



Occurrence of *Chlamydia trachomatis* and *Chlamydia pneumoniae* in paediatric respiratory infections

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ABSTRACT: An emerging body of evidence suggests that half of asthma in both children and adults is associated with chronic lung infection. The aim of the present study was to determine the frequency of viable *Chlamydia pneumoniae* (*Cp*) and *C. trachomatis* (*Ct*) in the respiratory tracts of paediatric patients with chronic respiratory diseases.

Bronchoalveolar lavage fluid (BALF) samples obtained from 182 children undergoing bronchoscopy for clinical reasons were assayed using PCR analysis, *in vitro* tissue culture and immunofluorescence staining for the presence of *Cp* and *Ct*.

Chlamydia-specific DNA was detected by PCR in 124 (68%) out of 182 patients; 79 were positive for *Cp*, 77 positive for *Ct* and 32 for both organisms; 75 patients had cultivable *Chlamydia*. *Ct* DNA prevalence decreased, whereas *Cp* positivity generally increased with age. A total of 59 out of 128 asthma patients and 16 out of 54 nonasthmatics were *Chlamydia* culture positive. When the patients were divided into inflammatory versus noninflammatory airway disease, there were 69 (46%) out of 150 and six (18%) out of 32 BALF samples with cultivable *Chlamydia*, respectively.

Viable *Chlamydia pneumoniae* and *Chlamydia trachomatis* occur frequently in children with chronic respiratory diseases and may be more prevalent in asthma patients. To the current authors' knowledge, this is the first report of viable *Chlamydia trachomatis* in the lungs of children.

KEYWORDS: Asthma, *Chlamydia*, chronic, lung disease

Respiratory diseases represent a major cause of disability and mortality among all age groups and races worldwide. They are also a leading cause of hospitalisation and morbidity in both adults and children, especially in developing countries [1–3]. Chronic respiratory diseases, which include asthma and chronic obstructive pulmonary disease (COPD), are just as prevalent as acute forms of these disorders [2]. In addition to known environmental and genetic contributors, there is growing evidence that pathogenic micro-organisms may play a role in diseases such as asthma [4–6]. It is often difficult, however, to identify the aetiological agent(s) of these respiratory infections, owing to a lack of standard diagnostic tools and the need for invasive procedures, such as lung aspirations or pulmonary biopsies, in order to confirm diagnosis. The worldwide increase in the incidence of asthma and the impact of the disease on public health, however, has led to renewed interest and investigations into its aetiopathogenesis.

Chlamydia trachomatis (*Ct*) and *C. pneumoniae* (*Cp*) are two of the most common members of the Chlamydiaceae family that infect humans. *Cp* is thought to be responsible for 10–15% of community-acquired pneumonia and 5% of pharyngitis and sinusitis cases [7, 8]. Approximately 50% of healthy young adults and 75% of elderly persons have serological evidence of previous *Cp* infection [9]. There is increasing evidence that *Cp* may play a role in paediatric asthma onset as well as in possible exacerbations of asthmatic symptoms [10, 11]. Conversely, *Ct* has been recognised as a pathogen in nongonococcal urethritis, salpingitis, endocervicitis, pelvic inflammatory disease, inclusion conjunctivitis of neonates, follicular conjunctivitis of adults, infantile pneumonia and associated diseases [12, 13]. Vertical transmission of infection from mother to the infant may result in the development of conjunctivitis and pneumonia [14, 15]. In the past, multiple investigations of paediatric pneumonia have emphasised the

