

## Epidemiology of respiratory syncytial virus bronchiolitis in hospitalized infants in Greece

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**Abstract.** New therapies have been introduced for the prophylaxis and treatment of respiratory syncytial virus (RSV) infection in recent years. The aim of the study was to determine the epidemiological and clinical characteristics of infants hospitalized with bronchiolitis in our area. All patients under 1 year of age admitted with acute bronchiolitis during four consecutive RSV seasons from February 1, 1997 to June 30, 2000 were enrolled in the study. The records of patients admitted during the first season were reviewed retrospectively while the rest were followed prospectively. A total of 636 infants with bronchiolitis were admitted and RSV infection was documented in 61% of those tested. Admission to intensive care unit (ICU) was required for 6.2% of them and was more common in premature infants (26%) ( $p < 0.001$ ). Case fatality rate was 0.7% (overall 0.3%). RSV

bronchiolitis accounted for about 12% of all infant admissions during the 5 months of the yearly outbreak. Patients with documented RSV infection had a more severe illness with a higher ICU admission rate (6 vs. 1%,  $p = 0.008$ ) and longer duration of hospitalization (mean 6.3 vs. 5.3 days,  $p < 0.001$ ) compared to those who tested negative. Although none of the patients had a positive blood culture on admission a considerable number of them (210/636, 33%) were treated with antibiotics. RSV infection has a significant impact on infant morbidity in our settings which is more serious among those born prematurely. Documentation of RSV infection may be a marker of more severe illness in infants hospitalized with bronchiolitis. Antibiotic use has to be restricted since the occurrence of a serious bacteraemic illness on admission is a very rare event.

**Key words:** Bronchiolitis, Epidemiology, Greece, Respiratory syncytial virus

**Abbreviations:** ICU – Intensive care unit; RSV – Respiratory syncytial virus

### Introduction

Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infection in infants worldwide. Infection by this virus most commonly manifests as acute bronchiolitis but it may also cause laryngotracheobronchitis and pneumonia [1]. New approaches have been introduced in recent years for the prevention and treatment of RSV infections mainly targeting infants at high risk for the development of severe illness such as those born prematurely, infants with chronic lung disease and other underlying conditions [2, 3]. Although research efforts for vaccine development have progressed slowly an RSV vaccine may become available in the future. The association of RSV infection with airway hyper-reactivity has been discussed over several decades, but is unresolved and requires continuing investigation [4]. For all these reasons there is increased interest lately in RSV epidemiology. In each community, the epidemiological impact of these infec-

tions has to be determined prior to the implementation of novel and costly therapies. This knowledge will contribute to the rationalization of antibiotic use in the management of infants with bronchiolitis.

Previous studies on RSV epidemiology from southern European and Mediterranean countries [5–8] have noted that the incidence of infection, age distribution and seasonality are similar to that recorded in northern and Central Europe [9–11] or the US [12, 13]. However, the impact of this infection has been thoroughly examined in only a few studies [7, 9, 14] and significant differences in disease severity have been found between different countries [14, 15] and even between different cities or populations within the same country [7]. In addition, considerable variation in the management of patients with bronchiolitis has been demonstrated between different European countries [16]. The aim of this study was to examine the frequency, clinical characteristics, severity and impact of RSV infection on infants under

1 year of age hospitalized with bronchiolitis in the Athens area. The rate of serious bacteraemic illness and the use of antibiotics in these infants was also evaluated.

## Methods

### *Patient enrollment*

Patients under 1 year of age who were admitted to the Second Department of Pediatrics of the University of Athens at the P. and A. Kyriakou Children's Hospital with the diagnosis of bronchiolitis were enrolled in the study. The study lasted from February 1, 1997 to June 30, 2000 and included four consecutive RSV seasons. The records of patients admitted between February 1, 1997 to December 31, 1997 were retrospectively reviewed while patients hospitalized during the following three RSV seasons were followed prospectively. The study was initiated in February 1997 when a rapid laboratory diagnostic procedure for RSV infections became available. Patients admitted with the diagnosis of bronchiolitis were included in the study if they had at least three of the following: tachypnea ( $>50$  respirations/min), use of accessory respiratory muscles, prolonged expiration or wheezing, diffuse crackling rales and hyperinflation with or without peribronchial thickening, atelectasis or infiltrates in case a chest-X-ray was obtained. Only patients with first episode of bronchiolitis were included. In each case a standardized form was filled with data extracted from each patient's chart.

