Role of Viruses and Atypical Bacteria in Exacerbations of Asthma in Hospitalized Children: A Prospective Study in the Nord-Pas de Calais Region (France)

C. Thumerelle, MD,[1]* A. Deschildre, MD,[1] C. Bouquillon, MD,[2] C. Santos, MD,[1]
A. Sardet, MD,[3] M. Scalbert, MD,[4] L. Delbecque, MD,[5] P. Debray, MD,[6] A. Dewilde, MD,[2]
D. Turck, MD,[1] and F. Leclerc, MD[1]

Summary. We studied the role of viruses and atypical bacteria in children hospitalized with exacerbated asthma by a prospective study of children with acute asthma admitted to the Department of Pediatrics in Lille, and to 15 hospitals in the Nord-Pas de Calais region, from October 1, 1998–June 30, 1999. We included children aged 2–16 years with active asthma, defined as three or more recurrent episodes of reversible wheezing. The severity of asthma and of asthmatic exacerbations was recorded. Immunofluorescence assays (IFA) on nasopharyngeal secretions (NPS), serological tests, or both, were used for detection of influenza virus, respiratory syncytial virus (RSV), adenovirus, parainfluenza virus, and coronavirus. Polymerase chain reaction (PCR) assays on NPS were used for rhinovirus and enterovirus. Serological tests for *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were performed. A control group of asymptomatic asthmatic outpatients was examined for respiratory viruses (using IFA and PCR). Eighty-two symptomatic children (mean age, 7.9 years) were examined. Viruses were detected in 38% (enterovirus, 15.8%; rhinovirus, 12%; RSV, 7.3%). Serological tests for atypical bacteria were positive in 10% of patients (*C. pneumoniae*, 5%; *M. pneumoniae*, 5%). Among the 27 control subjects (mean age, 7.9 years), one PCR was positive for enterovirus. There was no correlation between severity of chronic asthma or asthmatic exacerbations and the diagnosis of infection. Atypical bacterial pathogen infections were linked with prolonged asthmatic symptoms. In conclusion, we confirmed the high incidence of viral infection in acute exacerbations of asthma, especially enteroviruses or rhinoviruses. Persistent clinical features were more frequently associated with atypical bacterial infections, suggesting that these infections should be investigated and treated in cases of persistent asthmatic symptoms.
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INTRODUCTION

Asthma is the most common chronic disease in childhood. It is estimated that 10% of children in France will, at some time, have signs and symptoms compatible with asthma.[1] Numerous studies suggest that the prevalence of asthma has been increasing for 20 years.[1,2] Our understanding of the pathogenesis of asthma has improved, resulting in a new concept of asthma as a chronic inflammatory disorder of the airways. Control of the

[1]Department of Pediatrics, CHU Lille, Lille, France.

[2]Department of Virology, CHU Lille, Lille, France.

[3]Department of Pediatrics, Hôpital Lens, Lens, France.

[4]Department of Pediatrics, Hôpital St.-Antoine-Lille, Lille, France.

[5]Department of Pediatrics, Hôpital Tourcoing, Tourcoing, France.

[6]Department of Pediatrics, Hôpital Valenciennes, Valenciennes, France.

*Correspondence to: Dr. C. Thumerelle, Secrétariat de Pneumologie Infantile, Hôpital Jeanne de Flandre, CHU de Lille, 59037 Lille Cedex, France. E-mail: adeschildre@chru-lille.fr

inflammatory component has become one of the most important therapeutic targets of long-term treatment.[3] Despite improvements in treatment, asthmatic crises still occur, resulting in costly visits and hospitalizations.[2]

The link between respiratory tract infections and acute exacerbation of asthma has been recognized for a long time.[4–6] Through advances in microbiological diagnosis, recent studies have highlighted the role of upper respiratory tract viruses as probably the commonest cause of exacerbations of asthma.[7] Other research also suggests that atypical bacteria, such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*, may play a role in asthma.[6,8]

The purpose of this regional prospective study was to investigate the role of these organisms in acute exacerbations of asthma in children. To achieve this, we applied serological or more sensitive detection techniques to samples collected during a 9-month period in children with asthma exacerbations requiring hospitalization in 16 hospitals of the Nord-Pas de Calais region. We examined the possible link between clinical, radiological, or biological data, and the presence of virus or potentially treatable atypical bacteria.