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importance of infections with *Ct* in infants between 2 weeks and 4 months of age [16–18]. While stringent prenatal screening in the USA has greatly reduced the number of cases of neonatal conjunctivitis, there have been reports that ocular prophylaxis can fail to prevent neonatal chlamydial conjunctivitis and does not prevent colonisation or infection at other sites, such as the lungs [3, 19]. Approximately 5–22% of pregnant women are thought to have *Ct* infection of the cervix, and 30–50% of neonates born to infected mothers show culture evidence of infection. Of infected neonates, 15–25% present with clinical conjunctivitis and nasopharyngitis that in some cases develops into neonatal pneumonitis [9]. Published reports have documented that many infants infected with *Ct* at birth remain infected for months or years in the absence of specific antimicrobial therapy [20]. Indeed, some reports have suggested that wheezing may be another clinical expression of *Ct* infection and that this organism should be routinely assayed for in children who wheeze but have no demonstrable allergy and do not respond to the usual anti-asthmatic medications [21]. The current authors recently confirmed the presence of *Chlamydia* in bronchoalveolar lavage fluid (BALF) samples from paediatric patients with various chronic respiratory diseases using PCR and tissue culture techniques [22]. In the work presented herein, using established species specific PCR and culture techniques, these investigations were extended to an examination of the prevalence of both *Cp* and *Ct* in BALF samples collected from paediatric patients. It was shown that both infectious *Cp* and *Ct* are frequently present in lung washings from children with chronic respiratory diseases, and that both organisms may contribute to *Chlamydia*-mediated pneumonitis.

MATERIALS AND METHODS

Specimens

A prospective, consecutive, noninterventional cohort analysis of patients with various pulmonary disorders undergoing elective diagnostic bronchoscopy with BALF sample collection was conducted in a group of 184 patients from a community-based/academic hospital setting. Of these specimens, 70 were previously studied and reported on in a prior communication [22]. Two patients aged >20 yrs were excluded from the study because of the inclusion criteria of age ≤20 yrs for this paediatric cohort based on American Academy of Paediatrics accepted criteria [23]. Patients were recommended for bronchoscopy because they all met the criteria of having severe, persistent airway disease that was nonresponsive to therapy. Residual BALF samples obtained from study participants were de-identified in a manner compliant with the Health Insurance Portability and Accountability Act, and given alphanumeric codes prior to laboratory analysis. Approval for the study was obtained from the Institutional Review Board at Baystate Medical Center (Springfield, MA, USA). Written informed consent was obtained from the guardian of each patient prior to inclusion in the study and patients were not contacted during the course of the study, nor were they made aware of the results of the investigation. BALF was collected as previously reported [22, 24].

PCR analysis

Genomic DNA was isolated from BALF samples and PCR performed as previously described [22]. Initially, isolated DNA

was amplified using a 16S signature sequence to detect all strains of Chlamydiales, as previously reported [22, 25]. *Cp*-specific PCR was performed using the previously published primer pair Cpn 201 and 202 to generate a 207-bp product [26], while *Ct*-specific PCR was performed using the P1 and omp2 primer set that amplified a 1,100-bp segment of the omp1 gene [27]. The PCR products were separated by electrophoresis on a 2% agarose gel and visualised by staining with ethidium bromide. Photographs were taken with the Syngene GeneFlash gel documentation system (Syngene USA, Frederick, MD, USA).

Culture

Cells from BALF samples were pelleted, rinsed with sterile phosphate buffered saline and lysed with sterile glass beads in sucrose phosphate glutamate buffer. Cultures were performed as previously described [22] and cells were stained with a 1:100 dilution of a rabbit anti-*Chlamydia* antibody (BIODESIGN International, Saco, ME, USA) and visualised with a 1:1,000 dilution of Alexa-Fluor[®]488 goat anti-rabbit secondary antibody (Invitrogen, Molecular Probes, Carlsbad, CA, USA). The slides were then examined and photographs taken using a Zeiss LSM 510 Meta Confocal System (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA).

BALF cell counts

Upon recovery, BALF cell counts and differentials were performed according to standard techniques in the haematology clinical laboratory at the Baystate Medical Center. Cell enumeration was performed manually using a cell counting chamber (haemocytometer of Improved Neubauer type; Fisher Scientific, Pittsburgh, PA, USA) under phase microscopy with results expressed as number of cells per cubic millimetre. BALF differential counts were performed using Wright stained cytopsin preparations of BALF which were then examined under oil immersion microscopy (50× or 100× magnification). Results were expressed on the basis of a 100-cell count survey.

