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Poor Outcome of Acute Respiratory Infection in Young Children with Underlying Health Condition in Brazil

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SUMMARY

Objectives: It is well established that respiratory viruses are an important cause of hospitalizations in young children worldwide, but data are limited on the contribution of specific viruses to severe illness in South America. We describe clinical and laboratory findings from prospective surveillance for acute respiratory infections at a tertiary hospital in São Paulo, Brazil.
Methods: We screened children < 2 years old with acute respiratory tract infections admitted to an urban tertiary hospital for respiratory viruses from March 2008 through February 2010, using polymerase chain reaction assays.
Results: Respiratory viruses were identified in 378 (53%) of the 715 samples analyzed. Respiratory syncytial virus was the most commonly identified virus (52%), followed by adenovirus (27%) and Human metapneumovirus (12%). More than one virus was identified in 19% of specimens. Almost half of the samples (46%) were from children with underlying health conditions. We demonstrated that compared to the previously healthy group, those with comorbidities had a worse outcome in terms of severity, with prolonged hospital stay and more need of intensive care.
Conclusion: Identification of this high-risk population along with strategies for fast diagnosis might each help to reduce morbidity and mortality in this group.

1. Introduction

It is well established that respiratory viruses are an important cause of morbidity in young children worldwide.[1] While respiratory syncytial virus (RSV) remains the leading cause of lower respiratory tract infection (LRTI) in young children, especially those under 12 months,[2] other viruses, such as human metapneumovirus, influenza, parainfluenza and adenovirus, are also associated with LRTI.[3] Great effort has been done to improve viral diagnostic methods in an attempt to identify the causative agents of the most common clinical syndromes, such as bronchiolitis and pneumonia. In this scenario of multiple viruses causing the same spectrum of disease, diagnostic tests are helpful for identifying possible etiologies, which may ultimately help target prevention and treatment strategies. In addition to the agent, the host also plays an important role in determining outcomes.[4,5]

Therapeutic and preventive strategies available nowadays against respiratory viruses are still limited. Influenza vaccines

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and passive immunization with monoclonal antibodies against RSV (palivizumab) can help to reduce influenza and RSV infection and morbidity, but still leaves uncovered most of the acute respiratory virus infection burden. Moreover it's been demonstrated that influenza vaccine's efficacy is lower than desirable in young infants[6,7] and that palivizumab is cost-effective mostly in high-risk population.[8] Antivirals used for influenza treatment, such as neuraminidase inhibitors, can shorten the duration of symptoms and help to reduce related complications, but have their efficacy linked to the timing of drug prescription.[9]

Young children with underlying diseases or risk factors, with emphasis on those with a history of premature birth, congenital heart and lung disease, are at greater risk for unfavorable outcomes when infected with a respiratory virus.[10] Therefore, most of available preventive and treatment drugs are directed to this special population, although the majority of these strategies have high cost and /or limited efficacy. Active virus surveillance allowing good seasonality planning and accessible virus detection tools for etiological definition of the respiratory syndromes can lead to a more rational usage of these strategies.

We describe clinical and laboratory findings from a prospective surveillance of hospitalizations due to acute respiratory infection in young children at a tertiary hospital in São Paulo, Brazil, comparing outcomes in infants with underlying disease to those previously healthy.

2. Patients and Methods

2.1. Study design

In 2008, we started a prospective surveillance system of acute respiratory infections (ARI) in children under two years of age at a university hospital in São Paulo city, Brazil. This analysis includes clinical, epidemiological and laboratory data collected during the two-year time period from March 1, 2008 through February 28, 2010.

