up to 2 years of age in 93% of children [102]. However, only one-fourth of children between ages 6 - 23 months are fed appropriately with complementary foods [102]. The National Immunization Program (NIP) provides vaccines against 9 diseases free of cost to children. For most of the vaccines, coverage exceeds 85% (Table 2) [143] . The Hib vaccine was introduced in 2009 [144] and since January 2015 routine immunization with the 10-valent pneumococcal conjugate vaccine (PCV-10) is to be given at 6 weeks, 10 weeks and 9 months of age. The roll out of PCV has been initiated in some districts [136].

Table 2. Immunization schedule for children of Nepal with coverage

Vaccine	**Number of doses**	**Recommended**	**Immunization Coverage (2012 – 2013)**
BCG (Bacille Calmette Guerin)	1	At birth or first contact	98.9%
OPV (Oral Polio Vaccine)	3	6, 10 and 14 weeks of age	92.7%
DPT – HepB – Hib (Diptheria/Pertussis/Tetanus/Hepatitis B/Hemophilus influenza type b)	3	6, 10 and 14 weeks of age	92.8%
Measles	1	9 months of age	87.8%
TT (Tetanus toxoid)	2	To pregnant women	35.4%
JE (Japanese Encephalitis)	1	12 – 23 months (only in high risk districts)	87.3%

Adapted from Annual Report Department of Health Services (2012 - 2013)[133]

Study site and population

The clinical trial that generated the data on which this thesis is based was conducted at the Kanti Children's Hospital. It is a 300 bed tertiary referral care hospital under the Government of Nepal that provides services to children up to the age of 14 years from all parts of the country. The hospital is located in Maharajgunj in the Kathmandu District, which is one of three districts in the

Kathmandu valley (Figure 4). Almost half of the patients attending this hospital come from outside the valley. An average of 300 children visits the Medical Outpatient Department daily, which is open 6 days a week. The Emergency Department (daily) attends to at least 125 children 24 hours a day 7 days in a week. In the Nepali fiscal year 2067 – 2068 B.S. (17 July 2010 - 16 July 2011), of the 3933 patients admitted to the hospital for medical care, 1077 (27%) were diagnosed with pneumonia [145].

Figure 4. Kathmandu and its surrounding districts with location of study site

Reproduced from the webpage of the Local Governance & Community Development Programme under the Ministry of External Affairs and Local Development, Government of Nepal.

A summary of the methods in relation to the papers I-III is outlined below.

Table 3. Study design, period, topic and main analyses of studies presented in the papers of the thesis

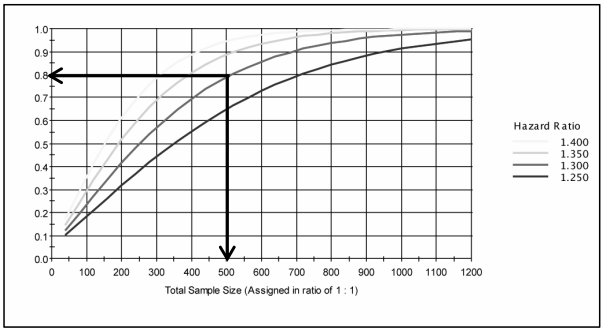
Paper	Study type	Study period	Topic	Main analyses
I	Double-blind, randomized, placebo-controlled hospital based trial (n = 610)	February 2006 to June 2008	Efficacy of oral zinc on recovery and treatment failure of severe pneumonia in hospitalized young children	1. Cox regression 2. Log-binomial regression
II	Cross sectional study (n = 641)	January 2006 to June 2008	Identification of RNA viruses in nasopharyngeal aspirates from young children hospitalized with severe pneumonia and their seasonality	Descriptive statistics
III	Secondary analyses of data from the clinical trial (Paper I) on zinc for severe pneumonia (n = 610)	February 2006 to June 2008	Predictors of time until recovery and treatment failure of severe pneumonia in hospitalized young children	1. Cox regression 2. Logistic regression

Study Methods

Estimation of Sample Size

In a previous comparable study in Bangladesh by Brooks *et al*. [115], the median time to resolution from severe pneumonia was 3 days in the zinc group and 4 days in the group receiving placebo. Based on a similar assumption, using minimal detectable hazard ratio of 1.30 for time to recovery from severe pneumonia, we calculated a total sample size of 500 children [146]. Calculations were done with 80% power and 5% alpha error. Assuming 10% loss to follow up, a total of 550 children with 275 in each group were required. We also explored different scenarios using different probabilities of type II errors, event probability estimates and effect sizes and finally agreed to enroll around 600 participants. The figure below is an example of one such scenario (Figure 5).

Figure 5. Power as a function of sample size and hazard ratio



Papers I, II and III

The data generated during the hospital-based clinical trial formed the basis for the three papers presented in this thesis.

Table 4. Case definitions

Acute episode of severe pneumonia	A child presenting with cough (duration <14 days) and/or difficulty in breathing of ≤ 72 hours' duration and lower chest indrawing (LCI).
Failure to improve	Persistence of LCI or of any danger signs* present at enrollment despite 48 hours of treatment or appearance of new danger signs or hypoxia with deterioration of a patient's clinical status any time after initiation of treatment.
Clinical improvement	Absence of danger signs, of hypoxia for a consecutive 24-hour period and of LCI for a 24-hour period.

***Danger signs –** were defined as the presence of:

- General danger signs – inability to breastfeed or drink, lethargy or unconsciousness and vomiting everything
- Other signs of severe pneumonia – nasal flaring, grunting, head nodding and central cyanosis

Recruitment of study participants – screening for inclusion and exclusion criteria

Severe pneumonia was defined using the classification suggested by WHO [21] (Table 4). All children 2-35 months of age who attended the Outpatient or Emergency departments of Kanti Children's Hospital with a history of cough lasting for no longer than 14 days and/or difficulty breathing for ≤ 72 hours and LCI were screened by trained study physicians. Children fulfilling these criteria were initially assessed for the presence of wheezing (audible or on auscultation) and hypoxia [defined as an oxygen saturation (SpO_2) of < 90% [21] measured using a portable pulse oximeter]. All children with hypoxia were given humidified oxygen using a nasal cannula before further evaluation. Children with recurrent wheezing (defined as > 3 episodes over the past 6 months) and on treatment with bronchodilators were not included. Children with first episode of wheezing or having had no more than two such episodes in the past 6 months were given up to three doses (2.5 mg each) of nebulized Salbutamol 15 minutes apart and reassessed. If LCI disappeared, they were not enrolled. For all eligible children, a history of the child's illness was taken and physical examination by a study physician was carried out using a standardized form. A chest radiograph was obtained in all children and haemoglobin measured. Children with severe wasting (< - 3z weight for length/height calculated using the WHO/NCHS normalized reference charts) [10], severe anemia (hemoglobin < 7 gm/dl) [21], heart disease, documented tuberculosis, concomitant diarrhea with dehydration, or other severe illness requiring special care and those needing surgical interventions were excluded. A child meeting inclusion criteria for severe pneumonia but with a history of having been previously enrolled in the study within the past 6 months was also not included.

For the studies reported both in Papers I and III, 610 children recruited between 1st February 2006 and 30th June 2008 were included.

For Paper II, an additional 31 children recruited from the first week of January 2006 to 31st January 2006 were also included.

Enrolment, randomization, administration of intervention and co-interventions and follow up

All children with severe pneumonia meeting requirements for inclusion were enrolled after informed consent was obtained from the parent or other caregiver of the child. Blood for investigations and nasopharyngeal aspirate for identification of respiratory viruses was collected by the study physician and treatment with intravenous antibiotics initiated.

Intervention

Dispersible tablets containing 10 mg of elemental zinc sulphate or placebo were available in a blister pack with 15 tablets. Each pack, labeled with a serial number to match the child's study identification number, was identical in packaging, appearance and inactive ingredients with no indication of group identity and content. The randomization list linking the treatment groups to these identification numbers was generated by and kept with a scientist not otherwise involved in the study. Children were allocated to either of the two intervention groups by randomization in blocks of 16 in a 1:1 ratio. Randomization was stratified on age < 12 months or ≥ 12 completed months and on whether the child had wheezing before nebulization. Study physicians selected blister packs with the lowest available number from a box specifying the stratum to which each child belonged.

Children <12 months were given 1 tablet and children ≥ 12 months 2 tablets dissolved in 5 mL of breast milk or clean water. The first dose was dispensed by the study physician and subsequently by trained study assistants who were not otherwise involved in patient care. The zinc or placebo dispersions were given as a single daily dose until discharge or for a maximum of 14 days. All children were observed for vomiting. For children who vomited within the first 15 minutes, a repeat

dose was given. Children who vomited the second time were given the required amount in two divided doses over a 24-hour period from the next day onwards.

Co-interventions

Enrolled children were admitted to the hospital and monitored at 8 hourly intervals by study physicians until discharge. The antibiotics, Benzyl penicillin (50,000 units/kg body weight every 6 hourly) and Gentamicin (7.5 mg/kg body weight once daily) were given intravenously until clinical improvement, following which children were discharged. At discharge parents were instructed to give oral Amoxicillin to the child to complete treatment for a total duration of 10 days. Those with failure to improve were switched to parenteral Cefotaxime after consultation with senior pediatricians involved in the study. These children were given oral Cefpodoxime at discharge.

For children unable to eat/drink or breast feed, intravenous fluids were initiated. Humidified oxygen provided by means of a nasal catheter was given to children with documented hypoxia and discontinued when oxygen saturation was > 90% with the child breathing room air. The absence of hypoxia was confirmed with a second reading taken 30 minutes later.

Children were discharged from the hospital if LCI had been absent for a consecutive 48 hour period, parenteral drugs or intravenous fluids were not needed and parents could be relied upon to continue treatment with oral antibiotics for the specified duration at home.

Data collection

The study physicians were trained by senior pediatricians and investigators to correctly identify, monitor and manage young children with severe pneumonia using the WHO guidelines on which the treatment protocol was based [21]. Their performance was monitored each day by experienced pediatricians. Standardization exercises were conducted before the start of the trial. Each physician

was assigned to record axillary temperature, count respiratory rate, observe for LCI, and listen for wheezing and crepitations in at least 10 children. Their findings were matched against those of an experienced pediatrician until the desired agreement was reached.

They were taught how to collect adequate samples of venous blood and nasopharyngeal aspirate with specific instructions for handling of clinical specimens and also to correctly fill in the standardized forms for data collection.

Demographic and Clinical variables

The age of each child recorded as completed months was confirmed by asking the parent/caretaker about the child's date of birth. The name of the district where the child was currently living was recorded. If the child resided in an area inside the Kathmandu valley or the main city of another district it was termed urban; rural if otherwise.

The weight of each child was measured using an Electronic Scale 890 (SECA, Hamburg, Germany) which measures until the nearest 100g. An infantometer was used to measure recumbent length in children < 2 years of age and those unable to stand, while in older children height was measured using a standard wooden height measuring board, both to the nearest 0.1cm. Stunting defined as length-for-age < -2z, wasting as weight-for-length <-2z and underweight as weight-for-age < -2z were calculated using the 2006 WHO Child Growth Standards [101]. SpO_2 was measured using a pulse oximeter (Nellcor Puritan Bennett NPB-40, Pleasanton, CA, USA) with a pediatric sensor (Nellcor Pedichek D-YSPD) placed over a finger or toe after stabilization of the reading for one minute and making sure the extremities were warm. SpO_2 was recorded twice and the higher of the two readings was used for the analyses.

Respiratory rate was counted twice using a UNICEF timer in a calm child. The mother was requested to breast feed a crying child and counting was deferred until the child had calmed down and no longer suckling. The counting of respiratory rate was repeated if there was a difference of 10 or more breaths per minute between the two measurements. The lower of the two readings was used for data analyses. The axillary temperature was measured once by keeping the tip of the thermometer in the roof of the axilla for at least 3 minutes and recorded to the nearest $0.1^{o}F$.

Collection of specimens and laboratory analyses

Study physicians collected 2 mL of venous blood from enrolled children using heparinized polypropylene syringes (Sarstedt, Numbrecht, Germany). A small amount of this blood was used for bedside measurement of hemoglobin concentration using Hemocue (Ångelholm, Sweden). The blood was then centrifuged, separated, the plasma transferred to Cryovials (Nunc A/S, Roskilde, Denmark) and stored at -70°C. The C-reactive protein (CRP) concentration was determined in this stored plasma using a particle-enhanced immunoturbidimetric assay on a Roche/Hitachi cobas c 501 analyzer (Roche, Germany).

In addition to the above, venous blood was collected and sent for culture from all study participants. About one mL of blood was transferred to a blood culture bottle containing brain heart infusion broth and transported to the laboratory where it was incubated for 12 hours. The specimens were then sub-cultured on chocolate agar, sheep blood agar and MacConkey agar following standard microbiology protocols. The specimens were discarded if no growth was detected after 4 days of incubation [147].

In each enrolled child, a nasopharyngeal aspirate (NPA) was collected using a sterile, disposable suction catheter (Pennine Healthcare Ltd., Derbyshire, United Kingdom) with a suction trap (trachea suction set, Unomedical a/s, Birkerød, Denmark) connected to a foot pump (Ambu Uni-suction

pump, Ambu A/S, Ballerup Denmark). The catheter was inserted through the child's nostril to a distance equivalent to that from the nostril to the earlobe and suction was applied for a minimum of ten seconds down to a maximum negative pressure of 200 mmHg. Secretions remaining in the catheter after suction was recovered by rinsing 2- 3 mL Universal Transport Medium (UTM) (Copan Diagnostics Inc., Corona, CA) through the catheter into the suction trap following which it was disconnected and sealed. The NPA specimens were refrigerated on site at 2°C to 8°C and later transported on ice to the PCR research laboratory at the Institute of Medicine, which is next door to the Kanti Children's hospital. In the laboratory, specimens were vortexed, divided into 3 aliquots, and stored at -70°C until analysis. One aliquot of each specimen was tested for RSV, influenza A and B, PIV type 1, 2, and 3, and hMPV using a commercially available multiplex reverse transcription PCR assay (Hexaplex Plus, Prodesse Inc., Waukesha, WI) in the research laboratory in Nepal. The Roche High Pure Viral Nucleic Acid Kit (Hoffman-La Roche Ltd., Basel, Switzerland) was used to extract nucleic acids from 360 µl of NPA (or plasmid RNA from positive control transcripts) following the manufacturer's instructions. Each run of the assay included a positive RNA control and a negative control (virus transport medium). Specimens and negative controls were individually spiked with 40 µl of internal control during nucleic acid isolation to identify any inhibitors. Complementary DNA (cDNA) was produced by reverse transcription using random hexamers, murine leukemia virus reverse transcriptase (ABI, Applied Biosystems, Foster City, CA), RNase inhibitor (ABI) and 3 µl of extracted viral RNA. Amplification reactions were performed using GeneAmp® PCR System 2700 (ABI). This was followed by purification of the PCR products using Qiagen QIAquick PCR Purification Kit (QIAGEN Inc., Valencia, CA). This purified product was denatured, analysed by enzyme hybridization assay [148] and the optical density at 450 nm (OD_{450}) measured using a micro-plate reader (Stat Fax® 2100, Awareness Technology Inc., Palm City, FL). The test result was labelled positive with NPA OD_{450} reading of ≥ 0.400 and at least four times

greater than the OD_{450} of the negative control [149]. For a given NPA, if the OD_{450} of the internal control was < 2.00 and sample absorbance was < 0.400 for all tested agents, the NPA was tested again. On repeated testing, if the same result was obtained it was interpreted as indeterminate due to potential inhibition and the case not included in the analyses.

Chest radiographs and their interpretation

All children with acute severe pneumonia had a chest x-ray (CXR) taken at the radiology department of the hospital using an X-ray machine (Shimadzu UD150B-10). The primary aim of this CXR was to detect any pleural effusion, pneumothorax or suspected heart disease, which, if present, made the children ineligible for the study. We also looked for presence of radiographic pneumonia using standardized WHO criteria [67]. The radiographs were not used to guide treatment and were repeated only in those who met criteria for failure to improve or developed complications while on treatment. For children with more than one CXR, only the first film was used for interpretation. With the parent's/caretaker's permission chest radiographs were kept with the study team when the children were discharged from hospital and later filed and kept in a locked cabinet by the principal investigator.

On completion of the study, 569 CXRs were available for interpretation. Our plan was to analyse radiographic findings in severe childhood pneumonia at a later stage and therefore the initial draft of Paper I that was submitted did not include CXR findings. However, during the process of peer review, we were advised to include radiographic findings by blinded pediatric radiologists. We were able to employ only one radiologist (identified as DM) due to constraints of time and funds. Before reading the CXRs of study participants, DM studied the WHO guidelines for standardized interpretation of chest radiographs that included a computer program for self-assessment of skills [67]. The form used to record findings was adapted from the guidelines [67] and reading of the

study CXRs was completed by June 2011. Readings were done in a 2-stage process. First the quality of the film was assessed and films classified as unreadable, suboptimal or adequate. Findings on the adequate and suboptimal films were then classified as endpoint consolidation, other infiltrates and in the absence of either, labelled as normal. Radiographic pneumonia was defined as the presence of endpoint consolidation. For Paper I, of the 564 CXRs interpreted by DM, 13(2.3%) were unreadable, 135 (23.9%) had endpoint consolidation, 218 (38.7%) had other infiltrates and 198 (35.1%) were normal.

For Paper III, a second radiologist (identified as RKG) already familiar with standardized interpretation of radiologic findings in childhood pneumonia using WHO guidelines was employed in April 2012. The same set of CXRs was provided and data entered in the form developed earlier. Radiologist RKG completed reading of CXRs by July 2012. Of the 569 films interpreted by radiologist RKG, 18 (3.2%) were unreadable, 233(40.9%) had endpoint consolidation, 168 (29.5%) had other infiltrates and 150 (26.4%) were normal.

On combining data of CXR findings between the radiologists we were able to identify only 546 films with identical child serial numbers for comparison and the agreement between the two is shown below (Table 5 a). We calculated a Kappa of 0.40 suggesting only fair agreement between the two readers [150]. Both radiologists then re-read the 152 films on which they did not agree and completed this assessment in October 2012. In this second set of CXRs, there were 148 films with identical serial numbers that could be compared, findings of which are shown (Table 5 b). Further consensus on disputed films was not attempted. Out of a total of 486 CXRs with findings that both radiologists agreed on, 177 (36.4%) were identified with endpoint consolidation.

Table 5. Agreement between radiologists on chest radiograph finding of endpoint consolidation

	Radiologist DM		
Radiologist RKG		Yes	No	**Total**
	Yes	105	127	**232**
	No	25	289	**314**
	Total	**130**	**416**	**546**

(a) First set of chest radiographs

	Radiologist DM		
Radiologist RKG		Yes	No	**Total**
	Yes	72	48	**120**
	No	8	20	**28**
	Total	**80**	**68**	**148**

(b) Second set of chest radiographs

Definitions of outcomes – Papers I and III

- **Time until cessation of severe pneumonia** – The period starting from enrolment to the beginning of a 24-hour consecutive period with absence of LCI, hypoxia and any general danger signs such as inability to breastfeed or drink or vomiting everything, convulsions, lethargy or unconsciousness.

  This was the primary outcome for Paper I.

- **Risk of treatment failure** – The proportion of study participants requiring a change in antibiotics for failure to improve or developing complications, such as empyema/pneumothorax requiring surgical intervention or needing admission to the intensive care unit for ventilator and/or inotropic support.

Definitions of secondary outcomes – Paper I

- **Risk of prolonged illness –** The proportion of study participants with duration of severe pneumonia beyond 72, 96 and 120 hours after enrolment.
- **Risk of vomiting –** The proportion of study participants with observed vomiting after administration of the first dose of supplement.