Statistics

Cross-tabs with the Fisher's exact test and Chi-squared test were used to determine significance. Univariate and bivariate analyses as well as logistic regression were also used to establish interactions between and among variables. For all analyses, tests were two sided and the level of significance was $p \leq 0.05$.

RESULTS

Patient demographics

The cohort of paediatric respiratory disease patients in the present study consisted of 100 males and 82 females. The average age of this group of patients was 8.7 yrs. Patients were from four different ethnic groups as follows: 121 white patients, 45 Hispanic patients, 15 black patients and one patient of Asian descent (table 1). Asthma diagnosis was made by a combination of family or personal history of atopy, elevated total immunoglobulin (Ig)E levels, positive skin or radioallergosorbent test, reversible flow limitation on spirometry, the presence of increased eosinophil levels, basement membrane thickening on bronchial biopsy, or positive methacholine challenge. All asthma patients met the definition of having severe persistent disease that was uncontrolled (according to Global Initiative for Asthma guidelines [28,

TABLE 1 Cohort demographics

Description	Asthma cohort	Nonasthma subcohort	Total
Patients with diagnosed disease	128	54	182
Average age yrs	8.7	12.5	8.7
Age range yrs			
0.0–2.0	11	18	29
2.1–5.0	27	13	40
5.1–10.0	41	5	46
10.1–15.0	33	13	46
15.1–17.0	16	5	21
Sex			
Male	68	32	100
Female	60	22	82
Ethnicity			
White	85	36	121
Black	13	2	15
Hispanic	30	15	45
Asian	0	1	1
Medication			
Yes	95	31	126
No	33	23	56

Data are presented as n, unless otherwise stated. The cohort consisted of 182 patients (100 males and 82 females). There were significant differences in the number of asthma versus nonasthma subjects in the 0–2.0 yrs age range (Fisher’s exact test, $p=0.001$), and the 5.1–10.0 yrs age range ($p=0.008$). There were significantly more patients in the nonasthma cohort in the 0–2.0 yrs age range than in the asthma cohort. Conversely, there were significantly more asthma patients in the 5.1–10.0 yrs age group than in the nonasthma cohort. No other age range, sex, ethnicity or medication use category had significant differences between the asthma and nonasthma cohorts.

29]). A diagnosis of asthma was confirmed in 128 (70%) out of 182 patients. Black patients were diagnosed with asthma more frequently (13 out of 15) compared with other ethnic groups (Hispanic patients: 30 out of 45; white patients: 85 out of 121). The lone Asian patient was nonasthmatic. Nonasthmatic disorders were gastro-oesophageal reflux disease (GERD), aspiration, bronchitis, bacterial bronchitis, structural anomalies (tracheomalacia, large-airway bronchomalacia and minor anatomic variants: accessory bronchi, tracheal bronchus and pinhole bronchus), chronic cough and vascular compression (innominate artery and pulmonary artery compression of left

mainstem artery), cystic fibrosis and recurrent pneumonia of unknown aetiology. Most of the diagnosed asthma patients also displayed GERD and bronchitis. There was no significant relationship between race or sex and BALF culture positivity for the infectious form of *Chlamydia*. Of the 182 patients, 126 were taking one or more medication(s) at the time of testing, including four patients on antibiotics (amoxicillin, Zithromax, trimethoprim–sulfamethoxazole (TMP–SMX), Cefdinir and Bactrim).

Detection of Chlamydia in BALF

PCR was performed on the BALF samples using a 16S signature ribosomal DNA sequence to determine the prevalence of chlamydial DNA carriage in these samples. PCR amplification of the target sequence resulted in a 298-bp product which was identified by electrophoresis. The data revealed that 124 (68%) patients were positive for the presence of chlamydial DNA (fig. 1a). The current authors have previously reported on the frequency of *Cp* in a similar cohort but did not specifically test all the samples for the presence of *Ct* [22]. This decision was mainly due to the fact that *Ct* is not routinely reported in association with lung infections or pneumonitis; rather, it is mainly observed in sexually transmitted infection cases, as well as conjunctivitis. With strong evidence from the literature that *Ct*-mediated pneumonitis is possible, especially in neonates [3, 12], all BALF samples that tested positive for chlamydial DNA using the 16S primers were re-tested with both *Cp*- and *Ct*-specific primers in order to determine the frequency of lung infection with these

