Adenovirus- associated pneumonia in South African children: presentation, clinical course and outcome.

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TITLE: Adenovirus-associated pneumonia in South African children: presentation, clinical course and outcome.

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Background: Pneumonia is an important cause of morbidity and mortality in children. Viruses have emerged as important aetiological agents in childhood pneumonia. The aim of this study was to document the clinical presentation, severity and outcome of adenoviral-associated pneumonia (AVP) in children and identify risk factors associated with poor outcome.

Methods: A retrospective study of laboratory-confirmed AVP cases was conducted between 1 January and 31 December 2011. The medical records of adenovirus PCR positive respiratory tract samples identified through the National Health Laboratory Service (NHLS) database were retrieved. Demographic, clinical and outcomes data of children with AVP was extracted and analysed. Outcome measures were death and development of chronic lung disease (CLD).

Results: 1910 respiratory samples were submitted to the NHLS from which 206/1910 (11%) AVP cases were identified. The median age was 12 months (IQR 6-24), 70 (34%) children were malnourished and 14 (7%) HIV-infected. Fever was the commonest presenting symptom occurring in 159 (77%) of cases. Seventy six (37%) required intensive care unit (ICU) admission. There was a high prevalence of co-morbid conditions with 98 (47%) having at least one; cardiac disease was the most common (48 (23%). Twenty nine (14%) developed CLD which was associated with hypoxia at presentation (26/29, 90%, $p = 0.01$) and admission to ICU (18/29, 62%, $p < 0.01$). Eighteen (9%) children died. Admission to ICU (OR 8.3, 95% CI 2.3- 29.0) and blood stream infection (OR 11.2; 95% CI 2.3-54.1) were independent risk factors for mortality.

Conclusion: Adenoviral-associated pneumonia is an important cause of pneumonia and CLD in young children in South Africa. Admission to ICU and blood stream infection were associated with poor outcome.

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Abbreviations:

AVP Adenoviral Pneumonia

BO Bronchiolitis Obliterans

CLD Chronic lung disease

ICU Intensive Care unit

WAZ weight for age z-score

Chapter 1

Introduction and literature Review

Worldwide, paediatric respiratory tract infections remain a common and significant source of morbidity and mortality. This is of particular importance in developing countries where pneumonia remains an important cause of death in children under the age of 5 years.[1]The WHO estimates 450 million cases of pneumonia are recorded annually with the most vulnerable groups being the under-fives and over75's.[2] Childhood pneumonia and diarrhoeal disease accounted for about 2 million deaths in 2011, with 81% of deaths due to pneumonia occurring in the first two years of life.[3] This remains concerning as most pneumonia deaths are preventable. Whilst paediatric pneumonia is a global problem, significant strides have been made in the developed world toward reducing both pneumonia incidence and pneumonia related mortality. Reduction in the under-5 mortality rate has dropped from 90 per 1000 live births in 1990 to 48 per 1000 in 2012. Despite this impressive statistic, 6,6million children under the age of five are still dying annually, with pneumonia the leading cause of death, accounting for approximately 1, 3 million childhood deaths per annum. [4] At this rate the Millennium Development Goal number 4(MDG4) is unlikely to be met by 2015, which aimed for a 2/3 reduction in under-5 mortality from the 1990 levels. Unless there is a dramatic change in policy, practice and prevention measures this target is only likely to be reached by 2028. African countries continue to lag behind significantly. Africa has one of the highest incidence rates of severe childhood pneumonia, accounting for almost half of all global cases of pneumonia. [5] In 2011, an estimated 120 million episodes of childhood pneumonia were reported globally. Of these, 14 million progressed to severe disease, with 1, 3 million deaths. Africa and Southeast Asia accounted for 69% of the global burden of severe cases. [4] In 2012, Nair et.al analysed data from 89 published and unpublished studies on acute lower respiratory infections, noting that almost 15 million admissions occurred worldwide in 2010. Of these, 96% were in developing countries. [6]

Evidence points to the fact that children in low and middle income countries are particularly at risk. Factors like poor nutrition, micronutrient deficiency, HIV infection, overcrowding and poor socio-economic circumstances, prematurity, exposure to smoke and other environmental pollutants all contribute to increased severity of disease. In Sub-Saharan Africa, HIV is of particular importance as this population of children have higher mortality, with increased severity of infections as well as increased risk of opportunistic infections. Early implementation of antiretroviral therapy in addition to cotrimoxazole prophylaxis can reduce the burden of both pneumonia and severe disease in this at-risk group. [5] In 2013, the WHO launched the Global action Plan for the Prevention and Control of Pneumonia and Diarrhoea (GAPPD), highlighting common strategies for prevention and treatment of these illnesses, as well as pneumonia-specific strategies.