MATERIALS AND METHODS

This was a prospective study performed at the University Hospital of Lille and at 15 community hospitals in the Nord-Pas de Calais region (France). The hospital's Research and Ethics Committee approved the study. Patients and control subjects were recruited between October 1, 1998–June 30, 1999. Parents gave consent for their children's participation.

Populations

Study population

We included children aged between 2–16 years with active asthma, defined as three or more recurrent episodes of reversible wheezing within the 2 years preceding the study. Children with exacerbations requiring hospitalization were enrolled in the study after the parents gave

ABBREVIATIONS	
5′NC region	5′ noncoding region
DNA	Desoxyribonucleic acid
HEPES	[2-hydroxyethyl]-1 piperazine ethanolsulfic acid
IFA	Immunofluorescence assay
Ig	Immunoglobulin
MEM	Minimum essential medium
MgSO$_4$	Magnesium sulfate
NPS	Nasopharyngeal secretions
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcriptase-polymerase chain reaction

consent. We excluded patients with a history of other chronic lung diseases or heart disease. Subjects or their parents were asked about the duration and frequency of asthmatic symptoms, medication, and hospitalization, and their history of allergy. Allergy was defined by one or more positive skin prick tests or specific IgE for inhalant allergen. Clinical features, including respiratory rate and chest examination, and associated digestive symptoms such as nausea, vomiting, diarrhea, or abdominal pain, were recorded. Oxymetry and peak expiratory flow rate were measured if the child was older than 5 years. Severity of asthma and asthmatic exacerbations were scored according to published guidelines.[3,9]

A baseline blood sample and nasal swabs were collected in the first 36 hours after admission, for serological and virological testing. At a follow-up visit three weeks after discharge from hospital, the children were examined again and a new blood sample was collected as a convalescent specimen. Clinical recovery was defined as absence of asthmatic symptoms with normal physical examination. A chest radiograph was taken early after admission for every patient. All chest radiograph readings were taken by the same radiologist.

Control population

We included asthmatic outpatients with no history of exacerbations or upper respiratory tract infection during the preceding 3-week period. They were all clinically investigated by the same physician to confirm that there were no respiratory signs suggestive of infection. Nasal swabs were collected without blood sampling.

Nasal Swabs

Nasal secretions were collected with swabs into two separate viral transport medium tubes (0.5% bovine serum albumin; antibiotics: 1,500 U of penicillin, 1 ng of streptomycin, minimal essential medium (MEM), and 4.76 mg of [2-hydroxyethyl]-1 piperazine ethanolsulfic acid (HEPES), in 2 mL of tryptose phosphate broth). One tube was used for immunofluorescence detection assay of viral antigens, and the second was frozen, transported at 4°C, and stored at −80°C in the 2–12 hr following samples before reverse transcriptase-polymerase chain reaction (RT-PCR) assays.[10]

Immunofluorescence Assay (IFA)

Cells were separated by centrifugation, washed in phosphate-buffered saline (PBS), and loaded onto microscope slides before being fixed with acetone.[10] Respiratory syncytial virus (RSV) and coronavirus 229E were detected by direct and indirect immunofluorescence (Argene Biosoft, France), respectively. Influenza A and B, parainfluenzae 1–3, and adenovirus were detected by indirect immunofluorescence with a commune test

(Sanofi-Pasteur, France). When the commune test was positive, specific immunofluorescence assays for each virus were performed (Argene Biosoft, France).

