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Defining RSV: clinical prediction of respiratory syncytial virus-associated disease in children under five years of age in Bondo District, Kenya, 2007-2009.

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An abstract of
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Abstract

Defining RSV: clinical prediction of respiratory syncytial virus-associated disease in children under five years of age in Bondo District, Kenya, 2007-2009.

By Lia N. Phillips

Pneumonia is one of the leading causes of global under five mortality, with respiratory syncytial virus (RSV) as a common etiology. Detection of RSV is especially challenging without laboratory access. Therefore we aimed to create a clinical case definition to identify RSV disease in resource poor settings. Data were collected from children under five years of age admitted to inpatient facilities in Bondo District, Kenya, between 2007-2009. Children were tested for RSV using real-time polymerase chain reaction if they presented with World Health Organization-classified severe or very severe pneumonia, influenza-like illness, or symptoms of an upper respiratory tract infection. We used logistic regression to find a model that predicted RSV disease in our study population and used this model to calculate odds ratios of disease according to different clinical patterns of presentation. Of the 2 970 children under five years admitted to an inpatient service, 46.3% were tested for RSV and of those, 10.7% had laboratory-confirmed RSV. The odds of disease were highest among those children presenting with difficulty breathing, chest wall indrawing, and hypoxemia (SpO_2<95%). The odds ratio was highest for children with all three of these symptoms, however was still significantly elevated when either two out of three symptoms or a single symptom were present. We suggest that a case definition that includes the clinical features of difficulty breathing, chest wall indrawing, and hypoxemia would allow for detection of RSV-associated disease in the absence of laboratory resources, which could decrease RSV-associated morbidity and mortality in developing countries.

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Chapter 1: Literature Review

Global Childhood Pneumonia

In 2013, an estimated 6.3 million children under five years of age died worldwide. About 50% of these deaths were due to infectious causes with pneumonia being the leading cause, or 15% of total deaths[1]. Although childhood mortality estimates have been decreasing in recent years, pneumonia remains one of the most common singular causes of death. Additionally, in China and India, the fastest growing populations in the world, pneumonia is the leading cause of under-five mortality [1, 2]. Between 2000-2013, there was a 4.1% average decrease in under five mortality per year, with most of these reductions in deaths due to infectious agents. Although impressive, if this trend continues, 4.4 million children under five years of age will still die in 2030 [1]. Likewise, pneumonia-specific mortality decreased by an average of 3.1% per year between 2000-2010, however will still remain a major cause of childhood mortality in the future [2].

The burden of disease falls disproportionately on developing countries. Almost 75% of under five deaths globally in 2010 were due to infectious causes in Africa. While the rate of pneumonia has decreased by about 3% per year globally, in Africa the rate has decreased by only 2% [2]. If current mortality trends continue, ¾ of the countries failing to achieve the world target development goals will be in sub-Saharan Africa [1]. Current estimates show that west and central Africa have the highest rates of child mortality, while all seven countries with an under five mortality rate over 100 deaths per 1000 are in sub-Saharan Africa [3]. About 12% of cases of pneumonia will progress to severe disease [4]. Looking more closely at developing countries, a recent systematic review revealed incidence of severe acute lower respiratory infection (ALRI) to be 51.8 per 1000 in children <1 year of age and 19.7 per 1000 in children <5 years of age, compared to 19.6 and 9.9 respectively in industrialized countries. Incidence of very severe ALRI

was 13.7 per 1000 and 5.1 per 1000 in developing countries compared to 8.6 and 3.0 per 1000 in industrialized countries [5]. Overall, there were 15 million admissions for severe or very severe ALRI globally in children under five years with an estimated 0.3 million deaths in hospital. Of these deaths, 99% occurred in developing countries [5]. Regionally, about 30% of pneumonia cases occur in African countries; more dramatically, Kenya is one of 15 countries that accounts for 65% of all episodes of pneumonia worldwide [4].

In settings where laboratory testing is unavailable, healthcare providers must rely on clinical judgement for diagnosis of disease such as pneumonia. The World Health Organization's Integrated Management of Childhood Illness (IMCI) provides methodology for identifying severe disease in children in resource-poor settings. Pneumonia as defined by the IMCI is cough or difficulty breathing with fast breathing or chest indrawing. Severe pneumonia is defined as cough or difficulty breathing with stridor or a danger sign (inability to drink/breastfeed, persistent vomiting, convulsions, lethargy, or unconsciousness) [6]. The IMCI is considered the major approach to childhood diseases in appropriate settings, but has been criticized for its facility-level approach and failure to identify community-level approaches [7]. Additionally, the IMCI is designed to cast a wide net in terms of diagnosis; it is unclear to what extent it is a useful tool for identifying pathogen-specific respiratory disease such as respiratory syncytial virus associated disease.

Burden of Respiratory Syncytial Virus

Since the introduction of the pneumococcal and Hib vaccines, a need has arisen to identify other common etiologies of childhood pneumonia globally. Feikin et al. examined viral etiology of children under five with severe acute respiratory illness in Bondo District, Kenya, and found that the most commonly detected viruses were rhinovirus/entervirus (50%), RSV (22%), and adenovirus (16%). Comparing cases to controls, RSV and influenza viruses were the only viruses more prevalent in cases than in controls. The highest incidence of pathogen-specific incidence of severe acute respiratory disease was for RSV (7.1 cases per 100 person-years),

giving further evidence to support RSV as a common pathogen associated with severe respiratory disease in young children [8]. Interestingly, the same authors found that pathogens implicated in severe acute respiratory disease in children >5 years and adults were different from those found in children <5 years.

In a systematic review estimating RSV-associated ALRI in children under five in 2005 globally, about 33.8 million new episodes occurred in 2005, with at least 3.4 million requiring hospital admission for severe ALRI. Incidence of RSV-associated ALRI in developing countries was twice that of industrialized countries. Morbidity was largely in the first year of life; incidence of RSV mortality was found to be 2-3 times greater in infants than in older children under five years. Even though these numbers may appear to be high, hospital-based passive case ascertainment as was performed in this systematic review likely underestimates RSV-associated ALRI incidence especially in developing countries due to low health service use or because children are often not tested [9]. Similarly, a different systematic review estimating respiratory viruses in general as etiology of ALRI found that RSV was significantly more common in children hospitalized with ALRI than asymptomatic controls with a statistically significant attributable fraction of 90% among the exposed. This study estimated that of children under five years of age hospitalized with ALRI in 2010, 2.9 million cases were attributable to RSV [10].