Surveillance was conducted in the Pediatrics Department of Santa Casa de Misericórdia Hospital; a tertiary care center that receives patients referred from other centers and serves as a primary hospital for surrounding communities. Enrollment and sample collection was performed Monday through Friday from 8:00 a.m. to 12:00 p.m. Children under two years-old were eligible for enrollment if they were admitted to the hospital and had acute respiratory symptoms of cough and/or difficult breathing or had an admitting diagnosis of bronchiolitis, pneumonia, wheezing, croup, pertussis, paroxysmal cough, apnea and cyanosis. An illness was considered associated with a specific pathogen if it was detected by polymerase chain reaction assays (PCR) as described below. The unit of analysis was hospitalizations, so individuals may be enrolled more than once over the surveillance period.

Information regarding symptoms, history of underlying health conditions and immunization status, were collected from parent/guardian interview and medical records. Antibiotic use, length of hospital stay, oxygen use, need for intensive care and mechanical ventilation data were retrieved from medical records.

After obtaining consent for enrollment and specimen collection from parents/guardians, a single nasopharyngeal aspirate (NPA) was obtained during each hospitalization by a respiratory technician. NPAs were kept under refrigeration at 4-6 °C within one to five hours and were then divided into three cryotubes per sample and stored in liquid nitrogen. Weekly, specimens were sent to the virology laboratory of the University of São Paulo for detection of respiratory viruses by PCR. All samples were tested for the following: respiratory syncytial virus (RSV), human metapneumovirus (hMPV), parainfluenza virus 1, 2 and 3 (PIV1-3), influenza virus A and B (IA, IB) and adenovirus (ADV). 2009 pandemic influenza A H1N1 (IA-pH1N1) was tested in samples obtained during the months of January 2009 through February 2010. PCR and reverse transcription (RT)-PCR assays were developed using Gene Scan analysis with primers previously described.[11–15] IA-p H1N1 was detected by real time PCR assay developed by the Center for Disease Control and Prevention (CDC RT-qPCR Swine Flu Panel).[16] The protocol specified that samples should be collected within 24 h of admission. If ordered by a clinician, the results of blood cultures were also recorded.

Vaccination in Brazil is public and accessible to all citizens. Private clinics are permitted and can provide vaccines and other prophylaxis not included in the National Immunization Program (NIP). The Brazilian NIP provides influenza vaccine to all children from 6 months to 5 years of age and all age groups with underlying conditions or risk factors.[17] There is a recent federal recommendation for RSV prophylaxis. Nevertheless, some states, including São Paulo where this study was carried out, have specific recommendations regarding RSV prophylaxis and provide, free of charge, palivizumab with 5 doses during the seasonality for infants born premature, under 12 months if $\leq$ 28 weeks of gestational age (WGA) and all children $<$ 2 years of age with chronic lung disease or severe congenital heart disease since 2007.[18] The Brazilian Pediatric Society[19] recommends universal influenza vaccination to all children older than 6 months and extends RSV prophylaxis to premature infants born $\leq$ 31 weeks and 6 days, infants with neuromuscular diseases, severe immunosuppression, and congenital abnormalities of the airways. Palivizumab should also be considered in a 3 doses regimen for those premature infants born with a gestational age of 32 weeks to 34 weeks and 6 days with at least one risk factor and born 3 months before or during RSV season.[19]

The study was approved by the Research Ethics Committee of Santa Casa de Misericórdia Hospital and by the University of São Paulo. Written informed consent was obtained from the parent or guardian of each child enrolled in the study.

2.2. Data analysis

Data analysis was limited to enrolled children who had a respiratory specimen collected according to the study protocol criteria. Categorical variables were compared using χ^2 test or Fischer's exact test, when appropriated. Student t test, Mann-Whitney test or the One-way ANOVA were used for the continuous variables. Odds ratio was determined to associate underlying conditions with hospitalization by specific respiratory virus. A multivariate logistic regression model was performed to take into account the other known risk factors for severe disease and need of intensive care. Age and hospital days were not normally distributed. Mann-Whitney U test was used to compare age and hospital days in the group of healthy versus underlying disease. All the analyses were performed with SPSS software, version 17.0. Statistical significance was considered when $p < 0.05$.