A blood and a urine culture was obtained from every patient who had fever ( $\geq 38$  °C, axillary) or ill appearance and whenever clinical deterioration was noted or treatment with antibiotics was initiated. A lumbar puncture was also performed in patients who appeared septic. Bacteraemia was defined by the isolation of a microorganism not considered to be a contaminant from a patient's blood culture. The BacT/Alert<sup>®</sup> PF and SN blood culture bottles were used with the BacT/Alert<sup>®</sup> microbial detection system (Organon Technika Corporation, USA).

Discharge diagnoses were reviewed for all infants under 1 year of age admitted to our pediatric Department during the months of the yearly outbreak between January and May each of the four study years. The proportion of infants admitted with the diagnosis of bronchiolitis was thus estimated.

The study was approved by the Hospital's Ethics Committee. Parents of patients were informed and written consent was obtained during the prospective part of the study in order to collect a nasopharyngeal aspirate from each infant.

The P. and A. Kyriakou Children's Hospital is one of the two largest tertiary care centers in the Athens area with about 23,000 admissions each year of which

4000 are admitted to our Department. The hospital admits patients on alternate days with the Aghia Sophia Children's Hospital and the two centers carry the main burden of acute care provided to pediatric patients in the area. According to data provided by the National Statistical Service the 1998 midyear estimate of the city population was about 3.5 million inhabitants. Athens is located in central Greece with geographic longitude 23°45' and latitude 37°54'.

### *Diagnosis of RSV infection*

A nasopharyngeal wash was obtained from each patient within 48 hours of admission. An 8 Fr size catheter (Vygon, France) was introduced through each nostril into the nasopharynx. The catheter was attached to a trap connected to electrical suction. Two milliliters of normal saline were instilled in each nostril and the suctioned mucus was collected into the trap. The specimen was kept at 4 °C for no longer than 48 hours and was tested for the presence of RSV antigen. RSV antigen was detected in exfoliated respiratory cells by direct immunofluorescence according to manufacturer's instructions (Monofluo Screen RSV, Sanofi Diagnostics, Pasteur).

### *Statistics*

Subjects were divided into two groups based on their RSV status. RSV positive infants were compared to those who tested negative according to their demographics, clinical and radiological characteristics. Continuous variables were compared by the Mann-Whitney test and categorical variables with the  $\chi^2$  or Fisher's exact test. A two tailed *p* value  $<0.05$  was considered significant. Odds ratios with 95% confidence intervals (CI) were calculated.

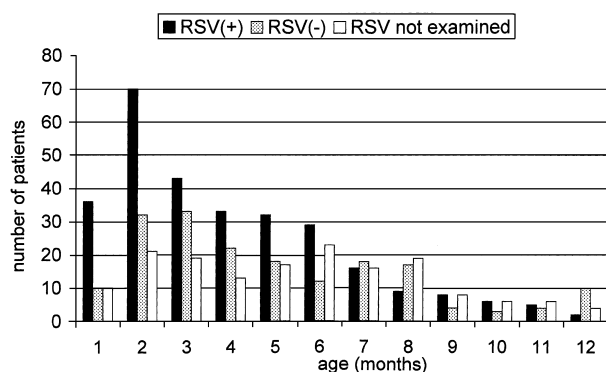
## Results

### *RSV infection in hospitalized infants with bronchiolitis*

A total of 636 patients were enrolled during the study period. Of them 473 were examined for RSV antigen and 291(61.5%) were positive. The proportions of patients with documented RSV infection during the four RSV seasons examined (from January to May of each year) were 84.6% (1997), 59.5% (1998), 56.7% (1999) and 63.3% (2000) respectively.

