Twenty Years of Outpatient Respiratory Syncytial Virus Infection: A Framework for Vaccine Efficacy Trials

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ABSTRACT. *Background.* **Respiratory syncytial virus (RSV) is the most important viral respiratory pathogen of infancy and childhood. Much has been written about inpatients with severe disease. Inpatients, however, represent only a minority of RSV-infected children. We studied the characteristics of symptomatic outpatient RSV infection in healthy children to gain a better understanding of RSV disease and to provide a background for the testing of intervention strategies in children without high-risk conditions.**

Methods. **A total of 1113 children were followed during 20 consecutive RSV seasons. Signs and symptoms of respiratory infection were monitored. Cultures were obtained for febrile upper respiratory infection, acute otitis media, and lower respiratory infection (LRI). Rates of febrile upper respiratory infection, acute otitis media, LRI, and hospitalization were calculated. Given those rates, numbers of children needed to demonstrate efficacy of a vaccine product were calculated.**

Results. **Mild disease from RSV infection lacked some of the classic features of RSV infection seen in hospitalized children. Involvement of the lower respiratory tract was, however, noted to be much higher in RSV infection than it was in infection with other viral respiratory pathogens. LRI was, therefore, considered the best candidate endpoint for vaccine trials. A product with 60% efficacy could be proven, with a power of 0.8, to be efficacious with as few as 1500 infants.**

Conclusions. **RSV infection is common and often involves the lower respiratory tract, even in outpatients. Our 20-year study of RSV infection provides a basis for calculation of sample sizes to be used in trials of vaccine candidates. *Pediatrics* 1997;99(2). URL: http://www.pediatrics.org/cgi/content/full/99/2/e7; *respiratory syncytial virus, outpatient, epidemiology, vaccine, bronchiolitis.***

ABBREVIATIONS. RSV, respiratory syncytial virus; URI, upper respiratory infection; LRI, lower respiratory infection; AOM, acute otitis media.

Respiratory syncytial virus (RSV) is widely recognized as the most important viral respiratory pathogen of infancy and childhood.[1] It causes distinct winter epidemics in a predictable fashion each year[2] and leads to frequent hospitalizations for bronchiolitis and pneumonia. Newborns and young infants are particularly prone to developing more severe lower respiratory tract disease.[3]

More than 50% of infants acquire the infection during their first RSV season,[4] and it is thought that most primary RSV infections are symptomatic.[4] By the time they have lived through two RSV seasons, more than 90% of children demonstrate serologic evidence of infection.[4]

Accordingly, the development of a vaccine for RSV has received a high priority.[5] Many vaccine products are currently undergoing animal studies and early clinical trials. A thorough understanding of the clinical syndrome of RSV infection, including attack rates and symptom frequency in an otherwise healthy outpatient population, will be critical to the design of such trials.

Although many studies of RSV infection in hospitalized patients have been reported,[6–12] surprisingly little is written about outpatient RSV infection.[13,14] We report a longitudinal study of RSV infection that spans 20 years and project, using symptomatic illness endpoints, population requirements for trials of RSV vaccine candidates.

METHODS

Data detailing RSV infection in the outpatient population were obtained from the Vanderbilt Vaccine Clinic of a National Institutes of Health-supported vaccine treatment and evaluation unit for a 20-year period from 1973 to 1993. Healthy full-term infants were enrolled into the clinic population at birth. Children in whom chronic diseases developed were excluded from the study. Children had all of their well and sick care provided by members of the pediatric infectious diseases faculty and staff. Well child care followed American Academy of Pediatrics guidelines as to timing of well infant examinations and routine vaccine administration. At enrollment in the clinic, they were encouraged to participate in vaccine trials as suitable candidates became available and agreed to surveillance for respiratory and enteric pathogens. Parents were instructed to bring their children to the clinic if runny nose, cough, fever, or symptoms suggesting ear infection developed. Members of the faculty and staff were available by telephone 24 hours a day, and patients were seen preferentially by our clinic rather than through emergency departments. During the study period, two trials of vaccine products to RSV were performed. Children who participated in either of these trials were excluded from this study. Enrolled infants represented a cross-section of the Nashville community. Fifty-one percent were male, 53.5% were white, 43.8% were African-American, and 2.8% were of other races. Just more than half had no siblings. Ninety percent were from urban sites, and 10% lived in rural areas. Children were followed for an average of 3.5 years, after which their care was transferred to other sources in the community. Children leaving the clinic were replaced by newborns, to maintain a population of approximately 200 children. An approximation of the age distribution of children in the clinic follows. During the 20-year period,