Although the children were monitored by the study physicians, the decision of whether a child had recovered or needed a change of antibiotics or referral for more specialized care was taken by

senior paediatricians who not only examined the study participants at least once daily but also were in regular contact with the junior doctors.

Ethical approval and informed consent

Ethical clearance was obtained from the ethical review board of the Institute of Medicine, Tribhuvan University and Nepal Health Research Council, Kathmandu, for conducting the hospital based clinical trial. The implementation of the study followed the international ethical principles for medical research involving human subjects as stated in the latest version of the Helsinki Declaration. Informed consent was obtained from at least one parent/guardian before a child was enrolled into the study. Literate parents first read a statement written in the local Nepali language before they signed the consent form. For parents who were unable to read/write, verbal informed consent was obtained in the presence of a witness, whose name and signature was recorded in a register along with the child's serial number.

Statistical analyses

Data management

The completed forms with patient data were collected by the study assistants on a daily basis. All forms were checked manually by one of the clinical supervisors before data entry, which was done within 48 hours. The data was double entered into a database (Visual FoxPro 6.0, Microsoft Corp, Redmond, WA) with inbuilt logic, range, and consistency checks. Data cleaning, definition of outcome variables, exclusion of cases as well as programming of scripts in the statistical packages were done before the analysis-files were merged with the randomization lists.

Paper I

Statistical analyses were undertaken using Stata version 10 (Stata Corp LP, College Station, TX) and differences between the treatment groups (zinc versus placebo) were considered significant when a two-sided *P*-value was < 0.05.

For the primary outcome, time to cessation of severe pneumonia, the Cox proportional hazards regression model was used to compare the treatment groups. The effect estimates were expressed as hazard ratios. Secondary outcomes, i.e. treatment failure, prolonged illness and vomiting after the first dose of the supplement, were compared using generalized linear regression models with log link functions and binomial distributions, yielding relative risks. We coded the outcomes and interventions so that hazard ratios > 1 and relative risks < 1 would represent a beneficial effect of zinc.

Paper II

We analysed the data using Stata/MP 10.0 for Macintosh (Stata Corporation, College Station, TX). The 95% confidence intervals for proportions were calculated using the "ci" command. The meteorological data we report were from the Kathmandu airport weather station that we obtained from Department of Hydrology and Meteorology, Ministry of Environment, Science and Technology in Kathmandu. The mean daily values for relative humidity and temperature that we calculated were the average of twice daily measurements of relative humidity at 8.45 AM and 5.45 PM, and maximum and minimum temperatures, respectively.

Paper III

Data were analyzed using Stata version 13 (Stata Corp, College Station, TX). We fitted three multiple regression models using 1) clinical, 2) clinical and radiological and 3) all variables (Table 6). Age was categorized into four groups (Table 6).

Predictors of time until cessation of a severe pneumonia episode were identified in Cox proportional hazards models with the "exactp" option to handle ties. For treatment failure, we used logistic regression. Outcomes were coded such that Hazard ratios (HR) < 1 for time until recovery indicated slower resolution of illness and Odds ratios (OR) > 1 increased odds of treatment failure. We first assessed the crude associations of relevant independent variables with the selected outcomes. Variables with $p< 0.25$ were included in the multivariable models and those variables which were still significant, i.e. being associated with the outcome with a p-value of < 0.05, were retained in the model. In these models we included the other variables one at a time and kept them if significant, in a manual stepwise approach outlined by Hosmer and Lemeshow [151].

We also tested the goodness-of-fit of the models by the method suggested by Hosmer and Lemeshow and for logistic regression by calculating the dfbetas and hat statistics [151, 152]. We assessed the assumptions for the cox models using tests of specification (plotting of Schoenfeld residuals) and goodness of fit.

Table 6. Variables assessed in the multiple regression models presented in Paper III

Variables	Continuous	Categorical
Clinical		
Age	Months	2 – 6 months: yes or no 7 – 11 months: yes or no 12 – 23 months: yes or no 24 – 35 months: yes or no
Gender	-	Male or female
Breast feeding status	-	Yes or no
Weight for height	Z scores	< -2 WHZ: yes or no
Height for age	Z scores	< -2 HAZ: yes or no
Hypoxia at enrolment (SpO_2 < 90%)	-	yes or no
Fever (Axillary temperature > 38.5 °C)	-	yes or no
Danger signs – Head nodding, nasal flaring, grunting, cyanosis	-	yes or no
Any one danger sign	-	yes or no
Radiological		
Radiographic pneumonia (Endpoint consolidation)	-	yes or no
Laboratory		
CRP (> 40 mg/L, > 80 mg/L)	mg/L	yes or no
NPA positive for respiratory syncytial virus		yes or no
NPA positive for parainfluenza type 1		yes or no
NPA positive for parainfluenza type 2		yes or no
NPA positive for parainfluenza type 3		yes or no
NPA positive for influenza A		yes or no
NPA positive for influenza B		yes or no
NPA positive for human metapneumovirus		yes or no
Supplemented with zinc	-	yes or no

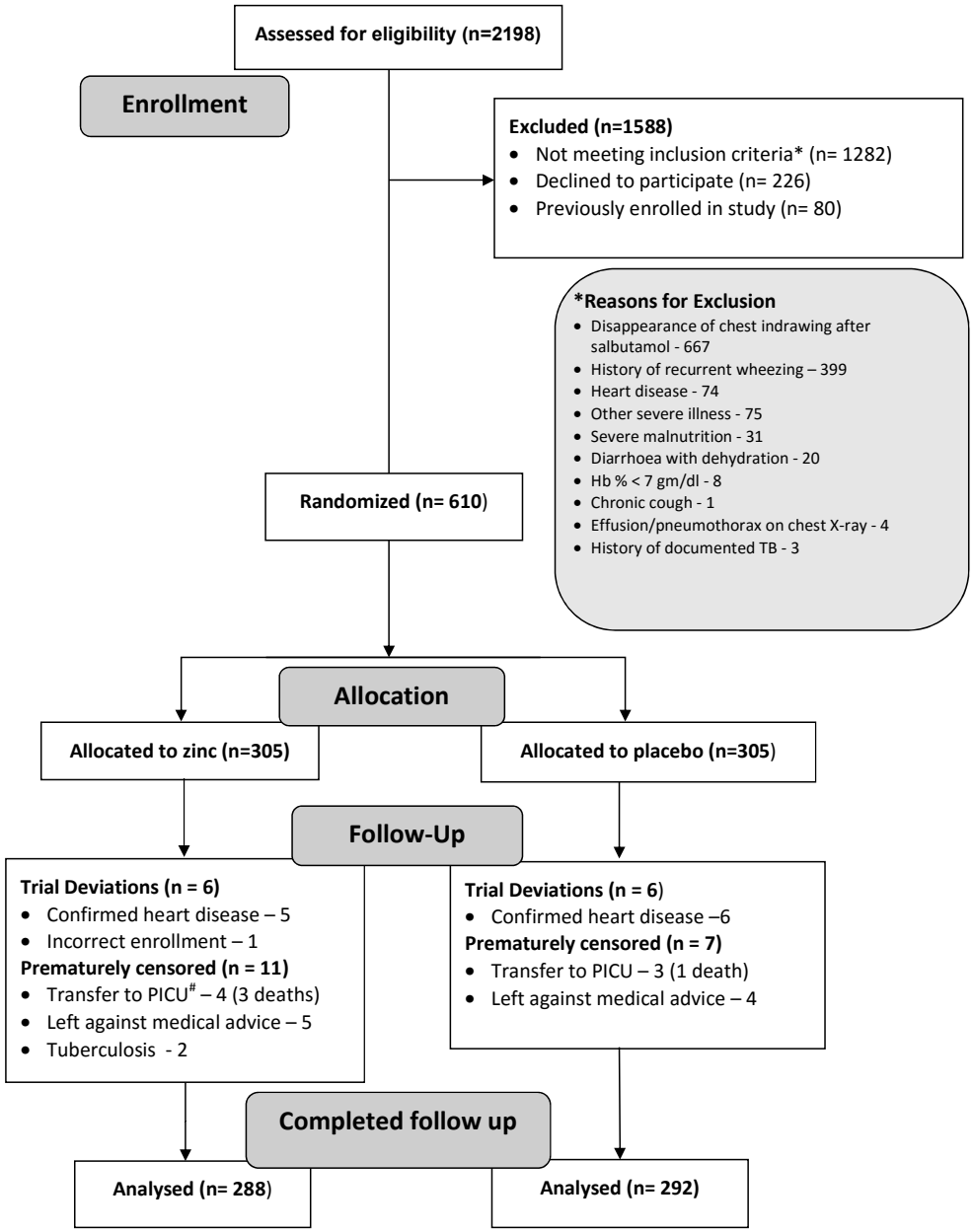
Results

From January 8, 2006 to June 30, 2008 we screened 2270 children aged 2-35 months with cough and/or difficulty in breathing of ≤ 72 hours duration and lower chest indrawing. The 31 children enrolled during the month of January 2006 were part of the pilot study and were included only in the results reported in Paper II. Between February 2006 to June 2008, 2198 children were screened for eligibility. There were 1588 (72%) children who were not eligible for randomization; 1282 (58%) fulfilled exclusion criteria, 226 (10%) did not give consent and 80 (4%) had been enrolled during the last 6 months. We enrolled 610 children that were randomized to receive zinc or placebo (Figure 6) but discovered later that 11 with heart disease and 1 with cough duration > 14 days had been included. These trial deviates, evenly distributed between the study arms, were excluded from the analysis. In the 598 children that we analysed, 18 children were lost to follow up or prematurely censored (Figure 6). The remaining 580 (288 in the zinc group and 292 in the placebo) stayed in the study until recovery from severe pneumonia (Figure 6).

Characteristics of the Study Population

The distribution of study participants according to place of residence was similar between the screened and enrolled children (Table 7). Ninety one percent of screened children were resident in the Bagmati zone and 9% were from other parts of Nepal (Table 7). Among the 8 districts in the Bagmati Zone, children residing in the Kathmandu district comprised two thirds of those that were screened. Similarly, 60% of enrolled children were residents of the Kathmandu District where the study site is also located (Figure 7). Only 9% of enrolled children were from the districts of Lalitpur and Bhaktapur within the Kathmandu valley as opposed to 21% from the districts of Dhading, Nuwakot, Sindhupalchok and Kavrepalanchok that surround it.

Figure 6. Trial Profile

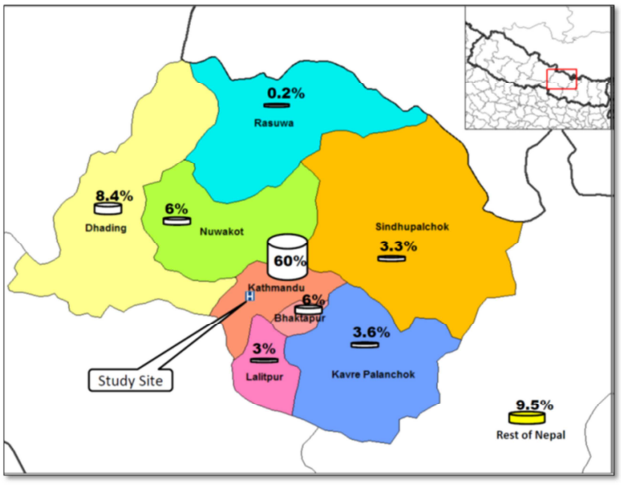


Pediatric Intensive Care Unit

Table 7. Distribution of children in study according to place of residence

Resident of:	Districts	Enrolled	Screened but not enrolled	Total
Bagmati Zone				
	Kathmandu	368 (60.3%)	1050 (66.1%)	1418
	Bhaktapur	37 (6.1%)	95 (6.0%)	132
	Lalitpur	17 (2.8%)	53 (3.3%)	70
	Dhading	51 (8.4%)	90 (5.7%)	141
	Nuwakot	36 (5.9%)	66 (4.2%)	102
	Kavrepalanchok	22 (3.6%)	47 (3.0%)	69
	Sindhupalchok	20 (3.3%)	46 (2.9%)	66
	Rasuwa	1 (0.2%)	4 (0.3%)	5
Other parts of Nepal		58 (9.5%)	137 (8.6%)	195
Total		**610**	**1588**	**2198**

Figure 7. Distribution of enrolled children in districts of the Bagmati Zone



Subject characteristics (Papers I and III)

In this clinical trial, we enrolled 610 (28%) of the 2198 that were assessed for eligibility. More than four-fifths (82%) of the children were less than one year old with more boys (61%) than girls (Table 8). The reported average duration of cough and difficulty breathing was 4 days and 30 hours,

respectively. On clinical examination at presentation, while axillary temperatures above 38.5^0C was found in only 15% of children, 90% infants and 98% older children had a respiratory rate above the WHO age-defined cut-offs for tachypnea (Table 8) and approximately two-thirds had oxygen saturation values measured with a pulse oximeter below 90%. Any danger sign, signifying breathing difficulty, was present in half of enrolled children. On chest auscultation, wheezing was detected in four-fifths and crepitations in every nine out of ten children (Table 8).

There were 226 (10%) eligible children whose parents/caregivers refused to participate in the study. The most frequent reason which was cited by 138 (61%) caregivers was unwillingness to give the supplement to the child. The characteristics of children in this group were fairly similar to the enrolled children. However, non-participating children were younger and the proportion that was stunted was almost twice that of enrolled children (Table 8).

Results of investigations done in enrolled children

A summary of the results are presented in Table 9. The mean CRP measured in 583 samples was 37.7 mg/L with 30% and 13% above 40 mg/L and 80 mg/L, respectively. Any virus was detected in 29% of NPAs of 596 children and RSV was the most frequent detected in 79 (13.3%). Blood culture was positive in 6.5% of the children and *Staphylococcus aureus* was the most common isolate (Table 9). Forty two percent (256/610) of children had history of being given antibiotics prior to enrolment in the study.

Radiographic pneumonia, defined as endpoint consolidation on a chest radiograph, identified and agreed upon by two radiologists blinded to study findings was found in 36% of children whose CXRs were available for interpretation (Table 9).

Table 8. Characteristics of enrolled children and screened but not enrolled due to non-consent

	Enrolled Children		Screened and no consent	
Background characteristics	**N**	**Value**	**N**	**Value**
Mean age in months (SD)	610	7.4 (5.8)	226	6.7 (5.9)
Median age in months (IQR)	610	6 (3, 10)	226	4 (3, 9)
Age categories				
• 2-6 months (%)		362 (59.3)		151 (66.8)
• 7-11 months (%)		142 (23.3)		36 (15.9)
• 12-23 months (%)		91 (14.9)		30 (13.3)
• 24-35months (%)		15 (2.5)		9 (4.0)
Boys (%)	610	373 (61.2)	226	152 (67.3)
Breastfed (%)	610	581 (95.3)	226	215 (95.1)
Wasted (%)	610	166 (27.2)	225	62 (27.6)
Stunted (%)	610	54 (8.9)	225	35 (15.6)
Mean age of mother (SD)	598	24.4 (4.2)	223	24.7 (4.3)
Illiterate mother (%) #	598	158 (26.4)	223	48 (21.5)
Illiterate father (%) #	594	38 (6.4)	219	13 (5.9)
Unemployed mother (%)*	596	428 (71.8)	223	168 (75.3)
Unemployed father (%)*	592	20 (3.4)	219	12 (5.5)
Residing in urban area (%)	610	430 (70.5)	226	168 (74.3)
Clinical characteristics				
Mean duration of cough in days (SD)	610	4.2 (2.1)	226	4.0 (2.0)
Mean duration of difficulty in breathing in hours (SD)	610	29.9 (19.3)	226	28.4 (18.6)
Mean axillary temperature in ^{o}C (SD)	610	37.7 (0.8)	225	37.7 (0.8)
Mean Respiratory rate (SD)				
• 2-11 months	504	65(12)	186	63 (10)
• 12-35 months	106	61(12)	39	65 (13)
Mean Oxygen saturation (SpO_2) (SD)	610	85.7 (8.5)	224	86.6 (7.5)
Hypoxia (SpO_2 < 90%) (%)	610	381 (62.5)	226	132 (58.4)
Febrile (Axillary temperature > 38.5 °C) (%)	610	93 (15.3)	226	40 (17.7)
Danger signs (%)				
- Nasal flaring	609	236 (38.8)	226	71 (31.4)
- Grunting	610	134 (22.0)	226	50 (22.1)
- Head nodding	610	141 (23.1)	226	44 (19.5)
- Cyanosis	610	3 (0.5)	226	1 (0.4)
• Any danger sign	610	299 (49.0)	226	92 (40.7)
• Presence of 3 danger signs	610	58 (9.5)	226	19 (8.4)
• Presence of 2 danger signs	610	99 (16.2)	226	36 (15.9)
• Presence of 1 danger sign	610	142 (23.3)	226	37 (16.3)
Wheezing (%)	610	500 (82.0)	226	180 (80.0)
Crepitations (%)	610	558 (91.5)	226	194 (86.0)
Hemoglobin in gram/dL at bedside (SD)	610	10.6 (1.3)	223	10.7 (1.2)

 # No schooling with inability to read part or whole of a sentence ** No work/housework*

Table 9. Results of diagnostic and biochemical tests (Papers I and III)

Test	N	Value
Mean CRP in mg/L (SD)	583	37.7 (50.9)
- CRP > 40 mg/L (%)	583	172 (29.5)
- CRP > 80 mg/L (%)	583	76 (13.0)
Nasopharyngeal aspirate positive for any virus (%)	596	175 (29.4)*
- Respiratory syncytial virus (%)	596	79 (13.3)
- Parainfluenza type 1 (%)	596	23 (3.9)
- Parainfluenza type 2 (%)	596	5 (0.8)
- Parainfluenza type 3 (%)	596	24 (4.0)
- Influenza A (%)	596	24 (4.0)
- Influenza B (%)	596	17 (2.9)
- Human metapneumovirus (%)	596	9 (1.5)
Blood culture positive for organisms (%)	596	39 (6.5)
- *Staphylococcus aureus* (%)	596	28 (4.7)
- *Streptococcus viridans* (%)	596	6 (1.0)
- *Escherichia coli* (%)	596	2 (0.3)
- *Klebsiella pneumoniae* (%)	596	2 (0.3)
- *Serratia marcescens* (%)	596	1 (0.2)
Radiographic Pneumonia (%)	457	164 (36.0)

** 6 children were positive for 2 viruses simultaneously*

Subject characteristics (Paper II)

For this paper, children enrolled in the pilot study in January 2006 were also included. There were 641 enrolled participants. After excluding 11 children with heart disease, one child that was incorrectly enrolled and also 2 specimens that did not yield a valid PCR result, we report the results of 627 NPAs collected from study participants. The demographic and clinical characteristics of these children were very similar to those described in the Table 8 above, and therefore details have not been provided.

Table 10. Demographic Characteristics of Trial Participants

Characteristics	Zinc group		Placebo group	
	N	Value	N	Value
Mean age in months (SD)	299	7.8 (6.0)	299	7.1 (5.6)
Age groups: 2 – 6 months (%)	299	167 (55.9)	299	185 (61.7)
7 – 11months (%)	299	78 (26.1)	299	63 (21.1)
12 – 23 months (%)	299	45 (15.1)	299	45 (15.0)
24 – 35 months (%)	299	9 (3.0)	299	6 (2.0)
Males (%)	299	177 (59.2)	299	190 (63.6)
Urban residence (%)	299	216 (72.2)	299	206 (68.9)
Mean age of mother in years (SD)	292	24.7 (4.2)	294	24.1 (4.1)
Illiterate[1] mother (%)	292	79 (27.1)	294	75 (25.5)
Illiterate[1] father (%)	293	20 (6.8)	295	18 (6.1)
Unemployed[2] mother (%)	290	205 (70.7)	294	213 (72.5)
Unemployed[2] father (%)	289	10 (3.5)	291	9 (3.1)
Child still breastfeeding	299	283 (94.6)	299	288 (96.3)

[1] No schooling with inability to read part or whole of a sentence [2] No work/housework

Summary of results (Paper I)

Tables 10, 11 and 12 summarize the baseline characteristics of enrolled children between the zinc and the placebo groups. While most demographic variables (Table 10) were similar across the two trial arms, there was a higher proportion (62% versus 56%) of infants between ages 2 – 6 months among placebo recipients and a higher proportion (26% vs 21%) of infants between ages 7 – 11 months among zinc recipients . The proportion of children with wasting was also slightly higher in the zinc arm (30% vs 24%) compared to the placebo arm of the trial cohort (Table 11).

