

# Bronchial *Chlamydia pneumoniae* infection, markers of allergic inflammation and lung function in children

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A relationship between respiratory *Chlamydia pneumoniae* infection (RCPI) and bronchial asthma is under discussion. Our objective was to study the frequency of RCPI and whether it is associated with markers of asthma in children with recurrent or chronic bronchitis as well as pneumonia. One-hundred and forty-eight children who underwent bronchoscopy were enrolled; 42 children with additional respiratory infections were excluded. Therefore, 106 children were examined, regarding a RCPI, by polymerase chain reaction (PCR) of tracheobronchial aspirate, eosinophilic inflammation of respiratory mucosa (cytology, eosinophilic cationic protein [ECP]), total serum immunoglobulin E (IgE) and specific IgE for six important allergens, as well as lung function tests if possible. There was a RCPI in 55 of 106 children (51.9%); 25.4% of PCR positives (14/55) were weakly positive (double cut-off), which was more prevalent in the 2–5-year age-group and teenagers. Children with RCPI, inclusive of weak positives, showed a milder eosinophilia of nasal mucosa than children without RCPI (5.58% vs. 9.35%,  $p=0.039$ ). Eosinophilia of  $\geq 13\%$  in nasal- and/or bronchial swab, as a marker for respiratory allergy, was less frequent in patients with RCPI too (7.3% vs. 21.6%,  $p=0.035$ ). There were no differences in ECP. Total IgE was lower in PCR-positive children (101 vs. 179 IU/ml,  $p=0.032$ ). Specific IgE with a radioallergosorbent test (RAST) of at least class 3 (as a marker for a relevant allergy), as well as any RAST above zero (to characterize early forms of allergy), were both less frequent in the RCPI group. In contrast, weak positives showed the highest rates of sensitization, surpassing RCPI negatives. In lung-function tests, vital capacity was lower in RCPI patients (87.5% vs. 95.3%,  $p=0.045$ ); all parameters characterizing obstructive disturbance tended to be higher. Weak positives had both the greatest reduction of vital capacity (75.3%) and the most impaired obstructive parameters. All differences were accentuated in children of 11–18 years of age. Hence, our results indicate that in the children selected, a RCPI is common and not associated with allergic respiratory inflammation. Weak positives, however, differ, having the highest rate of allergic sensitization, reduction of lung volume, and obstructive disturbance. This group might be important in clinically observed asthma after pneumonia caused by *C. pneumoniae*. In these children, early diagnosis and treatment of a RCPI is recommended.

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Bronchial asthma is a chronic inflammatory disease of the respiratory tract in which eosinophils, mast cells, and T cells play a very important role (1). The etiology of this disease is still unknown.

Children with frequent respiratory illness have a higher risk of suffering from bronchial asthma (2,3). Several pathogens are held responsible for acute asthma episodes and in the pathogenesis of

the disease (4). *Chlamydia pneumoniae*, a gram-negative bacterium acknowledged to exist as an individual species since 1989, is one of these pathogens. It causes acute respiratory infections but infection can be asymptomatic. The possibility of persistent respiratory infection (5) suggests a higher frequency of respiratory *C. pneumoniae* infection (RCPI) in patients with persistent inflammatory respiratory diseases. A RCPI can trigger acute asthma episodes (asthma exacerbation) or obstructive airway diseases (4,6). Bronchial asthma can be prolonged by a RCPI (asthma prolongation) (7): in children with more than three asthma exacerbations, *C. pneumoniae*-specific secretory immunoglobulin A antibodies in nasal aspirates were more than seven times higher than in children with only one asthma exacerbation (8).

Whether a persistent infection with *C. pneumoniae* is also responsible for the development of bronchial asthma (asthma initiation) remains to be established (9). Evidence for the possibility of this phenomenon is the fact that 23% of symptomatic and >28% of asymptomatic children (8), but only 5% of the healthy children (10,11), tested positive for *C. pneumoniae* when analysed using the polymerase chain reaction (PCR). In children with obstructive airway diseases, including bronchial asthma, *Chlamydia* antibodies have been detected at a higher frequency (12). A recently manifested asthma in adults with a RCPI was successfully treated with appropriate antibiotics. Only clinical improvement was achieved when the asthma persisted for a longer time before antibiotic therapy was started (13).