A 2010 study looking at patients 0-12 years old in the Kilifi District of rural Kenya who met the WHO severe or very severe pneumonia definition, RSV was the most commonly detected virus (34% of patients overall and 42% of infants). While RSV infected patients in this study population had less severe illness than admissions without RSV, admissions with RSV were associated with severe disease compared to controls. Viruses other than RSV were as common among well children as compared to sick children, suggesting that non-RSV viruses contribute minimally to virus-associated severe pneumonia [11]. Another cohort study done in Botswana found that children 1-23 months old with RSV infection have a higher risk of treatment failure, increased respiratory support requirements, and longer duration of hospitalization compared to

children with pneumonia of other etiologies. A case-control study of the same population found RSV to be more common in pneumonia cases than controls, while other viruses including influenza, parainfluenza, and rhinovirus were not [12]. Although RSV infection has been associated with severe respiratory disease in this and other studies, it does not seem to pose an increased risk of mortality compared to other lower respiratory viral infections [11, 12].

The majority of our knowledge of RSV comes from studies performed in industrialized countries such as the United States. A US 2009 study by Hall et al. found that of children with acute respiratory infections (ARI), 18% had RSV infections. RSV accounted for 20% of hospitalizations, 18% of ED visits, and 15% of office visits for children with ARI. Average annual hospitalization rates were dramatically higher for children younger than 6 months of age (17 per 1000) compared to children under five years of age (3 per 1000). Although the incidence of disease was higher for young children, the majority of cases still occurred in children over the age of 1 year. Most children were previously healthy, and the only risk factors for hospitalization in this population were prematurity and young age [13]. When comparing inpatient and office visits for RSV, inpatients were more likely to receive a specific diagnosis of RSV-associated illness; therefore the true burden of all RSV associated disease is likely underestimated even in developed countries [13]. Stockman et al. estimated US RSV lower respiratory tract infection (LRTI) and RSV rates via ICD-9 codes from the National Hospital Discharge Survey over a period of 9 years. They found that incidence of lower respiratory tract infections in children <5 years was 27.9 per 1000 per year. There were 547,000 hospitalizations for LRTI per year and 24% of those were due to RSV. Looking at age specific incidence rates, the authors found that infants 0-2 months of age had the highest rate of RSV hospitalization, followed by infants <6 months. These authors further suggest that given that the highest burden of disease falls to 0-3 month old infants, a vaccine given shortly after birth would have the highest preventative impact [14].

Risk of RSV associated hospitalization is associated with preterm birth, chronic lung disease, and congenital heart disease. Case fatality rates are also higher in children with these risk factors and RSV associated hospitalization than in previously healthy children [15]. Age at onset of RSV season is a well established risk factor for severe RSV disease, although studies differ on whether age <3 months or age <6 months is the more appropriate cut-off [16]. Other risk factors include low birth weight, young siblings in the household, daycare attendance, lack of exclusive breastfeeding, crowding, indoor air pollution, incomplete immunization, immunocompromised state, undernutrition, and HIV infection [16, 17].

Respiratory Syncytial Virus

RSV is an enveloped RNA virus belonging to the Paramyxoviridae family. There are two major strains, A and B, which differ by the variations of the large envelope G glycoprotein encoded by the viral genome. The F glycoprotein is another large envelope glycoprotein however this is common to both A and B strains. A and B strains usually circulate at the same time of year (November-May), with A as the usual dominant strain. RSV has an incubation period of 2-8 days, after which it replicates in the nasopharyngeal epithelium and spreads to the lower respiratory tract 1-3 days later. Disease may last as long as 4-8 weeks. Transmission occurs by large particle aerosol into the eyes and nose or by direct inoculation with secretions [18].

In bronchiolitis, infection of the upper airway spreads to the lower airway within a few days. Immunohistochemical investigations have found RSV-antigen extending from bronchial epithelial cells all the way to the alveolar level. Disease most severely affects distal medium and small bronchioles, with some less extensive proximal involvement. Inflammatory infiltrate is dependent on the distribution of the arterioles adjacent to the affected airway [19]. The final result is inflammation of the bronchiolar epithelium along with peribronchial infiltration of WBCs and edema of submucosa and adventitia. Debris of sloughed epithelium and fibrin causes partial or total obstruction of small distal airways. Air trapping in obstructed airways leads to ventilation-perfusion (V/Q) mismatch which in turn causes hypoxemia [20]. Young children are thought to

be predisposed to this condition for several reasons. The immature immune system may allow the virus to progress further than in adults. Anatomically, the small diameter of infant airways makes them more susceptible to obstruction and disease. Additionally, the infant lung has poor development of collateral alveolar regions for ventilation, ie. decreased ability to shunt [20, 21].

RSV Disease Presentation and Management

RSV infection is most commonly associated with bronchiolitis in infants. Bronchiolitis is the leading cause of hospitalization in infants in the US. The American Academy of Pediatrics defines bronchiolitis as "a constellation of clinical symptoms and signs including a viral upper respiratory prodrome followed by increased respiratory effort and wheezing in children less than 2 years of age" [21]. Studies in the US have reported common symptoms to be labored respirations, supplemental oxygen requirement, wheezing, fever, and cough [13]. Children older than two years presenting with recurrent wheezing usually have infection from viruses limited to the upper airway, such as rhinovirus. Risk factors for severe RSV disease include young age at time of infection (<3mo), underlying immunodeficiency, underlying cardiopulmonary disease, and prematurity [21]. Although RSV is associated with disease year-round, countries with lower annual temperature and precipitation have an RSV disease peak during months of temperature lows. Countries with higher annual temperatures have an RSV disease peak during the rainy season [22].

RSV causes the majority of bronchiolitis (50-80% of cases), however other causative viruses include human metapneumovirus, parainfluenza, and influenza. Co-infection is common, most frequently by RSV with hMPV or rhinovirus. In fact, the diagnosis of RSV bronchiolitis can be strongly suspected and empirically treated if the appropriate symptoms are present during wintertime months [21, 23]. Chest radiography may show hyperinflation or infiltrate, but may also be normal [23]. Oxygen saturation may be decreased; in a meta-analysis evaluating mortality in children under five years with pneumonia, multiple studies showed that hypoxemia was related to increased risk of mortality [24]. While traditional teachings say that oxygen saturation is

usually between 85-90% in RSV bronchiolitis, Lazzerini et al. suggest that it may be beneficial to consider children with SpO_2 as high as 92% [23, 24].

Treatment for RSV bronchiolitis is supportive therapy. Although various therapies have been tried, including bronchodilators, corticosteroids, leukotriene receptor antagonists, and hypertonic saline, none have shown to affect mortality or morbidity and thus are not routinely recommended for use in treatment of RSV [20].