Pneumonia aetiology remains a challenge in the paediatric population, as bacteraemic sepsis in children is relatively uncommon and nasopharyngeal colonisation by pathogenic bacteria limits the use of respiratory samples for diagnosing bacterial pneumonia. [7] Gilani et.al reviewed 10 years of literature and pneumonia aetiology studies from 2000-2010 and reported that Streptococcus Pneumoniae is implicated in 18% of severe pneumonia cases and 33% of deaths. This is followed by *Haemophilus influenzae* type B, which accounts for 4% of severe episodes and 16% of deaths. [4] These are all vaccine-

preventable causes of significant childhood morbidity and mortality. Pneumonia preventative measures have been bolstered by the introduction of conjugate vaccines against *H.influenzae* type B (Hib) and against S. Pneumoniae (PCV). In 2013, Bhutta et.al reviewed interventions to address deaths from childhood pneumonia and diarrhoea equitably and reported that the introduction of the Hib conjugated vaccine in low and middle income countries was associated with an 18% reduction in radiologically confirmed pneumonia, a 6% reduction in severe pneumonia and a 7% reduction in pneumonia-associated mortality.[8] By 2011, approximately 90% of low-income countries had introduced HIB into their immunisation programmes through support from Global alliance For Vaccines and Immunisation (GAVI). Furthermore they reported data from six studies in low and middle income countries which estimated a 29% reduction in radiologically confirmed pneumonia, 11% reduction in severe pneumonia and an 18,5% reduction in pneumonia-specific mortality with the introduction of PCV.[7] The effect of widespread immunisation has also led to a dramatic decline in pneumonia-related hospitalisation rates in adults, especially the elderly due to herd immunity as well as reduced transmission of pneumonia – specific serotypes in the United States. [7].PCV vaccination has also led to a reduction in hospitalisation for viral-associated pneumonia. Shabir Madhi's paper in 2004 revealed a 32% lower hospitalisation rate for viral associated pneumonia episodes in vaccinated children. [2] In the United States , a 41-50% reduction in influenza-virus attributable pneumonia hospitalisation is estimated for every 10% increment in childhood PCV immunisation.[7] Despite this, PCV introduction into national immunisation programmes is still suboptimal, with Wang et.al reporting in 2013 that only 31% of the world's birth cohort currently having access to PCV.[5] Only 36% of countries with the highest mortality and 37% of countries in which more than 10% of deaths were pneumonia-related, had introduced PCV by 2012. Less than 50% of countries eligible for support from the Global alliance for Vaccines and Immunisation(GAVI) had introduced PCV , with only 19 African countries introducing PCV into their national childhood immunisation programmes.[5].

Whilst global access to effective immunisation is still not 100%, improved immunisation against the main bacterial pathogens have resulted in respiratory viruses emerging as important aetiologic agents in childhood pneumonia. In the paediatric population in developed countries, respiratory viruses are the commonest cause of pneumonia. [3].Respiratory viruses were first discovered between 1933 and 1965, with the identification of the influenza virus, enterovirus, adenovirus, respiratory syncytial virus, rhinovirus, parainfluenza virus and coronaviruses by viral culture. In the early 2000's several new viruses were identified, and with the advent of molecular testing, the role of viruses in the aetiology of pneumonia has become clearer. [9] The incidence of viral pneumonia had hitherto been largely underestimated.[10] A majority of viral-associated lower respiratory tract infections are caused by Respiratory Syncytial virus (RSV), Human Metapneumovirus, Influenza A and B, adenovirus, Severe acute Respiratory Syndrome (SARS), Coronavirus and Bocavirus. [11] Adenovirus infections account for 5-15% of upper respiratory infections and 5% of childhood pneumonia. [11, 12].The discovery in the last decade of newer viruses like Human Metapneumovirus, Coronavirus, NL63 and HKU1, Human Bocavirus, new enterovirus, Polyomavirus, Parechovirus, SARS and H1N1 influenza virus have highlighted the fact that children remain a vulnerable population exposed to multiple viruses with similar seasonal patterns. [13] Whilst the role of RSV in childhood pneumonia is well established, the role of other viruses is less clear. Dual viral infections are common with a third of children having evidence of viral-bacterial co-infection.

[2] This is reflected in the findings by Niederman et.al who suggest that viruses account for up to 80% of infections in young children, with RSV and Rhinovirus being most commonly isolated. [14] With the emergence of severe acute respiratory syndrome (SARS), Avian Influenza A (H5N1) virus and the 2009 H1N1 influenza pandemic, the role of respiratory viruses as a cause of severe pneumonia was again highlighted. [2] A Ghanaian study published in 2012 demonstrated the importance of respiratory viruses in childhood pneumonia comparable to worldwide trends [15]. In South Africa, studies confirmed an increased burden of viral-associated respiratory tract infections in HIV-infected children. [14]. Furthermore, these children were shown to have increased severity of viral infections with higher morbidity and mortality. [14] More recently, Morrow et.al investigated the prevalence and outcome of patients admitted to a paediatric intensive care unit with viral respiratory tract infections in Cape Town. Their study showed that viral respiratory infections were common in their setting and a significant cause of morbidity and mortality. Rhinovirus incidence was highest, followed by RSV and adenovirus. Whilst RSV and Rhinovirus displayed a seasonal pattern, adenovirus did not and was present throughout the study period. [16] Comorbid factors like HIV exposure, HIV infection and congenital cardiac disease were significantly associated with poorer outcome. [16]