Picornavirus (Rhinovirus and Enterovirus) Reverse Transcriptase-Polymerase Chain Reaction Assay

Molecular techniques were applied to samples stored at $-80°C$. DNA and total RNA were extracted simultaneously from 200 µL of viral aspirate in viral transport medium, using a rapid extraction protocol on a silica column system (High Pure Viral Nucleic Acid Kit) according to the manufacturer's recommendations (Roche Molecular Biochemicals). RT-PCR assay was carried out with the "Access RT-PCR System," as described by the manufacturer (Promega, France). Briefly, each RT-PCR assay was performed in a total volume of 50 µL, using 0.25 µg nucleic acid extracted from nasal swabs, with 200 µM deoxynucleotide triphosphates, 25 mM MgSO$_4$, 2.5 IU *Tfl* DNA polymerase, 20 IU reverse transcriptase, 10 µL special 5α buffer (AMV/*Tfl* DNA polymerase), and 30 pmol of each primer (OL26: 5′-GCACTTCTGTTTC-CCC-3′; OL27: 5′-CGGACACCCAAAGTAG-3′), which recognize sequences located in the 5′ noncoding nucleotide sequences of human rhinovirus and enterovirus genomes.[11] Amplification cycling was performed in an MJ Research Thermocycler (Watertown, USA) according to the recommendations of the manufacturer, using 40 cycles (with a temperature of $55°C$ for primers' annealing phase). RNA-free negative controls containing only the RT-PCR reaction mix were included in each assay. Genomic viral RNA extracted from 10^4 pfu of coxsackievirus B3 or human rhinovirus 9 strain (ATCC), purified by CICs isopycnic ultracentrifugation, was used as a positive control. If positive, Picornavirus RT-PCR products (380 pb) were then subjected to agarose gel electrophoresis with ethidium bromide staining, and analyzed by hybridization in a microtiter plate with a digoxigenin-labelled probe (5′-GGCAGCCACGCAGGCT-3′), corresponding to a part of the human rhinovirus 5′ noncoding (5′NC) region.[10] We concluded that rhinovirus was present if detection of rhinovirus RNA sequences was positive by hybridization, and that enterovirus was present if detection of Picornavirus RNA sequences was positive without detection of rhinovirus RNA sequences.

Blood Samples

Leukocyte and platelet counts, and C-reactive protein levels, were determined.

Serology

Anti-virus (influenzae A–B, RSV, parainfluenzae 1–3, and adenovirus) antibodies and total antibodies against *M. pneumoniae* were assessed by complement fixation tests (Eurobio, Behring, Virion Ltd., Switzerland). A diagnosis of recent *M. pneumoniae* or viral infection was accepted if a 4-fold increase in antibody titer of paired sera occurred, or if the antibody titer was $\geq 1/80$ in the second serological test after a negative result in the first test. A microimmunofluorescence test was used to estimate levels of IgG, IgA, and IgM antibodies against *C. pneumoniae* (Savyon). Serum antibodies against *C. pneumoniae* elementary bodies were detected using fluorescein-conjugated monoclonal mouse anti-human Ig-subclass antibodies. A diagnosis of recent *C. pneumoniae* infection was defined by IgM titer equal to or greater than 1/16, or IgG titer equal to or greater than 1/512, or a 4-fold titer rise in IgM or IgG. The presence of IgA and low levels of IgG (between 1/16 and 1/512) were considered indicative of chronic infection.

Statistical Analysis

We analyzed the differences between patient and control groups for each parameter by Student's *t*-test for continuous variables, and a χ^2 test for categorical variables. To compare infected and noninfected patients, the same tests were used. To examine the differences between virus-infected patients and patients infected with atypical bacteria, we used Fisher's exact test for categorical variables, and a Wilcoxon test for continuous variables. We used a stepwise method to select clinical, biological, and/or radiological data linked to presence of infection. *P* values < 0.05 were considered significant. Values for all measurements are expressed as mean $\pm$ standard deviation (SD).

