

ORIGINAL ARTICLES

Role of viruses and atypical bacteria in asthma exacerbations among children in Oporto (Portugal)

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ABSTRACT

Background: Upper respiratory tract infections are known to be a significant precipitant of acute asthma exacerbations. The aim of this study was to evaluate seasonal trends and the role of these pathogens in asthma exacerbations in school-aged children from Oporto (Portugal).

Methods: Nasal aspirates were collected from children aged 6 to 12 years old with asthma exacerbations attended in the Pediatric Emergency Department one day per week from January 1 to December 31, 2003. Demographic data, severity of asthma and asthma exacerbations, and current treatment were recorded. Samples were obtained through nasal wash with 1 ml saline and were processed by immunofluorescence assays (respiratory syncytial virus, adenovirus, parainfluenza and influenza virus), retrotranscription polymerase chain reaction (rhinovirus) and polymerase chain reaction (enterovirus, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*).

Results: In 54 eligible children, 37 nasal samples were obtained. Infectious agents were detected in 78 %

of the patients. Rhinovirus was detected in 70.3 %, *Mycoplasma pneumoniae* in 16.2 %, enterovirus in 10.8 %, and *Chlamydia pneumoniae* in 2.7 %. Coinfection was identified in 21.6 % of the samples. There was no significant correlation between current treatment status, severity of asthma or exacerbations and the isolated agents. Two distinct peaks of asthma exacerbation were found: 27 % in autumn and 24.3 % in spring. The highest number of cases was recorded in March and the lowest in August. Rhinoviruses were detected between March and May in 46 % of the patients, and between September and November in 30.8 %.

Conclusions: These results confirm the previously reported high frequency of rhinovirus detection in asthma exacerbations in children and provide evidence that asthma exacerbations and rhinovirus infections follow a seasonal pattern, occurring mostly in spring and autumn. The findings also underscore the frequency of *Mycoplasma pneumoniae* detection, and emphasize the importance of this agent as a possible trigger of asthma exacerbations.

Key words: Asthma exacerbation. Children. Chlamydia. Mycoplasma. Rhinovirus.

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INTRODUCTION

Exacerbations are the main cause of quality of life impairment in asthmatic children. Clinical and experimental evidence suggests an important role for respiratory infections in the development of asthma attacks¹⁻⁵. The first epidemiological data establishing a relationship between viral infections and asthma

were obtained in the 1970s and 1980s by viral isolation and serologic techniques⁶. Viral infection was identified in 31.9 % of asthma exacerbations in childhood (Pattemore et al, 1992). The three most frequently isolated viruses were rhinovirus, respiratory syncytial virus (RSV) and parainfluenza virus, which were detected in 8.8, 6.4 and 6 % of cases, respectively. The development of polymerase chain reaction (PCR) for the diagnosis of respiratory viral infections has led to studies revealing the importance of viral infections in acute exacerbations of asthma⁷. An epidemiological study by Johnston et al⁸ using PCR and culture detected viral upper respiratory tract infections in 85 % of childhood asthma exacerbations and in over half of adult exacerbations. Rhinoviruses accounted for 61 % of the viruses detected (coronavirus 16 %, influenza 9 %, parainfluenza 9 % and RSV 5 %). These upper respiratory tract infections are probably responsible for seasonal peaks of asthma-related hospital admissions^{9,10}. Human rhinovirus has been implicated as the principal virus associated with asthma episodes in school children, especially during spring and fall^{1,11-13}. Co-infection with other virus may be important^{2,16}. RSV are more important in children under two years of age¹³⁻¹⁶. Recent attention has been directed to the role of infections with atypical bacteria such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*, as agents capable of triggering asthma exacerbations in older children^{1,3,6,13,14,17,18}.

The present study evaluates the seasonal trends and the role of these pathogens in asthma exacerbations in school children from Oporto (Portugal).

METHODS

Patients

The study comprised all children aged 6 to 13 years old with asthma exacerbation, attended in the Pediatrics Emergency Department during one day per week from January 1 to December 31, 2003. Asthma diagnosis was based on the criteria of the new 2002 Global Initiative for Asthma (GINA) guidelines¹⁹. The patients who did not meet the GINA criteria for asthma diagnosis, and patients whose parents did not give informed consent, were excluded.