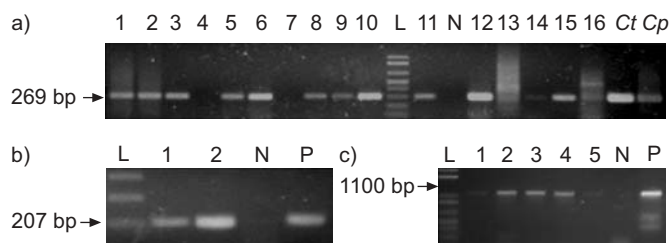


FIGURE 1. Representative agarose gels showing *Chlamydia* PCR products. a) 16S amplified DNA from both *Chlamydia*-positive (samples 1–3, 5, 6, 8–10, 11–13, 15 and 16) and -negative (4, 7, 14) bronchoalveolar lavage fluid (BALF) samples compared with the positive controls (for *Chlamydia trachomatis* (*Ct*) and *C. pneumoniae* (*Cp*)) and the negative control (N). b) *Cp* primers used to specifically amplify a 207-bp product and c) a *Ct*-specific primer pair used to amplify a 1,100-bp product from the BALF of paediatric patients tested. P: positive control; L: DNA ladder.

TABLE 2 Frequency of *Chlamydia* in asthma versus nonasthma patient groups and with corticosteroid administration

	All patients	Asthma	Nonasthma	Steroid treatment	No steroid treatment	Asthma		
						Oral and inhaled steroids	Oral steroids	Inhaled steroids
Subjects	182	128	54	103	79	21	22	39
<i>Chlamydia</i> DNA positive	124 (68)	86 (67)	41 (76)	70 (68)	54 (68)	17 (81) [†]	7 (32) [†]	24 (62) [†]
<i>Chlamydia</i> culture positive	75 (41)	59 (46) ^{#,†}	16 (30) ^{#,†}	42 (41)	33 (42)	12 (57)	13 (59)	13 (33)

Data are presented as n or n (%). #: p=0.048 for asthma versus nonasthma (Fisher's exact test); †: logistic regression confirmed association of asthma and culture positive (asthma (y/n)=culture (y/n)+steroids (y/n)+(culture × steroids (interaction)); p=0.033 for asthma; p=0.3171 for steroids; p=0.3945 for interaction).

two human pathogens. Of the 182 paediatric patient samples assayed, 79 (43.4%) were positive for the presence of *Cp*-specific DNA, while 77 (42.3%) were positive for *Ct*-specific DNA (fig. 1b and c). BALF samples from 32 (17.6%) paediatric patients contained both *Cp* and *Ct* DNA.

Having recovered both *Ct* and *Cp* DNA at high rates in these BALF samples, it was next attempted to determine the proportion of these organisms that were cultivable at the time of collection. All BALF samples were cultured on human or mouse macrophage cells using previously published protocols [22]. The results revealed that 75 (41%) of the 182 patient samples were positive for *Chlamydia* when the BALF was cultured (table 2). Since all culture-positive samples were also PCR positive, it is clear that 60% (75 out of 124) of all PCR-positive samples contained cultivable organisms. There was no significant difference in the finding of infectious *Chlamydia* between sexes; 33 of the BALF culture-positive samples were from female patients and 42 were from males. However, cultivable chlamydial organisms were found more frequently in the asthmatic population; 59 out of 128 asthmatics versus 16 out of 54 nonasthmatics were culture positive (p=0.048, Fisher's exact test; table 2). As an internal control, the patient cohort was divided into inflammatory respiratory disease (n=150) and noninflammatory airway diseases (n=32). The noninflammatory respiratory disease group was categorised as such based on normal bronchial biopsy and normal BALF. Aerobic cultures were also negative for this group, which

consisted of structural defects, IgG and IgA deficiencies, aspirations and GERD; the inflammatory group included asthma, bronchitis and pneumonia, and were also categorised based on the bronchial biopsy, BALF and aerobic cultures. When assessed for cultivable *Chlamydia*, 69 (46%) of the inflammatory respiratory disease patients harboured cultivable *Chlamydia*, versus six (18%) of the noninflammatory disease group (Fisher's exact test (two-sided), p=0.005). There was no significant association between these groups and the detection of chlamydial DNA (table 3). Evaluation of total serum IgE levels revealed no statistically significant association between patients with elevated serum IgE and the presence of *Chlamydia* DNA or cultivable organisms (table 3).