3. Results

3.1. Surveillance population and laboratory analysis

During the surveillance period, there were 760 hospitalized patients who met the eligibility criteria. Of those eligible, all had an NPA obtained, but 41 specimens were obtained >24 hours after admission and 4 specimens were excluded due to inadequate sample collection. Therefore, we included 715 (94%) specimens from all eligible patients, adding up to a total of 622 individuals.

Respiratory viruses were identified in 378 (53%) of the 715 specimens. RSV was the most commonly identified virus (52% of positive specimens), followed by adenovirus (27%), hMPV

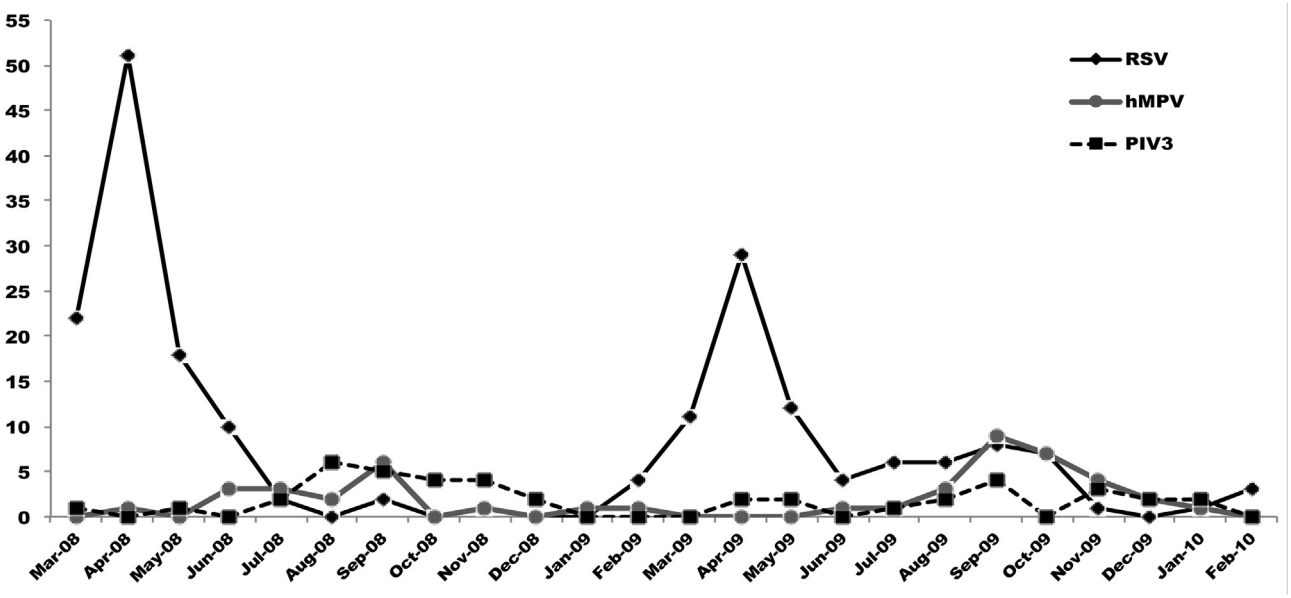

Figure 1. Monthly distribution of respiratory viruses (RSV, PIV3 and hMPV) among children hospitalized with acute respiratory tract infection from March 2008 to February 2010, in a tertiary hospital in São Paulo city, Brazil. Data are presented in total number of cases.
RSV: human respiratory syncytial virus; hMPV: human metapneumovirus; PIV3: parainfluenza virus.

(12%), PIV3 (11%), IA (7%), IA-pH1N1 (4%), IB (3%), PIV1 (3%) and PIV2 (1%). More than one virus was identified in 19% of specimens. A marked seasonality pattern could be observed in the RSV, hMPV and PIV3 infections (Figure 1).