Current Prophylactic Measures

Although we do not yet have a vaccine against RSV, some protection against RSV is afforded via passive immunization from the monoclonal antibody palivizumab. Because risk of hospitalization attributable to RSV infection is five times higher in high-risk infants who do not receive immunoprophylaxis as compared to non-high risk infants, passive immunization is warranted in high-risk infants [25]. The 2009 American Academy of Pediatrics policy statement regarding the use of palivizumab recommends targeting high-risk groups, ie. children with chronic lung disease, congenital heart disease, immunodeficiency, and prematurity if risk factors are present. Risk factors are defined as attendance of child care or more than 1 child <5 years in the household [26, 27].

Although randomized trials have shown that palivizumab reduces RSV hospitalization rates in high-risk infants by 39-78% [25], it is not a good candidate for widespread prophylaxis. First, it is very expensive. In cost-benefit analyses, results have shown that the cost of palivizumab exceeds the cost savings from reduced hospitalizations, even when considering potential cost savings from reduction in asthma rates. Looking at number needed to treat, it is estimated that the net cost of preventing one RSV hospitalization was $85,000 [25, 28]. Perhaps most importantly, palivizumab has not been shown to affect mortality [25]. Although the utility of palivizumab has proven itself in specific patient populations, it does not satisfy the need for comprehensive prevention of RSV disease. Most of the current prophylaxis methods are targeted

at high-risk children, however studies show that most children with RSV are previously healthy; therefore current prophylactic methods are unlikely to affect RSV disease burden [13].

RSV Vaccine Development

Given the demonstrated burden of RSV-associated respiratory disease that affects children worldwide but hits developing countries particularly hard, there is an immediate need for a method of prevention of disease acquisition and transmission. The presence of Hib and pneumococcal conjugate vaccines have at least partially reduced rates of pneumonia in children globally [29], therefore it is logical to address one of the most common viral etiologic pathogens of severe respiratory disease with a vaccine-centered approach as well.

Immunology

Unfortunately, naturally acquired immunity against RSV is not complete. Reinfection is common, although the likelihood of severe disease in recurrent infection is lower than in first-time infection [18]. One of the unusual features of RSV is that reinfection can occur even with the same strain of virus. Also unlike many other infectious agents, RSV is able to avoid maternal antibodies; this phenomenon manifests itself in the burden of disease we see in young infants [16]. Much of what we know about the immunologic response to RSV is based on the initial vaccine trials of the 1960s, in which children who had received the formalin-inactivated vaccine had worse outcomes than controls when exposed to RSV [30]. It was later determined that the vaccinated children did not have specific mucosal antibodies responsible for neutralizing and fusion-inhibiting activity. These children additionally had a more aggressive immune response compared to controls, with peripheral eosinophilia and increased lymphocytic proliferative response not seen in controls [18]. Thus there is a two-part approach to immunity – prevention of initial viral infection and controlling inflammation associated with infection.

Target Populations for Vaccine Development

In line with the age-specific burden of RSV disease, target populations for vaccine development fall along similar stratifications [31]. Given that RSV infection in the first six

months of life is associated with the highest hospitalization rates, it is reasonable that infants ≤ 6 months old make up one target group for vaccine development. The difficulty in targeting this group lies in the issues of an infant immune system that has not fully developed yet and maternal antibodies that may still be present. Maternal RSV-specific antibodies are present in term infants from birth, decline over the first 3 months life, and are undetectable in most infants by 6 months of age [32]. Infants who do not become infected with RSV in the first several months of life have higher titers of IgG at birth and were born to mothers with higher maternal RSV-specific IgG antibody levels than infants who develop RSV disease [33, 34]. The presence of maternal antibodies is a complication to delivering vaccine in this age group, however sufficient evidence suggests that immunologic protection during this time does reduce burden of severe disease.

When the target population is shifted to 6-24 months of age, many of the issues of the younger target population group are eliminated. The immune system becomes more mature and there are lower titers of maternal antibodies present. Although targeting this age group would not directly protect the age group with the highest rates of hospitalization (<6 months old), there are arguments for the possibility of indirectly protecting against RSV disease in <6 month olds by reducing transmission [31]. Targeting this age range would additionally address the large numbers of children who are hospitalized with RSV. Poletti et al. suggest that in low-income settings, it may be beneficial to target school-aged children since much of infection occurs via household transmission [35].

Another target population as per Anderson et al. is the pregnant woman; by inducing high levels of maternal antibodies that then are transferred to the fetus, the infant would be protected from severe disease during the early months at which he is most susceptible [31]. Passive prophylaxis with a high titer of neutralizing antibody has been shown to be protective. This, along with data showing that mothers with high titers of anti-RSV antibodies had infants with decreased risk of disease [16, 33], is supportive of the potential for maternal vaccination for infant protection against RSV infection. Poletti et al. suggest that RSV infant infection could be reduced

by 30% with vaccination of pregnant women [35]. From a public health standpoint, maternal vaccination would likely be successful in that it would take advantage of global mechanisms for delivery already in place [36].

RSV Vaccines in Development

Since the initial formalin inactivated RSV vaccine trials in the 1960s [30], vaccine developers have been cautious to avoid reproducing the enhanced RSV disease seen in those trials. In the years since, researchers have focused on other approaches to immunization. RSV glycoproteins F and G have shown to induce protective immunity. The F protein has several advantages over the G protein in terms of targeting for vaccine development. The G glycoprotein is highly glycosylated and can be quite variable between strains. In contrast, the F protein remains relatively stable among strains, induces higher levels of neutralizing antibodies, and offers better cross-protection against different strains [31]. Current RSV vaccines in development span a wide variety of type and target immunization group. Currently all live-attenuated vaccines in trial are targeted at the pediatric population [37]. Live attenuated vaccines in trial up to date have been challenged to find the right balance between attenuation and immunogenicity [38].

Several gene-based vector candidates are also being considered for the pediatric age group. Particle-based vaccine candidates are farthest along in development as of now (Phase 3); these RSV F nanoparticles are targeted at the maternal and elderly immunization groups. Additional candidates in trial include sub-unit and nucleic acid vaccines [37].