Table 12 describes the results of the investigations carried out and their distribution among the trial participants. While most results show minimal differences between the groups, the proportion of children with radiographic pneumonia is higher in the placebo recipients than in those that received zinc (38% vs 34%).

Table 11. Clinical characteristics of trial participants

	Zinc group		Placebo group	
Clinical characteristics	**N**	**Value**	**N**	**Value**
Mean duration of cough in days (SD)	299	4.0 (2.0)	299	4.2 (2.2)
Mean duration of difficulty breathing in hours (SD)	299	30.2 (20.2)	299	29.6 (18.3)
Mean duration of fever in days (SD)	264	3.5 (2.0)	279	3.5 (2.1)
Weight for length/height < -2z[1] (%)	299	89 (29.8)	299	72 (24.1)
Length/height for age < -2z[1] (%)	299	28 (9.4)	299	22(7.4)
Mean hemoglobin in g/dL (SD)	299	11.0 (1.4)	299	10.9 (1.4)
Mean axillary temperature in ^{o}C (SD)	299	37.7 (0.8)	299	37.7(0.9)
Febrile[2] (%)	299	46 (15.4)	299	46 (15.4)
Mean respiratory rate in breaths per minute (SD)				
2 to 11 months	245	64 (12)	248	65 (12)
12 to 35 months	54	60 (13)	51	63 (10)
Mean oxygen saturation in % (SD)	299	86 (8)	299	86 (9)
Oxygen saturation (%)				
< 90 %	299	186 (62.2)	299	187 (62.5)
< 93 %	299	255 (85.3)	299	249 (83.3)
Wheezing (%)	299	244 (81.6)	299	248 (82.9)
Crepitations (%)	299	273 (91.3)	299	276 (92.3)
Endpoint consolidation on chest radiograph (%)	230	78 (33.9)	227	86 (37.9)
Symptoms of severe pneumonia				
Child unable to drink/breastfeed (%)	299	30 (10.0)	299	24 (8.0)
History of convulsions (%)	299	1 (0.3)	299	2 (0.7)
Vomiting everything child eats (%)	294	9 (3.1)	292	5 (1.7)
Child unconscious/lethargic (%)	299	25 (8.4)	299	30 (10.0)
Signs of severe pneumonia				
Nasal flaring (%)	298	116 (38.9)	299	116 (38.8)
Grunting (%)	299	60 (20.1)	299	71 (23.8)
Head nodding (%)	299	65 (21.7)	299	73 (24.4)
Central cyanosis (%)	299	1 (0.3)	299	2 (0.7)

[1] Derived using the recent WHO Child Growth Standards [2] Defined as axillary temperature of > 38.5^{o}C

Table 12. Results of diagnostic and biochemical tests of trial participants

	Zinc Group		Placebo group	
Diagnostic Test	**N**	**Value**	**N**	**Value**
Mean CRP in mg/L (SD)	289	38.1(54.1)	294	37.2 (47.6)
- CRP > 40 mg/L (%)	289	82 (28.4)	294	90 (30.6)
- CRP > 80 mg/L (%)	289	36 (12.5)	294	40 (13.6)
Nasopharyngeal aspirate positive for any virus (%)	298	94 (31.5)	298	81 (27.2)
Mean Zinc in µg/L (SD)	202	63.1(16.6)	200	60.8 (15.7)
Radiographic Pneumonia (%)	230	78 (33.9)	227	86 (37.9)

The time for children to recover from a severe episode of pneumonia, the primary outcome, was not different between the zinc and placebo recipients (Figure 8). Zinc recipients recovered 10% earlier but this association was not statistically significant (HR 1.10; 95% CI 0.94, 1.30) (Table 13).

Figure 8. Time until cessation of severe pneumonia in trial participants

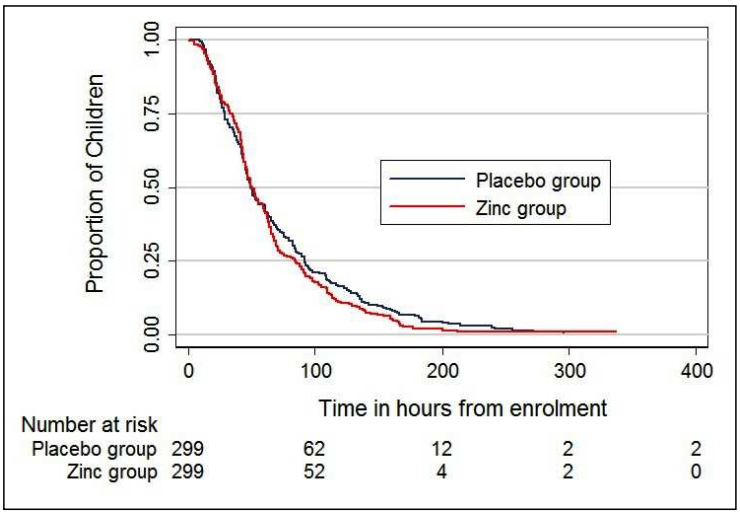


Table 13. Primary and secondary outcomes in clinical trial on oral zinc for severe pneumonia in children

	Zinc Group		Placebo Group			
	N	**Value (IQR)**	**N**	**Value (IQR)**	**Hazard Ratio[1] (95% CI)**	**P**
Median time to cessation of severe pneumonia in hours	288	49 (33, 77)	292	49 (29, 91)	1.10 (0.94 – 1.30)	0.22
Proportion with duration of severe pneumonia in hours	**N**	**Value (%)**	**N**	**Value (%)**	**Risk Ratio[2] (95%CI)**	**P**
> 72	295	83 (28)	297	104 (35)	0.80 (0.63 – 1.02)	0.07
> 96	294	56 (19)	296	64 (22)	0.88 (0.64 – 1.21)	0.44
> 120	293	31 (11)	294	46 (16)	0.67 (0.44 – 1.03)	0.07
Proportion with treatment failure	296	98 (33)	298	111 (37)	0.88 (0.71 – 1.10)	0.29
Proportion with vomiting after supplement [3]	299	41 (14)	299	26 (9)	1.57 (0.99 – 2.50)	0.05

[1] Hazard ratio for time until cessation of severe pneumonia was estimated using Cox proportional hazards models. A value of > 1 indicates beneficial effect of zinc.
[2] From generalized linear models with a log link function and binomial distribution.
[3] After the first dose

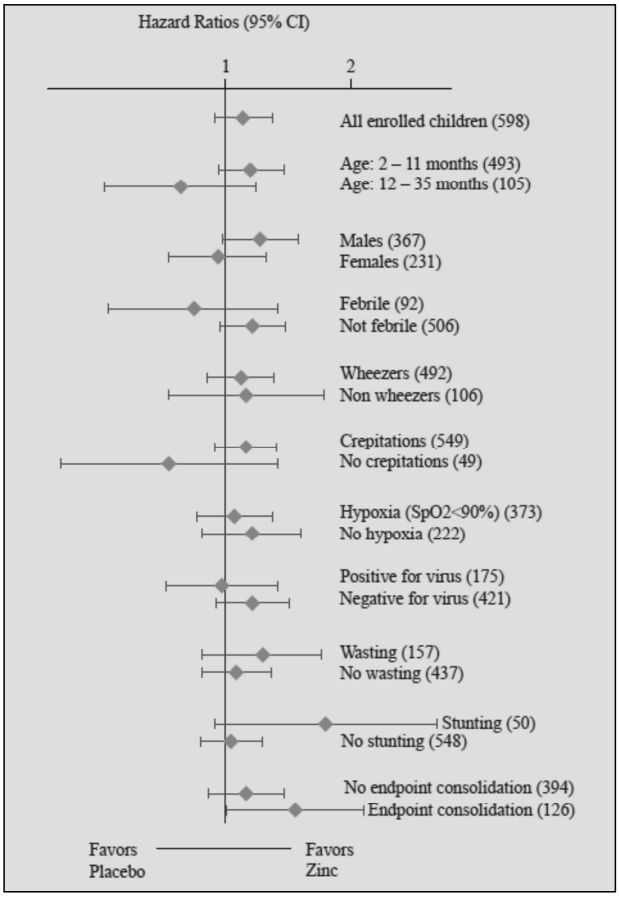
The secondary outcome, duration of severe pneumonia, was categorized to represent those children with persistent LCI with or without hypoxia at 72, 96 and 120 hours after enrolment. The proportion of children at risk of severe pneumonia at these time points was not significantly different between the zinc and placebo groups (Table 13). We repeated the analysis and adjusted for the stratum variables (age ≤/> 12 months and wheezing) and the two variables (age and wasting) that showed some differences at baseline. This, however, did not change either the estimates of association or their statistical precision. The other secondary outcome analyzed was the proportion of children with treatment failure. While children who received zinc were at a 12% lower risk of treatment failure (RR 0.88; 95% CI 0.71, 1.10), this association was also not statistically significant (Table 13).

Vomiting was recorded if this occurred 15 minutes after administration of supplement. The proportion of children who vomited was higher in those who received zinc (14%) versus those who received placebo (9%). Zinc recipients were at a 57% higher risk of vomiting compared to the placebo group (p = 0.05) (Table 13).

We explored whether the effect of zinc was different in selected subgroups. The groups we took into consideration were age category of ≤/> 12 months , gender, presence of fever, hypoxia, wheezing, crepitations, virus detected in nasopharyngeal aspirate, endpoint consolidation on CXR, wasting and stunting. In the subgroup consisting of children with endpoint consolidation, zinc recipients recovered significantly faster than those in the placebo group (Figure 9). The effect of zinc, however, was not significantly different between children with and those without radiographic pneumonia, i.e. the interaction was not statistically significant. The subgroup based on CXR finding of endpoint consolidation (Figure 9) represents numbers based on the reading of a single radiologist. A new variable for radiographic pneumonia was created after we analyzed the findings

of 2 radiologists at a later date. Both radiologists reached an agreement on findings of 457 CXRs of children included in the trial of which radiographic pneumonia was agreed upon in 164 (36%) films. Repeating the regression analysis with this new variable did not change the hazard ratios significantly. In children with radiographic pneumonia zinc had a beneficial effect (HR 1.40; 95% CI 1.01, 1.93) on time until recovery whereas no such association was observed among children without consolidation (HR 0.95; 95% CI 0.75, 1.20).

Figure 9. Efficacy of zinc on time until recovery from severe pneumonia in subgroups



Summary of results (Paper II)

In the nasopharyngeal aspirate specimens collected from 627 children, we detected at least one virus in 118 (30%; 95% CI 26.4%, 33.6%). In 6 of these children we detected 2 viruses (in the same child). RSV was the most frequent virus detected, and found in 88 (14%; 95% CI 11.4%, 17%) children. The number of specimens positive for the other viruses was much lower (Table 14). In this study conducted over a period of 30 months, severe pneumonia episodes occurred in 2 main annual peaks; but the timing of these peaks varied (Figure 10). The viral infections, mainly those caused by RSV, largely followed a similar epidemic pattern (Figure 10) with RSV contributing to the largest peaks. RSV was the predominant virus detected from July to October 2006 with influenza A, the second most frequent pathogen, also contributing to this epidemic. Influenza virus was also detected in the winter and pre-monsoon season, i.e. between January – April in 2006 (both A and B) and in 2007 (only B). While PIV 3 was not isolated in any particular season, PIV 1 was also more frequently found during the drier pre-monsoon months, with peaks in the month of April during 2006 and 2007. While hMPV was detected in sporadic cases between the months of November 2006 – October 2007, PIV 2 found in only 5 children with severe pneumonia is not shown. The relationship between monthly RSV infections and average monthly temperature and relative humidity and total monthly rainfall is shown in Figure 11.

Summary of Results (Paper III)

In this paper we used the same sample of 598 children with an acute episode of severe pneumonia enrolled in the clinical trial. During the study, 4 of the 7 children transferred to the pediatric intensive care unit died, parents of 9 (1.5%) children withdrew consent and 2 children were diagnosed with tuberculosis. Subject characteristics have been described earlier (Table 8).

Median time (IQR) until recovery from severe pneumonia was 49 (31, 87) hours while time until discharge (IQR) was 97 (83, 135). Treatment failure occurred in 209 (35%) of the 594 children.

There was a near linear relationship between age and both outcomes, i.e. time until recovery and risk of treatment failure (data not shown). An increment in age by one month was associated with a HR of 1.04 (95% CI 1.03, 1.06) for time until recovery (Table 15) and the OR for treatment failure was 0.93 (95% CI 0.90, 0.96) (Table 16), i.e. time until recovery and the odds of treatment failure decreased with increase in age of children.

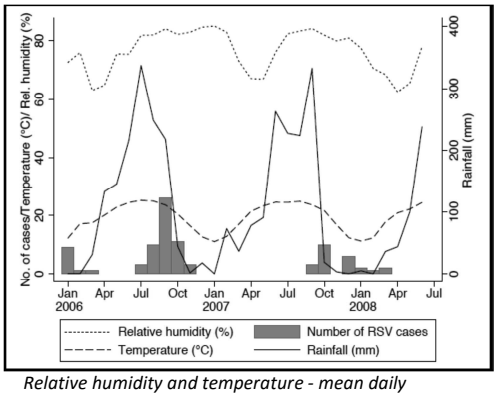
Wasting (HR 0.79; 95% CI 0.65, 0.96), hypoxia (HR 0.62; 95% CI 0.51, 0.74) and presence of any danger sign (HR 0.76; 95% CI 0.64, 0.91) were independently associated with slower recovery (Table 15). These associations did not change substantially with the addition of other variables (Models 2 and 3). Radiographic pneumonia was also a strong predictor of delayed recovery (HR O.58; 95% CI 0.47, 0.72). Presence of Parainfluenza type 1 (PIV 1) in the nasopharynx was associated with earlier recovery (HR 2.46; 95%CI 1.48, 4.09). Gender, being breastfed, stunting, high fever, CRP> 40 and > 80 mg/L, presence of other viruses and supplementation with oral zinc did not show any significant association with time until recovery (Table 15).

When estimating the association between child age categories and treatment failure, the older children had a lower risk of treatment failure (Table 16). While hypoxia and age categories up to 23 months remained as independent predictors, the oldest age group and any danger sign were no longer significant as indicators of treatment failure in models 2 and 3 (Table 16). Gender, being breastfed, stunted, having high fever, elevated CRP, viruses in the nasopharynx, and zinc supplementation were not significantly associated with treatment failure.

Table 14. Frequency of viruses from nasopharyngeal in aspirates of children in clinical trial

Viruses	N	Proportion of Pneumonia Cases (n = 627) % (95%CI)	Proportion of Virus positive cases (n = 188) %(95%CI)
RSV	88	14.0 (11.4, 17.0)	46.8 (39.5, 54.2)
Influenza A	28	4.5 (3.0, 6.0)	14.9 (10.1, 20.8)
PIV 3	24	3.8 (2.5, 5.6)	12.8 (8.4, 18.4)
PIV 1	23	3.7 (2.3, 5.4)	12.2 (7.9, 17.8)
Influenza B	17	2.7 (1.6, 4.3)	9.0 (5.4, 14.1)
HMPV	9	1.4 (0.70, 2.7)	4.8 (2.2, 8.9)
PIV 2	5	0.8 (0.26, 1.9)	2.7 (0.87, 6.1)

Figure 11. Relationship between monthly RSV infections and climatic conditions in Kathmandu, Nepal



Relative humidity and temperature - mean daily measurement. Rainfall calculated as the total measurement for the month.

Figure 10. Seasonal distribution of viruses detected in the clinical trial over a period of 30 months

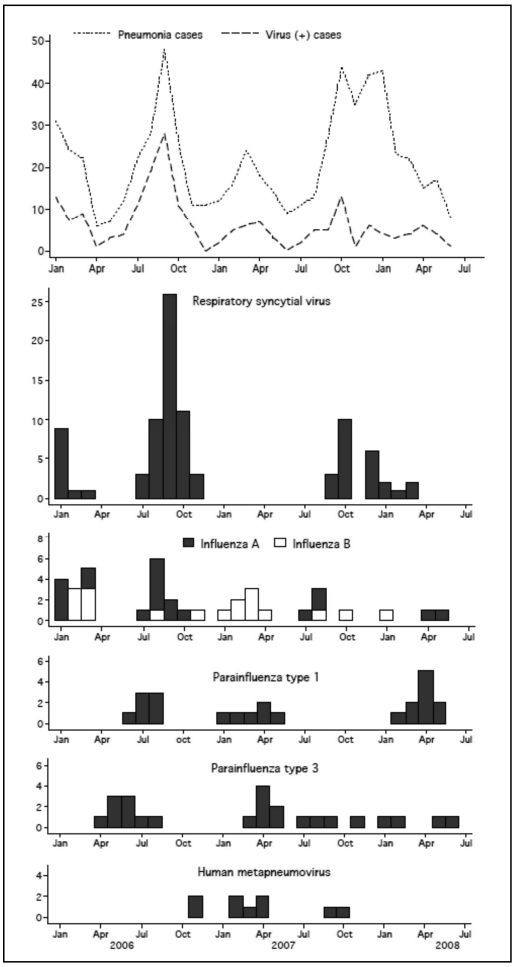


Table 15. Predictors of time until recovery of illness episode in children hospitalized with severe pneumonia

Variables	Crude Hazard Ratio*	Adjusted Hazard Ratio* (number of observations)		
Clinical		**Model 1 (598)**	**Model 2 (457)**	**Model 3 (456)**
Age in months	1.04 (1.03, 1.06)#			
Age categories				
2-6 months	1.00	1.00	1.00	1.00
7-11 months	1.43 (1.17, 1.75) #	1.48 (1.21, 1.82)	1.53 (1.21, 1.92)	1.53 (1.21, 1.93)
12-23 months	1.55 (1.21, 1.97) #	1.69 (1.31, 2.17)	1.45 (1.08, 1.95)	1.45 (1.08, 1.96)
24-35months	3.39 (2.01, 5.71) #	4.21 (2.45, 7.23)	3.31 (1.76, 6.22)	3.31 (1.76, 6.23)
Gender	1.11 (0.94, 1.31)			
Breastfed	0.97 (0.64, 1.47)			
Wasting (< -2 Weight for height/length)	0.77 (0.64, 0.93)#	0.80 (0.66, 0.76)	0.79 (0.64, 0.99)	0.79 (0.63, 0.97)
Stunting (< -2 Height/length for age)	0.84 (0.62, 1.14)			
Hypoxia (SpO_2 < 90%)	0.64 (0.54, 0.76)#	0.62 (0.52, 0.74)	0.72 (0.58, 0.88)	0.73 (0.59, 0.89)
Febrile (Axillary temperature > 38.5 °C)	1.14 (0.91, 1.43)			
Any danger sign	0.69 (0.59, 0.82) #	0.76 (0.64, 0.91)	0.74 (0.61, 0.90)	0.76 (0.62, 0.92)
Radiographic pneumonia	0.56 (0.46, 0.69)#		0.58 (0.47, 0.72)	0.55 (0.45, 0.68)
Laboratory				
C-Reactive Protein (CRP) > 40 mg/L	1.01 (0.84, 1.21)			
Respiratory syncytial virus (RSV)	0.79 (0.62, 0.99)			
Influenza A	0.78 (0.52, 1.19)			
Influenza B	0.81 (0.49, 1.33)			
Parainfluenza type 1 (PIV 1)	1.75 (1.14, 2.69) #			2.45 (1.48, 4.07)
Parainfluenza type 2 (PIV 2)	0.98 (0.40, 2.36)			
Parainfluenza type 3 (PIV 3)	1.44 (0.96, 2.17)			
Human metapneumovirus (hMPV)	0.64 (0.32, 1.28)			
Supplemented with zinc	1.11 (0.94, 1.31)			