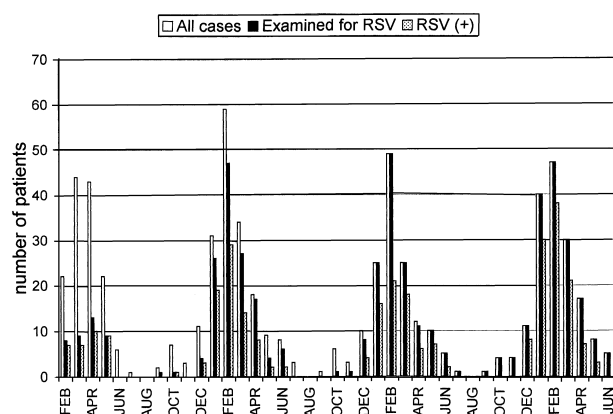
### *Age and sex of the patients*

Males were more commonly affected than females and the male to female ratio was 1.8 for both the RSV positive and negative patients. As shown in Figure 1 most (75%) of all patients with bronchiolitis were less than 6 months old. Of the infants with RSV infection 51% were under 3 months and 83% under 6 months



**Figure 1.** Age distribution of the 636 patients under 1 year of age admitted with the diagnosis of bronchiolitis.

of age compared with 39 and 68% of the RSV negative patients, respectively. Most cases of RSV bronchiolitis occurred in the second month while most of the RSV negative cases were noted in the third month of life. Median age of patients with RSV infection (2.8 months) was younger than that of the RSV negative ones (4.5 months) ( $p < 0.001$ , Table 1).



**Figure 2.** Seasonal distribution of the bronchiolitis cases admitted during three consecutive RSV seasons between February 1, 1997 and June 30, 2000.

#### Seasonal distribution

The RSV season started in December each year and lasted until May. Most cases occurred between January and March with the peak in February (Figure 2).

**Table 1.** Comparison of clinical and radiological characteristics of hospitalized infants with acute bronchiolitis who were examined for RSV antigen

Characteristic	RSV positive <sup>a</sup> N = 291	RSV negative <sup>a</sup> N = 182	p-Value	Odds ratio (95% CI)
Male/female ratio	1.8	1.8		
Age (months), median	2.8	4.5	<0.001	(0.477–1.517) <sup>b</sup>
Prematures $\leq$ 36 weeks	12	10.5	NS	
Hypoxemia				
$O_{2sat} < 95\%$	64 ( $n = 157$ )	52 ( $n = 95$ )	0.046	1.690 (1.01–2.84)
Tachypnea ( $\geq 50$ /min)	75.5	69.5	NS	
Retractions	71	65	NS	
Crackles	75	63	0.042	1.761 (1.13–2.74)
Fever ( $\geq 38$ °C, axillary)	30	25.5	NS	
Infants aged < 3 months	26	24	NS	
Infants aged $\geq$ 3 months	38	23.5	0.042	1.994 (1.16–3.43)
Chest-X-ray findings				
Abnormal	91	87	NS	
Hyperinflation	67	49	<0.001	2.135 (1.40–3.25)
Atelectasis	25	9.5	<0.001	3.173 (1.70–5.91)
Interstitial infiltrates	15	30	0.012	0.543 (0.34–0.88)
Consolidation	13	13.5	NS	
Received antibiotics	39	31	NS	
Parenterally	20	13	NS	
Otitis media	16	13	NS	
Abnormal chest-X-ray	11	12	NS	
Possible sepsis	10	4	0.028	2.407 (1.07–5.39)
Received corticosteroids	36	22.5	0.002	1.941 (1.27–2.96)
Received $\beta$ -agonists	55	52	NS	
(Third and fourth year only)	( $n = 185$ )	( $n = 131$ )		
Admitted to ICU	6.2	1	0.008	5.934 (1.36–25.89)
Mechanical ventilation	3.2	0	0.015	1.646 (1.53–1.77)
Hospitalization (days), Mean (SD)	6.3 (4.3)	5.3 (4.6)	<0.001	(0.191–1.817) <sup>b</sup>

CI – confidence interval.

<sup>a</sup> All expressed in percentages except for the male to female ratio, the median age and the median duration of hospitalization.