Challenges to Future RSV Vaccine Development

Aside from the science behind developing an effective and safe RSV vaccine, other challenges present barriers to global RSV immunization. Live attenuated vaccines will likely require a multiple dose schedule, presenting challenges to roll-out in resource-poor settings. Also there is limited data on how the live attenuated vaccine may interact with other vaccines if added on to coincide with the standard vaccination schedule. Additionally, the relationship of live vaccine in regards to potential sequelae of wheezing and asthma associated with RSV disease needs to be

studied. Practically, the vaccine may be expensive. An expert panel evaluating the likelihood of an RSV vaccine had a low level of optimism for low product cost and affordability for candidate vaccines [38]. Lastly, development of a vaccine alone will not necessarily address the inequity of disease burden around the world; the countries with the heaviest burden will require further innovation in vaccine delivery platforms.

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Chapter 2: Manuscript

Introduction

Of the 6.3 million children worldwide under five years of age who died in 2013, fifty percent died due to infectious causes. Pneumonia was the leading cause of infection-related death, causing approximately 15% of all under-five deaths [1]. The burden of under-five mortality falls disproportionately on developing countries; African countries in particular have the highest child mortality rates globally [2] as well as the highest global rates of childhood pneumonia [3].

With the success of Hib and pneumococcal vaccines in recent years, attention is turning to other pathogens responsible for childhood pneumonia. In a systematic review, Nair et al. estimated that in 2005, 33.8 million cases or 22% of all episodes worldwide of acute lower respiratory infection (ALRI) in children under five years old were attributable to respiratory syncytial virus (RSV), with 96% of cases and virtually all RSV-related deaths occurring in developing countries [4]. About 20% of acute respiratory infections in the United States can be linked to RSV [5], while studies in Kenya and Botswana found, respectively, that RSV was the most commonly detected virus in children with WHO-defined severe or very severe pneumonia [6] and predictive of severe ALRI [7]. Even so, reported global incidence of RSV-associated disease is likely underestimated, given low health service utilization and lack of laboratory availability in developing countries [4].

Given the demonstrated burden of RSV disease that falls on developing countries, there is an immediate need for a case definition that will allow identification of children with RSV disease in resource-poor settings. To address this knowledge gap, we evaluated signs and symptoms of children less than five years of age admitted to hospitals in Bondo District, Kenya between 2007-2009 to determine which clinical patterns were predictive of RSV-associated disease.

Methods

Data used for this retrospective study were collected by CDC's International Emerging Infections Program and the Kenya Medical Research Institute (KEMRI) as part of an infectious disease surveillance program, ongoing since 2005. All participating patients received free medical care at one of seven health facilities in Bondo District, Kenya. Bondo District is an impoverished, primarily rural area located in western Kenya with a doctor patient ratio estimated at 1:1700. Altitude in Bondo ranges from 1140-1400 meters above sea level [8]. This study was approved for IRB exemption.

Patients under five years of age admitted at participating facilities were tested for a panel of respiratory viruses including RSV if they presented with severe acute respiratory disease (SARD), influenza-like illness (ILI), or at least one of the following respiratory symptoms: cough, sore throat, difficulty breathing, chest pain, sneezing, or runny nose. Patients were excluded from surveillance if they had cough for >2 weeks or if previously hospitalized within 3 days of admission. A standardized form was used to collect demographic information, clinical signs and symptoms, medical history, medications, and outcomes.

Laboratory Methods

Nasopharyngeal and oropharyngeal swabs were collected from patients meeting inclusion criteria. Sample collection was only available during working hours and was occasionally limited by high patient volume. Specimens were placed in 1 ml virus transport media, brought to the laboratory and stored at -80^{o}C within 8 hours of collection. Total RNA extraction was performed using the QIAmp Viral RNA Minikit (Qiagen, Valencia, CA) according to manufacturer instruction. One-step RT-PCR was performed using the AgPath-ID One-Step RT-PCR Reagents (Applied Biosystems, Foster City, CA) to test for the presence of RSV. For further detail concerning laboratory collection, see Fuller et al. 2013 [9].

Definitions

We stratified our study population into three groups; 1) RSV test positive, those admitted with respiratory disease and a positive qRT-PCR for RSV, 2) RSV test negative, those admitted with respiratory disease and a negative qRT-PCR for RSV, and 3) those admitted without respiratory symptoms. Most analysis performed compared the RSV test positive and RSV test negative groups. Severe acute respiratory disease was defined as meeting requirements for IMCI severe or very severe pneumonia (cough or difficulty breathing accompanied with inability to breastfeed or feed, vomiting, convulsions, lethargy, stridor, chest indrawing, unconsciousness, or low oxygen saturation<90%). Hypoxemia was considered by two definitions; the first as defined by SpO2<90% and the second as defined by SpO2<95% and severe hypoxemia defined as SpO2<90%.

Data Analysis

All symptoms were defined as "yes/no" binomial variables with the exception of continuous variables respiratory rate (breaths per minute), temperature (degree Celsius), and oxygen saturation (%). We tested all variables for association with the outcome RSV test positive using chi-square or two-sample t-test and calculated crude relative risks. Variables with significant associations to RSV test positive status were considered for inclusion in the predictive model. We used the backwards selection method to select significant logistic regression models to predict RSV test positive in the all-patient population and tested population. Comparison models were created for four different age groups: all ages, 0-27 days, 0-14 weeks, and 0-2 years. We assessed predictive capability of the chosen models by generating ROC curves for each model. Logistic regression equation parameter estimates were used to generate odds ratios for RSV test positive status. We employed contrast statements to determine odds ratios for specific clinical patterns, using only symptoms included in predictive models. All analysis was conducted using SAS version 5.0 (SAS Institue Inc., Cary, NC).

Results

A total of 2 970 patients under five years of age were admitted to an inpatient facility in Bondo District between January 2007 and May 2009. Although more than half of these patients were between 0-2 years old (65%), only 35 (1.2%) fell into the neonatal age group (Table 1). Most patients (92.2%) originated from the same district as their admitting hospital. Of all admitted patients, 1 375 (46.3%) were tested for RSV (Table 1).

The most common admitting diagnosis in RSV test positive children was pneumonia/lower respiratory infection/acute chest infection (48.3%, Table 1). The most common presenting symptoms in RSV test positive children were cough, fever, and difficulty breathing with frequencies of 95.2%, 91.2% and 75.5% respectively (Table 2); the symptoms significantly associated with RSV test positive children included cough ($p<0.0001$) and difficulty breathing ($p<0.0001$) but not fever ($p=0.58$). Other associated symptoms included indrawing, stridor, wheezing, nasal flaring, hypoxemia, and sneezing ($p<0.001$). Crude risk ratio calculations showed risk of being RSV test positive to be increased by almost 13-fold if children presented with cough (RR 12.51; 95% confidence interval [CI] 5.88-26.63), by 5-fold if children presented with difficulty breathing (RR 5.17; 95%CI 3.57-7.47), and by 4-fold if children presented with indrawing (RR 4.24; 95%CI 3.00-6.00) [Table 2].