**Hazard ratios (95% CI) and P-value calculated using Cox proportional hazard model (exact p). Hazard ratios < 1 indicates slower resolution of illness*
P-values not reported. # Results with P-value < 0.05. Results of multiple regressions with P-value > 0.05 not shown in the table
Model 1 (Clinical variables): Adjusted for gender, breastfed, febrile and treatment with zinc
Model 2 (Clinical + Radiographic pneumonia): Adjusted for gender, breastfed, febrile and treatment with zinc
Model 3 (All variables): Adjusted for gender, breastfed, febrile, CRP, nasopharyngeal aspirate positive for RSV, influenza A and B, PIV 2 and 3 and hMPV and treatment with zinc

Table 16. Predictors of treatment failure of illness episode in children hospitalized with severe pneumonia

Variables	Crude Odds Ratio*	Adjusted Odds Ratio* (number of observations)		
Clinical				
Age in months	0.93 (0.90, 0.96)#	**Model 1 (594)**	**Model 2 (457)**	**Model 3 (456)**
Age categories				
2-6 months	1.00	1.00	1.00	1.00
7-11 months	0.67 (0.44, 1.01)	0.62 (0.41, 0.95)	0.57 (0.35, 0.93)	0.57 (0.35, 0.92)
12-23 months	0.37 (0.21, 0.64)#	0.33 (0.19, 0.58)	0.27 (0.13, 0.56)	0.28 (0.13, 0.57)
24-35months	0.22 (0.05, 0.99)#	0.18 (0.04, 0.84)	0.32 (0.07, 1.58)	0.35 (0.07, 1.77)
Gender	0.83 (0.59,1.18)			
Breastfed	0.67 (0.28,1.61)			
Wasting (< -2 Weight for height/length)	1.41 (0.97, 2.04)			
Stunting (< -2 Height/length for age)	1.25 (0.69, 2.27)			
Hypoxia (SpO_2 < 90%)	1.91 (1.33, 2.74)#	1.98 (1.35, 2.89)	1.76 (1.13, 2.75)	1.78 (1.14, 2.78)
Febrile (Axillary temperature > 38.5 °C)	0.94 (0.59, 1.51)			
Any danger sign	1.65 (1.18 , 2.32)#	1.44 (1.01, 2.06)		
Radiographic pneumonia	2.22 (1.49, 3.31)#		2.08 (1.36, 3.16)	2.10 (1.38, 3.21)
Laboratory				
C-Reactive Protein (CRP) > 40 mg/L	1.15 (0.79, 1.67)			
Respiratory syncytial virus (RSV)	1.47 (0.91, 2.38)			
Influenza A	0.75 (0.31, 1.84)			
Influenza B	1.01 (0.37, 2.76)			
Parainfluenza type 1 (PIV 1)	0.50 (0.18, 1.37)			
Parainfluenza type 2 (PIV 2)	1.23 (0.20 , 7.44)			
Parainfluenza type 3 (PIV 3)	0.36 (0.12, 1.06)			
Human metapneumovirus (hMPV)	2.34 (0.62, 8.81)			
Supplemented with zinc	0.83 (0.60, 1.17)			

**Odds ratios (95% CI) and P-value calculated using Logistic Regression. Odds ratios > 1 indicates increased odds of treatment failure.*

 # Results with P-value < 0.05

Results of multiple regressions with P-value > 0.05 not shown in the table with the exception of age analyzed as a categorical variable

Model 1 (Clinical variables): Adjusted for gender, breastfed, wasting, febrile and treatment with zinc

Model 2 (Clinical + Radiographic pneumonia): Adjusted for gender, breastfed, wasting, febrile, any danger sign and treatment with zinc

Model 3 (All variables): Adjusted for gender, breastfed, wasting, febrile, any danger sign, CRP, nasopharyngeal aspirate positive for RSV, PIV 1, 2 and 3, influenza A and B, hMPV and treatment with zinc

Discussion

Methodological discussion

Study Design

In Paper I we describe a hospital based double blind randomized clinical trial that was undertaken to evaluate the efficacy of oral zinc in reducing time until recovery from severe pneumonia.

Paper II, which describes common viral pathogens isolated from nasopharyngeal aspirates of hospitalized children with severe pneumonia, is a cross sectional analysis of baseline data collected during the clinical trial (including the pilot study) over a period of 30 months.

Paper III is a secondary analysis of the clinical trial data to identify predictors of duration and of treatment failure of severe pneumonia.

Assessment of internal validity: Can the results be trusted?

Sample size

Sample size calculations are recommended to determine the number of observations, or as in our study, participants needed to detect clinically relevant treatment effects [153] with a certain statistical power or precision. Studies with inadequate sample sizes may not reach the desired statistical precision needed to detect the presence of a true association with appropriate confidence. On the other hand, studies that recruit more participants than necessary may expose some to treatments and examinations that are potentially harmful or futile and may be a waste of resources [154]. For the clinical trial included in this thesis, we calculated the sample size based on a clinically relevant effect on the primary outcome,

more specifically on the time until cessation of severe pneumonia. Our effect measure, the hazard ratio (HR), is the ratio of the event rate between two exposures where HR = 1 indicates no difference. We used a HR of 1.3 as the minimum meaningful clinical difference when estimating the sample size for this trial. This means that children receiving zinc would on an average have a 30% increased probability of recovering at any time point compared to those in the placebo group, or in other words, 30% faster recovery. We explored various scenarios using different probabilities of type II error, event probability estimates and effect sizes and agreed on a sample size of 600 children. We recruited 610 participants and analyzed data from 598 (see trial profile) using the intention to treat approach. Providing zinc in addition to standard treatment to hospitalized children with severe pneumonia was associated with earlier resolution of the illness in our study (HR 1.10; 95% CI 0.94, 1.30) but the benefit was minimal and not statistically significant. Furthermore, the minimum clinically meaningful effect (HR of 1.3) was at the upper end of the CI, indicating that the effect, if any, was far from clinically relevant.

For Paper III we used the entire trial cohort of 598 children. While we were able to detect several predictors for duration and treatment failure, we did not find any association between exposure variables such as fever and breastfeeding and these outcomes. The distribution of these variables was such that the power to detect any significant association with the outcomes was low. The main study was designed to measure the effect of zinc supplementation during pneumonia and not to identify these predictors, which could have limited our ability to identify additional prognostic factors.

Definition of severe pneumonia and its implications for study outcomes

We chose the WHO diagnostic criteria requiring the presence of LCI to define severe pneumonia that were developed to identify U5 children that are to be treated in a health facility. The purpose of this highly sensitive clinical definition was to treat children empirically with antibiotics because studies had indicated that most of these episodes were caused by bacteria in LMICs [155]. While this algorithm identified more than 80% with severe pneumonia, there was still a sizeable proportion of children that did not need treatment with antibiotics [156]. Many children with non-recurrent wheeze following a viral infection were likely to receive unnecessary treatment with an antibiotic because they can have both fast breathing and LCI. Findings from studies by Hazir *et al.* [157] and Lochindarat *et al.* [158] indicated that 3 instead of the recommended 2 doses of rapid acting bronchodilator while screening helped to improve the specificity of the diagnostic guidelines and thereby reduce overuse of antibiotics [2]. We adopted this strategy of using up to 3 doses of bronchodilator while screening for eligibility. The 30% (667/2198) of children with wheezing and LCI that we excluded no longer had LCI and therefore did not fulfill criteria for inclusion as participants. In an attempt to make a more specific diagnosis of bacterial pneumonia, guidelines from HICs recommend a chest radiograph for hospitalized children with clinical signs and symptoms of CAP [4]. While there is no single test that reliably distinguishes the etiology, presence of significant alveolar consolidation on a chest radiograph is considered to be a specific predictor of bacterial pneumonia and used in many studies as the "gold standard" [67]. In our study all children fulfilling inclusion criteria had a CXR taken at the time of enrollment but we used these films to look for exclusion criteria and not to support our diagnosis of pneumonia. We did not have radiologists or physicians trained to interpret chest radiographs using standardized criteria at the start of our study

[67]. In addition, since many health facilities in resource limited settings of LMICs are not adequately equipped to either obtain or interpret chest radiographs, it would be difficult to extrapolate findings from our study to such settings. This suboptimal specificity in diagnostic criteria for measuring study outcomes may bias the results towards the null effect and might have led to an underestimation of our effect estimates[159]. After completion of the study, we did use the CXRs to improve our diagnosis of pneumonia and were able to identify 36% with radiographic consolidation. It is noteworthy that there was a beneficial effect of zinc in this subgroup.

Paper I

The primary objective, efficacy of zinc in reducing duration of severe pneumonia, was evaluated by comparing the outcomes between children who received zinc to those who received placebo. Randomization ensures that the observed differences in outcome between the treatment groups behave like differences between two independent random samples drawn from the same population[160]. Block randomization ensures equal numbers in each group and avoids that a run of participants with a certain, e.g. season-associated, factor is concentrated in one trial arm. Stratified randomization balances factors that may increase the risk of study outcomes in each group, with regard to the stratifying variable, and thus reduces confounding bias [161]. We used stratified block randomization and the baseline characteristics were well balanced between the zinc and placebo groups (Tables 10 - 12). There were, however, somewhat fewer infants between ages 2 – 6 months (56% versus 62%) and a higher proportion with wasting (30% versus 24%) among zinc recipients. Adjusting for these variables (age and wasting) in the regression analyses did not change the estimated association.

In an RCT, allocation concealment prevents preferential assignment to either intervention or control arm, which can occur if it is not disguised, because it would then be known by the investigator, study personnel responsible for recruiting subjects or participants themselves. Concealment thereby eliminates selection bias induced during enrollment [162]. Trials with inadequate allocation concealment have been shown to overestimate the effect of treatment by as much 37% compared to those using adequate methods [162]. The allocation sequence generation and concealment we describe in Paper I [122], is in accordance with the guidelines for reporting of randomization in RCTs [163].

Blinding/masking attempts to keep arm assignment unknown to key people in an RCT, i.e. study subjects, investigators, data collectors, study physicians and other persons assessing outcomes. The blinding of patients and study personnel avoids performance bias because it prevents the administration of co-interventions preferentially to one of the groups [164]. In our study, the active drug (zinc sulphate) and placebo were available as identical dispersible tablets in blister packs with no indication of group identity and content but labelled with the serial number that was used to identify the enrolled child. As an additional safeguard, after the first dose was dispensed by the study physician, the supplement was administered by trained study assistants not otherwise involved in patient care on subsequent days. This step was taken to prevent the study physicians from guessing group assignment following evidence of increased risk of vomiting in children administered zinc from previous trials [165, 166], and was necessary to minimize observer or ascertainment bias [167].

The assessment of important outcomes such as recovery from severe pneumonia, decision to change antibiotics or refer children for more specialized care, was done by study

physicians in consultation with senior paediatricians to ensure correct identification of these study endpoints [164].

Twelve randomized children were identified as trial deviates (because of heart disease and failure to identify an exclusion criterion) and excluded from the analyses. Amongst these children there were 6 with heart disease in the placebo arm, 5 with heart disease and 1 with cough of > 14 days' duration in the zinc arm (trial profile). The medical chart of each child was reviewed and the decision to exclude was made by adjudication between investigators blinded to the assignment and outcome and before the randomization code linking group assignment to individual patients was revealed. These post randomization exclusions are unlikely to have introduced bias because the decision was based on the children's clinical status before randomization [168], they were equally distributed in the two arms and account for only 2% (12/610) of the trial cohort. The inclusion criteria, difficulty breathing with LCI, also signs of congestive cardiac failure in young children with heart disease, could be difficult to distinguish by chest auscultation for presence of a cardiac murmur in a crowded and noisy emergency room where participants were screened and enrolled.

We performed a time to event analysis using Cox proportional hazards regression for the primary endpoint of our study because it takes censored data into account. Censoring occurs when information regarding time to outcome event, such as time until cessation of severe pneumonia in our study, is not available for all participants [169]. Children with acute severe pneumonia were enrolled, admitted and monitored until they fulfilled criteria for recovery and then sent home. Although we continued to collect some information on children that remained in the hospital, they were no longer given the intervention, i.e. zinc or placebo after day 14. The children lost to follow up were either those transferred for

intensive care or for whom parents withdrew consent. The trial participants that were lost to follow up were censored on the last day with information. In the zinc arm of our trial, there were 11 losses to follow up that included the 2 children with suspected tuberculosis. Similarly, in the placebo arm there were 7 losses to follow up. Thus, censoring was very limited and therefore very unlikely to have induced a selection bias.

The primary outcome of the study, time until cessation of severe pneumonia, defined as the period starting from enrolment to the beginning of a 24-hour consecutive period of absence of LCI, of hypoxia and of any general danger signs, such as inability to breastfeed or drink or vomiting everything, convulsions, lethargy or unconsciousness, was decided *a priori*. We used the WHO algorithm that requires presence of LCI in addition to cough and/or difficulty in breathing to diagnose severe pneumonia requiring hospitalization to define patient eligibility [21]. We chose disappearance of LCI, an accurate and reliable sign, to denote recovery from an episode of severe pneumonia in young children because LCI has a relatively high sensitivity and specificity in identifying children with severe pneumonia [170, 171]. In an earlier review on the diagnostic value of clinical signs in childhood pneumonia, while agreement for a sign that can be observed, such as LCI (kappa = 0.48) was better than the agreement for detection of adventitious sounds requiring chest auscultation (kappa = 0.3), observers were twice as likely to diagnose pneumonia if LCI was present [3]. However, according to a recent meta-analysis, the diagnostic accuracy of LCI for pneumonia in U5 children was poor with low pooled estimates of sensitivity [0.48 (0.16–0.82)] and specificity [0.2 (0.47–0.89)] [15]. We added absence of hypoxia and general danger signs to the definition of our primary study endpoint because these additional signs of severity might have been missed had we relied only on disappearance of LCI.

Oxygen saturation is a sensitive indicator of disease severity in instances where hypoxemia occurs as a result of ventilation- perfusion mismatch, such as in pneumonia. A reasonably accurate estimation of arterial oxygen saturation can be obtained by means of pulse oximetry, which is simple, non-invasive and especially suitable for children. SpO_2 measured by pulse oximetry has been tested against arterial oxygen saturation (SaO_2), which is the gold standard, and the standard deviation of differences between the two readings denotes the accuracy of the instrument [172]. The pulse oximeters (Nellcor Puritan Bennett-40) used in our study had an accuracy of ± 2 % without motion and ± 3 % with motion for SpO_2 within the range of 70 – 100% according to the manufacturer's instructions [173]. Studies on pulse oximeter accuracy in children have demonstrated that SpO_2 recorded by pulse oximeters tend to overestimate SaO_2 measured from arterial blood gas samples with readings from pulse oximeters becoming less accurate as SaO_2 falls below 90% [174, 175]. This limitation is more relevant when pulse oximeters are used to monitor oxygen saturation continuously in children with severe illness in an ICU setting. In our study, the pulse oximeters were used intermittently for the detection and treatment of hypoxemia and not for continuous monitoring of patients. We chose SpO_2 of < 90% for defining hypoxemia, which is the threshold for administering oxygen to children with severe pneumonia according to WHO recommendations [21] and also because this was already practiced at the study site.

Adverse effects of treatment

Oral zinc when given in therapeutic doses is safe. None of the numerous clinical trials that have tested zinc as treatment for diarrhea or pneumonia in children have reported severe adverse effects [176]. Symptoms related to the gastrointestinal system, nausea, metallic

taste and vomiting have been reported in adults given 50 – 150 mg/day of zinc [177], and vomiting is the most commonly reported side effect in clinical trials in children [108]. Each dispersible zinc sulfate tablet used in our study contained 10 mg elemental zinc. The doses used were 1 tablet for infants and 2 tablets for older children, administered either once daily or divided and given twice daily in case of vomiting, for a maximum duration of 14 days. Our study team, instructed to actively monitor adverse events, found that children receiving zinc had an increased risk of vomiting compared to the placebo group (RR 1.57; 95% CI 0.99, 2.50). As described earlier, we tried to maintain blinding by having study personnel not involved in assessing study outcomes administer the supplement to children and maintain a record of compliance and any reported or observed side effects.

Paper III

For this paper, existing data from the clinical trial was used to identify predictors of duration and treatment failure of severe pneumonia. Data from the trial cohort, excluding the pilot study, was utilized for this secondary analysis after verifying that treatment with zinc did not modify the association between the exposures of interest and the outcomes. The large sample size provided us with the opportunity to assess other factors associated with important outcomes of an acute episode of severe pneumonia in young children admitted to a hospital in an LMIC.

The discussion pertaining to the definition of severe pneumonia with its implications and data collection including definition of study outcomes as described for Paper I also apply to this Paper.

Confounding is the main reason why it is difficult to draw conclusions on causality from observational studies and something one endeavors to prevent or adjust for. For Paper III

we used multiple regression models when analyzing the data, which inherently controls for confounding. For the outcome, time until cessation of severe pneumonia, we used Cox regression and the independent variables that were assessed in these models are listed in Table 6. The association between treatment failure and the same independent variables was analyzed using logistic regression. Although the crude estimates of the association between the predictors and both these outcomes were not importantly different (i.e. > 10%) from the adjusted estimates for the variables we identified as potential confounders (Tables 15 &16), residual confounding, is still a possibility. Further, the observed associations could have been affected by confounding from variables we did not measure and, therefore, could not adjust for.

Paper II

We collected nasopharyngeal aspirate (NPA) specimens from 639 children, 2 – 35 completed months of age with severe pneumonia in a study spanning a 30 months' period from 8th January, 2006 to 30th June, 2008. Our objective was to identify common viral pathogens from the collected specimens using a multiplex reverse transcription PCR assay in a research laboratory at the Institute of Medicine in Kathmandu, Nepal, that was set up solely for this purpose. This assay was popular at the time we conducted the study because it took less time than conventional viral culture, was more sensitive than several immunofluorescent assays, optical immunoassays, and enzyme-linked immunosorbent assays, with the added advantage of the ability to detect infection with two or more viruses in the same patient [178]. Studies evaluating this Hexaplus assay against shell vial cell culture and/or direct fluorescent antibody staining, reported high overall sensitivity (95 – 100%) and specificity (90 – 95%) [178, 179]. In recent years this assay has been replaced by more advanced

methods, such as real-time PCR and the application of broader panels that typically detect more than 15-20 different pathogens in a single specimen obtained [180].

In this study we are faced with the question of whether detection of viruses from the upper airway reflects a viral infection of the lower airways. The highly sensitive PCR technique has increased detection rates of viruses not only in symptomatic but also in asymptomatic individuals making interpretation of results challenging. However, the detection rates are usually higher in those with than those without symptoms, at least for the viruses included in our assay [61]. The seven respiratory viruses we identified were indeed associated with pneumonia in our case control study in Nepal [60] and also in another case control study conducted in Thailand [181]. Moreover, in a study of hospitalized infants in Tunisia, there was an association between viral load measured by real-time PCR and severity of RSV induced bronchiolitis, suggesting that use of such quantitative assays may increase the specificity of the test [182].