<sup>b</sup> Numbers in parentheses indicate the 95% CI of the difference between means.

Year to year variations may be observed as in the first year of the study when the peak of bronchiolitis cases was noted in March and many cases continued to occur until May. The seasonal distribution of RSV positive and negative cases was similar.

#### *Prematurity/underlying illness*

A total of 61/636 (9.6%) of all patients with bronchiolitis were premature infants born at a gestational age  $\leq 36$  weeks. Fifteen of them were born at 36 weeks gestation, 38 at 32–35 weeks, seven at 28–31 weeks and one at 26 weeks, respectively. The rate of prematurity was 12% for the RSV positive and 8.3% for the RSV negative patients ( $p = 0.612$ ). Of those not tested for RSV infection 4.3% were premature infants. Only 12/636 (1.9%) of all patients had an underlying illness such as congenital heart disease (8), neurological disorder (1), tracheoesophageal fistula (2) and left lung hypoplasia (1). According to data provided by the National Statistical Service the rate of prematurity ( $\leq 36$  weeks) in Greece during 1998 among the 100,894 births was 4.14%.

#### *Clinical characteristics*

Fever ( $\geq 38$  °C, axillary) was noted in about one-third of the patients (174/636, 27%). Of all infants with fever 56 were younger than 3 months ( $< 1$  month, 10; 1 to  $< 2$  months, 16;  $\geq 2$  to  $< 3$  months, 14). There was no significant difference in the rate of fever between RSV positive (30%) and negative patients (25%). Infants  $\geq 3$  months of age with RSV infection were more likely to be febrile compared to younger ones ( $p = 0.042$ , Table 1).

The rate of hypoxemia, tachypnea, retractions and crackling on admission of the RSV positive and negative patients is shown in Table 1. Infants who tested positive for RSV were more likely to have hypoxemia (64 vs. 52%,  $p = 0.046$ ) and crackling rales (75 vs. 63%,  $p = 0.046$ ).

#### *Radiological findings*

A chest radiograph was obtained in 241/291(83%) RSV positive and 149/182 (82%) RSV negative patients. The radiograph was abnormal in the majority of both the RSV positive and RSV negative patients. Hyperinflation and atelectasis were found more frequently in infants with documented RSV infection compared to those who tested negative. Consolidation was noted at an equal rate and interstitial infiltrates were less common in the RSV positive patients (Table 1).

#### *Severity of illness and complications*

Overall 21/636 (3%) patients were admitted to the intensive care unit (ICU) and 10/636 (1.5%) required

mechanical ventilation. Twenty of the 21 infants admitted to ICU were tested for RSV infection and 18 were positive. The rate of ICU admission was significantly higher for the RSV positive patients (18/291, 6.2%) compared to those who tested negative (2/182, 1%,  $p = 0.008$ , Table 1). Nine RSV positive patients were intubated for 1–18 days and two of them died. Assisted ventilation was required for 9/291 (3.2%) of infants with documented RSV infection while none of the 182 negative infants was intubated ( $p = 0.015$ , Table 1).

Seven of the 18 RSV positive infants who required admission to ICU were premature infants born at 28–35 weeks and two at 36 weeks gestation. Five of these nine patients required mechanical ventilation. The rate of ICU admission for the RSV positive infants born at a gestational age  $\leq 36$  weeks was 26% (9/35) while that of the infants  $> 36$  weeks was 3.5% (9/256,  $p < 0.001$ ). Only three ICU admitted infants had an underlying illness.

Of all the 636 patients with bronchiolitis studied during the four RSV seasons there were two deaths from respiratory failure both in RSV positive infants born prematurely at 32 and 34 weeks, respectively. The overall case fatality rate (CFR) was 0.3% and it was 0.7% for the RSV positive patients. The CFR for RSV positive infants with gestational age  $\leq 36$  weeks was 2/35 (5.7%).

Mean duration of hospitalization of the RSV infected patients was longer (6.3 vs. 5.3 days,  $p < 0.001$ ) also indicating that the former had a more severe illness (Table 1).