Data collection and analysis

Participants were questioned in detail about common cold symptoms, previous asthma diagnosis, age at onset of asthma and rhinitis symptoms, and asthma management and control. The questionnaire was

composed of 5 demographic questions and 7 health related questions. The severity of asthma and of asthma exacerbation was recorded. Nasal aspirates were obtained through nasal wash with 1 ml saline and processed by immunofluorescence assays (IFA) for RSV, adenovirus, parainfluenza and influenza virus, retrotranscription polymerase chain reaction (RT-PCR) for rhinovirus, and polymerase chain reaction (PCR) for enterovirus, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. The diagnosis and classification of asthma, asthma severity and asthma exacerbation were based on the criteria of the new 2002 Global Initiative for Asthma (GINA) guidelines¹⁹. The statistical analysis was performed using the SPSS statistical package (Version 11.0 for Microsoft Windows; SPSS, Chicago, IL, USA). The associations between isolated pathogens and the demographic data, severity of asthma and asthma exacerbation and current therapy were determined using the χ^2 test. A p-value < 0.05 was considered statistically significant.

RESULTS

During the 12-month study period, 54 patients were screened for eligibility. Clinical reviews and agreement resulted in 17 exclusions. Data from the remaining 37 patients were analyzed. Twenty-one patients were males (56.8 %) with a mean age of 8.5 years (6 to 13). Asthma had already been diagnosed in 83.3 % of the children (in 43.2 % more than 5 years before). Fever was present in 32.4 %, and common cold symptoms in 51.4 % of the cases. Symptoms of rhinitis were recorded in 64.8 %. Asthma severity was intermittent in 21.6 %, mild-persistent in 27 %, moderate-persistent in 48.6 %, and severe-persistent in 2.7 % (table I). Asthma exacerbation was mild in 54.1 %, moderate in 37.8 % and severe in 8.1 % (table I). A quarter distribution was made for climatic reasons, since the weather in the last month of the season is more characteristic of the coming season. Two distinct peaks of asthma exacerbation were found, between September and November and between March and May. March had the highest and August and January the lowest number of cases (fig. 1). The seasonal distribution also showed two distinct peaks of asthma exacerbations: 40.5 % in spring and 32.4 % in autumn. The combination of conventional and molecular techniques detected infectious agents in 78.4 % of the cases. Rhinovirus was detected in 70.3 %, *Mycoplasma pneumoniae* in 16.2 %, enterovirus in 10.8 %, and *Chlamydia pneumoniae* in 2.7 % (fig. 2). Co-infection was identified in 21.6 % of the samples (rhinovirus + enterovirus in four patients; rhinovirus + *Mycoplasma pneumoniae*