Our aim was to ascertain, in frequently sick children with a higher risk for bronchial asthma, any association between respiratory tract infection with *C. pneumoniae* and markers of allergic inflammation and lung function. Therefore, allergic eosinophilic inflammation of the respiratory mucosa was measured by analysing nasal and bronchial swabs for eosinophils and eosinophilic cationic protein (ECP) in bronchoalveolar lavage (BAL) and serum; specific and total immunoglobulin E (IgE) measurements were made in serum; and lung function tests were performed (if possible). To identify a RCPI we used PCR amplification in combination with a DNA enzyme immunoassay detection.

## Materials and methods

### Patients

A total of 148 children who underwent bronchoscopy between January 1997 and May 1999 were

enrolled in the study. Indications for bronchoscopy were as follows:

- therapy-resistant pulmonary obstructive symptoms;
- recurrent or chronic bronchitis/pneumonia with suspicion of foreign body aspiration, malformation or a compression of the airways.

Children with autoimmune disease, infections with parasites, cancer or side-effects of drugs, were excluded.

**Role of other pathogens.** In 42 children, an acute infection with one of the following pathogens was proven serologically, by means of antigen detection or nucleic-acid amplification: influenza A and B, parainfluenza 1–3, respiratory syncytial virus (RSV), adenovirus, *Mycoplasma pneumoniae*. To avoid any influence on study parameters, these 42 children were excluded.

**Diagnoses.** In the remaining 106 children (66 male, 40 female) aged between 1 month and 17 years (median age 5.9 years), the following diagnoses were established: chronic bronchitis without wheezing (36.8%, 39/106), chronic bronchitis with wheezing (27.4%, 29/106), recurrent pneumonia without wheezing (30.2%, 32/106), and pneumonia with wheezing (5.7%, 6/106).

### Methods

**Bronchoscopy.** Bronchoscopy was performed with general anesthesia using the rigid bronchoscope (Storz, Tuttlingen, Germany) between 08.00 h and 11.00 h. At the start of the examination, swabs from the lower nasal region and from the bronchial mucosa were obtained for later cytological examination. After inspection of the bronchial system, tracheobronchial aspirate (TBA) was taken from the right main bronchus. Subsequently, BAL was performed in the middle lobe; three samples were obtained by washing with 1 ml/kg of 0.9% NaCl. The second sample was used for evaluation of ECP. A recovery of  $\approx 80\%$  was documented. Immediately after bronchoscopy blood samples were acquired.

**Sample preparation.** Blood and BAL samples were centrifuged within 60 min for 10 min at 1000 g. The aliquoted supernatants and the pellets of BAL, the aliquoted TBA, and the serum samples were frozen at  $-28^{\circ}\text{C}$  until testing.

**Analysis of *C. pneumoniae* infection by PCR.** DNA was extracted from TBA as follows: 50  $\mu\text{l}$

of TBA was treated with 150 µl of Chelex-100 (Bio-Rad, Hercules, CA, USA) suspension (10 mM Tris-HCl, pH 8.0; 0.1 mM EDTA, 0.1% NaN<sub>3</sub>, 20% Chelex). The mixture was then incubated at 56°C for 20 min, at 100°C for 15 min, and centrifuged at 3000 g for 4 min. Ten microlitres of the supernatant was subjected to PCR. Samples were either directly utilized for PCR or stored at -70°C until required.

**PCR amplification and detection.** For amplification of *C. pneumoniae* DNA, the primers HM1 (5'-GTGTCATTTCGCCAAGGTTAA-3') and HR1 (5'-TGCATAACCTACGGTGTGTT-3'), described by Campbell et al. (14), were used.