Human adenoviruses are non-enveloped double-stranded DNA viruses which were first isolated from adenoidal cells in 1953 as part of a project searching for the virus responsible for the common cold. The 57 recognised serotypes of Human Adenoviruses are divided into subgroups A to G. Tropism was found to exist amongst subgroups, with subgroup A and F typically causing GIT infection, subgroups B,C and E showing predilection for the respiratory tract and subgroup D associated with epidemic keratoconjunctivitis. Subspecies B includes serotypes 3, 7 and 21 which are associated with lower respiratory infections. [17] Adenovirus is typically transmitted from person to person by respiratory droplets and less commonly by the conjunctival and faecal-oral route. The incubation period is relatively short, ranging between 2 and 14 days. In normal hosts, illnesses usually last about 1 week, but asymptomatic shedding of virus can occur for months or years. Infections are commoner in winter and spring but may occur throughout the year. It is uncommon in the early neonatal period, suggesting that maternal antibodies confer protection. Neutralising antibodies develop in early childhood after symptomatic and asymptomatic infection which occur with equal frequency. By age 5, up to 75% of children have serologic evidence of adenovirus exposure. [17]These antibodies are thought to confer lifelong immunity against that particular serotype. [17]

The prevalence of adenoviral associated pneumonia is fairly low (range 2-12%) but it remains an important infection to identify because of its ability to cause severe and fatal necrotising pneumonia [18]. Despite this seemingly low incidence, adenoviruses may result in severe disease with resultant morbidity and mortality especially in high-risk patients. Serotypes 3, 7, 7a and 21 are the commonest cause of lower respiratory disease.[19] These serotypes are associated with severe disease as well as pulmonary sequelae such as bronchiectasis, bronchiolitis obliterans, (BO), unilateral hyperlucent lung and persistent abnormal pulmonary function.[11]

In severe adenoviral pneumonia there is destruction of the bronchi and bronchioles with necrosis spreading to the adjacent parenchyma. Resolution of the acute illness results in the development of BO with chronic sequelae. [20] Bronchiolitis obliterans is a rare form of chronic obstructive lung disease that

follows a severe insult to the lower respiratory tract resulting in fibrosis of the small airways. Severe adenovirus pneumonia is known to bronchiolitis obliterans. [21] In their systematic review on long term sequelae from childhood pneumonia in 2012, Edmund et.al reviewed published papers from 1970 to 2011 to determine the risks of long term sequelae from childhood pneumonia in children under 5 years of age. Standard global burden of disease categories (restrictive lung disease, obstructive lung disease, bronchiectasis) were labelled as "major sequelae" whilst chronic bronchitis, asthma, other abnormal pulmonary function were labelled as "minor sequelae". Their review showed that hospitalised children had a 13, 6 % risk of at least one major sequelae compared to non-hospitalised children where the risk was only 5, 5%. They also reported adenovirus pneumonia to be associated with the highest sequelae risk (54, 8%). [18]

The earliest review article on adenovirus pneumonia published in 1959 from Japan, described three cases of severe infantile pneumonia. [21] In 1973, an outbreak of adenovirus Type 7 was reported in Montreal, Canada. 13 infants and children with proven adenovirus type 7 were described; 4 were ventilated, 3 died and 1 developed chronic lung disease.[21] In Feb 1997 Canadian researchers published data on a 10year follow up study of 19 children who had been treated for pneumonia; 14 of them had developed severe pneumonia and had bronchiectatic changes at follow up. In all 14 cases adenovirus type 3 had been identified. This was one of the earliest studies suggesting a link between adenovirus infection and chronic lung disease. [22]

Hyperlucent lung was first described by Sawyer and James in 1953, followed by Mcleod in 1954. [23]Typically diagnosed radiologically as a unilateral lung appearing less dense on CXR. In addition, the pulmonary vessels are smaller than normal and bronchographic pictures suggest widespread obstruction of small bronchi with air trapping. This phenomenon was described by Canadian researchers in 1983 and again, adenovirus type7 was implicated in several cases. [23].A further study in 1984 had followed a cohort of 20 children admitted with confirmed adenovirus type 7 pneumonia. They were compared to 20 controls with adenovirus Type 7 upper respiratory tract infections: 65% of the pneumonia group had evidence of airway obstruction compared to 10% of the control group. Young age at time of infection was identified as an independent risk factor for long-term pulmonary function abnormality. [24] Most of the recent work on adenoviral infections, including complications comes from South America, particularly Argentina where adenoviruses are the second most prevalent cause of acute lower respiratory infection of viral origin in children under age of four, surpassed only by RSV.[11] One of the largest studies on post infectious BO came from Argentina in 2009, where Patricia Murtagh et.al did a retrospective observational study on 415 children hospitalised with adenoviral pneumonia over a 7 year period: 49% children recovered fully, 36% developed sequelae and 15% died. Independent risk factors for developing bronchiolitis obliterans were identified and included prolonged hospitalisation (more than 30 days), multifocal pneumonia and hypercapnia. Independent risk factors for death in the acute phase of the illness were mechanical ventilation, multifocal pneumonia, hypercapnia, coagulation disorders and co-infection with measles. [11] These results echoed the findings by Colom et.al in 2006 whose study also showed that early aggressive disease needing ventilatory assistance with prolonged hospitalisation was associated with subsequent development of bronchiolitis obliterans. [25] Castro Rodrigues et.al prospectively assessed 45 hospitalised children with adenovirus pneumonia over a 5 yr