Out of the 182 patients in the paediatric cohort, 103 (56.6%) were taking some form of steroid therapy at the time of BALF sample collection. In this cohort, 82 (64%) out of the 128 asthmatics were being prescribed corticosteroids; 23 nonasthmatics diagnosed with one or more of the other chronic respiratory diseases listed previously were also being prescribed corticosteroids at the time of sample collection. Of the 82 asthmatic patients taking corticosteroids, 52 (63%) were positive for the presence of chlamydial DNA and 38 tested positive for cultivable *Chlamydia* in culture. A total of 18 (78%) of the 23 nonasthma patients being administered corticosteroids were also positive for chlamydial DNA; five of these were *Chlamydia* culture positive. Asthma patients being prescribed both oral and inhaled corticosteroids were more likely to

TABLE 3 Prevalence of *Chlamydia* in inflammatory and noninflammatory airway disease

	Inflammatory airway disease	Noninflammatory airway disease	Elevated serum IgE	Normal serum IgE
Subjects	150	32	72	110
<i>Chlamydia</i> culture positive	69 (46) [#]	6 (18) [#]	43 (60)	81 (74)
<i>Chlamydia</i> DNA positive	104 (69.3)	20 (62.5)	26 (36)	49 (45)

Data are presented as n or n (%). Inflammatory airway disease included asthma, pneumonia and bacterial bronchitis; noninflammatory diseases included gastro-oesophageal reflux disease, aspirations, laryngomalacia, dyspnoea, structural anomalies, chronic cough and vascular compression. #: p=0.005, Fisher's exact test (two-sided). There was no significant association with PCR-positive samples (p=0.531). There was no statistically significant association between patients with elevated serum immunoglobulin (Ig)E and the presence of *Chlamydia* DNA or cultivable organisms.

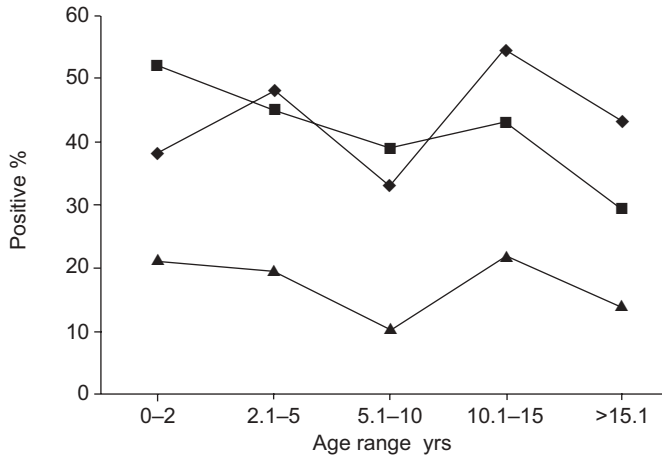


FIGURE 2. Age-based prevalence of chlamydial DNA in bronchoalveolar lavage fluid. The correlation between age and the prevalence of *Chlamydia trachomatis* (Ct) and *C. pneumoniae* (Cp) organisms determined by PCR analysis is shown. Percentages were calculated based on the number of patients positive for the particular organism and the total number of patients in that group. Note that patients in the 10.1–15 yrs age range accounted for the highest percentage (35 (76%) out of 46) of *Chlamydia* DNA-positive patients. The 0–2 yrs age group harboured the highest percentage of Ct organisms (15 (51.7%) out of 29). ♦: Cp; ■: Ct; ▲: Ct and Cp. See also table 4.

harbour chlamydial DNA in their BALF (17 out of 21) than those taking only oral (seven out of 22; $p=0.0461$, Fisher’s exact test) or inhaled corticosteroids (24 out of 39; $p=0.133$; table 2).