Overall, 73% of the children hospitalized were under 12 months and 49% six months or younger. Boys were predominant in this population (61%). Almost half of the cases (n=330) were from children with underlying health conditions including but not limited to history of premature birth (19.9%), congenital heart disease (12%), chronic lung disease (6.7%) and Down syndrome (1.7%). Young infants were less likely to have an underlying health condition than those older than 6 months (p=0.02).

Information regarding use of influenza vaccine and/or palivizumab for RSV prophylaxis was available for 588 of the 622 children included. We identified 102 (14%) patients who met eligibility criteria for RSV prophylaxis, considering recommendations from the Brazilian Pediatric Society.[19] In all, only nine patients had received palivizumab and 17 were previously vaccinated for influenza.

3.2. Clinical characteristics and outcomes

Of the 715 hospitalizations, 102 (14%) included intensive care and 92 (13%) required mechanical ventilation. Of those 102 children admitted to the intensive care unit (ICU), 58 had a respiratory virus detected with RSV being the most commonly detected among this group (57%), followed by ADV (36%) and PIV (15%).

The most frequent clinical diagnosis was bronchiolitis, attributed to 230 hospital admissions. Among these, 64% had a respiratory virus detected, RSV being the most frequent (42.6%). Detection of more than one virus occurred in 18.4% of cases. The mean age of the patients with bronchiolitis was 4.8 months, and only 15% had a history of underlying health condition. In this group 2.6% required intensive care.

Comparing healthy children (n=381) to those with risk factors or underlying disease (n=330), we demonstrated that children with at least one underlying condition were significantly older, stayed longer in the hospital, were more likely to be admitted to the ICU, were more likely to require mechanical ventilation and had less virus co-infection (Table 1). Young age and underlying diseases were both independent risk factors for hospitalizations and severe disease. Multivariate analysis with logistic regression demonstrated that although young age is a risk factor for admission in intensive care unit, when there is an underlying condition the risk is increased by 2.5 times.

Looking at only single-virus detections (n=292), a greater proportion of hospitalizations due to hMPV and PIV infections were in children with underlying health conditions compared to others (p=0.003 and p=0.03, respectively). The opposite was observed with the RSV-related hospitalizations, being the majority of the patients (58%) previously healthy (p<0.005) (Table 2).

A difference was also observed in the frequency of antibiotics prescription. Children admitted with an adenovirus (p<0.0005) or an influenza virus (p=0.01) received more antibiotics than the other single-virus detections. Descriptive analysis revealed a positive association between detection of an adenovirus and need of intensive care (20.6% ADV positive vs 13.1% ADV negative; chi-square=4.048; p=0.044). The same was not observed for influenza virus (4.9% FLU positive vs 7.5% FLU negative; chi-square=0.901; p=0.343)

Analyzing infants with diagnosis of bronchiolitis in the single-virus detection group and comparing situations where RSV was

Table 1
Comparison of severity measures in children hospitalized with acute respiratory tract infection from March 2008 to February 2010, in a tertiary hospital in São Paulo city, Brazil. N=711*.

	Underlying Disease (N=330)	Healthy (N=381)	p
Age (months)#	8	6	**0.000**
Hospital days#	7.5	5	**0.000**
Intensive Care Unit	63	39	**0.001**
Mechanical ventilation	55	37	**0.006**
Deaths	7	2	0.089
Antibiotics	178	180	0.074
Positive Blood Cultures	28/285	20/309	0.134
Positive Respiratory Virus	162	212	0.081
Virus Coinfection	25	47	**0.036**

* Four children from the total of 715, did not have information on history of underlying disease; # expressed in median;
Statistics: Age and Hospital days: Mann-Whitney U; others x^2 and Fisher exact test.

Table 2
Comparison of number of single viral infections regarding presence of underlying conditions in children hospitalized with acute respiratory tract infection from March 2008 to February 2010, in São Paulo city, Brazil. N=292.