period in an attempt to identify factors that potentially contribute to the development of Bronchiolitis Obliterans. At the end of their study period, 38 surviving patients were divided into 2 groups: those with CT evidence of BO and those without. The group who had CT evidence of BO had primary infection before age of 2 years. Those who developed BO had a more severe initial illness, requiring ICU, supplemental oxygen therapy, ventilatory assistance and steroids. [26] Finally, in 2009, Colom et.al devised a clinical prediction rule to diagnose post infectious BO in children. They developed and validated a clinical prediction rule (BO score) using multiple objectively measured parameters that were readily available; 125 patients were included in this study. The BO score was derived by assigning points to the following variables. Typical clinical history=4 points, adenoviral infection confirmed =3 points, CT showing mosaic lung perfusion pattern=4 points. A score of 7 or more predicted BO outcome with 100% specificity and 67% sensitivity. They concluded that the BO score is simple to use, and that a score of 7 or more predicts post infectious BO with high accuracy. [27]

The epidemiology, clinical presentation and complications of adenovirus –associated pneumonia in South African children is unknown. Like South American countries, local clinical experience would suggest that the morbidity and long-term sequelae of adenovirus-associated pneumonia are significant. Furthermore, the role of HIV infection on the outcome of adenovirus-associated pneumonia is unknown.

Multiplex PCR testing (which includes multiple adenovirus serotypes) was introduced in 2009 as the diagnostic test on all routine respiratory tract samples from Red Cross War Memorial Children's Hospital (RCWMCH).

The purpose of this study is to report the presentation, clinical course and outcome of laboratory-confirmed adenovirus associated pneumonia at RCWMCH.

Summary of literature review methods

This literature review aims to review the epidemiology of childhood pneumonia, summarise the aetiology of pneumonia, review the role of viruses in paediatric pneumonia in the developed as well as the developing world, review the epidemiology, prevalence , clinical features and outcome of adenovirus pneumonia in the literature.

Search strategy

The Literature review search was conducted on Pubmed (www.ncbi.nlm.nih.gov/pubmed) and Medline (www.ncbi.nlm.nih.gov) using the following search strings. PNEUMONIA and CHILDHOOD and AETIOLOGY or AETIOLOGY and AFRICA AND DEVELOPING COUNTRIES.

A second search was done using ADENOVIRUS and PNEUMONIA and CHILDREN and COMPLICATIONS

The inclusion criteria were: all English language articles, articles focusing on childhood pneumonia, studies looking specifically at Adenoviral infections in children. Articles reviewing the role of viruses were included as well. Articles specific to Africa were included. Abstracts derived from the above search were also reviewed. Those which met inclusion criteria were reviewed in full text. Those articles were

further filtered and references cited within the articles were searched and reviewed. Relevant articles identified by the supervisor were also included and cross-referenced.

Results

The initial search was conducted in May 2012 prior to writing the research proposal. This process was repeated in October 2014, prior to writing the final literature review.

The search strings (PNEUMONIA and CHILDHOOD and AETIOLOGY and AFRICA and DEVELOPING COUNTRIES plus ADENOVIRUS and PNEUMONIA and CHILDREN and COMPLICATIONS) yielded 214 articles. The inclusion and exclusion criteria were applied and 51 articles were reviewed in full text. 51 articles were included in this literature review, including studies from developed and developing countries. Most articles were recent, with publication between 2000 and 2014. Three relevant articles from 1959-1989 were included. All articles were relative to the paediatric population.

EPIDEMIOLOGY: 5 updated articles published between 2012 and 2014 were reviewed. These articles served to give the reader a clear understanding of the enormity of the pneumonia burden worldwide. They also highlight the contribution of pneumonia to under5 morbidity and mortality and how respiratory disease impacts on child health. 3 articles stressed the challenges pneumonia continues to pose in Africa, with Professor Zar's article in the Thorax journal of 2013 discussing progress that has been made in addition to the continued challenges we face in Africa. [1, 5,7]. One systematic review published in 2013 reviewed the global burden of hospital admissions for severe acute lower respiratory infections in children.[6]

PNEUMONIA AETIOLOGY: 21 articles pertaining to pneumonia aetiology were reviewed and analysed. The role of well -known bacteria, Streptococcus Pneumoniae and *Haemophilus influenzae* were studied.[5,7] Several articles reviewed the enormous strides made with improved immunisation schedules globally although many African countries still lag behind. [5] With the advancement of immunisation strategies, the role of viruses in childhood pneumonia aetiology was reviewed. [28]. Much has been studied and reported in the literature with regards to the role that viruses play in respiratory infections in the developed world, but a review of the literature revealed a scarcity of such studies in Africa. A total of 3 studies were identified in North Africa, with a few studies from South Africa, several slanted toward the effect of co-infection with HIV. [2,14,15,29,30,31,32] A review on the history of viral infections, their discovery and role in childhood pneumonia was completed. Studies allude to the fact that until multiplex testing for respiratory viruses became readily available, the contribution of viruses to pneumonia aetiology was largely underestimated. [9] The recent SARS and H1N1 outbreak again highlighted the important role viruses play in childhood pneumonia. The role of multiple viral infections as well as bacterial co-infection in childhood pneumonia was explored, revealing high incidence of both in the paediatric population. HIV exposure and infection were both identified as risk factors for severe pneumonia, with the added burden of opportunistic infections in this susceptible group. A study at Red Cross War Memorial Children's Hospital showed significant morbidity and mortality in their Paediatric ICU amongst children admitted with viral LRTI's. HIV, malnutrition and congenital cardiac disease were associated with poorer outcome. [16]

Adenovirus-associated pneumonia and its sequelae: Adenoviruses were reviewed. A Primary Pubmed search using ADENOVIRUS and PNEUMONIA and CHILDHOOD revealed 60 articles, 20 of which were foreign language articles and therefore excluded. The remaining articles were scanned and 14 articles included in the literature review. These articles looked at the history of adenoviral infections, epidemiology, and the contribution of adenoviruses to childhood morbidity and mortality.