RESULTS

Patient Characteristics

One hundred and thirteen children were included in this study. Thirty-one patients who did not attend the follow-up visit were subsequently excluded. Eighty-two patients remained for the entire analysis (26 females and 56 males; mean age, 7.75 years; range, 2.1–15.33 years). The distribution of patient participation over time was: October–December, 60.9%; January–March, 24.4%; and April–June, 14.7%. Asthma was classified according to the criteria of the Third International Pediatric Consensus Statement,[3] and showed infrequent episodic symptoms in 18% of patients, frequent episodic symptoms in 60%, and persistent symptoms in 22%. Allergy was noted in 77% of patients. Exacerbations began 1–360 hr before hospitalization (mean, 40 hr). Exacerbations were mild (21%), moderate (46%), or severe (33%).[9] The mean duration of hospitalization was 4.5 days (range, 2–12 days). Radiographic abnormalities of the chest, excluding hyperinflation, were present in 35.4% of patients (peribronchial thickening, 6%; opacities, 28%; pneumomediastinum, 2.5%).

TABLE 1—Detection of Viruses, *C. pneumoniae* and *M. pneumoniae*[1]

Exacerbations	Asthma	Controls
Number of children	82	27
Age (years)	7.75(±4.8)	7.75 (±3.6)
Gender	Girls, 32%; boys, 68%	Girls, 37%; boys, 63%
Severity of asthma	IES, 18%; FES, 60%; PS, 22%	IES, 18.5%; FES, 70%; PS, 11.5%
% positive nasal aspirates detected by immunofluorescence	Total, 8.5; RSV, 3.6; parainfluenza, 2.5; influenza, 2.5	0
% positive nasal aspirates detected by PCR	Total, 28; enterovirus, 15.8; rhinovirus, 12.2	Total, 3.7; enterovirus, 3.7
% positive viral serological tests	Total, 7.4; RSV, 3.6; adenovirus, 1.2; influenza, 2.5	Not done
% positive atypical bacteria serological tests	Total, 10 *C. pneumoniae*, 5; *M. pneumoniae*, 5	Not done
Total (%) positive children	45	3.7

[1]IES, infrequent episodic symptoms; FES, frequent episodic symptoms; PS, persistent symptoms.

Twenty-seven asymptomatic asthmatic outpatients (10 females and 17 males; mean age, 7.75 years; range, 3–15.25 years) were included in the control group. There was no statistical difference between the two groups in terms of sex, age, or severity of asthma (Table 1).

Laboratory Findings

Leukocyte counts were determined in 66 patients and revealed granulocytosis in 36 (55%) patients, neutropenia in 1 patient, lymphopenia in 27 (41%) patients, eosinophilia in 15 (23%) patients, and monocytosis in 14 (21.5%) patients. The C-reactive protein level was high (>20 mg/L) in 13 (16%) children.

Microbiological results were positive in 37 (45%) children. Viruses were detected in 31 (38%) patients with an asthma exacerbation (Table 1). For four samples, the Picornaviridae PCR assay could not be interpreted, and the corresponding patients were counted as not tested. Enteroviruses were detected in 13 (15.8%) patients, rhinovirus in 10 (12%) patients, RSV in 6 (7.3%) patients, influenza A and B in 3 (3.6%) patients, parainfluenza in 2 (2.5%) patients, and adenovirus in 1 (1.2%) patient.

Serological tests for atypical bacteria were positive in 8 patients with an asthmatic crisis (10%): 4 cases with *C. pneumoniae* infection (5%), and 4 with *M. pneumoniae* infection (5%). For *C. pneumoniae*, 1 patient had positive IgM at the second serology, suggesting recent infection, and the 3 others had two positive results with high IgA and IgG (>1/256), suggesting chronic infection. One of them had also a 4-fold rise of IgG. Two pathogens were found in 6 patients: 2 with RSV and rhinovirus, 1 with RSV and enterovirus, 1 with enterovirus and parainfluenza, 1 with *M. pneumoniae* and parainfluenza virus, and 1 with *M. pneumoniae* and enterovirus.