PCR amplification was performed in a volume of 50 µl containing 16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 50 mM Tris-HCl (pH 8.8), 1.5 mM MgCl<sub>2</sub>, 0.01% Tween-20, 0.2 mM of each dNTP, 0.5 U of *Taq* polymerase (InViTaq/InVitek, Berlin, Germany), 0.4 µM of each primer HR1 and HM1, and 10 µl of sample. The PCR was performed in an automated thermal cycler (TRIO, Biometra, Göttingen, Germany) with initial denaturation at 94°C for 3 min followed by 40 cycles of 1-min steps of annealing at 53°C, extension at 72°C, and denaturation at 94°C, terminated by a final extension at 72°C for 5 min.

The 437-bp amplification products were visualized by electrophoresis of 10 µl of reaction mixture on a 1.5% agarose gel, containing 0.5 µg/ml ethidium bromide, under UV illumination. The specificity of the PCR products was verified by hybridization with a 5'-biotinylated probe (5'-[Biot.]AGAGAAACTTCAAGTTGGAGAT-AAAATGGC-3') and detection by using a DNA enzyme immunoassay (GEN-ETI-K DEIA; DiaSorin, Saluggia, Italy). The assay was performed as recommended by the manufacturer. This method has a lower limit of detection of 0.01 inclusion-forming units/ml. All positive results were performed by repeated testing.

The PCR preparation and analysis procedures were carried out according to good laboratory practice, using standard precautions, in three different rooms. Numerous positive and negative controls were included in each run.

For demarcation of weak positives, the range from normal to double cut-off values, as defined by the DNA enzyme immunoassay, was used.

**Cytology.** The eosinophilic granulocyte count in the nasal and bronchial mucosa swabs was determined by light microscopy after Pappenheim staining. Both the determination

and the evaluation were carried out by the same experienced person.

The evaluation for diagnosis were carried out according to the following scheme (15):

- eosinophilic granulocytes ≤ 12%: no respiratory allergy;
- eosinophilic granulocytes 13–49%: potential respiratory allergy;
- eosinophilic granulocytes ≥ 50%: respiratory allergy.

**Assays.** *Chlamydia* antibodies (IgG/IgA/IgM) were detected using a recombinant enzyme-linked immunosorbent assay (rELISA) (Medac, Hamburg, Germany). Acute infection was defined as: a four-fold rise in IgG or IgA titers ≥ 1:100 (paired serum sample); a single IgG titer ≥ 1:500; or any IgM titer ≥ 1:50. Previous infection was defined as IgG or IgA titers of ≥ 1:100 that did not fulfil criteria for acute infection.

BAL and serum ECP was determined by double antibody radioimmunoassay (RIA) (Pharmacia, Uppsala, Sweden). ECP-RIA comprised an intra- and inter-assay coefficient of variation of 8.2% for BAL and 8.4% for serum. The lower detection limit was 1 µg/l for both BAL and serum.

Protein levels in BAL were used for adjusting the ECP levels for BAL. For determination of protein the Total protein test Kit (Bio-Rad) was used. This test has an intra-assay coefficient of variation of 9%; the inter-assay coefficient was 11.1%.

Results for BAL were expressed as quotient of ECP/protein and given as a percentage.

Both the total and the specific serum IgE were analysed by using the CAP-system IgE-FEIA (Pharmacia). The lower detection limit was 0.35 kU/l and coefficient of variation was 10%. The aim of the specific IgE detection was to find out whether a sensitization against the most common allergens was present. The level of specific IgE against birch, rye, mugwort, molds, *Dermatophagoides pteronyssinus*, and *D. farinae* was determined. The respective specific IgE serum levels were recorded in the six radioallergosorbent test (RAST) classes accordingly.