When considering RSV test positive and negative children only, we selected indrawing, difficulty breathing, and hypoxemia ($SpO2<95\%$) for inclusion in our predictive model. When considering all children (RSV test positive, negative and those without respiratory symptoms), cough was an additional variable included in our predictive model for outcome RSV test positive. Final models stratified by age are presented in Table 3. Interestingly, although wheezing and hypoxemia defined as $SpO2<90\%$ among other variables were considered for inclusion in our models, they did not add a significant effect to the model using our selection method. Among RSV test positive and negative children, we were able to correctly predict disease 64-65% of the time using our model (Area under ROC curve 0.64 (all ages); 0.65 (0-27 days); 0.65 (0-14

weeks); 0.66 (0-2 years) (Figure 1). Among all children admitted to an inpatient service, including those without respiratory symptoms, we were able to correctly predict RSV disease 78-79% of the time (Area under ROC curve 0.78 (all ages); 0.78 (0-27 days); 0.79 (0-14 weeks); 0.79 (0-2 years) (Figure 2).

Among RSV test positive and negative children, the odds of having a positive test was 5.57 times higher for children presenting with indrawing, difficulty breathing, and hypoxemia than children without those symptoms (OR 5.57; 95%CI 3.13-9.92). The odds ratio of a positive test was 2.85-3.78 when a child of any age presented with at least two out of three symptoms (indrawing and hypoxemia OR 2.88, 95%CI 1.72-4.82; difficulty breathing and hypoxemia OR2.85, 95%CI 1.69-4.83; indrawing and difficulty breathing OR 3.78, 95%CI 2.23-6.40). Considering the odds of positive RSV test in each symptom adjusted for all others, the odds of a positive RSV test was 200% higher if presenting with indrawing alone (OR 2.06; 95%CI 1.26-3.36), 77% higher if presenting with difficulty breathing alone (OR 1.77; 95%CI 1.13-2.78), and 82% higher if presenting with hypoxemia alone (OR 1.82; 95%CI 1.23-2.70) (Figure 3a).

The odds of being RSV test positive increase most dramatically when considering the 0-14 week age range, but also increase in the 0-2 year age range compared to all ages. The odds ratio of a positive RSV test was 8.81 and 7.90 for 0-14 weeks and 0-2 years respectively (95%CI 4.45-17.45; 95%CI 4.01-15.56) for a child presenting with all three symptoms, and similarly was higher in these specified age ranges as compared to all ages for a child presenting with two out of three symptoms or a single symptom (Figure 3c, 3d). Neonatal age did not significantly change odds of a positive RSV test.

Discussion

In this study, we found that the symptoms most likely to predict a positive qt-PCR test for RSV in a population of inpatients <5 years with respiratory complaints were indrawing, difficulty breathing, and hypoxemia. Odds of disease were further increased for children falling into the 0-14 week and 0-2 year age categories. Although guidelines exist for diagnosing pneumonia in children in developing countries [10], this is the first study to identify clinical patterns specific to RSV disease that can be used to develop a case definition in settings where laboratory methods of virus detection are unavailable. Like other studies examining RSV in young children [4, 11], we saw increased odds of RSV-attributable disease in children under 2 years of age which is consistent with the expected pathophysiology of bronchiolitis [12].

We were able to accurately predict a positive RSV test moderately well, about 78% of the time, when considering children <5 years with illness requiring inpatient services. When narrowing our scope to consider only those with respiratory symptoms, our ability to predict a positive RSV test decreased to about 65% accuracy. While we do not offer a strict case definition, we suggest that at least the presence of the aforementioned symptoms have utility in clinical prediction of RSV. The difference between the two models was the single variable cough, which was likely too common in both test positive and test negative groups to differentiate between the two.

Importantly, our proposed model is similar to the IMCI method for identifying cough or cold, pneumonia, and severe pneumonia. Our study supports the utility of the IMCI in identifying RSV in children <5 years in low-resource settings. However, there are some significant differences. First, our model utilizes fewer symptoms than the IMCI method to yield a similar capture of disease. We therefore provide a simpler method that can be used even more easily by healthcare workers to identify disease. Additionally, the IMCI guidelines suggest referring to a hospital if oxygen saturation falls below 90%; our analysis suggests that a more appropriate measure of hypoxemia is oxygen saturation below 95%. Although we were not able to directly

measure severity of disease, our study population was entirely inpatient, suggesting that a threshold of 95% is appropriate for identifying disease requiring inpatient services as well as less severe disease. Lazzerini et al. found that a threshold as high as 92% could be used for a definition of hypoxemia [13], we suggest that in the setting of identifying RSV disease a threshold of 95% is even more informative. Further studies examining this threshold for hypoxemia are warranted.

Interestingly, although wheezing is considered a hallmark of RSV-induced bronchiolitis [14-16], it was excluded as a predictor for RSV in our study after it did not have a significant effect during the model selection process. The percent of RSV-infected children with wheezing in our study was far less than expected; the most likely explanation for this is that health workers performing intake were not able to sufficiently recognize wheezing.

There were several limitations to our study. The number of children age 0-27 days in our study population was small and contributed to our inability to make any statements about this important age group. Additionally, we only evaluated RSV in an inpatient population, limiting our ability to generalize to the general pediatric population. Difficulty breathing was an important predictor in our model, however this is a subjective measure and difficult to define. Nonetheless, studies in the United States have also found difficulty breathing to be associated with RSV disease [5]. In practice, our model requires pulse oximetry for measurement of oxygen saturation, however this technology is often unavailable in the resource-poor settings that our model is designed to function within [17]. Lastly, we did not evaluate occurrence of symptoms in respiratory infection secondary to other pathogens, therefore we cannot comment on specificity of our model for RSV compared to other etiologies.

Overall, we provide a foundation to build a case definition for RSV disease. A definition should include some combination of difficulty breathing, chest wall indrawing, and hypoxemia defined as SpO_2<95%. This case definition could be used globally to help frontline health workers identify likely cases of RSV quickly and efficiently. With better detection of RSV

disease and proper referral to inpatient services and supportive care, we could potentially see a decrease in mortality of RSV-associated respiratory tract infection.

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Tables

Table 1. Characteristics of patients ages 0-59 months admitted to inpatient facilities in Bondo District, Kenya, between 2007-2009.