Except for one positive PCR for enterovirus, no virus was detected in the control group. Viral detection by IFA and PCR was significantly less frequent in controls than in hospitalized patients ($P < 0.05$).

Comparative Study

We compared infected patients with other children suffering asthmatic exacerbations (Table 2). The mean age of the 37 infected children was 6.9 years (range, 2.17–15.5 years), compared with 8.17 years for the others

TABLE 2—Comparison of Children With or Without Infection[1]

	Infections	No infections	P-values
N	37	41	
Age (years)	6.92 ± 4	8.2 ± 3.75	0.184
Gender	Girls, 35%; boys, 65%	Girls, 29%; boys, 71%	0.686
Severity of asthma	IES, 16%; FES, 65%; PS, 19%	IES, 20%; FES, 61%; PS, 17%	0.89
Exacerbations	Mild, 13%; moderate, 51%; severe, 35%	Mild, 27%; moderate, 41%; severe, 29%	0.287
Passive smoking	48.5%	39%	0.504
GI disturbance	35%	7%	0.004
Fever	57%	22%	0.011
Radiographic opacities	24%	29%	0.51
Hospitalization	4.75 days ± 2.6	4.3 days ± 1.1	0.394
Oxygen therapy	0.93 days ± 1.8	0.68 days ± 0.95	0.481
Improvement at 48 hr	Total, 43%; partial, 54%; none, 3%	Total, 63%; partial, 37%; none, 0%	0.131
Complete recovery at follow-up visit	78%	88%	0.085

[1]IES, infrequent episodic symptoms; FES, frequent episodic symptoms; PS, persistent symptoms; GI disturbance, gastrointestinal disturbances including nausea, vomiting, diarrhea, or abdominal pain.

(range, 2.08–14.25 years) ($P = 0.184$). There were no statistical differences in age, sex, period of inclusion, severity of asthma, inhaled steroids as long-term treatment, crisis severity, improvement at 48 hr, or recovery at 21 days. Fever (temperature higher than 38.5°C) was more frequent in infected than in uninfected children (57% vs. 22%; $P = 0.011$). The frequency of biological or radiological abnormalities was the same in both groups. Associated digestive symptoms were more frequent among infected patients than uninfected patients (35% vs. 7%; $P = 0.004$).

Finally, we compared children with *viral* infections (n = 29) and those with *atypical bacterial* infections (n = 8) (Table 3). There were no statistical differences in age, sex, period of inclusion, severity of asthma or crisis, fever, associated digestive disorders, or improvement at 48 hr. The frequency of biological or radiological abnormalities was the same in both groups. The only significant difference was a lower rate of recovery at 3 weeks in exacerbations associated with *atypical bacterial* infections than in those with *viral* infections (50% vs. 86%; $P = 0.028$).

DISCUSSION

Asthma and Infection: Epidemiological and Microbiological Data

In this study of 82 children with symptomatic asthma, viruses or evidence of viral infection were detected in 38% of patients, and evidence of atypical bacterial infection in 10%. Asthmatic patients often report a history of colds and upper respiratory symptoms in the days preceding the onset of an exacerbation. An association between respiratory infections and asthma attacks has also been suspected, and is supported by epidemiological data. Using conventional techniques (IFA, virus isolation, and serological methods), viruses were detected in about 30% of patients with asthmatic exacerbations.[5] By combining conventional and molecular techniques, Johnston et al. identified an infectious organism in 81.8% of patients with exacerbated asthma in a community study of 9–11-year-old asthmatic children.[7] The same authors reported that seasonal patterns in the prevalence of upper respiratory viruses correlated closely with hospital admissions for asthma in the area from which the cohort was drawn.[12] Finally, they confirmed a high frequency of viral infection, with positive findings in 68% of infants and children admitted to hospital for severe wheezing illnesses.[13] In a similar study over 4 years, Freymuth et al. identified infectious organisms in 77% of children aged between 3 months and 14 years and hospitalized for wheezing.[14]