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TITLE PAGE

Title: Adenovirus-associated pneumonia in South African children: presentation, clinical course and outcome.

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ABSTRACT

TITLE: Adenovirus-associated pneumonia in South African children: presentation, clinical course and outcome.

Background: Pneumonia is an important cause of morbidity and mortality in children. Viruses have emerged as important aetiological agents in childhood pneumonia. The aim of this study was to document the clinical presentation, severity and outcome of adenoviral-associated pneumonia (AVP) in children and identify risk factors associated with poor outcome.

Methods: A retrospective study of laboratory-confirmed AVP cases was conducted between 1 January and 31 December 2011. The medical records of adenovirus PCR positive respiratory tract samples identified through the National Health Laboratory Service (NHLS) database were retrieved. Demographic, clinical and outcomes data of children with AVP were extracted and analysed. Outcome measures were death and development of chronic lung disease (CLD).

Results: 1910 respiratory samples were submitted to the NHLS from which 206/1910 (11%) AVP cases were identified. The median age was 12 months (IQR 6-24), 70 (34%) children were malnourished and 14 (7%) HIV-infected. Fever was the commonest presenting symptom occurring in 159 (77%) of cases. Seventy six (37%) required intensive care unit (ICU) admission. There was a high prevalence of co-morbid conditions with 98 (47%) having at least one; cardiac disease was the most common (48 (23%). Twenty nine (14%) developed CLD which was associated with hypoxia at presentation (26/29, 90%, $p = 0.01$) and admission to ICU (18/29, 62%, $p < 0.01$). Eighteen (9%) children died. Admission to ICU (OR 8.3, 95% CI 2.3- 29.0) and blood stream infection (OR 11.2; 95% CI 2.3-54.1) were independent risk factors for mortality.

Conclusion: Adenoviral-associated pneumonia is an important cause of pneumonia and CLD in young children in South Africa. Admission to ICU and blood stream infection were associated with poor outcome.

CHAPTER 2

Introduction

Respiratory tract infections remain a common and significant source of morbidity and mortality in children worldwide. This is of particular importance in developing countries where pneumonia is an important cause of death in children under five years of age.[1] Whilst global access to effective immunisation against common bacterial pathogens slowly improves , respiratory viruses have emerged as important aetiologic agents in childhood pneumonia. This holds particularly true in developed countries, where respiratory viruses are the commonest cause of childhood pneumonia. [1] Most viral associated lower respiratory tract infections are caused by respiratory syncytial virus (RSV), Human metapneumovirus,influenza A and B, adenovirus, Severe Acute Respiratory Syndrome (SARS), coronavirus and bocavirus. [2] Data from an American study looking at community acquired peumonia requiring hospitalisation between 2010 and 2012 revealed that RSV, adenovirus and metapneumovirus are commoner in children under age of 5 than amongst older children. Adenovirus prevalence was 15% in the under -5 group compared to 3% in older children. [3]

Adenovirus infections account for 5-15% of upper respiratory infections and 5% of childhood lower respiratory tract infections (LRTI's). [4, 5]The ability of adenoviruses to cause severe and fatal necrotising pneumonia is well known and may result in significant morbidity and mortality especially in high risk patients. Serotypes 3, 7, 7a and 21 are the commonest cause of lower respiratory disease. [5] Their association with pulmonary sequelae such as bronchiectasis, BO, unilateral hyperlucent lung and persistent abnormal pulmonary function have been well described. [4] In a meta-analysis, adenovirus pneumonia in childhood was reported to be associated with the highest sequelae risk for chronic lung disease in adulthood. [6]

Whilst the role of RSV in childhood pneumonia is well established, the role of other viruses is less clear. Results from a Kenyan study from 2010 which aimed to determine the viral aetiology of severe pneumonia amongst infants and children revealed RSV to be most prevalent and associated with severe disease. Adenovirus and metapneumovirus accounted for only 3.8% of cases. [7]Dual viral infections are common with a third of children having evidence of viral-bacterial coinfection. [8] In South Africa, studies have confirmed an increased burden of viral associated respiratory tract infections in human immunodeficiency virus (HIV) infected children and children admitted to intensive care (ICU) with severe pneumonia.[9, 10 ,11] Morrow et al compared spectrum, course, seasonality and outcome of children with virus-associated respiratory symptoms (VARS) admitted to two paediatric ICUs in the United Kingdom (UK) and South Africa (SA). They found that the outcome of children with VARS was worse in SA compared with the UK. Adenovirus was more common in SA and also an independent risk factor associated with mortality. [11]

The epidemiology, clinical presentation and complications of adenovirus-associated pneumonia (AVP) in South African children is unknown. Furthermore, the role of HIV infection and malnutrition on the outcome of AVP is unknown. This study aimed to document the presentation, clinical course and outcome of AVP at a tertiary paediatric referral hospital in South Africa.

