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Aetiology of acute pharyngitis: the role of atypical bacteria

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In order to establish the role of atypical bacteria and compare characteristics of different infectious agents in acute pharyngitis, 127 patients with acute pharyngitis (66 males; median age, 5·33 years; range, 6 months to 14 years) and 130 healthy subjects of similar sex and age were studied. Serology with paired samples and PCR on nasopharyngeal aspirates and throat cultures were used to identify bacteria and viruses. Viruses were identified in 43 patients (33·8 %) and five controls (3·8 %; $P < 0{\cdot}0001$), potential bacterial pathogens in 34 patients (26·8 %) and 26 controls (20 %; $P = 0{\cdot}256$) and mixed viral/bacterial pathogens in 26 patients (20·5 %) and none of the controls ($P < 0{\cdot}0001$). The main aetiological agents were adenovirus, respiratory syncytial virus (RSV), *Mycoplasma pneumoniae*, *Streptococcus pyogenes* and *Chlamydia pneumoniae*. *M. pneumoniae* was the agent found most frequently as a single pathogen. A history of recurrent pharyngitis, having older siblings and a negative outcome were significantly more common among patients with acute *M. pneumoniae* infection than among those with infections due to other pathogens or healthy controls. This study demonstrates that: (i) adenovirus and RSV have a prominent role in acute pharyngitis; (ii) *S. pyogenes* is found frequently, but it is not possible to distinguish simple carriers from patients with a true infection; (iii) *M. pneumoniae* appears to be able to cause acute pharyngitis *per se*; and (iv) *C. pneumoniae* seems to be mainly a co-pathogen. To avoid the risk of an incorrect therapeutic approach, simple laboratory investigations that allow rapid identification of *M. pneumoniae* infections are urgently needed.

INTRODUCTION

Acute pharyngitis is one of the most common childhood illnesses to be diagnosed in an outpatient setting (Bisno *et al.*, 1997, 2002; Bisno, 2001). Viruses and *Streptococcus pyogenes* are considered to be the most frequent causes of this disease, but particular attention is usually given only to streptococcal cases, as they may be followed by severe early and late complications and are the only ones for which antibiotic treatment is definitely indicated (Bisno *et al.*, 1997, 2002; Bisno, 2001).

It has recently been demonstrated that a significant part of non-streptococcal acute pharyngitis may be associated with *Mycoplasma pneumoniae* and/or *Chlamydia pneumoniae* infection, but it has not been established whether these atypical bacteria act simply as co-pathogens or as primary aetiological agents, nor is it known what the outcome of such infections is when they are not treated with antibiotics (Principi & Esposito, 2001; Esposito *et al.*, 2002b). Definition of the real role of *M. pneumoniae* and *C. pneumoniae* in causing acute pharyngitis would be useful for deciding the best diagnostic and therapeutic approach. If these bacteria are primary causes of pharyngitis, and if the disease is likely to have a more complicated course unless treated with adequate antimicrobial agents, some cases that are not usually treated (as they are considered to be of viral origin) need to be identified and treated correctly. These bacteria can be eradicated safely in children only with macrolides (File *et al.*, 1998; Principi & Esposito, 1999; Hammerschlag, 2000, 2001), which, at least in some geographical areas, are not always active against streptococcal cases (Cornaglia *et al.*, 1998; Facinelli *et al.*, 2001; Martin *et al.*, 2002). On the other hand, penicillin V, amoxycillin and oral cephalosporins, which are currently considered to be the drugs of choice for streptococcal pharyngitis (Bisno *et al.*, 2002), are not active against atypical bacteria (File *et al.*, 1998; Principi & Esposito, 1999; Hammerschlag, 2000, 2001).

Although microbiological diagnostic methods have improved greatly over the last 20 years (Mäkelä *et al.*, 1998; Heikkinen *et al.*, 2002; Monto, 2002), no recent prospective and comprehensive study of the aetiology of acute pharyngitis has been published. The aim of this study was to

Abbreviations: EBV, Epstein–Barr virus; HSV-1, herpes simplex virus type 1; RSV, respiratory syncytial virus.

evaluate the aetiology of acute pharyngitis in childhood in order to establish the role of atypical bacteria and compare the characteristics of infections due to different pathogens.