Table I
Agents isolated and patients characteristics

Patients characteristics	n (%)	Agents isolated (percentages by row cells)							P
		No agent n (%)	Rhinovirus n (%)	Mycoplasma n (%)	Coinfection				
					n (%)	Rhino + Entero	Rhino + Myco	Myco + Chlam	
Sex									
Male	21 (56.8)	5 (23.8)	12 (57.1)	1 (4.8)	3 (14.3)	2	0	1	> 0.05
Female	16 (43.2)	3 (18.8)	7 (43.8)	1 (6.3)	5 (31.3)	2	3	0	
Asthma severity									
Intermittent	8 (21.6)	2 (25.0)	5 (62.5)	1 (12.5)	0 (0)	0	0	0	
Mild persistent	10 (27.0)	2 (20.0)	5 (50.0)	1 (10.0)	2 (20.0)	2	0	0	
Moderate persistent	18 (48.6)	4 (22.2)	9 (50.0)	0 (0)	5 (27.8)	2	3	0	
Severe persistent	1 (2.7)	0 (0)	0 (0)	0 (0)	1 (100)	0	0	1	
Asthma exacerbation severity									
Mild	20 (54.1)	3 (15.0)	10 (50.0)	2 (10.0)	5 (25.0)	3	2	0	> 0.05
Moderate	14 (37.8)	5 (35.7)	7 (50.0)	0 (0)	2 (14.3)	0	1	1	
Severe	3 (8.1)	0 (0)	2 (66.7)	0 (0)	1 (33.3)	1	0	0	
Current treatment step									
Intermittent	17 (45.9)	4 (23.5)	10 (58.8)	2 (11.8)	1 (5.9)	1	0	0	
Mild persistent 11 (29.7)	2 (18.2)	6 (54.5)	0 (0)	3 (27.3)	120				
Moderate persistent	9 (24.3)	2 (22.2)	3 (33.3)	0 (0)	4 (44.4)	2	1	1	> 0.05
Season distribution									
Autumn	12 (32.4)	0 (0)	9 (75)	2 (16.7)	1 (8.3)	0	1	0	0.02
Winter	4 (10.8)	1 (25)	0 (0)	0 (0)	3 (75)	2	0	1	
Spring	15 (40.5)	6 (40)	6 (40)	0 (0)	3 (20)	1	2	0	
Summer	6 (16.2)	1 (16.7)	4 (66.7)	0 (0)	1 (16.7)	1	0	0	
Common cold symptoms									
Present	19 (51.4)	6 (31.6)	10 (52.6)	0 (0)	3 (15.8)	1	1	1	> 0,05
Not present	18 (48.6)	2 (11.1)	9 (50.0)	2 (11.1)	5 (27.8)	3	2	0	
Fever									
Present	12 (32.4)	4 (33.3)	7 (58.3)	0 (0)	1 (8.3)	0	0	1	> 0.05
Not present	25 (67.6)	4 (16.0)	12 (48.0)	2 (8.0)	7 (28.0)	4	3	0	
Rhinitis previously diagnosed									
Present	23 (62.2)	6 (26.1)	11 (47.8)	1 (4.3)	5 (21.7)	3	2	0	> 0.05
Not present	14 (37.8)	2 (14.3)	8 (57.1)	1 (7.1)	3 (21.4)	1	1	1	
Asthma symptoms									
< 1 year	9 (24.3)	14 (22.2)	4 (44.4)	0 (0)	3 (33.3)	2	1	0	> 0.05
1 to 5 years	12 (32.4)	6 (16.7)	6 (50.0)	1 (8.3)	3 (25.0)	2	1	0	
> 5 years	16 (43.2)	17 (25.0)	9 (56.3)	1 (6.3)	2 (12.5)	0	1	1	

in two patients, and *Mycoplasma pneumoniae* + *Chlamydia pneumoniae* in one patient). There was no significant correlation between isolated agents and the month of year, though rhinoviruses were detected in the spring in 24.3 % of the cases, and in autumn in 27.0 % of the cases ($p = 0.02$) (table I; fig. 3). All isolated enteroviruses were associated with rhinovirus infection, and were mostly found in children with intermittent asthma (table I). Half of the cases of *Mycoplasma pneumoniae* isolation showed co-infec-

tion (rhinovirus in two cases and *Chlamydia pneumoniae* in one case). There was no significant correlation among sex, age, common cold symptoms, current therapy status, severity or exacerbation of asthma, age at onset of asthma symptoms, presence of fever or rhinitis and the isolated agents. Children with current treatment step ≥ 2 showed co-infection in a larger number of cases (35 % vs 5.9 %, $p = 0.09$). The only case of *Chlamydia pneumoniae* isolation showed co-infection with *Mycoplasma*

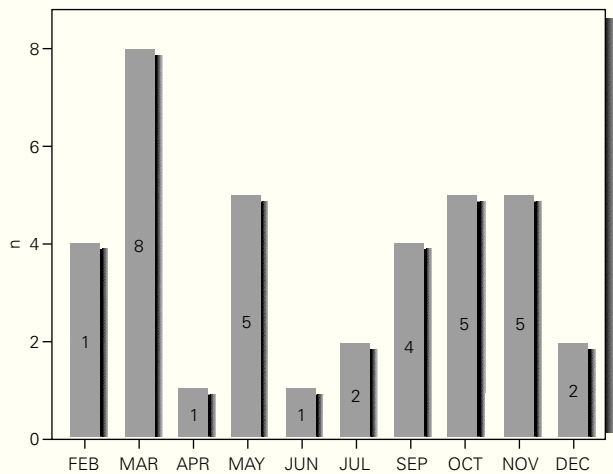


Figure 1.—Month distribution.

pneumoniae, and corresponded to a child with severe-persistent asthma.