METHODS

Study design and patient selection

A retrospective descriptive study was conducted at Red Cross War Memorial Children's Hospital (RCWMCH), a tertiary paediatric hospital in Cape Town, South Africa. Children 0-13 years of age admitted from 1st January 2011 to 31 December 2011 with laboratory- confirmed AVP were enrolled. For the purposes of this study, AVP was defined as pneumonia (cough, tachypnoea with or without chest indrawing) and isolation of adenovirus from any respiratory tract samples which included tracheal aspirates(TA's), bronchoalveolar lavage (BAL), induced sputums (IS), nose swabs or nasopharyngeal aspirates (NPA's). The National Health Laboratory Service (NHLS) database was searched to extract all PCR adenovirus positive respiratory samples collected in children admitted to RCWMCH within the study period. Respiratory viruses were identified using SeeplexRV7 Detection assay (Seegene, Seoul, Korea) which includes influenza A, influenza B, human metapneumovirus, respiratory syncytial virus, rhinovirus A, parainfluenza virus and adenovirus.

All laboratory identified adenovirus positive cases of children admitted to RCWMCH where medical records were available were included in the study.

Clinical and laboratory data

The medical records of identified patients were retrieved and relevant data recorded on a data capture form. Clinical and demographic information at the time of hospital admission or acquisition of hospital acquired infection was collected. The presence of fever more than 38 degree Celsius for more than 48 hours, diarrhoea, conjunctivitis and skin rash was recorded. Adenovirus-associated pneumonia cases were classified into community acquired or hospital acquired pneumonia. Community acquired AVP was defined as AVP confirmed within 48 hours of hospital admission. Hospital acquired AVP was defined as AVP acquired more than 48hours after hospital admission.

The HIV status of patients was classified as: HIV exposed but uninfected, HIV exposed and infected or HIV unexposed. In the HIV –infected group, CD4 count and percentage, HIV viral load and antiretroviral treatment details were recorded. The nutritional status of each child was determined by calculating the World Health Organisation (WHO) weight-for-age z-score (WAZ score) using igrowup macro for STATA (WHO Anthro version 3.2.2).Malnutrition was defined as a z-score of less than -2. The presence of an underlying medical condition was recorded and categorised as cardiac, respiratory, renal, neurologic, immune-suppression, prematurity or other.

Routine laboratory investigations that were recorded were the total white cell count, haemoglobin, C-reactive protein (CRP) and pro-calcitonin (PCT) at the time of hospital admission. The presence of significant laboratory-confirmed co-infections were documented and recorded as: other respiratory viruses, bloodstream infections (positive blood culture) and tuberculosis (positive culture of *M.Tuberculosis* from a site).

Pneumonia severity was established according to the presence or absence of hypoxia at admission, (oxygen saturation<90% in room air), need for ICU admission and need for ventilatory support. The ventilation modality and total duration of ventilation and ICU stay was recorded.

Outcome Measures

Two outcome measures were examined. Development of chronic lung disease (CLD) and in-hospital mortality. Chronic lung disease was defined as the ongoing need for supplemental oxygen beyond 30 days of hospital admission.

Data Analysis

Data was collected and entered into an EpiData (Entry version2.0, Denmark) programme. Thereafter statistical analysis was done using SPSS programme (version 12). Data was tested for normality using Shapiro-Wilks W-test. Data was not normally distributed and therefore presented as medians (interquartile ranges). Univariate analysis were conducted using Yates-corrected chi-square tests where cell values were <10. Logistic regression analysis was conducted for risk factors for death outcome using variables with p value < 0.05.

RESULTS

Between January 1st 2011 and December 31st 2011 a total of 1910 respiratory samples were submitted to the NHLS from RCWMCH for viral PCR testing. Of this group 206/1910 (11%) samples tested were positive for adenovirus, with 56/206 (27%) of these testing positive for additional respiratory viruses. A total of 206 patients with AVP were identified and included in this study.

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS (table1)

The median age was 12 months (IQR 6- 24) with a slight male predominance. Thirty four percent (70/206) of the study population was malnourished and 72% (148/206) were community acquired infections. Fever was the commonest presenting complaint, documented in 77% of cases. There was a high prevalence of underlying medical conditions with 47% of the study sample having at least one underlying medical condition, 16% having more than one and 37% having none. There were fourteen HIV infected children in the study sample. Twelve out of the fourteen children were either on antiretrovirals at the time of their infection or initiated on treatment during this admission.

The mean WCC on admission was 15 x10^9 /L (SD8) and mean Hb of 11g/dL (SD2). The mean CRP and PCT were 20mg/L (IQR 5-53 n=145) and 2,5ug/L (IQR 1-12, n=10) respectively.

PNEUMONIA SEVERITY: Hypoxia was a significant finding with 146(71%) children hypoxic on admission. 76(37%) needed ICU admission for respiratory support, of whom 54/76(71%) were mechanically ventilated. 31/76 (41%) needed CPPV (continuous positive pressure ventilation) and 13/76 (17%) needed HFOV (high frequency oscillatory ventilation). The median stay in ICU was 9 days (IQR 4-17). The median length of hospital stay was 10 days (IQR 3- 21).