In our study, the months of April and May 2006 had the lowest enrolment rate, which coincides with the period of political instability in Nepal. The month of April was marked by several days of nationwide strikes and street demonstrations followed by imposition of daytime curfew by the government, which impeded movement of people and possibly prevented many parents from accessing the hospital. We are not certain whether the low number of pneumonia admissions during this period was due to the situation in the country or the season (Figure 10).

Assessment of External Validity: To whom do the results apply?

The external validity, also known as generalizability, is the extent to which the study findings can be applied to the reference target population. For our study, the reference target

population included children in the age group 2 – 35 completed months after birth and residing in areas within the Kathmandu valley and districts surrounding it.

The study was conducted in a centrally located referral pediatric government hospital. This hospital also provides secondary level care and therefore, to a certain extent, represents other health facilities in a country where children with severe pneumonia would be referred to from the community.

Our study population comprised of children between 2 – 35 months of age who visited either the Outpatient or Emergency Department of a busy government hospital. Over the 30 month study period, 2198 children were screened for eligibility by study physicians who worked in shifts with one doctor present round the clock. Despite the urban location of the study site and a little over two-thirds of the children residing in urban areas, most came from households of lower socioeconomic status. Most of the parents/caregivers had not attained educational level beyond high school (95% mothers and 89% fathers) and were either unemployed (72% mothers), daily wage earners (19% fathers and 5% mothers) or employed to do skilled or unskilled work (36% fathers and 5% mothers); a situation not very different from that documented in a national survey and therefore reflecting the educational and occupational characteristics of Nepalese people in general [102].

The eligibility criteria, cough and/or difficulty in breathing in the presence of observed LCI, are 'entry criteria' in the WHO algorithm for identifying U5 children with severe pneumonia requiring hospitalization [155]. More than half of the screened population (58%) satisfied exclusion criteria but neither the sociodemographic characteristics (data not shown) nor the place of residence (Table 7) of these children was substantially different from those that were randomized. There were 226 (10%) children whose parents refused to participate.

These children were younger and the proportion that was stunted was two-fold higher compared to the enrolled participants (Table 8). It is possible that these children were either born with intrauterine growth retardation or preterm, conditions that put them at risk of infection severe enough to visit a hospital, but we do not have sufficient data on birth weight or gestational age at birth to explain this finding. However, other determinants, such as place of residence, severity of illness, gender, educational status and occupation of parents of these children were no different from that of the trial cohort (Table 8).

We restricted coughing to be of less than 2 weeks' duration and added difficulty breathing of no more than 72 hours in the inclusion criteria to ensure we captured an acute episode of severe illness and included only those who had LCI also after nebulization with a bronchodilator in order to avoid including children with asthma without severe pneumonia.

About two fifths (42%) of trial participants had a history of taking oral antibiotics prior to their arrival at the hospital, a common phenomenon in Nepal and many LMICs with easy access to drugs over the counter. We chose not to make this a criterion for exclusion for several reasons. The children had signs of severe pneumonia despite treatment and we did not have sufficient evidence to claim if what they had been prescribed was adequate. Being a large study, our randomization was designed to evenly distribute those with and without ongoing antibiotic intake between the two trial arms. Most importantly, excluding children on prior treatment with antibiotics would not only have increased the duration, and thereby the costs of the trial, but would have also reduced the external validity of our findings.

Children in our trial received treatment for severe pneumonia according to a standard protocol similar to the one followed at the study site and probably not very different from those in similar settings because we used the WHO guidelines. The presence of LCI in a child

with cough or difficulty in breathing, and criteria for severe pneumonia requiring hospital care were based on the WHO algorithm which was later revised in 2012 [24]. According to this new recommendation, children with “LCI pneumonia” can be treated at home with oral amoxicillin but only after hypoxia, presence of any sign of severe respiratory distress or general danger sign have been ruled out [23]. This new algorithm can still be applied to half of the trial participants who had at least one sign of severe respiratory distress and 62% that were hypoxic (SpO_2 < 90%) at enrolment.

Enrolled children were monitored thrice daily, at more frequent intervals if required, and any problem was almost immediately identified by our study physicians. This practice may well exceed that of the usual standards of a busy pediatric hospital in a LMIC with limited and over-worked healthcare personnel.

We waited for 48 hours after the first indication of disappearance of LCI and 24 hours following absence of hypoxia before we sent children home because we wanted to confirm that children were recovering. In a general children's hospital with high bed occupancy and patient turnover, this requirement of the trial protocol posed a challenge but was rigorously implemented. We therefore had very few parents that did not comply. Although parents were counseled to return to the hospital if there was a recurrence of severe symptoms we did not expect all of them to comply and following up children at home was not feasible.

Our observations for Papers II and III were limited to a carefully selected trial cohort using the WHO guidelines requiring only LCI in addition to symptoms to define severe pneumonia as inclusion criteria [21]. Therefore, while our findings can be generalized to settings where the approach to management of severe pneumonia in children is similar, our criteria for recruiting patients have limitations. Despite taking measures to not include children with

reactive airways disease, we may have included those with pneumonia that could have been treated as outpatients. We excluded children with pneumonia associated with complications such as pneumothorax or pleural effusion that needed surgical intervention or if they needed to be cared for in the intensive care unit. Children with other signs of severe pneumonia in the absence of LCI were not included. We were unable to recruit as much as 10% of the children who met the eligibility criteria because their parents did not want to be part of our study. The small proportion we did not obtain consent from is unlikely to seriously impede the external validity of the findings we report upon in Papers I and III.

The characteristics of the study population and setting are similar in many ways to those in other LMICs to which the results can be extrapolated. In our endeavor to conduct the trial with rigor, management of severe pneumonia may not be representative of many health care settings but this is a limitation, driven by the dedication and necessity to care for trial participants, of any RCT that is difficult to evade

Discussion of main findings

Efficacy of oral zinc in addition to standard treatment for severe childhood pneumonia

In our hospital based clinical trial we found a statistically precise but clinically not relevant and not statistically significant effect of daily zinc administration in reducing time to cessation of severe pneumonia defined using the WHO criteria (Table 13). Similarly, neither the proportion of children who still had LCI with/without hypoxia at 72, 96 and 120 hours after enrolment or the risk of treatment failure was significantly different between the zinc and placebo recipients (Table 13).

Since the first published report on clinical trials estimating the efficacy of zinc as adjunct therapy for severe pneumonia in 2004, there have been 13 publications addressing this important topic [115-125, 127-129]. According to the International clinical trials registry platform (http://www.who.int/ictrp/en/), four additional trials (ISRCTN33548493, IRCT201103025951N1, IRCT201103025951N1, NCT00142285) have completed recruiting participants and the published results from these studies are awaited. The therapeutic effect of zinc in hospitalized children with severe pneumonia, however, is still not clear. Most studies have used modified versions of the WHO criteria to define severe pneumonia, i.e. history of cough and/or difficulty in breathing with a general danger sign, cyanosis or documented hypoxia with pulse oximetry and signs of severe respiratory distress, but the definitions vary across studies. The definitions for study endpoints also differ and those reporting time until recovery as an outcome have shown different results. Two studies, one carried out in Bangladesh and the other in Iran, have demonstrated a shorter duration of illness in zinc recipients [115, 120]. While the mean reduction in duration of hospitalization was equivalent to 1 hospital day amongst the children that received zinc in the Bangladesh

trial, in the study from Iran it was equivalent to only half a day and maybe not clinically relevant [115, 120]. The results of our study and the other community based trial in Nepal [117, 122] were in the same direction, but the effect on average duration, if any at all, was precisely estimated as being of no clinical importance. The remaining trials, 6 from the Indian subcontinent, 2 from Tanzania and Uganda, one from Ecuador and the other in indigenous children in the Northern territory of Australia, found no significant beneficial effect of zinc on duration of illness in young children with severe pneumonia [116, 118, 119, 121, 123-125, 127-129]. While inherent differences in the populations studied, including differences in the illness characteristics and definition of recovery discussed above may explain the discrepancy between studies, many were also statistically underpowered because of small sample sizes.

Therapeutic zinc was beneficial in certain subgroups of children in several trials and some studies have even reported negative effects. The criteria used to define subgroups/strata according to etiology or severity, however, was not uniform across the various trials. In a study undertaken in Kolkatta, India, there was no overall effect, but zinc was efficacious in boys [129]. In the subgroup of Bangladeshi children without wheezing, Brooks *et al.* reported that administration of zinc resulted in earlier resolution of clinical signs and suggested that these children had bacterial pneumonia because the wheezing is more likely to be a sign associated with a viral infection [115]. In contrast, in South India, children receiving zinc had slower recovery during the hot season compared to those getting placebo, while children who were recruited during the rest of the year had no effect of zinc. Earlier studies from this region indicated that the incidence of viral respiratory infections is low during the hot season [116], which led the authors to hypothesize that the etiology of

severe pneumonia was likely to be bacterial in these children. A secondary analysis of data from this trial in South India concluded that the treatment effect of zinc was modified by etiology because recovery amongst zinc recipients with severe pneumonia of suspected bacterial etiology, defined using a serum CRP concentration > 40 mg/L, was slower than zinc recipients with CRP < 40 mg/L, defined as non-bacterial pneumonia [183]. However, in another trial using PCR assays to describe etiology, Sempertegui *et al*. found no difference between time to resolution of illness between the zinc and placebo groups for each of the commonly associated respiratory pathogens (RSV, hMPV, adenovirus, and *S.pneumoniae*) [128]. Our findings in the subgroup of patients with viruses detected in the nasopharynx were similar to that in the study from Ecuador. In our subgroup analysis, we did find that children with consolidation on a chest radiograph, a proxy for bacterial pneumonia [67], recovered earlier when they received zinc compared to those that were given placebo. The effect of zinc, however, was not significantly different among children with versus in those without radiographic consolidation, i.e. the interaction was not statistically significant. In the study in Uganda that enrolled children without wheezing, there was no difference in time until recovery between the zinc and placebo recipients [123]. In our study, 82% children had wheezing, the highest proportion compared to 63% in the South Indian and 37% in the Bangladesh trial [115, 116]. The effect of zinc was not modified by wheezing status in our subgroup analysis (Figure 9) and that reported upon in the trial in South India [116]. Moreover, in the 106 children without wheezing we had insufficient power to detect any beneficial effect of zinc. In the Indian study by Wadhwa, zinc recipients in the category with very severe pneumonia (defined as any sign of pneumonia in addition to presence of cyanosis or any general danger sign) recovered earlier than those that received placebo (HR: 1.52; 95% CI 1.03, 2.23) but this finding was no longer statistically significant when adjusting

for baseline differences between children that were severely underweight in the treatment arms [124]. In our study, although we collected data on these signs, central cyanosis was detected in only three and any general danger sign was present in less than 10% of enrolled children and therefore not meaningful to analyze as a subgroup. It is likely that the very ill or severely malnourished children that we excluded prior to randomization had either one or more of these signs of very severe pneumonia defined by Wadhwa *et al [124]* .

The lack of consistent beneficial effects of zinc in many therapeutic trials of children with severe pneumonia is proposed by many to be related to the fact that the population studied was not zinc deficient to start with. In the absence of a better indicator, plasma zinc concentration continues to be used as a biomarker of zinc status in individuals [103] and the suggested lower cut-offs for children is < 9.9 µmol/L [99]. The baseline plasma zinc levels reported in many trials however does not modify the effect of zinc. While the plasma level of zinc was 10.1 µmol/L in the study in Bangladesh that reported a beneficial effect [115], levels ranging from the lowest (4.5 µmol/L) in Uganda to the highest (22.9 µmol/L) in India were not associated with time until recovery in either trial [118, 123]. A low zinc concentration can be a part of a physiological response to an acute infection or inflammation, wherein zinc is redistributed from the plasma to the liver [99]. It is therefore worthwhile to consider elevated concentrations of acute phase reactants, such as CRP, while interpreting the results of plasma zinc in studies enrolling hospitalized children with an infectious illness. In fact, Sempertegui *et al*. demonstrated that higher baseline zinc levels adjusted for CRP concentration was associated with shorter time until remission of severe pneumonia [128]. The mean baseline plasma zinc concentration of our trial participants was 9.5 µmol/L. Other studies from our region indicate that the populations are deficient in zinc

[184, 185]. Moreover, according to national estimates, approximately 2 of 5 children are stunted, and approximately one fourth of Nepalese citizens are likely to have inadequate intake of zinc [100, 102].

With no dose-response studies to support findings, our results could be attributed to the dose of zinc (10 mg) that was used to supplement children < 12 months, which was lower than that given to participants of several other trial. However, zinc was still ineffective in reducing illness duration even in trials that used a higher dose of 20 mg in infants [116, 118, 121, 124, 127, 128].

The inconsistency in observed effects in therapeutic trials of zinc in children with pneumonia from LMICs at increased risk of zinc deficiency may have alternative explanations. While knowledge on mechanism of action of zinc in childhood pneumonia is limited, the theory that it may enhance the protective immune response irrespective of zinc status requires further consideration. The way forward could be well planned and conducted intervention studies with nested mechanistic studies that are aimed at understanding the role of zinc in immunologic pathways, specifically T cell immunity, in order to realize its possible potential as an adjunct to treatment of severe pneumonia in children [186].

While the evidence from clinical trials in the past decade points towards a limited role of therapeutic zinc for severe pneumonia in children, the challenges of defining the disease in the absence of a 'gold standard' diagnostic test, especially in LMICs, may be a factor responsible for this observed suboptimal effect. There are indications that children with pneumonia of increasing severity and/or bacterial etiology may benefit from supplementation with zinc. Future trials assessing the efficacy of zinc need to focus on diagnostic criteria for severe pneumonia in children with high specificity and reproducibility.

Viruses in respiratory specimens of hospitalized children with severe pneumonia

We detected at least one of the seven respiratory viruses in 188 (30%) of the 627 young children 2 – 35 months of age in our study of hospitalized children with severe pneumonia [21]. Respiratory syncytial virus was the most frequently found pathogen in 88 (14%) of our participants. This virus is an important respiratory pathogen in the etiology of childhood CAP [47] and estimated to account for 34 million (22%) of new ALRI episodes with 10% episodes severe enough to require hospital admission in U5 children worldwide [187]. The development of novel molecular assays has not only enabled diagnostic testing of respiratory specimens for many new and emerging viruses simultaneously but also made it feasible in research settings of LMICs. Among the studies in LMICs that have used PCR based methods to detect viruses, many have used extensive panels to detect pathogens [181, 188-192]. The study in India, however, used an in-house multiplex assay that detected the same seven RNA viruses we identified in our study [190]. In this hospital – based 2 year study of 301 Indian children with age ranging from 1 – 72 months with clinical features of ALRI, a virus was detected in 35% with RSV the most frequently identified in 20% of patients [190]. RSV was still the most frequently detected organism even among those studies in LMICs that used assays to detect a wider range of pathogens [181, 188, 189]. We detected viruses in 30% and RSV in 14% of our study participants, proportions that are lower than the other studies in LMICs [181, 188-192]. While specific diagnostic assays used in studies may differ, differences in study participants, epidemiological setting, method of case ascertainment and case definitions between studies may be additional factors to explain the variability of the findings. We included only those children who came to the hospital and provide data on those fulfilling eligibility to participate in the clinical trial. We did not include 667 children with wheezing whose LCI disappeared after administration of bronchodilators. Moreover,

we did not include children less than 2 months of age in whom RSV infection is very common, with hospitalization rates between 10 – 28% in infants aged below 6 weeks [193, 194] . After RSV, the most frequently detected viruses among our participants were influenza A in 5.4% and PIV type 3 in 3.8% of children. This is in line with findings from other studies; while PIV type 3 was the other common virus detected in studies on hospitalized children in Kenya, India and Korea [188-190], influenza A was also a common pathogen detected in the study from Kenya [188]. These three viruses were also the most common in the community based study of mostly non-severe pneumonia conducted by our research group in Bhaktapur, one of the districts within the Kathmandu valley (Figure 4), over a 3 year period extending from January 2004 to June 2007 [58].

We collected data over a period of 30 months and have demonstrated seasonal variations for the individual viruses (Figure 10). The overall proportion of severe pneumonia cases positive for viruses was 43% during the first half compared to 19% in the second half of the study, while corresponding proportions for RSV were 22% and 7%, respectively. The year to year variation in the magnitude of the RSV epidemic that we identified corroborates with findings from other studies [195-197].

The seasonality of respiratory viral infections differs between temperate and tropical climates. While RSV epidemics occur in winter in temperate regions, the peak timing of RSV infection is more diverse in tropical climates [198]. The detection of RSV in our study was common in the transition period at the end of monsoon and during winter months, a pattern similar to that observed in the community setting in Bhaktapur [58]. Semi-annual peaks of RSV have also been observed in several other locations in South East Asia [198]. On combining data from the two studies, assuming that the peaks of hospital RSV admissions

reflect the RSV activity in the wider community residing within the Kathmandu valley, we were able to show that in a four year period (July 2004 – June 2008), there were four RSV epidemics with alternating early and late onset in a biennial rhythm similar to those observed in Europe [196, 199, 200]. However, a longer observation time is needed for the confirmation of such an epidemic pattern of RSV in the Kathmandu valley. During the time period when the two studies overlapped, January 2006 to June 2007, we observed that peak occurrence of detections for RSV, influenza A, PIV types 1 and 3 in hospitalized children coincided with similar outbreaks in the community-based study [58]. This important observation implies that establishing sentinel virus surveillance, especially of RSV, in a centrally located general hospital, such as our study site, may give a good indication of viral activity in the wider community. Such surveillance could be useful in estimating RSV disease burden using an approach similar to that described in the WHO generic protocol for hospital-based surveillance of rotavirus gastroenteritis in children [201] and may also help to guide the need for vaccination against RSV in Nepal.

Predictors of time until recovery and treatment failure of acute severe pneumonia in hospitalized children

Pneumonia in Nepalese U5 children continues to be a significant burden to health services with a little over one million children classified with severe pneumonia in a report by the Ministry of Health for the fiscal year 2069/70 B.S. (16 July 2012 - 15 July 2013 A.D.) [133]. The community based – Integrated Management of Childhood Illness (CB-IMCI) program, which is promoted by the Nepal government, recommends children with severe pneumonia to be referred to and treated in a health facility [132]. While uncertainty regarding the numbers that actually reach a health facility remains, this is still a significant load on the

existing health infrastructure, which has limited resources. The early identification of factors associated with poor outcomes among children with severe pneumonia may help to prioritize the management of children at risk and thereby increase their chances of survival. The meticulously collected data from the clinical trial enabled us to assess the associations between prognostic factors and the outcomes, i.e. time until recovery and treatment failure. We used the entire trial cohort of 598 children, 2 – 35 months of age. In this large prospective cohort study with median time until recovery of 2 days and treatment failure in 35%, independent predictors for both outcomes were younger age, radiographic consolidation and hypoxia on admission.

The age groups, study setting, definition of severe pneumonia, exposures and outcomes vary between the few studies from LMICs on predictors of poor outcomes of severe pneumonia in hospitalized children [33, 80-83, 85, 202-204]. Many of these studies explore the relationship between factors associated with an increased risk of death [33, 80-82, 203]. Severe respiratory illness is more likely in younger children with an estimated incidence of hospital admissions for severe ALRI increasing from 20 episodes per 1000 U5 children to 52 episodes per 1000 infants (1 -11 months) per child year in a review of studies from LMICs [130]. In studies exploring the association between age and death, younger children with severe pneumonia are at a higher risk of death [33, 82]. We found slower time until recovery with decreasing age (Table 15) and to our knowledge, ours is the first study to identify age as an independent predictor of the duration of severe pneumonia.