DISCUSSION

Previous studies have examined the importance of viral infections in acute exacerbations of asthma. Using conventional techniques alone, the detection rate for infections agents did not exceed 30 % (Pattemore et al, 1992). With the development and use of highly sensitive molecular techniques, it was hypothesized that the rate, as well as the range, of infectious agents associated with exacerbations of asthma, would increase^{5,20}. Indeed, as confirmed by Johnston et al (1995), the rate of viral detection by PCR-based assays increased to 77.3 %. In the present study, infectious agents were detected in 78.4 % of the cases. In 79.4 % of these cases a virus was detected (rhinoviruses, enteroviruses or both), while in 10.3 % of the cases a bacterium was isolated (*Mycoplasma pneumoniae* or *Chlamydia pneumoniae*), and in 10.3 % both a virus (rhinovirus) and a bacterium (*Mycoplasma pneumoniae*) were found (fig. 2). In addition, we noted that rhinovirus and *Mycoplasma pneumoniae*, found in 70.3 % and 16.2 % of the cases, respectively, were detected more frequently than other agents.

Rhinovirus was the most common agent found in the nasal samples studied (70.3 %), this being in accordance with several other studies that define this agent as the major cause of asthma exacerbations in school children^{5,7,15,20,21}. Co-infection with other viruses or bacteria may be important, and has been found in other studies^{4,20}. Co-infection was detected in 21.6 % of the cases, all of them corresponding to children with severe-persistent asthma.

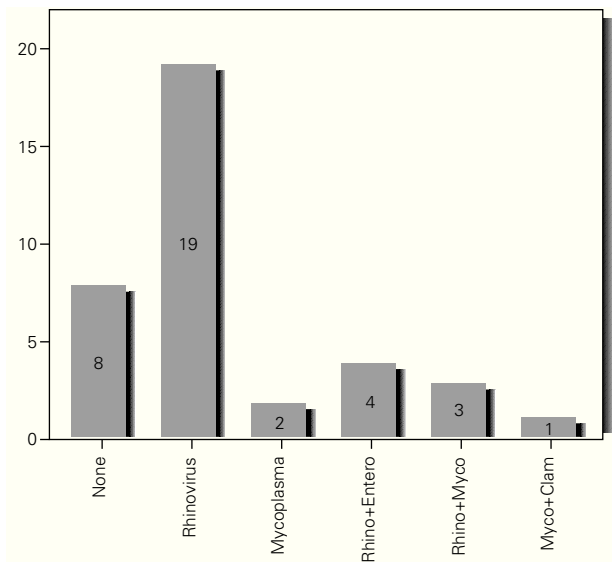


Figure 2.—Agents isolated.

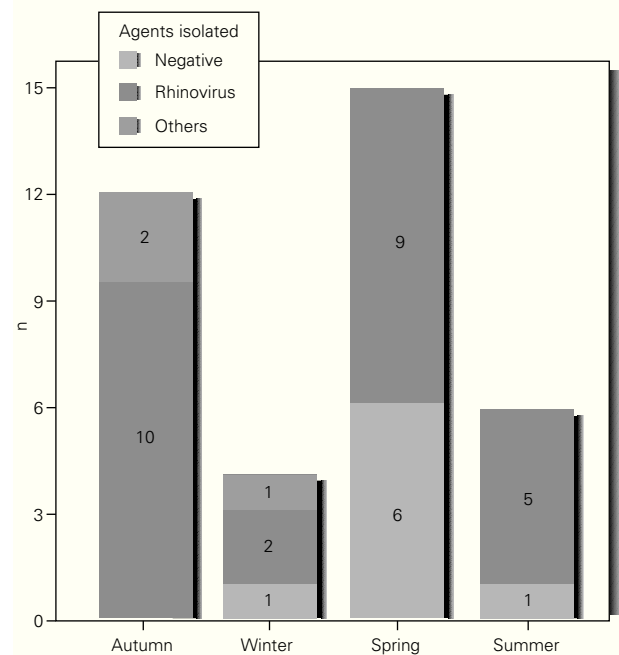


Figure 3.—Agents isolated by season.