OUTCOMES

Chronic lung disease: 29 (14%) children developed CLD). Hypoxia (p=0,016) and admission to ICU (p=0,002)was significantly associated with the development of CLD. Compared to children without CLD, children with CLD had a longer median ICU stay of 9 days vs 5 days (p=0,06) and a longer median hospital stay of 41 days vs 9 days (p<0,001).

In-hospital mortality: **(table 2)** Eighteen (9%) children died. 10/18(56%) of the deaths were adenovirus related and 8/18 (46%) were unrelated. Most children made a full recovery 168/206(82%) and were discharged with no sequelae. Hypoxia (p=0, 02) and ICU admission (p=0, 01) were associated with in-hospital mortality on univariate analysis (table 2). Furthermore, underlying cardiac disease (p=0, 02)and blood stream infection (p=0,001) were additional factors associated with mortality. Logistic regression analysis revealed that ICU admission (OR 8.3, 95% CI 2.3- 29.0) and blood stream infection (OR 11.2; OR 2.3-54.1) were independently associated with mortality; underlying cardiac disease trended towards significant association with mortality (OR 2.88, 95% CI 0.98-8.5).

DISCUSSION

This is the first study undertaken looking at adenoviral respiratory infections in children specifically in a South African context. Much of the literature on this subject comes from South America and Asia where adenoviral infections and longterm sequelae thereof are commonly described. [4, 6,12,13,14,15] In this study, adenovirus was isolated in 11% of all respiratory tract samples that were tested thus highlighting the contribution of adenovirus in pneumonia aetiology in our setting. Furthermore, this study confirms previous reports of AVP causing severe pneumonia and significant morbidity in children as supported by our data documenting 37% of patients needing ICU admission and 14% developing chronic lung disease. Outcome was associated with pneumonia severity, ICU admission and bloodstream infections.

Adenoviral viraemia may be associated with prolonged high fever as was observed in our study. [5, 16]. It is thought that 20% of all cases of paediatric conjunctivitis is due to adenovirus with seasonal predilection for autumn and winter. [17] Conjunctivitis in our study was less common than expected and can likely be explained by lower prevalence of adenovirus serotypes which cause conjunctivitis in our population.

An underlying medical condition and malnutrition was found in almost half and one third of patients respectively. Prematurity and congenital cardiac disease were the most common underlying conditions which highlights the vulnerability of these conditions to severe adenovirus-associated pneumonia. Underlying cardiac disease tended towards a significant risk factor associated with death. Adenovirus is directly cardiotoxic and is known to cause myocarditis and dilated cardiomyopathy in children. [18,19] Children with cardiomyopathy are therefore likely to be at greater risk of acquiring severe pneumonia

with or without further myocardial impairment secondary to the direct effects of adenovirus infection on the myocardium.

HIV infection in a South African context has been associated with severe pneumonia but in contrast to other pneumonia studies, the HIV prevalence in this study was relatively low (7%). HIV was not found to be associated with poor outcome in this study. Explanations for this observation include improved access to effective perinatal mother to child transmission (PMTCT) programmes which have reduced the rate of perinatal HIV infections in South Africa. [19] Furthermore, early initiation of antiretroviral treatment in HIV infected children is now the standard of care in South Africa. [20]. That said, severe pneumonia is common in HIV infected children and adenovirus should be considered in the aetiological diagnosis along with other viruses and opportunistic infections such as *Pneumocystis jeroveci* and cytomegalovirus (CMV). [21] Positive bloodstream infection was associated with increased mortality in this study. The synergistic effect produced by bacterial and viral coinfection resulting in more severe illness than either factor independently has been documented. The primary viral infection alters the respiratory mucosa predisposing it to colonisation by secondary bacteria which alters the immune responses resulting in severe secondary bacterial infection. [4]

The severity of AVP as measured by hypoxia and need for ICU admission was associated with development of both CLD and death. This finding was not unique to this study and is consistent with previous studies looking at outcomes following adenoviral infections [4,6,12,13,14,15] In their 2006 study reviewing risk factors for BO following acute bronchiolitis, Colom et.al showed that adenoviral infection and mechanical ventilation were both risk factors for developing BO, a common chronic sequelae following adenovirus pneumonia. [15]Castro Rodriguez compared 38 patients with AVP who developed BO to those who did not develop BO in a five year follow up period and reported that mechanical ventilation, prolonged requirement of oxygen, more severe respiratory compromise and extrapulmonary manifestations of adenovirus infection with prolonged hospitalisation were risk factors for the development of BO which occurred in 47,4% of cases. [12] A study by Murtagh et.al from Argentina in 2009 identified mechanical ventilation as a risk factor for death but not for the development of BO. In their study of 415 children hospitalised with AVP, 49% recovered, 36% had chronic sequelae and 15% died.[4] In our study over a one year period, 14% developed CLD and 9% died. The lower incidence of CLD and death in our cohort compared to the Argentinian study could be explained by different study design, different definition of CLD or different epidemiology of adenovirus serotypes and disease spectrum in our setting.