METHODS

Study population and follow-up. The study was conducted at the primary care centre of the Institute of Paediatrics, University of Milan, Italy, between February 2000 and March 2002. The level of pharyngitis over time was not concurrent with epidemic peaks of viral or bacterial infections. During this period, we enrolled 127 patients aged between 6 months and 14 years (66 males; median age of all enrolled children, 5·33 years) with an acute episode of pure pharyngitis, defined as evidence of uvular and pharyngeal or tonsillar inflammation in the presence of fever, sore throat or dysphagia, without any signs or symptoms of lower respiratory tract infection (Kaplan & Johnson, 2001). Exclusion criteria included severe concomitant diseases (e.g. neoplasia, kidney or liver disease, immunodepression, cardiovascular disease or malabsorption syndrome) and systemic antibiotic treatment in the 48 h preceding study entry or therapy with azithromycin during the previous week or with intramuscular benzathine penicillin G during the previous month. During the same period, 130 healthy subjects of similar sex and age (67 males; median age, 5·11 years) without any history of respiratory tract infection or antibiotic treatment in the 3 months before enrolment, who were seen for minor surgical problems, were enrolled as a control group. The study protocol was approved by the institutional review board of the University of Milan and written informed consent was obtained from the parents or legal guardians of all participants.

Upon enrolment, systematic recordings were made of the children's demographic characteristics and medical history by using standardized written questionnaires (Principi *et al.*, 1999; Esposito *et al.*, 2002b). Questions included duration of breast-feeding, living conditions, childcare attendance, number and age of family members, birth rank, smoking habits of family members living together, number and type of respiratory infections in the previous 6 months, presence of a history of recurrent pharyngitis (defined as at least three acute episodes in the 6 months preceding enrolment) (Korppi, 1997; Esposito *et al.*, 2002b) and number and type of antimicrobials administered during the previous 6 months.

After a complete physical examination, laboratory samples were taken from all patients and controls, including venous blood specimens for haematological and blood chemistry tests (haemoglobin, leukocyte differential count, erythrocyte sedimentation rate and C-reactive protein), acute serum for assay of antibodies to *M. pneumoniae*, *C. pneumoniae*, adenovirus, influenza A and B viruses, parainfluenza virus types 1, 2 and 3, respiratory syncytial virus (RSV), Epstein–Barr virus (EBV) and herpes simplex virus type 1 (HSV-1), nasopharyngeal aspirates for detection of *M. pneumoniae* and *C. pneumoniae* DNA and a pharyngeal swab for *S. pyogenes* culture. Children with acute pharyngitis whose throat swabs were positive for *S. pyogenes* received 50 mg amoxycillin kg^{-1} day^{-1} (divided between three daily doses) for 10 days, whereas other patients received only symptomatic therapy; no other antibiotic treatment was given during the study period to any patients without streptococcal infection and no therapy was administered to controls, even when throat swab results were positive for *S. pyogenes*. The children's caregivers were asked to complete a diary card concerning the child's signs and symptoms of pharyngitis and any other medical problems. Parents were also asked to return immediately to the study centre if the children experienced any persistent, recurrent or worsening signs or symptoms.

All children were scheduled to return on days 11–15 after enrolment for an additional visit, at which their signs and symptoms were assessed. Between 4 and 5 weeks after enrolment, medical history, general physical condition and clinical symptoms of each child were re-evaluated and a second serum sample was obtained, to assay convalescent antibody titres against *M. pneumoniae*, *C. pneumoniae* and viruses.

Each child's clinical outcome was defined at each visit as 'positive' (absence of signs and symptoms of pharyngitis) or 'negative' (persistence or progression of signs and symptoms of pharyngitis or development of new clinical findings consistent with acute pharyngitis) (Kaplan & Johnson, 2001; Principi *et al.*, 2001; Esposito *et al.*, 2002b).