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of 3615 child-years followed in the clinic, 17% were of children younger than 6 months, 15% were of children from 6 to 12 months of age, 25% were of children 1 to 2 years of age, 19% were of children between the ages of 2 and 3 years, and 24% were of children 3 years or older. All symptoms and signs of respiratory illness were recorded on a standardized clinical form; one upper respiratory tract infection (URI) diagnosis and/or one lower respiratory infection (LRI) diagnosis was made at each respiratory illness visit. Informed consent for participation in surveillance for respiratory and enteric pathogens was obtained with the approval of the Vanderbilt Institutional Review Board.

Nasal wash cultures for virus isolation were obtained for any of the following indications: URI accompanied by fever of 38.4°C or greater, signs or symptoms suggesting LRI, and acute otitis media (AOM). Because this study was begun before the availability of rapid antigen detection tests, these were not performed. Wheezing, rales, rhonchi, respiratory distress, and retractions were considered signs of LRI. AOM was defined as redness and bulging of the tympanic membrane, with loss of normal light reflex and decreased mobility on pneumatic otoscopy. Bronchiolitis was defined as a wheezing illness without evidence of focal infiltrate on a chest roentgenogram. Pneumonia was defined as presence of infiltrate or consolidation on a chest roentgenogram in a patient with rales or decreased breath sounds at physical examination. Nasal washes were performed by instilling 15 mL of sterile saline into one nostril and collecting the wash in a medicine cup. Contents of the cup were immediately transferred to a vial containing 0.5 mL of Pen-Genta-Gel (200 000 units of penicillin and 50 mg of gentamicin in 100 mL of sterile water and 10% gelatin) and placed on ice. Specimens were taken to the laboratory within 3 hours, where they were cultured on human neonatal kidney, human embryonic lung, HEp 2, rhesus monkey kidney, Maden-Darby canine kidney, and Vero cells.

The RSV season was defined as the time between the first RSV isolate after November 1 and the last isolate before April 30 obtained from the clinic population.

Statistical Methods

Relative risks and corresponding confidence intervals are Mantel-Haenszel estimates computed using SAS PROC FREQ (version 6.10; SAS Institute, Cary, NC). Comparison of rates of hospitalization and LRI in RSV-positive children by age group were made using Fisher's exact test computed with StatXact version 2 (CYTEL Software Corp, Cambridge, MA). Power calculations were done using a public domain program by Dupont and Plummer[15] as well as Monte Carlo simulations to confirm the sample sizes for Table 5. A significance level of $P = .05$ was assumed in all calculations.

TABLE 1. Presenting Signs and Symptoms in 241 Outpatients With Respiratory Syncytial Virus Infection

Symptoms	%	Signs	%
Runny nose	89	Rhinitis	84
Cough	86	Abnormal TM	71
Irritability	53	Pharyngitis	55
Anorexia	44	Wheezing	34
Vomiting	31	Fever	33
Wheezing	22	Rhonchi	18
Earache	16	Rales	17
Diarrhea	15		

RESULTS

Demographics

A total of 1427 children were followed over 20 years in the Vanderbilt Vaccine Evaluation Unit. Of these, 1113 were followed during one or more yearly RSV epidemics.

The mean age of patients with positive RSV cultures was 15.7 months. The median age was 13.4 months, with a range of from 2 weeks to 5 years. There was a total of 241 positive cultures. Fourteen cultures were positive in months outside of seasonal outbreaks. Seventeen of the cultures were second isolates.