In addition to corticosteroids, four of these patients were being prescribed antibiotics at the time of sample collection. Three of these four patients were diagnosed with asthma (one patient was diagnosed with both asthma and bronchitis) and one patient had bronchitis. The BALF from two of these patients taking Bactrim and TMP–SMX harboured chlamydial DNA and one was culture positive for *Chlamydia*; patients on amoxicillin and Zithromax were both negative for *Chlamydia*.

Chlamydia, age and disease associations

The cohort of 182 respiratory disease patients consisted of 128 (70%) diagnosed asthmatics. Chlamydial DNA was recovered by PCR amplification from 86 (67%) out of 128 patients diagnosed with asthma (table 2). The remaining 41 patients were *Chlamydia* negative by PCR. Chlamydial DNA was, however, not exclusively found in the asthma population, with 41 (76%) out of 54 nonasthma patient samples also PCR positive. Of the 32 patients who harboured both Ct and Cp DNA in their lungs, 20 out of 128 were asthmatic and 12 out of 54 were nonasthmatic. Patients in the 10.1–15 yrs age range accounted for the highest prevalence (35 (76%) out of 46) of *Chlamydia* infection (fig. 2 and table 4). The 0–2 yrs age group harboured the highest percentage of Ct DNA samples (15 (51.7%) out of 29). The percentage of patient samples testing positive for Cp DNA increased from 11 (38%) out of 29 in the 0–2 yrs age group to 19 (47.5%) out of 40 in the 2.1–5 yrs age range. The number of patients testing positive for the presence of Ct DNA decreased over time with increasing age, while the inverse was generally true for Cp. The prevalence of both Cp and Ct decreased after age 15 yrs (fig. 2 and table 4).

TABLE 4 Age-based prevalence of chlamydial DNA in bronchoalveolar lavage fluid: correlation between age and the prevalence of *Chlamydia trachomatis* (Ct) and *C. pneumoniae* (Cp) organisms determined by PCR analysis

	Age range yrs				
	0–2	2.1–5	5.1–10	10.1–15	>15.1
Total patients	29	40	46	46	21
Total PCR positive	20 (69)	29 (73)	28 (61)	35 (76)	12 (57)
Ct PCR positive	15 (52)	18 (45)	18 (39)	20 (43)	6 (29)
Cp PCR positive	11 (38)	19 (48)	15 (33)	25 (54)	9 (43)
Ct and Cp positive	6 (21)	8 (20)	5 (11)	10 (22)	3 (14)
Culture positive	14 (48)	14 (35)	18 (39)	20 (43)	9 (43)

Data are present as n or n (%). Percentages are calculated based on the number of patients positive for the particular organism and the total number of patients in that group. Note that patients in the 10.1–15 yr age range accounted for the highest percentage (35 (76%) out of 46) of *Chlamydia* DNA-positive patients. The 0–2 yr age group harboured the highest percentage of Ct organisms (15 (51.7%) out of 29). See also figure 2.

BALF cellularity and Chlamydia

BALF collected from each patient was analysed in a clinical setting for the presence of various cell types. Specifically, cell counts were performed in order to determine the number of lipid-laden macrophages (LLMs)/monocytes, eosinophils, lymphocytes and neutrophils. A total of 45 patient samples showed elevated levels of eosinophils (table 5), 149 contained lymphocytes, and monocytes were found in 89 patient samples. There was no significant difference in percentages of alveolar macrophages, lymphocytes and monocytes in the asthma versus nonasthma group. The mean cell counts for monocytes, eosinophils, lymphocytes and neutrophils are presented for *Chlamydia*-positive versus -negative samples (DNA and culture) and asthma versus nonasthma subcohorts (table 5). The mean eosinophil count was significantly higher in asthmatics (5.5%) versus nonasthmatics (0.06%; $p=0.001$, Fisher’s exact test). The range of BALF eosinophil counts in asthmatic patients was 1–25%. The mean neutrophil count in *Chlamydia* culture-positive patients was 68.39% compared to 30.87% in culture-negative subjects ($p=0.001$, Fisher’s exact test (two-tailed)). Since this group of children all had severe respiratory diseases and many had GERD, it was not surprising to discover that 175 out of the 182 BAL samples contained at least some LLMs. The cytological evaluation did not reveal a statistically significant association between the finding of LLMs and *Chlamydia* organisms. In total, 21 patients harboured few LLMs (1–3 per field) in their BALF, 66 patients had moderate numbers (4–6 per field) and 95 patients had many LLMs (≥ 6 LLMs per field) in their BALF. Overall, 68 (90%) out of 75 culture-positive versus 93 (87%) out of 107 culture-negative patient samples displayed moderate to many LLM (table 5).