Antibody assays and PCR for atypical bacteria. Each acute and convalescent serum sample was tested after absorption for IgM and IgG antibodies to *M. pneumoniae* (ELISA; Pantec), and IgM, IgA and IgG antibodies to *C. pneumoniae* (microimmunofluorescence; Labsystems) as described previously (Wang & Grayston, 1970; Principi *et al.*, 2001; Esposito *et al.*, 2002a). Nasopharyngeal aspirates were evaluated for the presence of *M. pneumoniae* and *C. pneumoniae* DNA by using validated nested PCR for both pathogens, as described previously (Tong & Sillis, 1993; Abele-Horn *et al.*, 1998; Blasi *et al.*, 1999; Principi *et al.*, 2001; Esposito *et al.*, 2002a).

The nested PCR technique for *M. pneumoniae* was developed by Abele-Horn *et al.* (1998), and that for *C. pneumoniae* by Tong & Sillis (1993); both techniques have been found to correlate well with cultures (Tong & Sillis, 1993; Abele-Horn *et al.*, 1998). To avoid risk of contamination, sample preparation, PCR amplification and product analysis were performed in separate rooms. Positive and negative controls were included in each assay. Negative controls contained all PCR reagents and sterile distilled water; in the case of *M. pneumoniae*, we also used DNA from the reference strains *Mycoplasma orale* T519, *Mycoplasma salivarium* A889 and *Mycoplasma genitalium* G37c, as described previously (Abele-Horn *et al.*, 1998). Serial dilutions of purified *M. pneumoniae* and *C. pneumoniae* were used as positive controls for all runs in order to ensure successful nucleic acid amplification. All PCR-negative samples were analysed by PCR for the presence of β-actin DNA, to confirm the presence of DNA in the samples.

Primers MP-1 and MP-2 were used for *M. pneumoniae*-specific amplification (Abele-Horn *et al.*, 1998). For the first and second rounds of amplification, reactions were carried out in 50 µl volumes, with 0·1 µM each primer (Abele-Horn *et al.*, 1998). Amplification was carried out for 40 cycles, each consisting of 20 s at 95 °C, 2 min at 63 °C and 1 min at 72 °C (Principi *et al.*, 2001). For *M. pneumoniae* nested PCR, primers MUH-1 and MUH-2 were used (Abele-Horn *et al.*, 1998). Nested amplification was performed by using 5 µl of a 1 : 10 dilution of PCR product (5 µl in 45 µl sterile water) from the first round of amplification, under identical conditions (Abele-Horn *et al.*, 1998).

Nested touchdown PCR for detection of *C. pneumoniae* DNA was performed by using primers that were designed to detect the major outer-membrane protein (Tong & Sillis, 1993; Blasi *et al.*, 1999). Extracted DNA solution (10 µl in a total volume of 50 µl) was used in the first round of PCR, then 5 µl PCR product that was amplified by the outer primers was transferred to a new PCR mix (total volume, 50 µl) for a second amplification using the inner primers (Blasi *et al.*, 1999). The first round consisted of 40 cycles and the second of 35 cycles (Blasi *et al.*, 1999).

Acute *M. pneumoniae* and/or *C. pneumoniae* infection was diagnosed if the child had a significant antibody response to one of the pathogens in paired sera (*M. pneumoniae*: an IgM-specific antibody titre of $\geqslant$1 : 100 or a fourfold increase in IgG antibody; *C. pneumoniae*: an IgM-specific antibody titre of $\geqslant$1 : 16 or a fourfold increase in IgG antibody) and/or if nasopharyngeal aspirates were PCR-positive, according to previously established criteria (Principi *et al.*, 2001; Esposito *et al.*, 2002a, b).

Viral antibody assays. Acute and convalescent serum samples were also tested by ELISA for IgM and IgG antibodies to adenovirus,

influenza A and B viruses, parainfluenza virus types 1, 2 and 3, RSV, EBV and HSV-1, according to the manufacturer's instructions (Pantec).