In our study, only 38% of children had a proven viral infection. The population's characteristics, and our diagnostic methods, may explain this low frequency. We only included children with a previous diagnosis of asthma, or with a history of three or more episodes of wheezing, and we excluded children under 2 years of age, to avoid any confusion with bronchiolitis. In their study of hospitalized children, Johnston et al. included all children with wheezing, including those with bronchiolitis and pneumonia.[13] Freymuth et al. did not describe the criteria used to define asthma, and toddlers (and therefore cases of bronchiolitis) were included in their study.[14] A weakness in the diagnostic methods of our study was the absence of PCR detection of adenovirus, RSV, parainfluenza, and coronavirus, as used in previous studies.[7,13,14] Molecular techniques are more sensitive than IFA and viral isolation for the detection of these viruses, as previously reported.[15] Community studies, involving intensive monitoring of children and their parents, allow early sampling, which probably increases the rate of detection of viruses compared with hospital-based studies.[5] In fact, several days may elapse between rhinitis and cough and the onset of wheezing, and between the onset of wheezing and hospital admission.[5] In our study, the delay between the onset of exacerbation and viral investigations could explain, in part,

TABLE 3—Comparison of Groups With Viral Infections and Atypical Bacteria Infections[1]

	Viral infections only	Atypical bacteria infections	P-values
N	29	8	
Age (years)	6.25 ± 3.8	9.1 ± 4.3	0.214
Severity of asthma disease	IES, 20.5%; FES, 65.5%; PS, 13.5%	IES, 0%; FES, 62.5%; PS, 37.5%	0.213
Exacerbations (9)	Mild, 13.8%; moderate, 58.5%; severe, 27.6%	Mild, 12.5%; moderate: 25%; severe, 62.5%	0.138
Prodromes	93%	87.5%	0.607
GI disturbance	27.6%	62.5%	0.067
Fever	52%	75%	0.380
Radiographic opacities	24%	50%	0.157
Hospitalization	4.3 days ± 2.3	6.25 days ± 3.2	0.096
Oxygen therapy	0.6 days ± 1.1	2.1 days ± 3	0.1528
Improvement at 48 hr	Total, 45%; partial, 55%; none, 0%	Total, 37.5%; partial, 50%; none, 12.5%	0.297
Complete recovery at follow-up visit	86%	50%	0.028

[1]IES, infrequent episodic symptoms; FES, frequent episodic symptoms; PS, persistent symptoms; GI disturbance, gastrointestinal disturbances including nausea, vomiting, diarrhea, or abdominal pain.

our lower rate of viral detection using the same methods. Finally, the exclusion of September, due to a delay in obtaining Ethics Committee approval, could have contributed to this low rate.

The viruses predominantly detected in our study were the Picornaviridae, which represented 28% of positive samples, of which 12.2% were rhinovirus and 15.8% enterovirus, in agreement with previous studies.[7,14] The PCR method is 3-fold more sensitive than cell culture techniques.[15,16] In the Southampton community study, rhinoviruses were detected in 50% of children.[7] In hospital-based studies, similar results were reported for children older than 2 years, with detection rates of 46%[14] and 50%.[13] Our lower detection rate might be explained by variations in viral ecology between areas, or by the different sensitivities of the assays used. The first hypothesis appears unlikely, because rhinoviruses are ubiquitous, and have been detected with the same prevalence in the United States, Finland, England, and France.[7,14,17,18] To detect Picornaviridae, we used the same primers (OL26 and OL27) used in similar studies.[7,13,14] Rhinovirus was specifically identified by Freymuth et al.[14] and by us with a specific primer. In the community study,[7] rhinovirus was identified in 29.8% of patients, but in 21.2% of patients, picornaviruses were classified as rhinovirus without confirmation with a specific primer. Therefore, these viruses may correspond to rhinovirus or enterovirus. Indeed, enterovirus is frequently detected, occurring in 15.8% of patients in our study. Freymuth et al. identified enterovirus in 9.8% of patients,[14] and Johnston et al. identified it in 10% of patients in their hospital-based study.[13] Enterovirus was not specifically identified in the community study.[7]