Presenting Signs and Symptoms

The presenting signs and symptoms of children whose cultures were positive for RSV are summarized in Table 1. Coryza and cough were the most common respiratory symptoms. About half of the patients were irritable and had decreased appetite. Vomiting was reported in about one third of patients. Pharyngitis was found by physical examination in more than half of the patients; it was much more common in older children and was not seen in infants.

Comparison With Other Respiratory Viruses

Ninety-two percent of RSV-positive patients were given a diagnosis of URI. This figure did not differ from that associated with other respiratory viruses isolated, ie, influenza A and parainfluenza types 1 through 3. Not surprisingly, because AOM was a criterion for obtaining viral cultures, AOM was the most common upper respiratory diagnosis, at 51.5%. However, the frequency of associated AOM was relatively even spaced among the common respiratory viruses (Table 2).

In contrast, RSV was found to be much more likely than other respiratory viruses to involve the lower respiratory tract. Table 2 shows the percentage of RSV-infected patients who had LRI and the relative risk compared with that of other respiratory viruses. Of children who presented with wheezing during an RSV season and had a culture positive for any respiratory virus; 70% grew RSV. Physical examination signs of LRI, ie, wheezing, rhonchi, and rales, were seen in 34%, 18%, and 17% of RSV-infected children, respectively. Involvement of the lower respiratory tract in RSV infection was age related and exceeded 50% in early infancy, as data on the very young age of hospitalization would suggest. After a steep de-

TABLE 2. Incidence and Relative Risk of Acute Otitis Media (AOM) and Lower Respiratory Infection (LRI) in Respiratory Syncytial Virus (RSV) Versus Other Respiratory Viral Infections

	RSV (n = 241)	Influenza (n = 268)	Parainfluenza 1 (n = 83)	Parainfluenza 2 (n = 33)	Parainfluenza 3 (n = 158)
AOM, %	51.5	41.8	37.3	30.3	51.3
Relative risk	1.0	0.81	0.72	0.59	1.0
95% confidence interval		0.67–0.98	0.53–0.98	0.35–1.01	0.83–1.2
LRI, %	37.8	10.8	16.8	15.1	15.1
Relative risk	1.0	0.29	0.44	0.40	0.39
95% confidence interval		0.19–0.42	0.27–0.74	0.18–0.9	0.28–0.63

cline, the incidence of LRI in RSV culture-positive children did not change significantly from 18 months to 4 years of age (Figure; $P = .78$). Of RSV-positive patients who had an LRI diagnosis, 67% had bronchiolitis, and 23% had pneumonia. Young infants were more likely to be given a diagnosis of bronchiolitis. The median age of patients with a diagnosis of bronchiolitis was 7.9 months; for pneumonia, it was 14.2 months.

Primary Versus Secondary Infection

Of the 241 positive cultures, all but 17 were primary isolates. Nine of the 17 had LRI at the time of their first RSV isolation. Of those nine, 7 had a URI diagnosis on second infection. Of the 8 patients whose primary infection caused a URI, only 1 had a LRI diagnosis on second infection.

Hospitalization

Almost 5% of all RSV culture-positive patients (n = 227) required hospitalization for their disease. Forty-two percent of the hospitalized patients were younger than 3 months, although they made up only 9% of the clinic population with positive cultures. Table 3 compares hospitalization rates of RSV-positive children by age.

Table 4 shows AOM, URI, LRI, and hospitalization rates by age in patients with positive RSV cultures. Of those four possible endpoints, LRI and hospitalization are the most practical. Using the rate of LRI with RSV infection for the 20-year period as an endpoint, it becomes possible to calculate sample sizes of healthy children needed to show efficacy of an investigational vaccine product or immunoprophylaxis in a randomized, placebo-controlled, prospective clinical trial. Table 5 depicts sample sizes needed to show vaccine efficacy rates of 40% to 80% against RSV LRI with a power of 0.8 or 0.9.