Acute adenovirus, influenza A and B viruses, parainfluenza viruses types 1, 2 and 3, RSV, EBV and/or HSV-1 infection was diagnosed if the child had a significant antibody response to one of the viruses in paired sera (for each virus, an IgM-specific antibody titre of $\geqslant$1 : 100 or a fourfold increase in IgG antibody), according to previously established criteria (Heiskanen-Kosma *et al.*, 1998). For EBV, IgM and IgG antibodies against viral capsid antigen were evaluated.

***S. pyogenes* culture.** Throat cultures were obtained by swabbing both tonsils and the posterior pharynx with a rayon-tipped swab (BBL Becton Dickinson). The swab was then placed in Amies' medium without charcoal and plated within 2 h onto 5 % sheep's blood agar; the agar was stabbed and a bacitracin disc was placed in the primary streak (BBL Becton Dickinson). Plates were incubated at 37 °C in 5 % carbon dioxide and were examined after 24 and 48 h, according to previously described procedures (Stevens & Kaplan, 2000; Martin *et al.*, 2002). Any β-haemolytic colonies were subcultured, isolated and typed (PathoDx).

Acute *S. pyogenes* infection was diagnosed by the presence of *S. pyogenes* in throat cultures of patients with acute pharyngitis; presence of *S. pyogenes* in throat cultures of healthy controls was considered to be a carrier state.

Statistical analysis. Data entry and statistical analyses were carried out by using SAS software (version 8.0; SAS Institute). A P value of $<$0·05 was considered to be statistically significant for all statistical tests. Parametric data were compared by using analysis of variance (ANOVA) with terms for treatment and tests for multiple comparisons; when data were not normally distributed or were non-parametric, the Kruskal–Wallis test was used. Categorical data were analysed by using contingency analysis and a χ^2 or Fisher's test.

RESULTS

Bacterial and viral findings

At least one of the bacteria or viruses studied was found in 103 patients (81·1 %) and 31 controls (23·8 %; $P < 0{\cdot}0001$) (Table 1). Viruses were demonstrated in 43 patients (33·8 %) and five controls (3·8 %; $P < 0{\cdot}0001$), bacteria in 34 patients (26·8 %) and 26 controls (20 %; $P = 0{\cdot}256$) and mixed viral/bacterial pathogens in 26 patients (20·5 %) and none of the controls ($P < 0{\cdot}0001$). The agents found most frequently were adenovirus (34 patients versus four controls; $P < 0{\cdot}0001$), RSV (27 patients versus one control; $P < 0{\cdot}0001$), *M. pneumoniae* (25 patients versus three controls; $P < 0{\cdot}0001$), *S. pyogenes* (24 patients versus 21 controls; $P = 0{\cdot}678$) and *C. pneumoniae* (17 patients versus two controls; $P = 0{\cdot}0006$). Acute *M. pneumoniae* infection was

Table 1. Microbiological findings in 127 children with signs and symptoms of acute pharyngitis and 130 healthy controls

Infection or pathogen	No. (%) of patients with pharyngitis	No. (%) of healthy controls	*P* value
Single viral infection:	37 (29·1)	5 (3·8)	$<$ 0·0001
Adenovirus	16 (12·6)	4 (3·1)	0·008
RSV	10 (7·9)	1 (0·8)	0·012
Parainfluenza virus type 3	5 (3·9)	0	0·028
Influenza B virus	3 (2·4)	0	0·119
EBV	2 (1·5)	0	0·243
Influenza A virus	1 (0·8)	0	0·494
Double viral infection:	6 (4·7)	0	0·013
Adenovirus + RSV	6 (4·7)	0	0·013
Single bacterial infection:	28 (22·0)	26 (20·0)	0·802
M. pneumoniae	18 (14·2)	3 (2·3)	0·001
S. pyogenes	6 (4·7)	21 (16·2)	0·005
C. pneumoniae	4 (3·1)	2 (1·5)	0·443
Double bacterial infection:	6 (4·7)	0	0·013
M. pneumoniae + *C. pneumoniae*	3 (2·4)	0	0·119
C. pneumoniae + *S. pyogenes*	2 (1·5)	0	0·243
M. pneumoniae + *S. pyogenes*	1 (0·8)	0	0·494
Viral and bacterial infections:	26 (20·5)	0	$<$ 0·0001
Adenovirus + *S. pyogenes*	8 (6·3)	0	0·003
RSV + *C. pneumoniae*	7 (5·6)	0	0·006
RSV + *S. pyogenes*	4 (3·1)	0	0·058
Adenovirus + *M. pneumoniae*	3 (2·4)	0	0·119
Influenza B virus + *S. pyogenes*	2 (1·5)	0	0·243
Adenovirus + *C. pneumoniae*	1 (0·8)	0	0·494
Parainfluenza virus type 1 + *S. pyogenes*	1 (0·8)	0	0·494
No pathogen	24 (18·9)	99 (76·2)	$<$ 0·0001