	Underlying Disease (N=131)	Healthy (N=161)	p*	OR	95% CI
RSV	48	94	**<0.0005**	0.41	0.26–0.66
hMPV	24	11	**0.003**	3.06	1.44–6.51
PIV	21	12	**0.03**	2.37	1.12–5.02
FLU	17	13	0.18	1.70	0.79–3.64
ADV	21	31	0.54	0.81	0.43–1.47

* p chi-square
RSV: human respiratory syncytial virus; hMPV: human metapneumovirus; PIV: parainfluenza virus; FLU: influenza virus; ADV: adenovirus.

the sole virus identified (n=76) with other single-virus etiologies (n=49), those with RSV had a tendency to be younger (mean of 4.4 months *vs* 5.5 months; p=0.07) and had fewer comorbidities (18.4% of underlying conditions in RSV positive *vs* 36.7% non-RSV; p=0.004). No difference was found in the need for intensive care (4% in both groups).

Twelve children had Down syndrome (DS). Congenital heart disease was present in 11 patients and three patients had a history of premature birth. Two children, with need of mechanical ventilation, demanded intensive care. Half of patients had a respiratory virus detected: hMPV (n=2); IA (n=2); ADV (n=1); IA + PIV3 + ADV coinfection (n=1). One child died. She had a cyanotic congenital heart disease and was admitted with a non-alveolar pneumonia. Blood cultures were negative and hMPV was detected in the NPA.

Death occurred in nine (1%) of the 622 children. Six of the nine infants that died had a respiratory virus detected, distributed as follow: PIV 1 (n=2); hMPV (n=1); RSV (n=1); ADV (n=1) and one coinfection ADV+RSV. All six had an underlying condition and required intensive care with mechanical ventilation.

4. Discussion

In this study we evaluated acute respiratory tract infection leading to hospitalization in young children in a large urban center in Brazil. We found that a large proportion had underlying conditions (46%) and that compared to the previously healthy group, those with comorbidities had more severe outcomes, with prolonged hospital stay and more need of intensive care.

This special population also behaved in a different manner than usually described in healthy children. Viruses more associated with mild symptoms were responsible for acute respiratory failure and death. This was demonstrated with human metapneumovirus and parainfluenza viruses, usually associated with less severe disease.

Since its discovery, human metapneumovirus has been related to acute respiratory tract infection in infants worldwide. Heikkinen et al in a study with 1,338 children under 13 years old with acute respiratory infection demonstrated a higher incidence rate in children less than 24 months. In this cohort of previously healthy children, most of the infected with hMPV had mild or moderate symptoms, with no need of hospitalization.[20]

Whereas a high-risk population has a greater chance of having a poor outcome with viruses that are usually associated with mild disease (ex: hMPV), it is possible that some respiratory viruses, such as RSV and ADV, are indeed causing more severe infections, irrespectively of health condition status.

Marguet et al, in a study evaluating the role of each respiratory virus in the severity of bronchiolitis, showed that young infants (median age 2.4 months), previously healthy, had a more severe presentation when RSV was causing the disease, compared to other respiratory viruses. On the other hand, when hMPV was causing bronchiolitis, there was a lower risk of prolonged hospital stay.[21]

We observed significant clinical and demographic differences among the different etiologies in the infants with a diagnosis of bronchiolitis. When RSV was the causative agent, patients had fewer comorbidities (p=0.004) and had a tendency of being younger (p=0.07) than patients with other viruses. Garcia et al in a publication of risk factors in 4,285 children hospitalized due to bronchiolitis, comparing RSV *versus* non-RSV cases, reported similar results, with more underlying diseases in the group without RSV (37.5% *vs* 27% of the RSV positives).[22]