#### *Frequency of bacteraemic illness and use of antibiotics*

Of the 636 infants admitted with acute bronchiolitis (174 febrile) none had a positive blood culture on admission. Five patients subsequently acquired bacteraemia during their period of stay in hospital. *Staphylococcus aureus* was isolated in blood cultures from two full term infants, who were  $< 2$  weeks old, on days 6 and 15 following their admission. The RSV status of these patients was not known. One week later the first patient developed septic arthritis of the shoulder. Another two RSV positive, 35 week premature infants, 28 and 30 days old respectively, were admitted to ICU where they developed *Klebsiella pneumoniae* sepsis. A fifth RSV positive full term infant who was also admitted to ICU at 25 days of age and was intubated developed *Stenotrophomonas maltophilia* sepsis. Urinary tract infection was documented in six RSV infected (2%) and two RSV negative (1%) infants.

Antibiotics were administered to 39% of the RSV positive infants and to 31% of the negative ones (Table 1). About half of the patients in each group received them parenterally. Common reasons for administration of antibiotics to RSV positive infants included acute otitis media (AOM) (16%) followed

by possible bacterial pneumonia (11%) and possible sepsis (10%). Infants with RSV infection received antibiotics more frequently for possible sepsis compared to those who tested negative (Table 1).

#### *Use of corticosteroids and beta agonists*

Beta agonists were administered to almost all patients during the first two seasons but there was an effort to restrict their use during the second half of the study. During this last part  $\beta$ -agonists were given to 102/185 (55%) patients with RSV infection and to 68/131 (52%) of the negative ones. Corticosteroids were administered to 105/291 (36%) of the RSV infected patients and to 41/141 (22.3%) of those who tested negative ( $p < 0.001$ ).

#### *Impact of RSV bronchiolitis on infant morbidity*

Bronchiolitis accounted for 19% and RSV bronchiolitis for 12% of all infant (<1 year of age) admissions during the 5 months of the yearly RSV outbreak from January to May during the three study years in our pediatric department.

## **Discussion**

Infants with acute bronchiolitis may be sent to one of two major pediatric hospitals for inpatient care within the Athens area. Infants admitted to the P. and A. Kyriakou Children's Hospital therefore, provide a profile of the burden of RSV infection in the source population of infants experiencing their first RSV infection. Sixty percent of cases of bronchiolitis admitted to hospital were shown to have laboratory proven evidence of RSV infection. The higher rate of RSV infection in the first season (1997) may be attributed to the selective testing for RSV infection of more seriously affected infants during this retrospective part. Previous studies have demonstrated this virus as the main causative agent of bronchiolitis [1, 5–22] and it has been found to account for 40–90% of the cases [23].

In this geographical region, the first RSV cases are admitted to hospital in December with the highest prevalence rates in February and March. In this study, the epidemics lasted 5 months with the last few cases appearing in May. A similar seasonal distribution during the cold months of the year was observed in other countries with temperate climates [1, 6–14]. In tropical climates annual outbreaks occur during the rainy season [19–22]. It is interesting that the RSV negative bronchiolitis has a similar seasonal distribution. Many of these cases may represent RSV infection that was not detected because the test was performed late in the course of the illness or the viral load was lower. Other viruses such as parainfluenza or influenza may have caused some of them but the

fact that their temporal distribution exactly coincided each year with that of RSV disease indicates that an appreciable number of the negative cases must have also been caused by this virus. A newly discovered human metapneumovirus has recently been shown to cause bronchiolitis in infants and its exact role remains to be investigated [24].