determined serologically in all infected subjects (specific IgM $\geqslant$1 : 100 in 21 patients and two controls, and a fourfold increase in IgG in four patients and one control), and confirmed by PCR in 16 patients (64 %) and none of the controls. Acute *C. pneumoniae* infection was determined serologically in 10 of 17 infected patients and in the two infected controls (all with a fourfold increase in IgG; none was positive for IgM), and confirmed by PCR in six patients (60 %) and none of the controls. *C. pneumoniae* DNA was detected in a further seven patients, who showed no serological signs of acute infection.

A single pathogen was demonstrated in 65 patients (51·1 %) and 31 controls (23·8 %; $P < 0{\cdot}0001$), whereas two pathogens were diagnosed in 38 patients (29·9 %) and none of the controls ($P < 0{\cdot}0001$). Among patients, *M. pneumoniae* was the single pathogen that was found most frequently (18/25 cases of *M. pneumoniae* infection, 72 %), followed by adenovirus (16/34 cases of adenoviral infection, 47·1 %) and RSV (10/27 cases of RSV infection, 37 %). *S. pyogenes* and *C. pneumoniae* were found as single pathogens in a minority of patients [6/24 cases of *S. pyogenes* infection (25 %) and 4/17 cases of *C. pneumoniae* infection (23·5 %)].

Clinical and laboratory findings

Table 2 shows the epidemiological and clinical characteristics of patients with acute pharyngitis and healthy controls in whom a single infectious agent was demonstrated. A history of recurrent pharyngitis and having older siblings were significantly more frequent in patients with acute *M. pneumoniae* infection than in those with pharyngitis associated with other infections and healthy controls. No other significant difference between groups of patients was observed; none of the controls had signs or symptoms of acute pharyngitis. Similar results were obtained when patients with infections due to *M. pneumoniae* together with other pathogens were compared with other multiple infections, thus confirming the relationship between *M. pneumoniae* and history of recurrent pharyngitis and having older siblings.

Table 3 summarizes the laboratory data at enrolment in patients with acute pharyngitis and healthy controls in whom a single infectious agent was demonstrated. There were no significant differences between patients with pharyngitis due to the various infectious agents and these parameters were also not significantly different among patients with multiple infections. Total white blood cell counts, C-reactive protein concentrations and erythrocyte sedimentation rates were significantly higher in all groups of patients with pharyngitis than in healthy controls.

Clinical outcome

Table 4 compares the clinical outcome of patients with acute pharyngitis and healthy controls in whom a single infectious agent was demonstrated. Duration of fever was significantly longer in patients with acute *M. pneumoniae* infection than in those with infections due to other pathogens. There were no significant inter-group differences in duration of the other symptoms of pharyngitis, but there was a trend toward longer persistence of all symptoms among patients with *M. pneumoniae* infection. A negative outcome, i.e. recurrence of acute pharyngitis after an initial cure, was significantly more frequent among patients with acute *M. pneumoniae* infection than among those with infections due to other single pathogens. No patient developed different respiratory tract infections or progressed to more serious lower respiratory tract disease or other systemic infection during the study period. Similar results were observed when patients with infections due to *M. pneumoniae* plus other pathogens were compared with those with other multiple infections. None of the healthy controls showed any signs or symptoms of disease during the study period.