To detect RSV, we coupled IFA on nasopharyngeal secretions and serological tests, as in the Southampton studies.[7,13] In our study, RSV was detected in 7.3% of patients, and only during November and December, confirming an epidemic variation for this virus. RSV infections were found principally in the youngest children, and the RSV detection rate increased to 15% in children less than 7 years old. RSV was identified in 18% of children older than 2 years in the study of Freymuth et al. (using IFA and PCR),[14] in 12.5% of children in the hospital-based study of Johnston et al. (using IFA, culture, and serology),[13] and in only 4.1% in the community-based study of older children (IFA, culture, and serology).[3]

Detection levels for other viruses were 3.6% for influenza, 2.4% for parainfluenza, and 1.2% for adenovirus. Our results for the influenza virus are similar to those of previous studies.[7,13,14] In our study, parainfluenza virus was always associated with another pathogen: enterovirus in one patient, and *M. pneumoniae* in the other. Adenovirus was identified in just one patient. However, Freymuth et al., using molecular techniques, detected adenovirus in just 1.5% of children older than 2 years. Adenoviral infections seem to play a minor role in the exacerbation of asthma. Finally, we did not detect coronavirus with IFA. Freymuth et al. identified this virus in 4.5% of patients using IFA,[14] and Johnston et al. identified it in 13% by culture and molecular amplification.[7]

Viruses are not the only organisms incriminated in the acute exacerbation of asthma. Previous studies suggested that common bacterial infections do not play such a role.[5] However, atypical bacteria that can develop intracellularly, such as *C. pneumoniae* and *M. pneumoniae*,[19] may be associated with the exacerbation of asthma.[20,21] Dallo and Baseman[19] demonstrated intracellular development of *M. pneumoniae* in human cells cultures, but this has not been reported in patients with respiratory disease. Future research may clarify whether or not this organism, generally recognized as a surface parasite, can also play an intracellular role. *C. pneumoniae*, an obligate intracellular organism, is a common respiratory pathogen associated with atypical pneumonia, bronchitis, rhinitis, or sinusitis in children and adolescents.[22] *C. pneumoniae* infection is associated with wheezing in both children and adults.[21] The diagnosis of *C. pneumoniae* infection remains difficult. The PCR technique is more sensitive than other techniques, but is susceptible to false-positive diagnoses in cases of asymptomatic carriers or environmental contamination. Serological tests have a poor sensitivity because of the difficulty in detecting specific IgM by immunofluorescence techniques and the delayed rise of antibodies. In fact, IgM appears after 3 weeks in *C. pneumoniae* infections, and IgG after 6–8 weeks.[23] The half-life of serological IgA is short (less than 1 week), and the presence of IgA may correspond to persistent antigenic stimulation and reflect chronic infection.[23,24] Emre et al. identified *C. pneumoniae* in 11% of patients by culture and in 25% by serological testing in children with exacerbated asthma, and reported improvement in respiratory symptoms after treatment with macrolides.[25] Cunningham et al.,[8] using PCR and specific secretory IgA in nasal aspirates, showed similar results in asthmatic children with or without exacerbations (23% and 28%, respectively). However, patients with repeatedly positive PCR and high local IgA presented with more frequent acute exacerbations, suggesting a link between asthma symptoms and chronic *C. pneumoniae* infection.[8] Four *C. pneumoniae* serological tests were positive in our study (5%), of which three might be suggestive of chronic infections, according to our definition.

M. pneumoniae is the primary causative agent of community-acquired pneumonia in children aged 2–15 years in industrialized countries, and is more common after 3 years of age.[20] Asymptomatic carriers are not infrequent, and represent 20% of positive PCR results in cases of familial epidemics.[26] As in previous studies, our detection

rate was low in exacerbated asthma. We detected four *M. pneumoniae* infections (5%) using serological tests, including two dual infections with virus. Using the more sensitive PCR techniques, Freymuth et al. reported *M. pneumoniae* infections in 2.2% of patients,[14] and Cunningham et al. in only 1%.[8] These studies and our data suggest a minor role for *M. pneumoniae* in the exacerbation of asthma.