DISCUSSION

Chronic lung disease affects an increasingly wide cross-section of the world’s population, manifesting itself mainly as asthma,

TABLE 5 Bronchoalveolar lavage fluid cellularity and the presence of *Chlamydia*

	<i>Chlamydia</i> culture positive	<i>Chlamydia</i> culture negative	<i>Chlamydia</i> DNA positive	<i>Chlamydia</i> DNA negative	Asthma	Nonasthma
Subjects n	75	107	124	58	128	54
LLM						
Many	44 (58.7)	51 (48)	60 (48)	35 (60.3)	73 (57)	21 (39)
Moderate	24 (32)	42 (39)	49 (40.0)	17 (29.4)	42 (33)	24 (44)
Few/none	7 (9.3)	14 (13)	15 (12)	6 (10.3)	13 (10)	9 (17)
Mean cell count per mL %						
Macrophages/monocytes	2.84	2.79	2.63	3.26	3.11	2.11
Eosinophils	1.45	1.16	1.10	1.64	5.5 [#]	0.06 [#]
Lymphocytes	8.04	7.83	8.67	6.30	7.68	8.48
Neutrophils	68.39 [*]	30.87 [*]	33.85	29.4	29.0	40.59
Other	19.28	57.35	46.25	59.4	54.71	51.24

Data are presented as n (%), unless otherwise stated. LLM: lipid-laden macrophage. [#]: p=0.001, Fisher's exact test; ^{*}: p=0.001, Fisher's exact test (two-tailed). There was no statistically significant correlation between any other cell types and the detection of *Chlamydia* organism or DNA.

COPD, pneumonia and bronchitis [2, 18]. Globally, respiratory infections in childhood are a leading cause of disease and substantially contribute to school absence and severe economic strain on healthcare resources [30]. In the developing world, respiratory infections are also a major cause of childhood mortality [31]. Respiratory diseases of infancy and childhood are predominantly infectious in nature and can be caused by either viruses, bacteria or parasites [32].

In the current observational study it was demonstrated that human strains of *Chlamydia*, *Ct* and *Cp*, can be isolated from the lungs of children with chronic respiratory disease. *Cp* has long been reported as an aetiological agent of community-acquired pneumonia and has been found in high prevalence in the lungs of adults [33], and also in the respiratory secretions of adult asthmatics, and lungs of COPD patients [34]. Recently, the present authors reported that *Cp* can also infect the lung tissue of children and might contribute to the pathology commonly seen in a variety of chronic respiratory diseases [22]. While the number of patients with chlamydial DNA in their lower respiratory tract analysed in the current study is surprisingly high compared with those in some recent studies [35, 36], the cohort is significantly larger. Furthermore, the type of samples collected differs from most published work to date, which utilises samples from the upper respiratory tract. It is also noteworthy that the present cohort is a select group of severely ill children with chronic respiratory disease and, as such, may not be reflective of the general population. Most other studies that report a prevalence of *Cp* in the 5–30% range assess sputum, nasal aspirates or throat swab samples [35, 37, 38] as opposed to bronchial washings, a more invasive procedure with which samples were obtained in the current study. In a 2001 study where *Cp* PCR was performed on tracheobronchial aspirate, the authors reported a 51.9% prevalence was reported [39]. It should again be noted that the samples used in the present study were residual in nature and not obtained for the purpose of research, but as a part of the diagnostic evaluation of each patient. Importantly, most studies by others have only tested for the presence of *Cp* DNA, while the presence of both *Cp* and *Ct* DNA was assayed for by the current authors.