DISCUSSION

This study highlights the prominent role of viruses, mainly adenovirus and RSV, in acute childhood pharyngitis (Putto *et al.*, 1986; Putto, 1987; Tsai *et al.*, 2001; Nokso-Koivisto *et al.*, 2002). It also shows that, among the bacteria studied, *S. pyogenes* is found frequently in acute pharyngitis (Bisno *et al.*, 1997, 2002; Bisno, 2001) but, as it is often present together with other viruses or bacteria that are involved in the aetiology of the disease and may also be present in healthy subjects, it is not possible to distinguish carriers from patients with a true infection. Finally, our results indicate that atypical bacteria play a role in causing acute pharyngitis, but show that this is much more relevant for *M. pneumoniae* than for *C. pneumoniae*. These data, supported by the findings that atypical bacterial infections are very rare in healthy controls, suggest that *M. pneumoniae* is able to cause acute pharyngitis *per se*, whereas *C. pneumoniae* seems to be mainly a co-pathogen.

The importance of *M. pneumoniae* as a primary cause of acute pharyngitis appears to be further demonstrated by the history and clinical outcome in patients with this infection. Previous recurrent episodes of pharyngitis seem to be specific for acute *M. pneumoniae* infection; this may be considered to be a useful parameter for differentiating *M. pneumoniae* from other pathogens in acute pharyngitis. Similar data have been observed in wheezing; a history of recurrent wheezing is significantly more frequent in patients infected by *M. pneumoniae* (Esposito *et al.*, 2000). Presence of older siblings also seems to be more common in children with acute *M. pneumoniae* infection, a finding that concurs with data that show that transmission of the pathogen occurs frequently in the household, with school-age children being the main reservoir (Dorigo-Zetsma *et al.*, 2001). Moreover, most episodes of non-streptococcal pharyngitis associated with infections due to *C. pneumoniae* and/or viruses resolved spontaneously in our patients and did not relapse, despite absence of antibiotic treatment, whereas a significant proportion of pharyngitis associated with *M. pneumoniae* infections had a negative course, with longer duration of fever and recurrence of symptoms within a short time. This seems to confirm the role of *M. pneumoniae* in the pathogen-

Table 2. Epidemiological and clinical characteristics of 65 children with acute pharyngitis and 31 healthy controls in whom a single infectious agent was demonstrated

Numbers in parentheses are percentages.