Relation Between Infections and Asthma

Contrary to an increase in antibodies or positive IFA results on nasal aspirates, PCR may detect virus not only in recent infections, but also in asymptomatic carriers or during latent viral infections. In Freymuth et al.[14] and Johnston et al.,[7] nasal aspirates were repeated in some children at different times, and no chronic infections were detected. In our study, we examined a control population of 27 asthmatic outpatients with demographic characteristics similar to those of the hospitalized children. Only one PCR was positive for enterovirus in the control group. In this patient, subsequent examination revealed the presence of rhinitis at the time of sampling, with no symptoms of asthma. Therefore, our study provides further supportive evidence that acute infection is a significant cause of exacerbations of asthma. Viruses can induce asthmatic crises by two different pathways: directly, by infection of the lower airways; or indirectly, by an infection limited to the upper airways, which induces lower airway inflammation by immunological and neurogenic mechanisms, and consequently asthma symptoms.[27] Recent studies have shown positive signals for rhinovirus in bronchial biopsies[28] or bronchiolar lavage fluid,[29] strongly suggesting that rhinovirus replicates in the lower airways, as do parainfluenza and influenza viruses, adenovirus, and RSV. These results support the hypothesis of a direct effect on the lower respiratory tract. Although the precise mechanisms by which respiratory viruses cause symptoms are unknown, there is evidence to suggest that the immune response to the virus plays a major role in symptom pathogenesis.[27,30–32] This response is complex and involves many airway cells, cytokines, and mediators, but few of these are likely to play key roles in this process.[30–32] The degree of chronic allergic inflammation may influence the type of immune response to viral infection.[17]

Therapeutic Implications

We tried to determine features characterizing patients with infection. There were no significant differences between the two groups regarding severity of asthma, severity of asthma exacerbations, or presence of radiological infiltrates or biological changes. Parameters statistically linked to infection were the presence of digestive symptoms and fever. Digestive symptoms are frequently associated with respiratory infections, especially in the case of enterovirus, rhinovirus, or atypical bacteria. In spite of discriminative statistical analyses, we were unable to estimate a predictive score for viral or atypical bacterial infection from the characteristics of patients at hospital admission. The determination of such a score would require a larger study with optimal microbiological assays.

Antibiotic treatment may be effective in atypical bacterial infections. In our study, the initial clinical or radiographic features in children with *M. pneumoniae* or *C. pneumoniae* infections did not differ from those of children without atypical bacterial infections. Therefore, the low incidence does not justify a systematic antibiotic treatment in all children with asthma exacerbations. However, our study suggests that atypical bacterial infections are linked to the persistence of respiratory symptoms 3 weeks after the onset of an exacerbation. More than *M. pneumoniae*, *C. pneumoniae* may contribute to the persistence of asthmatic symptoms poorly controlled by steroids,[8,21,33] and these atypical bacteria should be sought in such cases. These infections are difficult to eradicate and seem to require prolonged use (4–6 weeks) of macrolides.[34]

CONCLUSIONS

Although microbiological diagnosis remains difficult, especially for viruses and atypical bacteria, we confirm the high incidence of viral infections in acute exacerbations of asthma. Atypical bacteria, and especially *C. pneumoniae*, are incriminated in the persistence of asthmatic symptoms, and chronic infection should be investigated when prolonged asthmatic symptoms are poorly controlled by steroids. The high incidence of viral infections justifies research into preventive treatment. Currently, only influenza vaccine is effective. Strategies to prevent or cure viral respiratory infection, either through vaccination or medication, could potentially reduce the occurrence of exacerbations of asthma, improve the quality of life of asthmatic patients, and reduce the cost of asthmatic disease.

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