TABLE 3. Hospitalization Rate of Respiratory Syncytial Virus-infected Patients by Age Groups*

<3 mo	3–6 mo	6–15 mo	>15 mo
5/21	3/34	3/72	0/100
23.8%	8.8%	5.3%	0.0%

* $P < .0001$ for full table (Fisher's exact test); $P = .023$ for 0 to 15-month table.

TABLE 4. Seasonal Illness and Hospitalization Rates Attributable to Respiratory Syncytial Virus

Illness*	0–6 mo, per 100 Child-Seasons	0–12 mo, per 100 Child-Seasons	0–24 mo, per 100 Child-Seasons
URI	7.0	7.3	8.2
LRI	4.5	4.4	3.7
AOM	3.5	4.4	4.7
Hospitalization	1.1	0.8	0.6

* URI indicates upper respiratory infection; LRI, lower respiratory infection; and AOM, acute otitis media.

DISCUSSION

The characteristics of symptomatic RSV infection in outpatient populations have not been well described. Our outpatient evaluation in the Vanderbilt Vaccine Clinic for 20 years defines the spectrum of RSV disease and provides help in designing clinical trials in healthy infants.

Isolation of RSV from a nasal wash was associated with upper respiratory tract disease in virtually all children. Afebrile children with mild cold symptoms and asymptomatic children were not cultured. Prior studies have shown that recovery of RSV from well children is rare.[4] RSV was largely an afebrile illness, with only 33% of patients having fever at the time of their clinic visits. Physical examination revealed coryza in most. The tympanic membrane was seen to be abnormal in 71%, although only 50% met the diagnostic criteria of AOM. The incidence of AOM

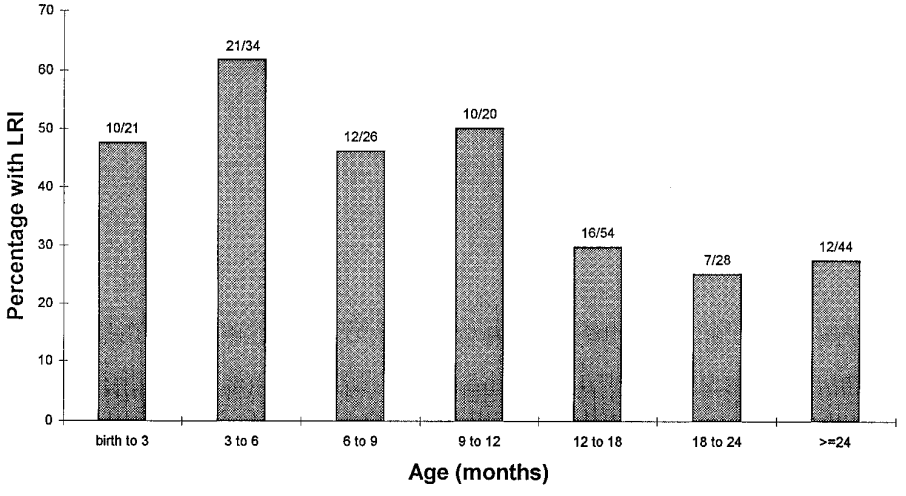

Figure. Graph showing the proportion of lower respiratory tract infection in subjects with cultures positive for respiratory syncytial virus, by age. Please note that children are grouped by 3-month intervals to 12 months of age, then by 6-month intervals to 24 months of age, and finally all children 24 months or older are grouped together. The fractions above each bar indicate the number of children who had lower respiratory infection, over the total number of respiratory syncytial virus isolates. Nonseasonal isolates were excluded. The difference in proportion with lower respiratory infection between those younger than 12 months and those older than 12 months is significant ($P = .0007$).