Characteristic	Patients with pharyngitis in whom a single infectious agent was demonstrated				Healthy controls
	M. pneumoniae ($n = 18$)	*C. pneumoniae* ($n = 4$)	*S. pyogenes* ($n = 6$)	Single virus ($n = 37$)	Single infectious agent ($n = 31$)
Male	8 (44·4)	2 (50·0)	3 (50·0)	19 (51·3)	16 (51·6)
Race (white)	17 (94·4)	4 (100·0)	6 (100·0)	36 (97·3)	30 (96·8)
Age:					
<2 years	4 (22·2)	1 (25·0)	0	10 (27·0)	6 (19·4)
2–5 years	6 (33·3)	1 (25·0)	3 (50·0)	18 (48·7)	13 (41·9)
6–10 years	7 (38·9)	1 (25·0)	2 (33·3)	7 (18·9)	9 (29·0)
>10 years	1 (5·6)	1 (25·0)	1 (16·7)	2 (5·4)	3 (9·7)
Breast-feeding for ⩾3 months	13 (72·2)	3 (75·5)	4 (66·6)	26 (70·3)	22 (70·9)
Urban residence	17 (94·4)	3 (75·5)	5 (83·3)	35 (94·5)	27 (87·1)
Full-time attendance at childcare centre*	16 (88·8)	3 (75·5)	5 (83·3)	30 (81·1)	25 (80·6)
No. subjects in each childcare centre:					
<20	6 (33·3)	2 (50·0)	2 (33·4)	13 (35·1)	12 (38·7)
⩾20	12 (66·7)	2 (50·0)	4 (66·6)	24 (64·9)	19 (61·3)
Passive smoking	10 (55·6)	2 (50·0)	3 (50·0)	20 (54·1)	16 (51·6)
Recurrent episodes of pharyngitis†	15 (83·3)‡§	0	1 (16·6)	5 (13·6)	0
Having older sibling(s)	15 (83·3)‡§	1 (25·0)	2 (33·4)	2 (5·4)	3 (9·7)
Antibiotic therapy in the previous 6 months	4 (22·2)	1 (25·0)	1 (16·6)	8 (21·6)	3 (9·7)
Axillary temperature ⩾38 °C	16 (88·8)‖	3 (75·5)¶	5 (83·3)‖	32 (86·5)	0
Cough	9 (50·0)‖	2 (50·0)¶	3 (50·0)#	18 (48·6)‖	0
Rhinitis	8 (44·5)‖	2 (50·0)¶	3 (50·0)#	20 (54·1)‖	0
Sore throat#	12 (85·7)‖	2 (66·7)¶	5 (83·3)‖	22 (78·6)‖	0
Dysphagia#	5 (35·7)¶	1 (25·0)	2 (33·3)#	9 (24·3)#	0
Pharyngeal erythema	18 (100·0)‖	4 (100·0)‖	6 (100·0)‖	37 (100·0)‖	0
Pharyngeal exudate	7 (38·9)¶	1 (25·0)	2 (33·4)#	12 (32·4)#	0
Tonsillar enlargement	15 (83·3)‖	3 (75·5)¶	5 (83·3)‖	30 (81·1)‖	0
Cervical lymphadenopathy	10 (55·6)‖	2 (50·0)¶	3 (50·0)#	20 (54·1)‖	0

*5–6 days week^{-1}, 6–8 h day^{-1}.

†Defined as at least three acute episodes of pharyngitis in the 6 months preceding enrolment.

‡$P < 0{\cdot}05$: patients with *M. pneumoniae* infection versus patients with *C. pneumoniae* and *S. pyogenes* infections.

§$P < 0{\cdot}05$: patients with *M. pneumoniae*, *C. pneumoniae*, *S. pyogenes* and single virus infections versus healthy controls.

‖$P < 0{\cdot}0001$: patients with *M. pneumoniae*, *C. pneumoniae*, *S. pyogenes* and single virus infections versus healthy controls.

¶$P < 0{\cdot}0001$: patients with *M. pneumoniae* infection versus patients with infection due to single virus and healthy controls.

#Evaluated only in children aged >2 years.

esis of pharyngitis and suggests that antibiotic therapy is required for *M. pneumoniae* infection, in order to minimize the risk of recurrence and reduce transmission of the pathogen within households.

Unfortunately, none of the clinical or laboratory findings in children with acute pharyngitis was associated with a particular aetiological agent and none was sufficient to distinguish one infection from another. However, it is well-known that the presence of *S. pyogenes* cannot be diagnosed on the basis of presenting manifestations or non-specific laboratory findings, but can only be identified by means of a throat culture or rapid antigen detection test (Putto *et al.*, 1986; Putto, 1987; Bisno *et al.*, 1997, 2002; Bisno, 2001). However, diagnosis of acute *M. pneumoniae* infection currently depends on non-routine laboratory methods (File *et al.*, 1998; Layani-Milon *et al.*, 1999; Apfalter *et al.*, 2001; Dowell *et al.*, 2001; Esposito & Principi, 2002), which make it difficult to identify in outpatient practice. To avoid risk of an inappropriate therapeutic approach to acute

Table 3. Laboratory data of 65 children with acute pharyngitis and 31 healthy controls in whom a single infectious agent was demonstrated

Values are means ± SD. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell count.