The detection rate of each of the respiratory viruses studied was similar to other studies that used comparable diagnostic methods and study population.[23,24] We found a high prevalence of adenoviruses in our samples, corresponding for the second most frequent virus detected (27% of the positive samples). The frequency of adenoviruses varies in the different studies depending on population selected for screening and on the method applied for diagnosis. Studies that compare the sensitivity of the different diagnostic methods, demonstrated that PCR increased the number of positive cases by three to four times when compared to immunofluorescent assays.[25,26] Nevertheless, local epidemiological characteristics, such as overcrowding or poor sanitary conditions, may be involved, considering the results published by Moura et al in Brazil, where 26.6% of the positive samples in children hospitalized with LRTI had an adenovirus. They screened for respiratory viruses in a hospital in São Paulo city, during the years of 1995 and 2000 using immunofluorescent assays.[27]

Bezerra PGM et al.,[28] also reported similar results in a surveillance study carried out in children under 5 years old in a Northeastern region in Brazil. They collected samples from 407 children (median age 8 months) including hospitalized and outpatients. RSV was the most frequent pathogen (37.3%), followed by adenovirus (24.8%) and hMPV (10.3%). Severity analysis revealed that children with RSV were more likely to be hospitalized than those infected with other viruses.

Down syndrome is associated with a worse outcome in respiratory viral infections when compared to healthy infants. Population-based cohort studies demonstrated a rate of hospitalization in children with DS five times greater, with a longer hospital stay than general population, even in the absence of concurrent risk factors.[29,30] Respiratory infections are considered to be the second cause of death in children with DS.[31]

There was an underuse of the already available and accessible prophylaxis for RSV and influenza viruses in the study population. Several studies have demonstrated a reduction on hospitalization rates and mortality in high-risk patients that receive passive prophylaxis against RSV infection.[32,33] In the last five years there was an extension in the inclusion criteria of these prophylaxes in Brazil, with major changes in 2014. Influenza vaccine was given to all children under 5 years of age, rather than the previous 2 years of age limit and palivizumab was incorporated as a national program and not limited any more to only a minority of the Brazilian states. These changes, probably a result of improvement in the respiratory virus surveillance system throughout the country that occurred as a response to the 2009 pandemic influenza A H1N1 epidemic, promoted awareness to health care professionals and the population to these existing prophylaxes. Some studies already demonstrate an increment in the number of doses of influenza vaccine during the last five years.[14]

Our study had some limitations. Samples were not screened for all of respiratory viruses known to cause lower respiratory tract infection in this age group, such as rhinovirus, other enteroviruses, coronaviruses and human bocavirus, which probably explains the low virus detection rate. Other respiratory pathogens that cause severe respiratory disease in young infants, such as *Bordetella pertussis*, were also not included.[34] Therefore, a broader diagnostic panel, including these agents, might have provided a more precise

analysis. However, the viruses included in this study are well-established causes of respiratory infections, with low rates of viral coinfection and, except from adenoviruses, low rates of viral persistence, making it easier to compare the differences in etiology.[3]

In conclusion, in this study we found that young age and underlying diseases are both independent risk factors for worse outcome in children hospitalized with a respiratory virus. Identification of this special population by health care professionals along with fast diagnosis of the viral nature of the disease, allow a more aggressive approach that may help to reduce morbidity and mortality in this group. Recent changes in Brazil, with broader access of the available prevention tools, through directed programs for high-risk population, will probably result in the reduction of severe respiratory infections in children. Future studies evaluating this new scenario are necessary to confirm the positive impact of these prophylaxes.

Ethical Approval

The study was approved by the Research Ethics Committee of Santa Casa de Misericórdia Hospital and by the University of São Paulo. Written informed consent was obtained from the parent or guardian of each child enrolled in the study.

Conflict of Interest Statement

Some of the authors have financial competing interests. Giuliana Stravinskas Durigon and Edison Luiz Durigon received lecturing fees from Abbott Brazil. Eitan Naaman Berezin received lecturing fees from Wyeth Pharmaceuticals Inc.

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