This study has several limitations. First, the retrospective search for eligible patients may not have represented all the AVP cases as testing for respiratory viruses was not routinely performed on all children admitted with pneumonia. Bias toward more severe pneumonia cases may be present in this study as clinicians were likely to request viral testing when pneumonia was more severe. Secondly, we could not diagnose BO by standard high resolution computed tomography (HRCT) criteria as HRCT is not routinely performed in our setting in children recovering from severe pneumonia due to resource constraints and to limitation of unnecessary radiation exposure in children. We elected to categorise children with CLD if they remained oxygen dependent after 30 days of hospitalisation as this approach would capture the majority of children who would develop BO. Lastly, we were unable to identify

adenovirus serotypes in this study as testing for serotypes is not routinely offered in our setting. Knowledge of serotype patterns may explain some observed differences in the spectrum of AVP disease severity as described elsewhere. [23]

In conclusion, AVP is an important cause of pneumonia and CLD in young children in South Africa. Severe AVP needing ICU admission and blood stream infection is associated with poor outcome. Early recognition of severe AVP is important to identify children at risk of developing chronic lung disease.

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Table 1: Demographic, clinical and co-infection data in children with adenovirus-associated pneumonia, January 2011-December 2011 (n=206)

DEMOGRAPHIC DATA	n=206	%
Age(months) median IQR	12 (6-24)	
Male (n, %)	116	56%
Female (n, %)	90	44%
WAZ (mean,SD)	1,38	(1,91)
WAZ<-2 (n/N, %)	70	34%
Community Acquired (n/N, %)	148	72%
Hospital Acquired (n/N ,%)	58	28%
CLINICAL FEATURES		
Rash	5	2%
Conjunctivitis	34	17%
Fever more than 38 degree Celsius	159	77%
Diarrhoea	31	15%
UNDERLYING CONDITIONS		
Cardiac	48	23%
Respiratory	26	13%
Neurologic	15	7%
Renal	4	2%
Immune suppression	19	9%
Prematurity	36	18%
Other(including tracheostomy)	27	13%
HIV STATUS		
HIV exposed, uninfected	42	20%
HIV infected	14	7%
CD4 % (median, IQR) n=14	18,1 (8-20.3)	
HIV viral load (log x10 6) (median IQR)	6,4 (5.1-6.6)	
COINFECTIONS		
Viral, TB &positive blood culture	65	32%
Rhinovirus	29	14%
Parainfluenza virus	6	3%
Influenza virus	8	4%
Tuberculosis	11	5%
Bloodstream infections	11	5%

WAZ: weight-for-age z-score.
HIV: Human immunodeficiency virus

Table 2: Clinical factors associated with in-hospital mortality in children with adenovirus-associated pneumonia

	DIED	ALIVE	p-value
Age (median, IQR)	19 (10-36)	2 (6-24)	P=0,06
WAZ <-2	7/15 (47%)	63/77 (36%)	P=0,7
Cardiac	8/18 (44%)	40/188 (21%)	P=0,02
Respiratory	1/18 (5,6 %)	25/188 (13%)	P=0,35
Immune Suppression	2/18 (11%)	17/188 (9%)	P=0,70
Prematurity	3/18 (17%)	33/188 (17%)	P=0,92
HIV infected	2/18 (11%)	54/188 (29%)	P=0,78
Hypoxia	17/18 (94%)	129/188 (69%)	P=0,02
ICU admission	14/18 (78%)	62/188 (32%)	P=0,01
Bloodstream infection	4/18 (22%)	7/188 (41%)	P=0,01

WAZ: weight-for-age

ICU: intensive care unit

Appendices: DATA COLLECTION SHEET

INITIALS: FOLDER NUMBER:

DOB: AGE: M/F: RACE

DATE OF RCH ADMISSION:

DATE OF POSITIVE ISOLATE:

CLINICAL INFORMATION REGARDING ILLNESS

COMMUNITY (1) OR HOSPITAL ACQUIRED (2):

WEIGHT:

RASH: Y/N

CONJUNCTIVITIS: Y/N

TEMP>38*> 48HRS : Y/N

DIARRHOEA : Y/N

UNDERLYING CHRONIC ILLNESS: 1-CARDIAC, 2-RESP, 3-GIT, 4-RENAL,5-NEURO 6-IMMUNOSUPPRESSED, 7-EXPREM,8-TRACHEOSTOMY,9-OTHER

HIV STATUS: 0-UNEXPOSED 1-EXPOSED,UNINFECTED 2-INFECTED

ART: Y/N

LABS:

WCC ;

Hb CRP PCT

CD4: COUNT %:

VIRAL LOAD :

CO-INFECTIONS:

OTHER VIRUSES:

TB:

BLOOD CULTURE:

SEVERITY:

HYPOXIA : Y/N

ICU ADMISSION: Y/N DURATION:

VENTILATORY SUPPORT: Y/N

1-CPAP, 2-IPPV, 3-HFOV 4-NPO2

OUTCOMES:

O2 DEPENDENT > 1 MONTH : Y/N

DAYS IN HOSPITAL:

DEATH: Y/N CAUSE OF DEATH:

DISCHARGED: Y/N

FULL RECOVERY: Y/N

CHRONIC LUNG DISEASE: Y/N

O2 DEPENDENT > 1 MONTH: Y/N

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