Parameter	Patients with pharyngitis in whom a single infectious agent was demonstrated				Healthy controls
	M. pneumoniae ($n = 18$)	*C. pneumoniae* ($n = 4$)	*S. pyogenes* ($n = 6$)	Single virus ($n = 37$)	Single infectious agent ($n = 31$)
WBC (cells μl^{-1})	11 504 ± 5182*	11 573 ± 5859*	12 889 ± 4534*	12 530 ± 4731*	6460 ± 2960
Neutrophils (%)	61 ± 16	60 ± 20	64 ± 15	62 ± 16	55 ± 15
Lymphocytes (%)	30 ± 16	30 ± 18	27 ± 16	28 ± 17	36 ± 15
Monocytes (%)	7 ± 3	8 ± 3	7 ± 3	8 ± 3	7 ± 3
Eosinophils (%)	1 ± 2	1 ± 1	1 ± 2	1 ± 2	1 ± 2
Basophils (%)	0·3 ± 0·6	0·4 ± 0·7	0·3 ± 0·4	0·3 ± 0·6	0·4 ± 0·6
CRP ($\mu g\ dl^{-1}$)	38 ± 32*	34 ± 29*	43 ± 40*	39 ± 34*	7 ± 3
ESR ($mm\ h^{-1}$)	34 ± 15*	31 ± 16*	34 ± 19*	36 ± 18*	9 ± 4

*$P < 0·05$: patients with *M. pneumoniae*, *C. pneumoniae*, *S. pyogenes* and single virus infections versus healthy controls.

Table 4. Comparison of the clinical outcome of 65 children with acute pharyngitis and 31 healthy controls in whom a single infectious agent was demonstrated

No patient or healthy control had a negative outcome on day 11–15.

Clinical outcome	Patients with pharyngitis in whom a single infectious agent was demonstrated				Healthy controls
	M. pneumoniae ($n = 18$)	*C. pneumoniae* ($n = 4$)	*S. pyogenes** ($n = 6$)	Single virus ($n = 37$)	Single infectious agent ($n = 31$)
Duration of axillary temperature ⩾38 °C (mean days ± SD)	3·76 ± 2·28†‡	2·19 ± 1·40‡	2·14 ± 1·25‡	2·53 ± 1·55§	0
Duration of cough (mean days ± SD)	3·10 ± 1·88‡	3·01 ± 1·70‡	2·99 ± 1·77‡	2·78 ± 1·96‡	0
Duration of rhinitis (mean days ± SD)	4·16 ± 2·10‡	3·96 ± 2·28‡	3·69 ± 2·40‡	4·10 ± 2·19‡	0
Duration of sore throat (mean days ± SD)‖	2·48 ± 1·58‡	2·27 ± 1·61‡	1·81 ± 1·69‡	1·96 ± 1·55‡	0
Duration of dysphagia (mean days ± SD)‖	2·36 ± 1·70‡	2·10 ± 1·88‡	1·76 ± 1·49‡	1·82 ± 1·79‡	0
Negative outcome on day 28–35	14 (77·8)§¶	0	1 (16·7)	2 (5·4)	0

*Patients with acute pharyngitis and *S. pyogenes* infection were treated with 50 mg amoxycillin $kg^{-1}\ day^{-1}$ (divided between three daily doses) for 10 days, whereas other patients and healthy controls did not receive any antimicrobials.

†$P < 0·05$: patients with *M. pneumoniae* infection versus patients with *C. pneumoniae*, *S. pyogenes* and single virus infections.

‡$P < 0·0001$: patients with *M. pneumoniae*, *C. pneumoniae*, *S. pyogenes* and single virus infections versus healthy controls.

§$P < 0·0001$ patients with *M. pneumoniae* infection versus patients with single virus infection and healthy controls.

‖Evaluated only in children aged >2 years.

¶$P < 0·05$ patients with *M. pneumoniae* infection versus patients with *C. pneumoniae* and *S. pyogenes* infections.

pharyngitis, simple laboratory investigations that allow rapid identification of infections due to *M. pneumoniae* are urgently needed.

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