The observation that hypoxia on admission was independently associated with treatment failure was also identified in studies by Ashgar *et al* [202] and Patel *et al* [204]. Similarly, the

association between age and treatment failure, where younger children were at a higher risk of treatment failure, was also found in the study by Fu. *et al.* [85]

In children with pneumonia, hypoxia and danger signs are both indicators of severity. The measurement of oxygen saturation requires a pulse oximeter that may not be available in many health facilities treating children with pneumonia in LMICs. While hypoxia was an independent predictor of both outcomes, presence of any danger sign predicted treatment failure only when hypoxia was removed as a covariate in the models. Clinical predictors may not be easily recognized, whereas measurement of oxygen saturation with a pulse oximeter, when performed correctly, to document hypoxia is a more reliable and objective sign to assess severity. Pulse oximetry, the 'fifth pediatric vital sign' [30] supplemented by treatment with oxygen was found to be a feasible and cost effective intervention for childhood pneumonia with 35% reduction in mortality in Papua New Guinea [34]. Pulse oximetry to document hypoxia in CAP is not only endorsed by professional societies in developed countries [4, 205] but also stated in recent WHO guidelines [23] and our findings support these recommendations.

The finding from studies reporting how radiographic pneumonia identifies those children with severe pneumonia likely to have poor outcomes [80, 82, 83, 203, 204] may not to be applicable to settings with limited resources to neither get a good quality film nor have one correctly interpreted. In our study, chest radiographs were taken to screen for exclusion criteria and had no role in the initial diagnosis and management of enrolled patients. We found that addition of radiographic pneumonia to the analyses did not substantially change the estimates of independent clinical predictors of neither time until recovery nor treatment failure. In resource poor settings, diagnosis of severe pneumonia relies heavily on clinical

signs and a chest radiograph in uncomplicated but severe CAP would not change initial management with empirical antibiotic therapy in such settings. Our findings support the WHO guidelines, which state that radiographs in severe pneumonia should be done only if it is possible to do so [23].

As this is the only study until date to report on predictors of illness duration, we believe our findings to be relevant to characterize and thereby guide the management of severe pneumonia in hospitalized children in resource poor settings. Our results support the understanding that while a chest radiograph may not always be indicated, the detection and treatment of hypoxia is a crucial step in the management of children admitted to health care settings with pneumonia.

Conclusion

The studies presented in this thesis contribute to the understanding of the epidemiology of community acquired pneumonia (CAP) with lower chest indrawing in hospitalized young children in an LMIC, such as Nepal. Our observations adds to the growing body of literature suggesting that there is limited evidence to support the use of zinc as adjunct treatment in children with pneumonia. In fact, we generated statistically precise evidence that, in our setting, zinc did not benefit children treated for CAP. We identified young age, hypoxia and radiographic consolidation as independent prognostic factors for time until recovery from an acute episode of CAP. This is the first study to report on predictors of time until recovery in children with pneumonia. In the study of respiratory viruses, we demonstrated a distinct seasonal variation in hospital admissions with severe pneumonia, with respiratory syncytial virus (RSV) contributing to the biennial peaks. RSV was the most commonly detected pathogen from NPA. These findings in Nepalese children add to the knowledge on viral etiology and contribution of RSV to the burden of pneumonia in hospitalized children from an LMIC.

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Errata

Page 17 - Reference 4 which was incorrectly placed, removed from middle of sentence in line 3.

Page 56 - Figure 6. Trial profile corrected. Data added to box with incomplete information on follow up of participants allotted to zinc

Page 94 - Incorrectly quoted Table Y changed to Table 15 in line 19.

PAPER III

PLOS | ONE

RESEARCH ARTICLE

Predictors of Duration and Treatment Failure of Severe Pneumonia in Hospitalized Young Nepalese Children

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Abstract

Background

Pneumonia in young children is still the most frequent cause of death in developing countries. We aimed to identify predictors for recovery and treatment failure in children hospitalized with severe pneumonia.

Methods

We enrolled 610 Nepalese children, aged 2 – 35 months from February 2006 to June 2008. Study participants were provided with standard treatment for pneumonia and followed up until discharge. Three multiple regression models representing clinical variables, clinical and radiological combined and all variables, including C-reactive protein (CRP) and viral etiology were used to assess the associations.

Results

The median age of study participants was 6 months with 493 (82%) infants and 367 (61%) males. The median time (IQR) till recovery was 49 (31, 87) hours and treatment failure was experienced by 209 (35%) of the children. Younger age, hypoxia on admission and radiographic pneumonia were independent predictors for both prolonged recovery and risk of treatment failure. While wasting and presence of any danger sign also predicted slower recovery, Parainfluenza type 1 isolated from the nasopharynx was associated with earlier resolution of illness. Gender, being breastfed, stunting, high fever, elevated CRP, presence of other viruses and supplementation with oral zinc did not show any significant association with these outcomes.

numbers: 151054 and 172226) and a grant from the South-Eastern Norway Regional Health Authority (grant number: 2012090) The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and took the final decision to submit this report for publication.

Competing Interests: The authors have declared that no competing interests exist.

Conclusion

Age, hypoxia and consolidation on chest radiograph were significant predictors for time till recovery and treatment failure in children with severe pneumonia. While chest radiograph is not always needed, detection and treatment of hypoxia is a crucial step to guide the management of hospitalized children with pneumonia.

Introduction

Pneumonia is an important cause of illness and leading cause of death in young children in developing countries [1]. More than 99% of pneumonia deaths occur in low- and middle-income countries (LMICs) [2]. The recent estimate is a median incidence of 0.22 episodes per child year (e/cy) with severe pneumonia contributing to 11.5% in LMICs [3]. In Nepal the estimated incidence of pneumonia is 0.24 e/cy with 11.5% severe episodes in children under 5 years of age [3]. While several preventable risk factors for severe pneumonia in developing countries have been identified [4] there is limited data on predictors of illness duration. Because the diagnosis of severe pneumonia in low income settings to a large extent relies on clinical criteria, it is important to identify objective clinical signs at baseline that may guide subsequent management of a severe pneumonia episode. The World Health Organization (WHO) uses evidence based clinical guidelines for the care of sick children in hospitals of resource poor settings [5,6]. The aim of this study is to identify predictors for duration and treatment failure of WHO-defined severe pneumonia in hospitalized young children in Nepal.

Methods

The study participants were recruited into a clinical trial [7] of zinc as adjuvant therapy to standard treatment of an acute episode of severe pneumonia, defined using WHO guidelines [8] and registered at clinicaltrials.gov as NCT00252304. Clearances were obtained from the ethics board of the Institute of Medicine, Tribhuvan University and Nepal Health Research Council, Kathmandu. We enrolled 610 children, 2–35 months of age from February 2006 to June 2008 at the Kanti Children's referral hospital in Kathmandu, Nepal. The Kathmandu valley lies at an altitude of approximately 1400 meters above sea level.

Eligible children presenting to the Kanti Children's hospital Emergency or Outpatient departments were screened for enrollment by trained physicians and first assessed for hypoxemia using a pulse oximeter (Nellcor Puritan Bennett NPB-40, Pleasanton, CA, USA) with a pediatric sensor (Nellcor Pedichek D-YSPD) and for presence of wheezing. Oxygen saturation (SpO_2) was recorded twice after stabilization of the reading for one minute. The higher of the two readings was used. For children with SpO_2 <90%, oxygen was provided prior to further evaluation. This oxygen saturation of <90% was the threshold used to define hypoxia based on WHO guidelines [8]. Children with wheezing were given up to three doses of nebulized Salbutamol 15 minutes apart, reassessed and excluded if lower chest indrawing (LCI) disappeared. History of illness was recorded and the findings of the physical examination were captured in a standardized form. Children were weighed using an Electronic Scale 890 (SECA, Hamburg, Germany) to the nearest 100 grams. Height in children 2 years of age or older was measured using a standard wooden height measuring board, while recumbent length in children less than 2 years old was measured using an infantometer, both to the nearest 0.1cm. Length-for-age and weight-for-length z scores were calculated using the 2006 WHO Child Growth Standards when

these parameters were below-2z, the children were considered to be stunted or wasted, respectively. We measured hemoglobin concentrations using Hemocue (Ångelholm, Sweden). Chest x-rays (CXR) were taken in all children, not only to identify infiltrations, but also to detect pleural effusion, pneumothorax or suspected heart disease, which would make a child ineligible for the study. Children with recurrent wheezing (defined as >3 episodes over the past 6 months and on treatment with bronchodilators), disappearance of lower chest indrawing (LCI) after nebulized Salbutamol, severe wasting (weight-for-length <-3z), severe anemia (hemoglobin <7 g/dL), heart disease, documented tuberculosis, concomitant diarrhea with dehydration and those with severe illness requiring special care or surgical intervention were also excluded. We used standardized WHO criteria to identify radiographic pneumonia [9]. The CXRs were interpreted independently by two radiologists blinded to clinical data, classifying findings as endpoint consolidation, other infiltrates or normal. If the two radiologists did not reach an agreement on consolidation, a second round of interpretation was carried out to arrive at a consensus. Informed consent was obtained for eligible children. Parents who could read and write signed the consent statement after they had read a written statement in the local Nepali language. For parents who were unable to read/write, verbal informed consent was obtained in the presence of a witness, whose name and signature was recorded in a register along with the child serial number of that particular participant. This procedure was approved by the ethics committee for the study. After obtaining consent, blood was collected and the first dose of intravenous antibiotics administered. This was followed by collection of nasopharyngeal aspirates for identification of 7 respiratory viruses, namely respiratory syncytial (RSV), Influenza A and B, parainfluenza (PIV) type 1, 2, and 3, and human metapneumovirus, using a commercially available multiplex reverse transcription polymerase chain reaction (PCR) assay. Details of the sampling technique and analyses have been described elsewhere [10].

Definitions

Acute episode of severe pneumonia—A child presenting with cough (duration <14 days) and/or difficult breathing of </ = 72 hours duration with LCI.

Time till cessation of severe pneumonia—The period starting from enrollment to the beginning of a 24-hour consecutive period of absence of LCI, of hypoxia and of any danger signs.

Treatment failure—A requirement for a change in antibiotics to second line therapy in those with failure to improve, development of complications such as empyema/pneumothorax requiring surgical intervention or admission to the intensive care unit for ventilator and/or inotropic support.

Failure to improve—Persistence of LCI or of any danger signs present at enrollment despite 48 hours of treatment or appearance of new danger signs or hypoxia with deterioration of a patient's clinical status any time after initiation of treatment.

Clinical improvement—Absence of danger signs, of hypoxia for a consecutive 24-hour, and of LCI for a 48-hour period.

Duration of hypoxia—The period from enrolment with SpO_2 < 90% on pulse oximetry to the beginning of a 24 hour consecutive period of SpO_2>90%, when breathing unassisted in room air.

Enrolled children were admitted to the hospital and monitored at 8 hourly intervals by study physicians until discharge. Benzyl penicillin (50,000 units/kg IV every 6 hourly) and Gentamicin (7.5 mg/kg IV once daily) were given till clinical improvement, following which children were discharged with advice to continue oral Amoxicillin to complete treatment for a total duration of 10 days. In addition, each participant received daily supplements (dispersible

tablets with either zinc or placebo) during their stay in the hospital and up to a maximum of 14 days [7].

Antibiotics were changed to Cefotaxime in children who did not improve. A decision to change antibiotics was made only after consultation with senior pediatricians involved in the study. For children unable to eat/drink or breast feed, intravenous fluids were initiated. Humidified oxygen was given to children with documented hypoxia and discontinued when they were no longer hypoxic. The absence of hypoxia was confirmed after a second reading taken 30 minutes later.

Statistical Analyses

Data were analyzed using Stata version 13 (Stata Corp, College Station, TX). We fitted multiple regression models using clinical (Model 1), clinical and radiological (Model 2) and all (Model 3) variables. Age was used as a categorical variable. Predictors of time till cessation of a severe pneumonia episode were identified in Cox proportional hazards models with the "exactp" option to handle ties. For treatment failure, we used logistic regression. Outcomes were coded such that Hazard ratios (HR) <1 for time till recovery indicates slower resolution of illness and Odds ratios (OR) > 1 increased odds of treatment failure. Initially we assessed the crude associations of relevant independent variables with the selected outcomes. Variables with $p< 0.25$ were included in the multivariable models and those variables which were still significant, i.e. being associated with the outcome with a p-value of < 0.05 retained in the model. In these models we included the other variables one at a time and kept if significant. This manual stepwise approach were outlined by Hosmer and Lemeshow [11].

We tested the goodness-of-fit of the models by the method suggested by Hosmer and Lemeshow [11] and for logistic regression by calculating the dfbetas and hat statistics. We assessed the assumptions for the cox models using tests of specification (plotting of Schoenfeld residuals) and goodness of fit.

Results

We enrolled 610 children meeting criteria for an acute episode of severe pneumonia. We excluded from the analyses 11 children with heart disease and 1 child with chronic cough discovered after inclusion. During the study, 4 of the 7 children transferred to the pediatric intensive care unit died, 9 (1.5%) parents withdrew consent and 2 were diagnosed with tuberculosis. Demographic, clinical and laboratory characteristics of study participants are outlined in Table 1. The median age of the 598 children was 6[Interquartile range (IQR) 3–10] months, 61% were boys and 82% infants (<12 months). Mean respiratory rate [Standard deviation (SD)] was 64 (12), 62% were hypoxic and 49% had a danger sign, while 10% had 3 danger signs. On chest auscultation, 82% of the children had wheezing and 92% had crepitations. C-reactive protein (CRP)> 40mg/L was detected in 30%, 13% had CRP> 80mg/L. At least one virus was isolated from 29% of the nasopharyngeal specimens; RSV being the most frequent. Radiographic pneumonia, defined as endpoint consolidation on chest X-ray, was detected in 164 of 457 (36%) chest radiographs available for interpretation.

Median time (IQR) till recovery, using predefined criteria, was 49 (31, 87) hours while time till discharge (IQR) was 97 (83, 135). Treatment failure occurred in 209 of the 594 children (35%).

There was a near linear relationship between age and both outcomes, i.e. time till recovery and the risk of treatment failure (data not shown). An increment in age by one month was associated with an HR of 1.04 (95% CI: 1.03–1.06) for time till recovery and the OR for treatment failure was 0.93 (95% CI: 0.90–0.96).

Table 1. Baseline Characteristics of children ages 2–35 months with WHO defined severe pneumonia.

Background Characteristics	N	Value
Median age in months (IQR)	598	6 (3, 10)
Age categories		
• 2–6 months (%)	352	58.9
• 7–11 months (%)	141	23.5
• 12–23 months (%)	90	15.1
• 24–35months (%)	15	2.5
Boys (%)	598	367 (61)
Breastfed (%)	598	571 (96)
Wasted (Weight for height/length Z score < -2) (%)[#]	594	157 (26)
Stunted (Height for age Z score <- 2) (%)[#]	598	50 (8)
Underweight (Weight for age Z score <- 2) (%) [#]	598	102 (17)
Mean age of mother (SD)	586	24.4 (4.1)
Illiterate mother (%)[##]	586	154 (26)
Illiterate father (%)[##]	588	38 (7)
Unemployed mother (%)**	584	418 (72)
Unemployed father (%)**	580	19 (3)
Clinical Characteristics		
Mean Respiratory rate as breaths per minute (SD)		
• 2–11 months	493	65(12)
• 12–35 months	105	61(12)
Hypoxia (SpO_2 < 90%) at enrolment (%)	598	373 (62)
Febrile (Axillary temperature > 38.5°C) (%)	598	92 (15)
Danger signs (%)		
-Nasal flaring	597	232 (39)
-Grunting	598	131 (22)
-Head nodding	598	138 (23)
-Cyanosis	598	3 (0.5)
• Any one danger sign	598	294 (49)
• Presence of 3 danger signs	598	57 (10)
• Presence of 2 danger signs	598	96 (16)
• Presence of 1 danger sign	598	141 (24)
Wheezing (%)	598	492 (82)
Crepitations (%)	598	549 (92)
Laboratory Characteristics		
Mean CRP in mg/L (SD)	572	37.9 (51.2)
-CRP > 40mg/L	572	170 (30)
-CRP > 80mg/L	572	75 (13)
Nasopharyngeal aspirate positive for any virus (%)	596	175 (29)*
-Respiratory syncytial virus (%)	596	79 (13)
-Parainfluenza type 1 (%)	596	23 (4)
-Parainfluenza type 2 (%)	596	5 (1)
-Parainfluenza type 3 (%)	596	24 (4)
-Influenza A (%)	596	24 (4)
-Influenza B (%)	596	17 (2)
-Human metapneumovirus (%)	596	9 (1)
Radiographic Pneumonia (%)	457	164 (36)

(*Continued*)

Table 1. (*Continued*)

Background Characteristics	N	Value
Supplemented with zinc (%)	598	299 (50)

[#] Calculated using WHO Growth standards.

*6 children were positive for 2 viruses simultaneously.

[##] No schooling with inability to read part or whole of a sentence.

** No work/housework.

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Wasting (HR 0.79 95% CI: 0.65, 0.96), hypoxia (HR 0.62 95% CI: 0.51, 0.74) and presence of any danger sign (HR 0.76 95% CI: 0.64, 0.91) were independently associated with slower recovery (Table 2). These associations did not change substantially with the addition of other variables (Models 2 & 3). Radiographic pneumonia was also a significant predictor of delayed recovery (HR O.58 95% CI: 0.47, 0.72). Presence of Parainfluenza type 1 (PIV 1) in the nasopharynx was associated with earlier recovery (HR 2.46 95%CI 1.48, 4.09). Gender, being breastfed, stunting, high fever, CRP> 40 and >80 mg/L, presence of other viruses and supplementation with oral zinc did not show any significant association with time till recovery (Table 2).

When estimating the association between child age categories and treatment failure, the older children had a lower risk of treatment failure (Table 3). While hypoxia and age categories up to 23 months remained as independent predictors, the oldest age group and any danger sign were no longer significant as indicators of treatment failure in models 2 and 3 (Table 3). Gender, being breastfed, stunted, having high fever, elevated CRP, viruses in the nasopharynx, and zinc supplementation were not significantly associated with treatment failure.

We repeated our regression analysis excluding hypoxia as a covariate in all three models and found very little change in the hazard and odds ratios of predictors, age and radiographic pneumonia identified earlier (data not shown). However, in the absence of hypoxia as a covariate, presence of at least one danger sign was a significant predictor of treatment failure in all models (data not shown). We also repeated the analyses using lower cut offs to define hypoxemia i.e. SpO2 of 88% and 85% but the regression coefficients were only marginally altered (data not shown).

We explored possible interactions between independent variables using interaction terms (age x breastfeeding, age x wasting and age x any danger sign). Age did not modify the effect of breastfeeding, wasting or any danger sign in any of the models.

Discussion

We report findings from secondary analyses of data from a clinical trial assessing the efficacy of zinc in childhood pneumonia. In this large prospective study of hospitalized young Nepalese children with acute severe pneumonia, median time till recovery of 2 days and treatment failure in 35%, independent predictors for both outcomes were younger age, radiographic consolidation and hypoxia on admission. In addition, wasting and any danger sign on admission was associated with prolonged duration of illness. With the exception of detecting PIV 1 from the nasopharynx, which signaled earlier recovery, presence of other viruses and raised CRP levels were not associated with either outcome. As this was a clinical trial on the efficacy of zinc in children with severe pneumonia, we also explored the possibility of differences in the outcomes between those who received the drug versus those who did not, and found no such interaction.

Table 2. Predictors of Time till Recovery of illness episode in children 2–35 months hospitalized with WHO defined Severe Pneumonia.