	All patients n=2970		RSV test positive n=147	
	n	%	n	%
Sex				
Female	1346	45.3	83	56.5
Male	1624	54.7	64	43.5
Age				
0-28 days	35	1.2	1	0.7
0-14 weeks	186	6.3	24	16.3
0-23 months	1952	65.7	96	65.3
24-59 months	1018	34.3	26	17.7
District				
Bondo	2738	92.2	134	91.2
Siaya	82	2.8	4	2.7
Kisumu	75	2.5	6	4.1
Other	74	2.5	3	2.0
Diagnosis[a]				
Pneumonia/LRI/Acute chest infection	632	21.3	71	48.3
URTI	295	9.9	15	10.2
Gastroenteritis/Diarrhea	590	19.9	21	14.3
Dehydration	323	10.9	12	8.2
Other	1130	38.0	28	19.0
Tested for RSV	1375	46.3		

[a]Diagnosis at time of hospital admission.

Table 2. Comparative frequencies and means of symptoms in children with positive RSV test compared to all others admitted to inpatient services.[a]

	RSV test positive	Non-RSV test positive	RR[b]	95% Confidence Interval		Test of Association p-value[c]
	n (%)	n (%)		Lower Limit	Upper Limit	
Cough	140 (95.25)	1687 (59.76)	12.51	5.88	26.63	<.0001
Difficulty breathing	111 (75.51)	998 (35.36)	5.17	3.57	7.47	<.0001
Indrawing	37 (25.17)	181 (6.41)	4.24	3.00	6.00	<.0001
Stridor	15 (10.20)	85 (3.01)	3.26	1.99	5.35	<.0001
Wheezing	15 (10.20)	101 (3.58)	2.79	1.69	4.61	<.0001
Nasal flaring	24 (16.33)	186 (6.59)	2.56	1.69	3.88	<.0001
Hypoxemia	93 (63.27)	1200 (42.51)	2.23	1.61	3.10	<.0001
Sneezing	92 (62.59)	1311 (46.46)	1.85	1.33	2.56	0.0002
Fever	134 (91.16)	2533 (89.73)	1.17	0.67	2.04	0.58
Vomiting	102 (69.39)	1859 (65.88)	1.17	0.83	1.64	0.38
Hemoptysis	1 (0.71)	17 (1.01)	0.72	0.11	4.88	0.73
Sore throat	8 (5.44)	253 (8.97)	1.08	0.50	2.32	0.84
Inability to drink	31 (21.09)	454 (16.09)	1.37	0.93	2.01	0.11
Convulsions	34 (23.13)	821 (29.09)	0.74	0.51	1.08	0.12
Lethargy	26 (17.69)	467 (16.55)	1.08	0.71	1.63	0.72
Coma	1 (0.68)	14 (0.50)	1.35	0.20	9.02	0.76
Mean respiratory rate (breaths per minute)[d]	46.03 (14.37)	40.35 (13.08)				<0.0001
Mean temperature (degree Celsius)[d,e]	37.46 (1.05)	37.29 (1.12)				0.08
Mean oxygen saturation (%)[d]	93.31 (4.78)	95.57 (3.69)				<0.0001

[a]Frequencies are described for binomial variables, means are described for continuous variables.
[b]Relative risk of RSV test positive status in patients with symptom versus patients without symptom
[c]p-value reflects chi-square test of association statistic for binomial variables and two-sample t-test statistic for continuous variables.
[d] Values presented are unweighted mean (standard deviation)
[e]Oxygen saturation determined by pulse oximetry.

Table 3a. Beta-estimates of parameters in models predictive of RSV test positive compared to RSV test negative, by age grouping.

	Intercept	Indrawing	Difficulty breathing	Hypoxemia	Age group
All ages	-2.89 (p<0.0001)	0.67 (p<0.01)	0.66 (p<0.001)	0.39 (p<0.05)	--
0-27 days	-2.88 (p<0.0001)	0.69 (p<0.01)	0.66 (p<0.01)	0.38 (p<0.05)	-0.97 (p=0.35)
0-14 weeks	-2.87 (p<0.0001)	0.55 (p<0.05)	0.54 (p<0.05)	0.58 (p<0.01))	0.80 (p<0.01)
0-2 years	-3.17 (p<0.0001)	0.69 (p<0.01))	0.50 (p<0.05)	0.56 (p<0.01)	0.52 (p<0.05)

Table 3b. Beta-estimates of parameters in models predictive of RSV test positive status compared to all other children admitted to hospital, by age grouping.

	Intercept	Indrawing	Difficulty breathing	Hypoxemia	Cough	Age group
All ages	-5.44 (p<0.0001)	0.86 (p<0.001)	0.96 (p<0.0001)	0.36 (p=0.05)	1.98 (p<0.0001)	--
0-27 days	-5.43 (p<0.0001)	0.87 (p<0.0001)	0.96 (p<0.0001)	0.35 (p=0.06)	1.96 (p<0.0001)	-0.57 (p=0.58)
0-14 weeks	-5.48 (p<0.0001)	0.72 (p<0.005)	0.91 (p<0.0001)	0.35 (p=0.06)	2.01 (p<0.0001)	0.63 (p<0.05)
0-2 years	-5.75 (p<0.0001)	0.82 (p<0.001)	0.91 (p<0.0001)	0.32 (p=0.08)	1.96 (p<0.0001)	0.49 (p<0.05)