The data revealed a significant association between cultivable *Chlamydia* and asthma diagnosis, consistent with earlier findings. Importantly, the data also confirm that at earlier ages of life, *Ct* appears more prevalent than *Cp* in BALF samples. Although the organisms were also found in the neonate to 2 yrs old age group, it is not until age 5–10 yrs that an increased prevalence of *Cp* is observed. This suggests that these later infections may have been contracted through increased social interactions, possibly in pre-school or day care settings. These findings agree with previously published data, suggesting that *Ct* can be found in the lower respiratory tract of newborns and can lead to pneumonitis [12, 15, 21]. Evidence for the presence of *Ct* in the human placental tissue also exists [40–42].

Increased lipid content in alveolar macrophages of BALF is thought to be a useful indicator for recurrent pulmonary aspiration [43, 44]. Previous studies have confirmed that *Chlamydia* has the ability to survive, and even thrive, in alveolar macrophages [45, 46]. It has been previously reported that *Cp* induces foam cell formation by human monocyte-derived macrophages [47]. Exposure of macrophages to *Cp*, followed by the addition of low-density lipoprotein in tissue culture, caused a marked increase in the number of foam cells and accumulation of cholesteryl esters [47]. While not statistically significant, the current authors observed a correlation between the finding of moderate to many LLM (*i.e.* >4 cells per high power field) and *Chlamydia* positivity by PCR and culture. Infection of macrophages by *Chlamydia*, coupled with epithelial cell damage in the airways, could increase the inflammatory response in the lungs. With increased oxidative bursts by these phagocytes and the release of proinflammatory cytokines such as tumour necrosis factor- α , interleukin (IL)-1 β , IL-6 and IL-8, airway hyperreactivity and pulmonary inflammation might be significantly increased.

In the current study, asthma patients treated with a combination of oral and inhaled corticosteroids were more likely to harbour chlamydial DNA (table 5). Inhaled glucocorticoids are a mainstay of asthma therapy. Oral steroid treatment is the most

potent therapeutic intervention available for the effective relief of symptoms in acute and chronic asthma, especially for patients with severe disease. However, corticosteroids negatively affect many aspects of cell-mediated immunity and favour the shift from a T-helper type-1 response towards a T-helper type-2 response. Therefore, corticosteroids may severely impact the host's ability to eradicate an intracellular pathogen, such as *Chlamydia*, which requires properly functioning cell-mediated (T-helper type-1) immune responses for pathogen clearance. Previous *in vitro* studies confirm that persistent *Chlamydia* in macrophages are reactivated when corticosteroid treatment is administered, resulting in the release of infectious elementary body particles into the immediate surroundings, whereby new cells are infected [48, 49]. Corticosteroids have also been shown to reactivate persistent *Chlamydia* carriage leading to an active growth phase, thus increasing the production of proinflammatory cytokines at the site of infection and further amplifying inflammation in the airways of patients with asthma [50–53]. The combination of both inhaled and oral steroid treatment might represent an increased amount of corticosteroids in the circulation leading to increased reactivation of *Chlamydia*. While antibiotic treatment of the patients in the present study was not attempted, previously published work by others has demonstrated that clarithromycin therapy improves lung function in subjects with positive PCR findings for *Chlamydia* and *Mycoplasma* [52, 54, 55].

Along with previously published studies, the present work suggests that further in-depth investigations of the involvement of the Chlamydiaceae family of obligate intracellular pathogens in the aetiology and exacerbation of asthma and other chronic respiratory diseases, particularly in paediatric populations, are needed. The current data confirms the presence of both *Chlamydia trachomatis* and *Chlamydia pneumoniae* organisms in the lungs of these patients. Importantly, patients with asthma and other inflammatory airway disease were more likely to harbour cultivable chlamydial organisms in their lower respiratory tract.

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