Variables	Crude Hazard Ratio*	Adjusted Hazard Ratio* (number of observations)		
Age in months	1.04 (1.03, 1.06) < 0.001			
Age categories		**Model 1 (594)**	**Model 2 (455)**	**Model 3 (454)**
• 2–6 months	1.00	1.00	1.00	1.00
• 7–11 months	1.43 (1.17, 1.75) < 0.001	1.49 (1.21, 1.83) < 0.001	1.53 (1.22, 1.94) < 0.001	1.22, 1.94) < 0.001
• 12–23 months	1.55 (1.21, 1.97) < 0.001	1.68 (1.30, 2.16) < 0.001	1.46 (1.09, 1.97) 0.012	1.09, 1.97) 0.012
• 24–35months	3.39 (2.01, 5.71) < 0.001	4.22 (2.46, 7.25) < 0.001	3.33 (1.77, 6.27) < 0.001	3.33 (1.77, 6.27) < 0.001
Gender	1.11 (0.94, 1.31) 0.223			
Breastfed	0.97 (0.64, 1.47) 0.882			
Wasting (< -2 Weight for height/length)	0.76 (0.63, 0.91) 0.004	0.79 (0.65, 0.96) 0.017	0.79 (0.64, 0.98) 0.030	0.78 (0.63, 0.96) 0.022
Stunting (< -2 Height/length for age)	0.84 (0.62, 1.14) 0.261			
Hypoxia (SpO2 < 90%)	0.64 (0.54, 0.76) < 0.001	0.62 (0.51, 0.74) <0.001	0.71 (0.58, 0.88) 0.001	0.72 (0.58, 0.89) 0.002
Febrile (Axillary temperature > 38.5°C)	1.14 (0.91, 1.43) 0.248			
Any danger sign	0.69 (0.59, 0.82) <0.001	0.76 (0.64, 0.91) 0.003	0.74 (0.60, 0.90) 0.003	0.76 (0.62, 0.92) 0.006
Radiographic pneumonia	0.56 (0.46, 0.69) <0.001		0.58 (0.47, 0.72) <0.001	0.55 (0.45, 0.68) <0.001
C-Reactive Protein (CRP) > 40 mg/L	1.01 (0.84, 1.21) 0.918			
Nasopharyngeal aspirate positive for virus				
• Respiratory syncytial virus (RSV)	(0.62, 0.99) 0.049			
• Influenza A	0.78 (0.52, 1.19) 0.252			
• Influenza B	(0.49, 1.33) 0.399			
• Parainfluenza type 1 (PIV 1)	1.75 (1.14, 2.69) 0.010			2.46 (1.48, 4.09) < 0.001
• Parainfluenza type 2 (PIV 2)	(0.40, 2.36) 0.957			
• Parainfluenza type 3 (PIV 3)	(0.96, 2.17) 0.082			
• Human metapneumovirus (hMPV)	0.64 (0.32, 1.28) 0.203			
Supplemented with zinc	1.11 (0.94, 1.31) 0.221			

*Hazard ratios (95% CI) and P-value calculated using Cox Regression with exact p option. Hazard ratios <1 for time till recovery indicates slower resolution of illness.

Results of multiple regressions with P-value > 0.05 not shown in the table.

Model 1 (Clinical variables): Adjusted for gender, breastfed, febrile and treatment with zinc.

Model 2 (Clinical + Radiographic pneumonia): Adjusted for gender, breastfed, febrile and treatment with zinc.

Model 3 (All variables): Adjusted for gender, breastfed, febrile, CRP, nasopharyngeal aspirate positive for RSV, influenza A and B, PIV 2 and 3 and hMPV and treatment with zinc.

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While several studies report on predictors of death [12–15], we were unable to find any exploring clinical risk factors for duration of illness of a severe pneumonia episode in young children. In our study only 4 children died in the hospital, and mortality was not an outcome. Hypoxia, a significant predictor of death in most of the studies [12,13,15] was an independent predictor of time till resolution of illness. While studies have identified younger children to be at a higher risk of death [14,15], we found slower time till recovery with decreasing age (Table 2). To our knowledge, this is the first study to identify age as an independent predictor of the duration of severe pneumonia.

The proportions with treatment failure in large multicenter clinical trials conducted in hospitalized children with severe pneumonia were 13.6% [16] and 20% [17]. These estimates are different from our study (35%) and may partly be due to the differences in outcome definition. Similar to the present study, hypoxia on admission was identified as an independent predictor

Table 3. Predictors of Treatment failure of illness episode in children 2–35 months hospitalized with WHO defined Severe Pneumonia

Variables	Crude Odds Ratio*	Adjusted Odds ratio* (number of observations per model)		
Age in months	0.93 (0.90, 0.96) <0.001			
Age categories		**Model 1 (590)**	**Model 2 (455)**	**Model 3 (454)**
• 2–6 months	1.00	1.00	1.00	1.00
• 7–11 months	0.67 (0.44, 1.01) 0.058	0.61 (0.40, 0.94) 0.024	0.56 (0.34, 0.91) 0.019	0.55 (0.34, 0.90) 0.018
• 12–23 months	0.37 (0.21, 0.64) <0.001	0.33 (0.19, 0.58) <0.001	0.27 (0.13, 0.55) <0.001	0.27 (0.13, 0.55) <0.001
• 24–35months	0.22 (0.05, 0.99) 0.049	0.18 (0.04, 0.83) 0.028	0.31 (0.06, 1.54) 0.153	0.34 (0.07, 1.72) 0.194
Gender	0.83 (0.59,1.18) 0.296			
Breastfed	0.67 (0.28,1.61) 0.370			
Wasting (594) (< -2 Weight for height/ length)	1.49 (1.02, 2.16) 0.038			
Stunting (598) (< -2 Height/Length for age)	1.25 (0.69, 2.27) 0.457			
Hypoxia (SpO2 < 90%)	1.91 (1.33, 2.74) <0.001	2.00 (1.36, 2.93) <0.001	1.80 (1.15, 2.80) 0.010	1.81 (1.16, 2.83) 0.009
Febrile (Axillary temperature > 38.5°C)	0.94 (0.59, 1.51) 0.808			
Any danger sign	1.65 (1.18, 2.32) 0.004	1.44 (1.01, 2.07) 0.045		
Radiographic pneumonia	2.22 (1.49, 3.31) < 0.001		2.09 (1.37, 3.19) 0.001	2.12 (1.39, 3.24) 0.001
C-Reactive Protein (CRP) > 40 mg/L	1.15 (0.79, 1.67) 0.472			
Nasopharyngeal aspirate positive for virus				
• Respiratory syncytial virus (RSV)	1.47 (0.91, 2.38) 0.115			
• Influenza A	0.31, 1.84) 0.533			
• Influenza B	0.37, 2.76) 0.989			
• Parainfluenza type 1 (PIV 1)	0.18, 1.37) 0.178			
• Parainfluenza type 2 (PIV 2)	0.20, 7.44) 0.819			
• Parainfluenza type 3 (PIV 3)	0.12, 1.06) 0.063			
• Human metapneumovirus (hMPV)	2.34 (0.62, 8.81) 0.209			
Supplemented with zinc	0.83 (0.60, 1.17) 0.291			

*Odds ratios (95% CI) and P-value calculated using Logistic Regression. Odds ratios > 1 indicates increased odds of treatment failure.

Results of multiple regressions with P-value >0.05 not shown in the table with the exception of age analyzed as a categorical variable.

Model 1 (Clinical variables): Adjusted for gender, breastfed, wasting, febrile and treatment with zinc.

Model 2 (Clinical + Radiographic pneumonia): Adjusted for gender, breastfed, wasting, febrile, any danger sign and treatment with zinc.

Model 3 (All variables): Adjusted for gender, breastfed, wasting, febrile, any danger sign, CRP, nasopharyngeal aspirate positive for RSV, PIV 1, 2 and 3, influenza A and B, hMPV and treatment with zinc.

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of treatment failure in both studies. The trends for age as a predictor were similar in our study and the study by Fu. *et al.* [18], with younger children having a higher risk of treatment failure.

We found hypoxia to be a predictor of both treatment failure and illness duration in our study. With hypoxia removed as a covariate, presence of any danger sign was a significant predictor of treatment failure in all three models. The variables hypoxia and any danger sign were correlated, which is expected as both are known indicators of severe pneumonia. Clinical predictors may not be easily recognized whereas measurement of oxygen saturation with a pulse oximeter, when performed correctly, to document hypoxia seems to be a more reliable and objective sign to assess severity. Pulse oximetry, the 'fifth pediatric vital sign' [19] supplemented by treatment with oxygen was found to be a feasible and cost effective intervention for childhood pneumonia with 35% reduction in mortality in Papua New Guinea [20]. Unfortunately even though pulse oximeters and oxygen are available, they are underutilized by health workers

in developing countries [21,22]. Pulse oximetry to document hypoxia in community acquired pneumonia is not only endorsed by professional societies in developed countries [23,24] but also stated in recent WHO guidelines[6] and our findings support these recommendations.

We identified one study in India [25] reporting lobar consolidation as a predictor of longer stay in hospital but this was based on unadjusted analysis and therefore not comparable to the results of our study. The two studies that report an association between radiographic consolidation and mortality in hospitalized children with severe pneumonia have conflicting results. While Lupisan *et al*. [14] found dense infiltrates to be an independent predictor, Reed *et al*. [12] report that alveolar consolidation on multiple regression analysis was not significant. Primary end point consolidation was an independent predictor of treatment failure at 48 hours of hospitalization especially among penicillin recipients (RR 3.58 95% CI: 1.47, 8.75) in the study by Patel *et al*. [17], a finding very similar to what we found. In our study, chest radiograph was done to screen for exclusion criteria and had no role in the initial diagnosis and management of enrolled patients. We found that addition of radiographic pneumonia to the analyses (Models 2 and 3) did not substantially change the estimates of independent clinical predictors of both outcomes (Tables 2 and 3). In resource poor settings, diagnosis of severe pneumonia relies heavily on clinical signs. An initial chest radiograph in uncomplicated but severe community acquired pneumonia would not change initial management with empirical antibiotic therapy in such settings. Our findings support the WHO guidelines [6] which state that radiographs in severe pneumonia should be done only when possible.

We report findings from a randomized clinical trial [7] conducted under controlled settings. Our findings may therefore not be directly applicable to other hospitals caring for children in developing countries. We used previous WHO guidelines [8]requiring only lower chest indrawing in addition to symptoms to define severe pneumonia as inclusion criteria. Although we took measures to screen out children with reactive airways, we may have included those with pneumonia that could have been treated as outpatients. While recruiting, we excluded very sick children with pneumonia and probably missed those with other signs of severe pneumonia in the absence of LCI and this may also have affected our observed associations. While we were able to detect several predictors for duration and treatment failure, for many of the selected independent variables such as fever and breastfeeding, the power to detect any significant association with the outcomes was low. It should be noted that the study was designed to measure the effect of zinc supplementation during pneumonia and not to identify these predictors.

This large study on children aged between 2–35 months, with acute severe pneumonia found younger age, hypoxia on admission and radiographic pneumonia to independently predict both time to resolution and treatment failure. As it is the only study till date to report on predictors of illness duration we believe our findings to be relevant, not only to characterize but also manage a severe pneumonia episode in hospitalized children from resource poor settings. Our results indicate that a chest radiograph is not always needed but the detection and treatment of hypoxia is a crucial step in the management of children admitted to health care settings with pneumonia.

Supporting Information

S1 Dataset.
(XLSX)

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RESEARCH ARTICLE

Predictors of Duration and Treatment Failure of Severe Pneumonia in Hospitalized Young Nepalese Children

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Abstract

Background

Pneumonia in young children is still the most frequent cause of death in developing countries. We aimed to identify predictors for recovery and treatment failure in children hospitalized with severe pneumonia.

Methods

We enrolled 610 Nepalese children, aged 2 – 35 months from February 2006 to June 2008. Study participants were provided with standard treatment for pneumonia and followed up until discharge. Three multiple regression models representing clinical variables, clinical and radiological combined and all variables, including C-reactive protein (CRP) and viral etiology were used to assess the associations.

Results

The median age of study participants was 6 months with 493 (82%) infants and 367 (61%) males. The median time (IQR) till recovery was 49 (31, 87) hours and treatment failure was experienced by 209 (35%) of the children. Younger age, hypoxia on admission and radiographic pneumonia were independent predictors for both prolonged recovery and risk of treatment failure. While wasting and presence of any danger sign also predicted slower recovery, Parainfluenza type 1 isolated from the nasopharynx was associated with earlier resolution of illness. Gender, being breastfed, stunting, high fever, elevated CRP, presence of other viruses and supplementation with oral zinc did not show any significant association with these outcomes.

numbers: 151054 and 172226) and a grant from the South-Eastern Norway Regional Health Authority (grant number: 2012090) The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and took the final decision to submit this report for publication.

Competing Interests: The authors have declared that no competing interests exist.

Conclusion

Age, hypoxia and consolidation on chest radiograph were significant predictors for time till recovery and treatment failure in children with severe pneumonia. While chest radiograph is not always needed, detection and treatment of hypoxia is a crucial step to guide the management of hospitalized children with pneumonia.

Introduction

Pneumonia is an important cause of illness and leading cause of death in young children in developing countries [1]. More than 99% of pneumonia deaths occur in low- and middle-income countries (LMICs) [2]. The recent estimate is a median incidence of 0.22 episodes per child year (e/cy) with severe pneumonia contributing to 11.5% in LMICs [3]. In Nepal the estimated incidence of pneumonia is 0.24 e/cy with 11.5% severe episodes in children under 5 years of age [3]. While several preventable risk factors for severe pneumonia in developing countries have been identified [4] there is limited data on predictors of illness duration. Because the diagnosis of severe pneumonia in low income settings to a large extent relies on clinical criteria, it is important to identify objective clinical signs at baseline that may guide subsequent management of a severe pneumonia episode. The World Health Organization (WHO) uses evidence based clinical guidelines for the care of sick children in hospitals of resource poor settings [5,6]. The aim of this study is to identify predictors for duration and treatment failure of WHO-defined severe pneumonia in hospitalized young children in Nepal.

Methods

The study participants were recruited into a clinical trial [7] of zinc as adjuvant therapy to standard treatment of an acute episode of severe pneumonia, defined using WHO guidelines [8] and registered at clinicaltrials.gov as NCT00252304. Clearances were obtained from the ethics board of the Institute of Medicine, Tribhuvan University and Nepal Health Research Council, Kathmandu. We enrolled 610 children, 2–35 months of age from February 2006 to June 2008 at the Kanti Children's referral hospital in Kathmandu, Nepal. The Kathmandu valley lies at an altitude of approximately 1400 meters above sea level.

Eligible children presenting to the Kanti Children's hospital Emergency or Outpatient departments were screened for enrollment by trained physicians and first assessed for hypoxemia using a pulse oximeter (Nellcor Puritan Bennett NPB-40, Pleasanton, CA, USA) with a pediatric sensor (Nellcor Pedichek D-YSPD) and for presence of wheezing. Oxygen saturation (SpO_2) was recorded twice after stabilization of the reading for one minute. The higher of the two readings was used. For children with SpO_2 <90%, oxygen was provided prior to further evaluation. This oxygen saturation of <90% was the threshold used to define hypoxia based on WHO guidelines [8]. Children with wheezing were given up to three doses of nebulized Salbutamol 15 minutes apart, reassessed and excluded if lower chest indrawing (LCI) disappeared. History of illness was recorded and the findings of the physical examination were captured in a standardized form. Children were weighed using an Electronic Scale 890 (SECA, Hamburg, Germany) to the nearest 100 grams. Height in children 2 years of age or older was measured using a standard wooden height measuring board, while recumbent length in children less than 2 years old was measured using an infantometer, both to the nearest 0.1cm. Length-for-age and weight-for-length z scores were calculated using the 2006 WHO Child Growth Standards when

these parameters were below-2z, the children were considered to be stunted or wasted, respectively. We measured hemoglobin concentrations using Hemocue (Ångelholm, Sweden). Chest x-rays (CXR) were taken in all children, not only to identify infiltrations, but also to detect pleural effusion, pneumothorax or suspected heart disease, which would make a child ineligible for the study. Children with recurrent wheezing (defined as >3 episodes over the past 6 months and on treatment with bronchodilators), disappearance of lower chest indrawing (LCI) after nebulized Salbutamol, severe wasting (weight-for-length <-3z), severe anemia (hemoglobin <7 g/dL), heart disease, documented tuberculosis, concomitant diarrhea with dehydration and those with severe illness requiring special care or surgical intervention were also excluded. We used standardized WHO criteria to identify radiographic pneumonia [9]. The CXRs were interpreted independently by two radiologists blinded to clinical data, classifying findings as endpoint consolidation, other infiltrates or normal. If the two radiologists did not reach an agreement on consolidation, a second round of interpretation was carried out to arrive at a consensus. Informed consent was obtained for eligible children. Parents who could read and write signed the consent statement after they had read a written statement in the local Nepali language. For parents who were unable to read/write, verbal informed consent was obtained in the presence of a witness, whose name and signature was recorded in a register along with the child serial number of that particular participant. This procedure was approved by the ethics committee for the study. After obtaining consent, blood was collected and the first dose of intravenous antibiotics administered. This was followed by collection of nasopharyngeal aspirates for identification of 7 respiratory viruses, namely respiratory syncytial (RSV), Influenza A and B, parainfluenza (PIV) type 1, 2, and 3, and human metapneumovirus, using a commercially available multiplex reverse transcription polymerase chain reaction (PCR) assay. Details of the sampling technique and analyses have been described elsewhere [10].

Definitions

Acute episode of severe pneumonia—A child presenting with cough (duration <14 days) and/or difficult breathing of </ = 72 hours duration with LCI.

Time till cessation of severe pneumonia—The period starting from enrollment to the beginning of a 24-hour consecutive period of absence of LCI, of hypoxia and of any danger signs.

Treatment failure—A requirement for a change in antibiotics to second line therapy in those with failure to improve, development of complications such as empyema/pneumothorax requiring surgical intervention or admission to the intensive care unit for ventilator and/or inotropic support.

Failure to improve—Persistence of LCI or of any danger signs present at enrollment despite 48 hours of treatment or appearance of new danger signs or hypoxia with deterioration of a patient's clinical status any time after initiation of treatment.

Clinical improvement—Absence of danger signs, of hypoxia for a consecutive 24-hour, and of LCI for a 48-hour period.

Duration of hypoxia—The period from enrolment with $SpO_2 < 90\%$ on pulse oximetry to the beginning of a 24 hour consecutive period of $SpO_2 > 90\%$, when breathing unassisted in room air.

Enrolled children were admitted to the hospital and monitored at 8 hourly intervals by study physicians until discharge. Benzyl penicillin (50,000 units/kg IV every 6 hourly) and Gentamicin (7.5 mg/kg IV once daily) were given till clinical improvement, following which children were discharged with advice to continue oral Amoxicillin to complete treatment for a total duration of 10 days. In addition, each participant received daily supplements (dispersible

tablets with either zinc or placebo) during their stay in the hospital and up to a maximum of 14 days [7].

Antibiotics were changed to Cefotaxime in children who did not improve. A decision to change antibiotics was made only after consultation with senior pediatricians involved in the study. For children unable to eat/drink or breast feed, intravenous fluids were initiated. Humidified oxygen was given to children with documented hypoxia and discontinued when they were no longer hypoxic. The absence of hypoxia was confirmed after a second reading taken 30 minutes later.