Figures

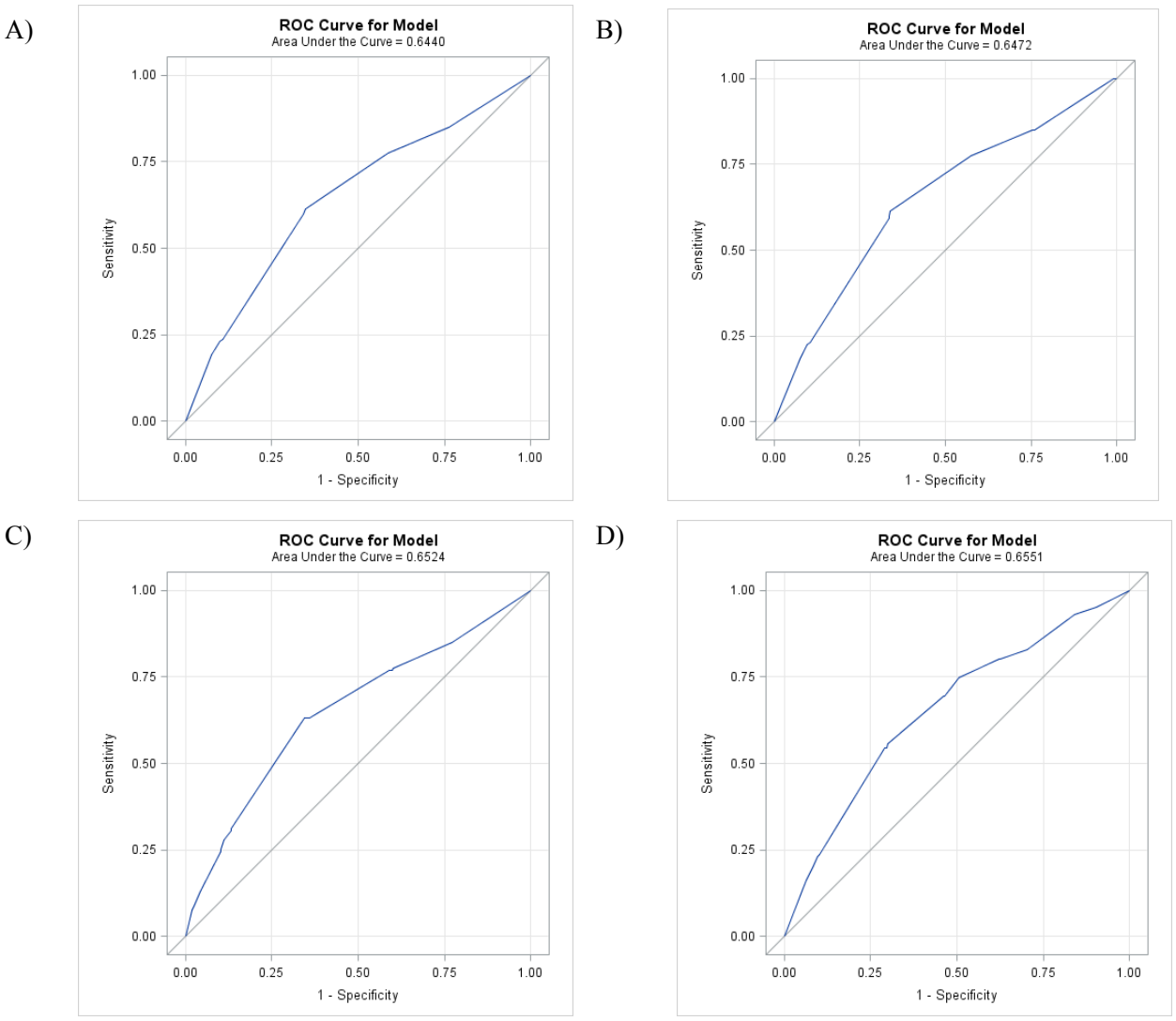
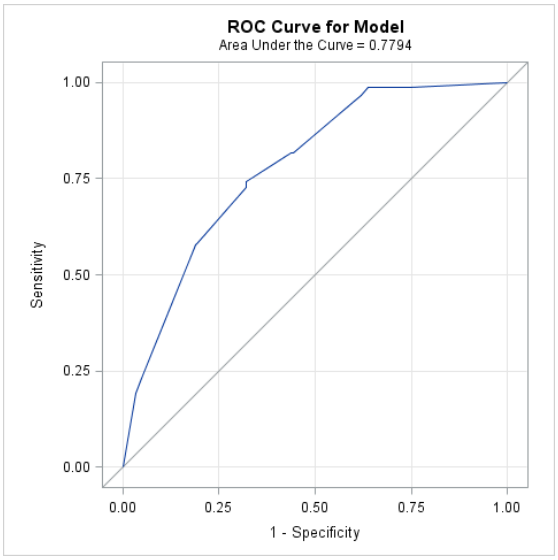
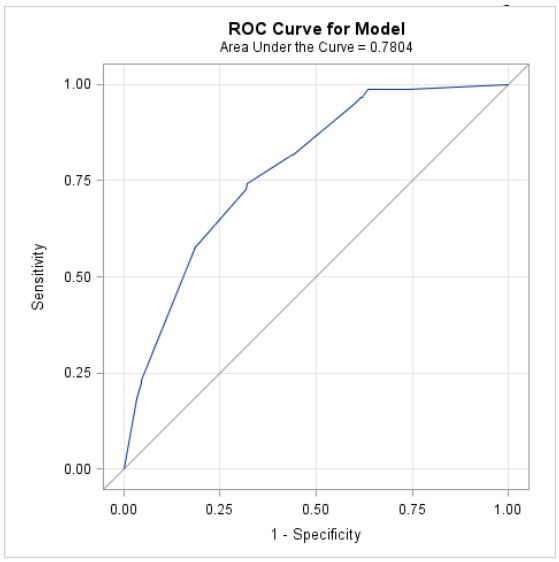


Figure 1. Sensitivity and specificity as demonstrated by ROC curves for models predicting RSV test positive status among children admitted for respiratory symptoms in children A) 0-5 years of age, B) 0-27 days of age, C) 0-14 weeks of age, D) 0-2 years of age. All models contain predictive variables indrawing, difficulty breathing, and hypoxemia (SpO_2<95%).

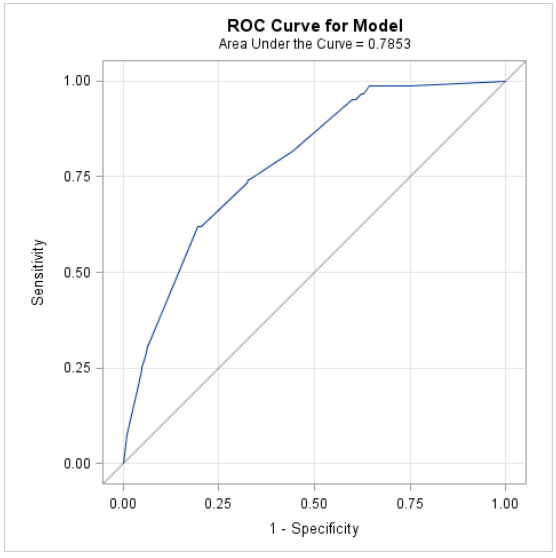
A)



B)



C)



D)