A significant seasonal pattern in rhinovirus detection (27 % in autumn and 24.3 % in spring) was observed. These results support the fact that upper respiratory tract infections, especially by rhinovirus, are probably responsible for seasonal peaks of asthma-related hospital admissions, as suggested by several studies⁸⁻¹⁰ – occurring mostly in spring and fall. Eight patients that were excluded because they did not give informed consent came to the Pediatric Emergencies Department with asthma exacerbation in March and April, which explains the low

number of cases included corresponding to these months.

The ability of rhinovirus to produce common cold-like symptoms in healthy individuals while producing significant alterations in lower airway physiology in asthmatic patients, would indicate that either the immunoinflammatory response to the virus is different in patients with asthma, or the consequences of this response in the lower airway is altered, or both¹⁴. This is supported by the observation in this study that 14 patients with rhinovirus isolation did not have common cold symptoms.

Experimentally induced rhinovirus infections in patients with allergic rhinitis can alter the pattern of their lower airway response to allergen challenge, by significantly enhancing their propensity to develop late asthmatic responses^{14,22}. The interaction between atopia and asthma and viral infections appears to be bidirectional and dynamic in that the atopic state can influence the lower airway response to viral infections^{23,24}. The atopia state was not questioned in this study, but there was no correlation between the presence of previously diagnosed rhinitis and the agents isolated.

No RSV, adenoviruses, influenza viruses or parainfluenza viruses were isolated. RSV is a common cold virus and a major cause of acute asthma exacerbations, predominantly in children under two years of age^{8,13,25,26}. The fact that no RSV was found in nasal samples in our study can be explained by the weaker detection rate of the isolation method used: all these viruses were assessed by IFA. Another possible explanation is the lesser importance of this agent as a cause of asthma exacerbation in school children.

Since bacterial infections are known to impair mucociliary clearance and to increase mucus production in the lung, it has been proposed that certain bacterial infections may cause chronic lower airway inflammation²⁷. The organisms primarily implicated in this process include *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*^{27,28}. These atypical bacterial infections may precipitate asthma exacerbations, and were linked to prolonged asthmatic symptoms^{1,2,7,18}. In school children with wheezing, an unexpectedly high prevalence of low-grade *Chlamydia pneumoniae* infection also has been reported²⁹. Cunningham et al²⁹ studied 108 children (age range 9-11 years) with asthma symptoms longitudinally followed-up on (for 13 months) by means of a daily diary of respiratory symptoms and peak flow rates. Nasal aspirates were obtained when respiratory symptoms were reported. The presence of infection was investigated by PCR for *Chlamydia pneumoniae*, and detections were similar in the symptomatic and asymptomatic epi-

sodes (23 % vs 28 %, respectively). Children who reported multiple episodes also tended to remain PCR-positive for this agent, suggesting the presence of chronic infection. *Mycoplasma pneumoniae* also has been associated with chronic asthma, and infection has been documented in about 25 % of children with wheezing^{7,30}. In the present study we found a lower prevalence of these agents – *Mycoplasma pneumoniae* being found in 16.4 % and *Chlamydia pneumoniae* in only 2.7 % of the cases. *Mycoplasma pneumoniae* was the second most frequently isolated agent, but *Chlamydia pneumoniae* did not seem particularly involved in children with acute asthma exacerbation, as found in other studies¹⁴. There was no significant correlation to asthma severity, although the only case of *Chlamydia pneumoniae* detection corresponded to a co-infection with *Mycoplasma pneumoniae* in a child presenting severe-persistent asthma. This is an isolated case, but suggests that this agent may be associated with a more severe asthma course.

CONCLUSION

Our results confirm a high frequency of rhinovirus detection in asthmatic exacerbations in school children, and support the fact that the seasonal pattern of asthma exacerbations is due to rhinovirus infection – occurring mostly in spring and fall. The findings also underscore the frequency of *Mycoplasma pneumoniae* detection, and emphasize the importance of this agent as a possible trigger of asthma exacerbations.

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