Statistical Analyses

Data were analyzed using Stata version 13 (Stata Corp, College Station, TX). We fitted multiple regression models using clinical (Model 1), clinical and radiological (Model 2) and all (Model 3) variables. Age was used as a categorical variable. Predictors of time till cessation of a severe pneumonia episode were identified in Cox proportional hazards models with the "exactp" option to handle ties. For treatment failure, we used logistic regression. Outcomes were coded such that Hazard ratios (HR) <1 for time till recovery indicates slower resolution of illness and Odds ratios (OR) > 1 increased odds of treatment failure. Initially we assessed the crude associations of relevant independent variables with the selected outcomes. Variables with $p< 0.25$ were included in the multivariable models and those variables which were still significant, i.e. being associated with the outcome with a p-value of < 0.05 retained in the model. In these models we included the other variables one at a time and kept if significant. This manual stepwise approach were outlined by Hosmer and Lemeshow [11].

We tested the goodness-of-fit of the models by the method suggested by Hosmer and Lemeshow [11] and for logistic regression by calculating the dfbetas and hat statistics. We assessed the assumptions for the cox models using tests of specification (plotting of Schoenfeld residuals) and goodness of fit.

Results

We enrolled 610 children meeting criteria for an acute episode of severe pneumonia. We excluded from the analyses 11 children with heart disease and 1 child with chronic cough discovered after inclusion. During the study, 4 of the 7 children transferred to the pediatric intensive care unit died, 9 (1.5%) parents withdrew consent and 2 were diagnosed with tuberculosis. Demographic, clinical and laboratory characteristics of study participants are outlined in Table 1. The median age of the 598 children was 6[Interquartile range (IQR) 3–10] months, 61% were boys and 82% infants (<12 months). Mean respiratory rate [Standard deviation (SD)] was 64 (12), 62% were hypoxic and 49% had a danger sign, while 10% had 3 danger signs. On chest auscultation, 82% of the children had wheezing and 92% had crepitations. C-reactive protein (CRP)> 40mg/L was detected in 30%, 13% had CRP> 80mg/L. At least one virus was isolated from 29% of the nasopharyngeal specimens; RSV being the most frequent. Radiographic pneumonia, defined as endpoint consolidation on chest X-ray, was detected in 164 of 457 (36%) chest radiographs available for interpretation.

Median time (IQR) till recovery, using predefined criteria, was 49 (31, 87) hours while time till discharge (IQR) was 97 (83, 135). Treatment failure occurred in 209 of the 594 children (35%).

There was a near linear relationship between age and both outcomes, i.e. time till recovery and the risk of treatment failure (data not shown). An increment in age by one month was associated with an HR of 1.04 (95% CI: 1.03–1.06) for time till recovery and the OR for treatment failure was 0.93 (95% CI: 0.90–0.96).

Table 1. Baseline Characteristics of children ages 2–35 months with WHO defined severe pneumonia.

Background Characteristics	N	Value
Median age in months (IQR)	598	6 (3, 10)
Age categories		
• 2–6 months (%)	352	58.9
• 7–11 months (%)	141	23.5
• 12–23 months (%)	90	15.1
• 24–35months (%)	15	2.5
Boys (%)	598	367 (61)
Breastfed (%)	598	571 (96)
Wasted (Weight for height/length Z score < -2) (%)[#]	594	157 (26)
Stunted (Height for age Z score <- 2) (%)[#]	598	50 (8)
Underweight (Weight for age Z score <- 2) (%) [#]	598	102 (17)
Mean age of mother (SD)	586	24.4 (4.1)
Illiterate mother (%)[##]	586	154 (26)
Illiterate father (%)[##]	588	38 (7)
Unemployed mother (%)**	584	418 (72)
Unemployed father (%)**	580	19 (3)
Clinical Characteristics		
Mean Respiratory rate as breaths per minute (SD)		
• 2–11 months	493	65(12)
• 12–35 months	105	61(12)
Hypoxia (SpO_2 < 90%) at enrolment (%)	598	373 (62)
Febrile (Axillary temperature > 38.5°C) (%)	598	92 (15)
Danger signs (%)		
-Nasal flaring	597	232 (39)
-Grunting	598	131 (22)
-Head nodding	598	138 (23)
-Cyanosis	598	3 (0.5)
• Any one danger sign	598	294 (49)
• Presence of 3 danger signs	598	57 (10)
• Presence of 2 danger signs	598	96 (16)
• Presence of 1 danger sign	598	141 (24)
Wheezing (%)	598	492 (82)
Crepitations (%)	598	549 (92)
Laboratory Characteristics		
Mean CRP in mg/L (SD)	572	37.9 (51.2)
-CRP > 40mg/L	572	170 (30)
-CRP > 80mg/L	572	75 (13)
Nasopharyngeal aspirate positive for any virus (%)	596	175 (29)*
-Respiratory syncytial virus (%)	596	79 (13)
-Parainfluenza type 1 (%)	596	23 (4)
-Parainfluenza type 2 (%)	596	5 (1)
-Parainfluenza type 3 (%)	596	24 (4)
-Influenza A (%)	596	24 (4)
-Influenza B (%)	596	17 (2)
-Human metapneumovirus (%)	596	9 (1)
Radiographic Pneumonia (%)	457	164 (36)

(*Continued*)

Table 1. (*Continued*)

Background Characteristics	N	Value
Supplemented with zinc (%)	598	299 (50)

[#] Calculated using WHO Growth standards.

*6 children were positive for 2 viruses simultaneously.

[##] No schooling with inability to read part or whole of a sentence.

** No work/housework.

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Wasting (HR 0.79 95% CI: 0.65, 0.96), hypoxia (HR 0.62 95% CI: 0.51, 0.74) and presence of any danger sign (HR 0.76 95% CI: 0.64, 0.91) were independently associated with slower recovery (Table 2). These associations did not change substantially with the addition of other variables (Models 2 & 3). Radiographic pneumonia was also a significant predictor of delayed recovery (HR O.58 95% CI: 0.47, 0.72). Presence of Parainfluenza type 1 (PIV 1) in the nasopharynx was associated with earlier recovery (HR 2.46 95%CI 1.48, 4.09). Gender, being breastfed, stunting, high fever, CRP> 40 and >80 mg/L, presence of other viruses and supplementation with oral zinc did not show any significant association with time till recovery (Table 2).

When estimating the association between child age categories and treatment failure, the older children had a lower risk of treatment failure (Table 3). While hypoxia and age categories up to 23 months remained as independent predictors, the oldest age group and any danger sign were no longer significant as indicators of treatment failure in models 2 and 3 (Table 3). Gender, being breastfed, stunted, having high fever, elevated CRP, viruses in the nasopharynx, and zinc supplementation were not significantly associated with treatment failure.

We repeated our regression analysis excluding hypoxia as a covariate in all three models and found very little change in the hazard and odds ratios of predictors, age and radiographic pneumonia identified earlier (data not shown). However, in the absence of hypoxia as a covariate, presence of at least one danger sign was a significant predictor of treatment failure in all models (data not shown). We also repeated the analyses using lower cut offs to define hypoxemia i.e. SpO2 of 88% and 85% but the regression coefficients were only marginally altered (data not shown).

We explored possible interactions between independent variables using interaction terms (age x breastfeeding, age x wasting and age x any danger sign). Age did not modify the effect of breastfeeding, wasting or any danger sign in any of the models.

Discussion

We report findings from secondary analyses of data from a clinical trial assessing the efficacy of zinc in childhood pneumonia. In this large prospective study of hospitalized young Nepalese children with acute severe pneumonia, median time till recovery of 2 days and treatment failure in 35%, independent predictors for both outcomes were younger age, radiographic consolidation and hypoxia on admission. In addition, wasting and any danger sign on admission was associated with prolonged duration of illness. With the exception of detecting PIV 1 from the nasopharynx, which signaled earlier recovery, presence of other viruses and raised CRP levels were not associated with either outcome. As this was a clinical trial on the efficacy of zinc in children with severe pneumonia, we also explored the possibility of differences in the outcomes between those who received the drug versus those who did not, and found no such interaction.

Table 2. Predictors of Time till Recovery of illness episode in children 2–35 months hospitalized with WHO defined Severe Pneumonia.

Variables	Crude Hazard Ratio*	Adjusted Hazard Ratio* (number of observations)		
Age in months	1.04 (1.03, 1.06) < 0.001			
Age categories		**Model 1 (594)**	**Model 2 (455)**	**Model 3 (454)**
• 2–6 months	1.00	1.00	1.00	1.00
• 7–11 months	1.43 (1.17, 1.75) < 0.001	1.49 (1.21, 1.83) < 0.001	1.53 (1.22, 1.94) < 0.001	1.22, 1.94) < 0.001
• 12–23 months	1.55 (1.21, 1.97) < 0.001	1.68 (1.30, 2.16) < 0.001	1.46 (1.09, 1.97) 0.012	1.09, 1.97) 0.012
• 24–35months	3.39 (2.01, 5.71) < 0.001	4.22 (2.46, 7.25) < 0.001	3.33 (1.77, 6.27) < 0.001	3.33 (1.77, 6.27) < 0.001
Gender	1.11 (0.94, 1.31) 0.223			
Breastfed	0.97 (0.64, 1.47) 0.882			
Wasting (< -2 Weight for height/length)	0.76 (0.63, 0.91) 0.004	0.79 (0.65, 0.96) 0.017	0.79 (0.64, 0.98) 0.030	0.78 (0.63, 0.96) 0.022
Stunting (< -2 Height/length for age)	0.84 (0.62, 1.14) 0.261			
Hypoxia (SpO2 < 90%)	0.64 (0.54, 0.76) < 0.001	0.62 (0.51, 0.74) <0.001	0.71 (0.58, 0.88) 0.001	0.72 (0.58, 0.89) 0.002
Febrile (Axillary temperature > 38.5°C)	1.14 (0.91, 1.43) 0.248			
Any danger sign	0.69 (0.59, 0.82) <0.001	0.76 (0.64, 0.91) 0.003	0.74 (0.60, 0.90) 0.003	0.76 (0.62, 0.92) 0.006
Radiographic pneumonia	0.56 (0.46, 0.69) <0.001		0.58 (0.47, 0.72) <0.001	0.55 (0.45, 0.68) <0.001
C-Reactive Protein (CRP) > 40 mg/L	1.01 (0.84, 1.21) 0.918			
Nasopharyngeal aspirate positive for virus				
• Respiratory syncytial virus (RSV)	(0.62, 0.99) 0.049			
• Influenza A	0.78 (0.52, 1.19) 0.252			
• Influenza B	(0.49, 1.33) 0.399			
• Parainfluenza type 1 (PIV 1)	1.75 (1.14, 2.69) 0.010			2.46 (1.48, 4.09) < 0.001
• Parainfluenza type 2 (PIV 2)	(0.40, 2.36) 0.957			
• Parainfluenza type 3 (PIV 3)	(0.96, 2.17) 0.082			
• Human metapneumovirus (hMPV)	0.64 (0.32, 1.28) 0.203			
Supplemented with zinc	1.11 (0.94, 1.31) 0.221			

*Hazard ratios (95% CI) and P-value calculated using Cox Regression with exact p option. Hazard ratios <1 for time till recovery indicates slower resolution of illness.

Results of multiple regressions with P-value > 0.05 not shown in the table.

Model 1 (Clinical variables): Adjusted for gender, breastfed, febrile and treatment with zinc.

Model 2 (Clinical + Radiographic pneumonia): Adjusted for gender, breastfed, febrile and treatment with zinc.

Model 3 (All variables): Adjusted for gender, breastfed, febrile, CRP, nasopharyngeal aspirate positive for RSV, influenza A and B, PIV 2 and 3 and hMPV and treatment with zinc.

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While several studies report on predictors of death [12–15], we were unable to find any exploring clinical risk factors for duration of illness of a severe pneumonia episode in young children. In our study only 4 children died in the hospital, and mortality was not an outcome. Hypoxia, a significant predictor of death in most of the studies [12,13,15] was an independent predictor of time till resolution of illness. While studies have identified younger children to be at a higher risk of death [14,15], we found slower time till recovery with decreasing age (Table 2). To our knowledge, this is the first study to identify age as an independent predictor of the duration of severe pneumonia.

The proportions with treatment failure in large multicenter clinical trials conducted in hospitalized children with severe pneumonia were 13.6% [16] and 20% [17]. These estimates are different from our study (35%) and may partly be due to the differences in outcome definition. Similar to the present study, hypoxia on admission was identified as an independent predictor

Table 3. Predictors of Treatment failure of illness episode in children 2–35 months hospitalized with WHO defined Severe Pneumonia

Variables	Crude Odds Ratio*	Adjusted Odds ratio* (number of observations per model)		
Age in months	0.93 (0.90, 0.96) <0.001			
Age categories		**Model 1 (590)**	**Model 2 (455)**	**Model 3 (454)**
• 2–6 months	1.00	1.00	1.00	1.00
• 7–11 months	0.67 (0.44, 1.01) 0.058	0.61 (0.40, 0.94) 0.024	0.56 (0.34, 0.91) 0.019	0.55 (0.34, 0.90) 0.018
• 12–23 months	0.37 (0.21, 0.64) <0.001	0.33 (0.19, 0.58) <0.001	0.27 (0.13, 0.55) <0.001	0.27 (0.13, 0.55) <0.001
• 24–35months	0.22 (0.05, 0.99) 0.049	0.18 (0.04, 0.83) 0.028	0.31 (0.06, 1.54) 0.153	0.34 (0.07, 1.72) 0.194
Gender	0.83 (0.59,1.18) 0.296			
Breastfed	0.67 (0.28,1.61) 0.370			
Wasting (594) (< -2 Weight for height/ length)	1.49 (1.02, 2.16) 0.038			
Stunting (598) (< -2 Height/Length for age)	1.25 (0.69, 2.27) 0.457			
Hypoxia (SpO2 < 90%)	1.91 (1.33, 2.74) <0.001	2.00 (1.36, 2.93) <0.001	1.80 (1.15, 2.80) 0.010	1.81 (1.16, 2.83) 0.009
Febrile (Axillary temperature > 38.5°C)	0.94 (0.59, 1.51) 0.808			
Any danger sign	1.65 (1.18, 2.32) 0.004	1.44 (1.01, 2.07) 0.045		
Radiographic pneumonia	2.22 (1.49, 3.31) < 0.001		2.09 (1.37, 3.19) 0.001	2.12 (1.39, 3.24) 0.001
C-Reactive Protein (CRP) > 40 mg/L	1.15 (0.79, 1.67) 0.472			
Nasopharyngeal aspirate positive for virus				
• Respiratory syncytial virus (RSV)	1.47 (0.91, 2.38) 0.115			
• Influenza A	0.31, 1.84) 0.533			
• Influenza B	0.37, 2.76) 0.989			
• Parainfluenza type 1 (PIV 1)	0.18, 1.37) 0.178			
• Parainfluenza type 2 (PIV 2)	0.20, 7.44) 0.819			
• Parainfluenza type 3 (PIV 3)	0.12, 1.06) 0.063			
• Human metapneumovirus (hMPV)	2.34 (0.62, 8.81) 0.209			
Supplemented with zinc	0.83 (0.60, 1.17) 0.291			

*Odds ratios (95% CI) and P-value calculated using Logistic Regression. Odds ratios > 1 indicates increased odds of treatment failure.

Results of multiple regressions with P-value >0.05 not shown in the table with the exception of age analyzed as a categorical variable.

Model 1 (Clinical variables): Adjusted for gender, breastfed, wasting, febrile and treatment with zinc.

Model 2 (Clinical + Radiographic pneumonia): Adjusted for gender, breastfed, wasting, febrile, any danger sign and treatment with zinc.

Model 3 (All variables): Adjusted for gender, breastfed, wasting, febrile, any danger sign, CRP, nasopharyngeal aspirate positive for RSV, PIV 1, 2 and 3, influenza A and B, hMPV and treatment with zinc.

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of treatment failure in both studies. The trends for age as a predictor were similar in our study and the study by Fu. *et al.* [18], with younger children having a higher risk of treatment failure.

We found hypoxia to be a predictor of both treatment failure and illness duration in our study. With hypoxia removed as a covariate, presence of any danger sign was a significant predictor of treatment failure in all three models. The variables hypoxia and any danger sign were correlated, which is expected as both are known indicators of severe pneumonia. Clinical predictors may not be easily recognized whereas measurement of oxygen saturation with a pulse oximeter, when performed correctly, to document hypoxia seems to be a more reliable and objective sign to assess severity. Pulse oximetry, the 'fifth pediatric vital sign' [19] supplemented by treatment with oxygen was found to be a feasible and cost effective intervention for childhood pneumonia with 35% reduction in mortality in Papua New Guinea [20]. Unfortunately even though pulse oximeters and oxygen are available, they are underutilized by health workers

in developing countries [21,22]. Pulse oximetry to document hypoxia in community acquired pneumonia is not only endorsed by professional societies in developed countries [23,24] but also stated in recent WHO guidelines[6] and our findings support these recommendations.

We identified one study in India [25] reporting lobar consolidation as a predictor of longer stay in hospital but this was based on unadjusted analysis and therefore not comparable to the results of our study. The two studies that report an association between radiographic consolidation and mortality in hospitalized children with severe pneumonia have conflicting results. While Lupisan *et al.* [14] found dense infiltrates to be an independent predictor, Reed *et al.* [12] report that alveolar consolidation on multiple regression analysis was not significant. Primary end point consolidation was an independent predictor of treatment failure at 48 hours of hospitalization especially among penicillin recipients (RR 3.58 95% CI: 1.47, 8.75) in the study by Patel *et al.* [17], a finding very similar to what we found. In our study, chest radiograph was done to screen for exclusion criteria and had no role in the initial diagnosis and management of enrolled patients. We found that addition of radiographic pneumonia to the analyses (Models 2 and 3) did not substantially change the estimates of independent clinical predictors of both outcomes (Tables 2 and 3). In resource poor settings, diagnosis of severe pneumonia relies heavily on clinical signs. An initial chest radiograph in uncomplicated but severe community acquired pneumonia would not change initial management with empirical antibiotic therapy in such settings. Our findings support the WHO guidelines [6] which state that radiographs in severe pneumonia should be done only when possible.

We report findings from a randomized clinical trial [7] conducted under controlled settings. Our findings may therefore not be directly applicable to other hospitals caring for children in developing countries. We used previous WHO guidelines [8]requiring only lower chest indrawing in addition to symptoms to define severe pneumonia as inclusion criteria. Although we took measures to screen out children with reactive airways, we may have included those with pneumonia that could have been treated as outpatients. While recruiting, we excluded very sick children with pneumonia and probably missed those with other signs of severe pneumonia in the absence of LCI and this may also have affected our observed associations. While we were able to detect several predictors for duration and treatment failure, for many of the selected independent variables such as fever and breastfeeding, the power to detect any significant association with the outcomes was low. It should be noted that the study was designed to measure the effect of zinc supplementation during pneumonia and not to identify these predictors.

This large study on children aged between 2–35 months, with acute severe pneumonia found younger age, hypoxia on admission and radiographic pneumonia to independently predict both time to resolution and treatment failure. As it is the only study till date to report on predictors of illness duration we believe our findings to be relevant, not only to characterize but also manage a severe pneumonia episode in hospitalized children from resource poor settings. Our results indicate that a chest radiograph is not always needed but the detection and treatment of hypoxia is a crucial step in the management of children admitted to health care settings with pneumonia.

Supporting Information

S1 Dataset.
(XLSX)

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Author Contributions

Conceived and designed the experiments: SB MM HS TAS AS PSS PVB. Performed the experiments: SB MM HS TAS AS PSS PVB RKG DMS. Analyzed the data: SB MM HS TAS. Contributed reagents/materials/analysis tools: MM TAS. Wrote the paper: SB MM HS TAS AS PSS PVB RKG DMS. Collection of biological specimens: MM. Virus analyses: MM. Interpretation of chest radiographs: RKG DMS. Interpretation of the manuscript: AS PSS PVB RKG DMS.

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