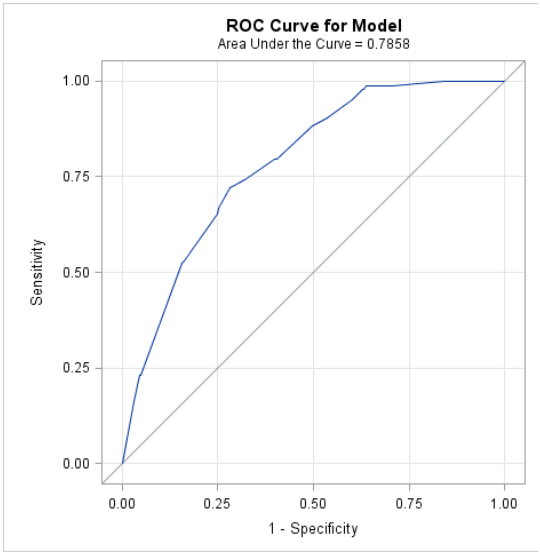
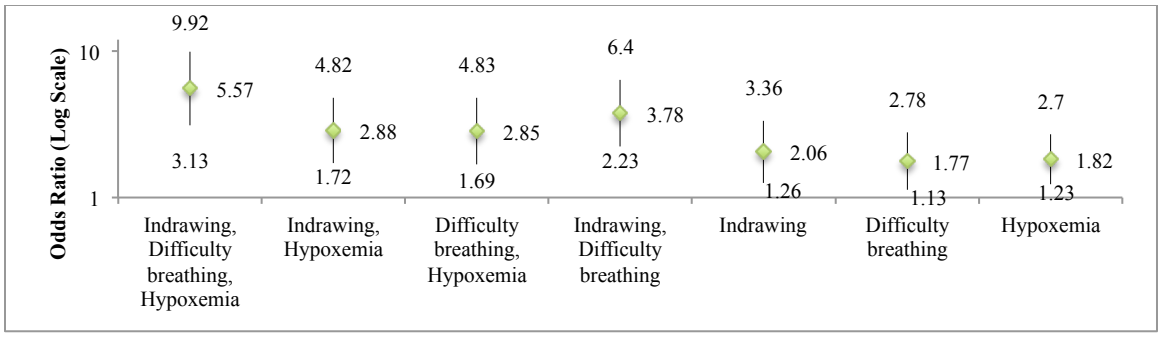
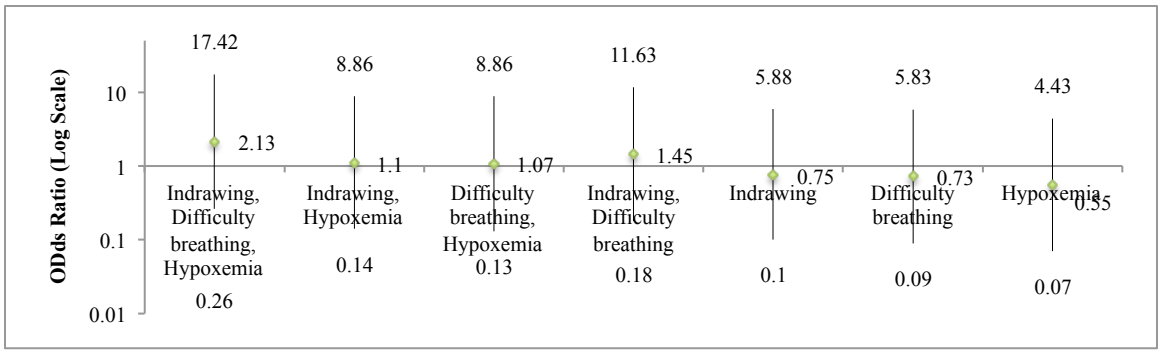


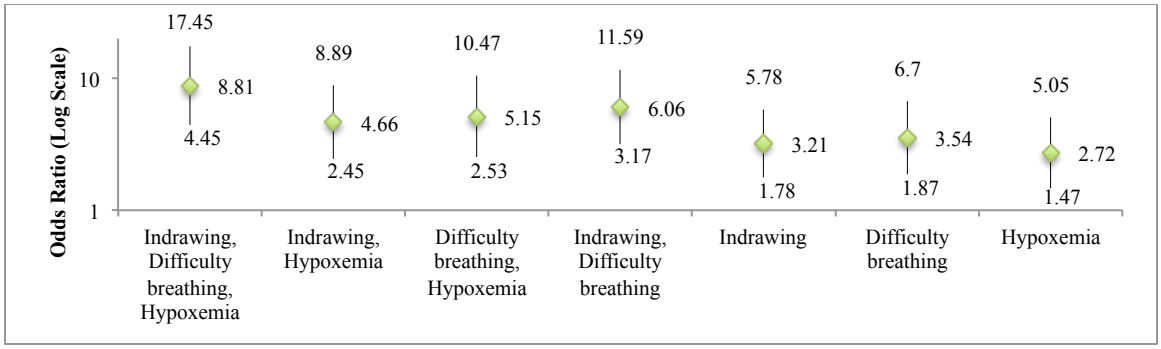
Figure 2. Sensitivity and specificity of models predicting RSV test positive status among all inpatients <5 years old for those children A) 0-5 years of age, B) 0-27 days of age, C) 0-14 weeks of age, D) 0-2 years of age. All models contained predictive variables indrawing, difficulty breathing, hypoxemia (SpO_2<95), and cough.



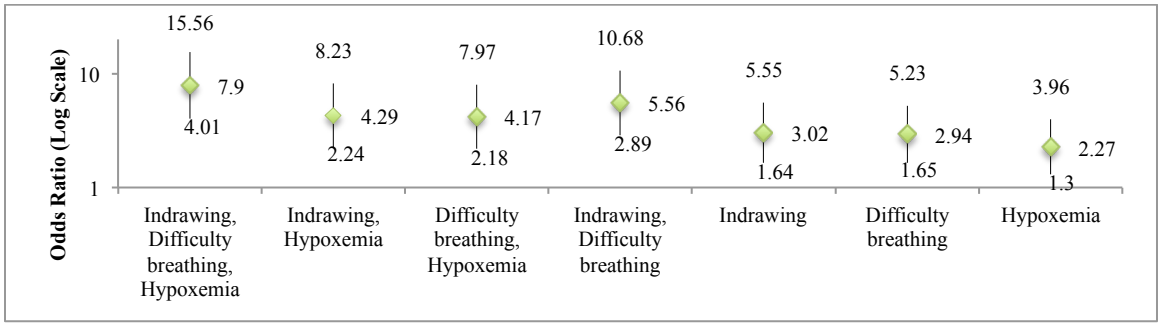
A) All ages



B) Neonate, 0-27 days



C) 0-14 weeks



D) 0-2 years

Figure 3. Odds ratios of RSV test positive status among those tested for RSV depending on pattern of clinical presentation, according to predictive models. Odds ratios are stratified by age range of A) all ages, B) neonatal age, 0-27 days, C) 0-14 weeks, and D) 0-2 years.