TABLE 5. Sample Sizes Needed for Vaccine Trials Using Respiratory Syncytial Virus Lower Respiratory Infection as the Endpoint

Vaccine Efficacy, %	Children 0–12 mo				Children 0–24 mo,	
	Power of 0.8		Power of 0.9		Power of 0.8	
	n/Group	Total n	n/Group	Total n	n/Group	Total n
40	1840*	3680	2420	4840	2200	4400
50	1130	2260	1480	2960	1350	2700
60	750	1500	980	1960	900	1800
70	530	1060	680	1360	630	1260
80	380	760	490	980	460	920

* Numbers have been rounded up to even 10s.

was highest in late infancy and the early toddler months (9 to 15 months), a time that parallels the increased incidence of AOM seen in conjunction with a variety of viral agents. Because we did not routinely perform tympanocentesis, we cannot define the microbiologic causes of these infections. The bacteriology of AOM is well known; the possible role of RSV in the middle ear is not clearly understood. In one study, RSV antigens were found in the middle ear fluid of 15% of children with AOM, and in 7% RSV was the sole pathogen found.[16] Studies in both hospitalized patients[17] and in a day-care setting[18] have suggested that the incidence of AOM is higher in RSV infection than it is in infection with other respiratory viruses (especially parainfluenza 1 through 3 and rhinovirus). Our outpatient data do not reveal striking relative differences among viruses in viral-associated AOM. In the present study, cultures were obtained during acute illness rather than as surveillance. This difference in study design may explain the disparate results.

One third of outpatients with culture-proven RSV disease had wheezing either by history or by physical examination. Our culture criteria, which emphasize obtaining cultures in children with signs of LRI, may tend to bias the data toward an overestimation of RSV-associated wheezing. However, the propensity of RSV to involve the lower respiratory tract is unique among the common respiratory viruses and lends support to the idea that when young children, especially infants, present during RSV season with signs and symptoms suggesting LRI, RSV is by far the most likely pathogen. In fact, the incidence of influenza A and parainfluenza types 1 and 2 often drop precipitously when RSV is circulating.[1] The incidence of LRI is highest in early infancy. After an initial drop, however, it remains steady through at least 4 years of age (Figure).

Although our number of second infections was too small to establish statistical significance, the trend toward less severe disease with subsequent reinfection was clear and parallels data from experimental infection in adult volunteers.[19] This is also consistent with the observation that most hospitalization occurs after primary exposure to RSV during the first year of life.[20]

Using our data to establish endpoints of RSV-associated disease in a group of unimmunized healthy controls allows the determination of power calculations for clinical trials of investigational vaccines. URI is unlikely to be a satisfactory endpoint because of its frequency and the variety of causative agents. Culturing all patients with URI would be burdensome; our experience shows a yield of only 13.4% in culturing those with febrile URI. URI without fever would likely be even less efficient. AOM would be a poor choice for an endpoint measure as well, owing to the fact that even during RSV season patients with AOM are not more likely to have RSV than they are to have other respiratory pathogens. Because previously published[4,10] and our hospitalization rates attributable to RSV infection are low (only 0.9% in patients from birth to age 12 months and 0.58% in patients from birth to 24 months in our study), we view hospitalization as an impractical endpoint; very large numbers of children would need to be enrolled to prove efficacy. Effectiveness of intervention strategies against hospitalization for RSV infection could be more easily evaluated in cardiac and pulmonary patients because of their higher risk of severe disease.[21] LRI, on the other hand, is a common enough occurrence in RSV infection to be a useful clinical endpoint, especially in children younger than 1 year. Fewer than 1500 infants would need to be enrolled in a trial to demonstrate 60% or greater efficacy of an investigational vaccine product against culture-proven LRI.

In summary, our data underscore the enormity of the public health problem created by RSV. We more fully describe the characteristics of RSV infection in nonhospitalized infants and young children and provide a numerical framework for vaccine trials. It is this group of young children, at highest risk for severe RSV disease, that will need to be targeted for intervention, both prophylactic and therapeutic. It is a complex problem; immunization will have to be completed in the first few months of life. It may prove more difficult to stimulate immunity in very young infants. Additionally, the exact immune correlates of protection and the degree of protection afforded by a single exposure are not clearly elucidated. Our experience with prior RSV vaccine preparations, as well as with influenza vaccines,[22] suggests that two doses may be required to stimulate protective immunity. Ongoing studies of novel treatment strategies such as passive immunization offer possible alternatives, especially for those in the highest risk group for universal vaccination.

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