The sensitivity and specificity of immunofluorescence for the diagnosis of RSV infection when compared to viral culture has been in the range of 70–90% [10, 23, 25–27]. Detection of RSV antigen in the nasopharynx of infants hospitalized with bronchiolitis may be a marker of more severe disease compared to those who test negative. Infants who tested positive were more likely to have hypoxemia, the rate of ICU admission among them was higher, requirement for assisted ventilation was more frequent and duration of hospitalization longer. In a recent study, a centrifugation enhanced cellular immunofluorescence assay was used for multiple viral pathogens in addition to RSV EIA. The presence of other viruses was documented in a small proportion of the RSV negative patients (21%) [28]. RSV positive patients had a higher respiratory rate on admission and were more likely to receive supplemental oxygen [28]. These findings may truly mean that RSV bronchiolitis is a more severe illness compared to that caused by other viruses. Alternatively, infants with a negative RSV rapid antigen test may still be infected by this virus but carry a lower viral load or they may be more efficient in controlling the infection resulting in a less severe illness. It has been shown that the sensitivity of antigen detection is affected by the viral load in adult patients [29]. Simultaneous examination of a large number of patients for RSV and other respiratory viruses and comparison of the severity of lower respiratory tract illness produced in the two groups may provide an answer. In addition, long term follow-up of the RSV positive and negative patients and comparison of the rate of future wheezing and atopy may also be of interest.

The CFR of RSV bronchiolitis (0.7%) was low and comparable to that reported from other developed countries [5, 10], however, the proportion of high risk patients in our patient population was small. Higher CFRs up to 7% have been reported from developing countries, although the disease burden in them is under study [30]. Low CFRs ( $\leq 3.5\%$ ) have been reported in recent years from developed countries even among patients who belong to high-risk groups [31].

Although a large number of infants were admitted each season the course of RSV infection was mild since about 6% of the RSV positive infants were admitted to the ICU and only 3% required mechanical ventilation. Much higher rates of intubation have been reported from other industrialized countries [31].

RSV positive, premature infants were more likely to be admitted to the ICU compared to those born at

term and about half of the patients admitted there were premature infants  $\leq 36$  weeks. Case fatality was high in this group of patients but their total number was too small for firm conclusions to be drawn.

The risk of a serious bacteraemic illness in infants with bronchiolitis was found in this study to be very low. None of the 636 infants of which 174 were febrile was found to have a bacteremic illness on admission. All five patients with a positive blood culture had a nosocomial infection. They were young infants less than 30 days old and two of them were premature. However, about 39% of the RSV infected infants received antibiotics. Similar and even higher rates of antibiotic use have been reported in previous studies from academic institutions in Europe, US and Canada [17, 32, 33]. The most common reason for their administration was acute otitis media which was diagnosed in 16% of the cases. Other reasons included possible sepsis and an infiltrate in the chest radiograph. Acute otitis media is known to accompany RSV bronchiolitis in up to 67% of the cases and it was recently shown that in addition to RSV, bacteria are commonly found in the middle ear fluid obtained by tympanocentesis in these patients [34]. However, the diagnosis must be relied on stringent criteria [35] and antibiotic use avoided in cases of otitis media with effusion. Antibiotic use may be restricted in previously healthy infants with bronchiolitis, even in those younger than 2–3 months of age, with documented RSV infection if they do not appear ill and fulfill the low-risk criteria [36] despite the finding of an abnormal chest radiograph unless the latter is strongly suggestive of bacterial pneumonia.

Bronchiolitis was the single most common cause for admission of infants to the hospital during the three RSV seasons examined and accounted for about 20% of all admissions of patients less than 1 year of age. RSV was the cause in about 60% of the cases and the impact of this infection would have been even higher if patients with croup or pneumonia and older children or those with recurrent episodes of wheezing were included.

It is concluded that the impact of annual RSV epidemics in the Athens conurbation is similar to that described in other western industrialized cities. Glenzen et al. estimated that approximately two-thirds of all infants are infected with RSV during the first year of life, and almost all have been infected by the time they are 2 years old [37]. A Canadian based study estimated that the annual cost of RSV-associated illness was \$18 million [38]. The largest component of direct expenditure (62%) was for inpatient care for the estimated 0.7% of all children in the study who were ill enough to require hospital admission. As expected, premature infants tended suffer higher levels of morbidity but accounted for a small proportion of bronchiolitis patients. A cost-benefit analysis will be required to assess the value of instituting prophylaxis with palivizumab (Synagis) to this

high-risk group. The introduction of a safe and effective RSV vaccine will have a dramatic beneficial effect on the public health of infants and others vulnerable to this infection.

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