**Lung function.** Lung function tests were performed if children were co-operative, and were measured by maximum forced expiratory and inspiratory flow-volume curves and body-plethysmography (Compact Lab., Erich Jaeger, Würzburg). Calibration was performed daily. The room temperature (21–23°C) and humidity

Table 1. Frequency of bronchial *Chlamydia pneumoniae* infection in 106 children, according to clinical presentation, age, and gender, assessed by using the polymerase chain reaction (PCR) and serology

|                              | PCR                  |                                    | Serology<br>Acute infection, % (n) |
|------------------------------|----------------------|------------------------------------|------------------------------------|
|                              | All positives, % (n) | Weak positives, % of positives (n) |                                    |
| Without wheezing             |                      |                                    |                                    |
| Recurrent/chronic bronchitis | 53.8 (21/39)         | 28.6 (6/21)                        | 5.1 (2/39)                         |
| Recurrent pneumonia          | 53.1 (17/32)         | 23.5 (4/17)                        | 9.4 (3/32)                         |
| With wheezing                |                      |                                    |                                    |
| Recurrent/chronic bronchitis | 51.7 (15/29)         | 20.0 (3/15)                        | 10.3 (3/29)                        |
| Recurrent pneumonia          | 33.3 (2/6)           | 50.0 (1/2)                         | 16.7 (1/6)                         |
| Age-group                    |                      |                                    |                                    |
| 0–1 years                    | 53.8 (7/13)          | 14.3 (1/7)                         | 23.1 (3/13)                        |
| 2–5 years                    | 52.0 (26/50)         | 34.6 (9/26)                        | 4.0 (2/50)                         |
| 6–10 years                   | 47.4 (9/19)          | 11.1 (1/9)                         | 10.5 (2/19)                        |
| 11–18 years                  | 54.2 (13/24)         | 23.1 (3/13)                        | 8.3 (2/24)                         |
| Gender                       |                      |                                    |                                    |
| Female                       | 55.0 (22/40)         | 27.3 (6/22)                        | 10.0 (4/40)                        |
| Male                         | 50.0 (33/66)         | 24.2 (8/33)                        | 7.6 (5/66)                         |

(60–65%) were constant during the test period. The reference values described by Zapletal et al. (16) were used. Measurements were obtained from a seated child wearing a nose clip. Each child had to practise the technique until the variation of lung function values was less than 5%. The test with highest value of forced expiratory volume in 1 s (FEV<sub>1</sub>) was used. To characterize lung volume we used total lung capacity (TLC) and vital capacity (VC). Parameters to describe an obstructive disturbance were specific resistance (SR), intra-thoracic gas volume (ITGV), FEV<sub>1</sub>, FEV<sub>1</sub> in relation to forced vital capacity (FEV<sub>1</sub> % FVC), maximum expiratory flow at 50% (MEF<sub>50</sub>) and 25% (MEF<sub>25</sub>) of vital capacity, and maximum mid-expiratory flow between 75 and 25% of vital capacity (MMEF<sub>75–25</sub>).

#### Statistical analysis

The Kolmogorov-Smirnov test (Lilliefors) was initially used to determine the normal distribution of the parameters. When a normal distribution was documented, the *t*-test was carried out; for non-normal distributions, the Mann-Whitney *U*-test and Kruskal-Wallis tests were used. The chi-squared test was used to detect differences in groups. A *p*-value of <0.05 was considered significant. Data were analyzed using the statistical package for the social sciences (spss for Windows, version 10.0; SPSS Inc., Chicago, IL, USA).

## Results

### *Chlamydia pneumoniae* infection

*Chlamydia pneumoniae* infection was detected in TBA (by using the PCR) in 51.9% (55/106) of

subjects. Of all positives, 25.5% (14/55) were weak positives. A weak infection detected by PCR was most common in children of the 2–5 yr age group, followed by children of the 11–18 yr age group.

Acute *Chlamydia* infection was found in 8.5% (9/106) by means of serology; 7.3% of PCR positives (4/55) and 9.8% of PCR negatives (5/51). A serological acute infection was not found in PCR weak positives. Serology revealed acute infection most frequently in babyhood. Using serological acute infection as a discriminator there were no differences, as next shown by PCR.

Evidence of previous infection was demonstrated in 10.4% (11/106), 14% in both PCR negatives (7/51) and weak positives (2/14), and 5% (2/41) in strong positives. Differences in relation to diagnoses, age and gender (Table 1) were not significant.

### Eosinophilic inflammation of respiratory mucosa

Chronically ill children with RCPI developed a milder eosinophilia of the respiratory mucosa as compared to those without (Table 2). Differences were significant for nasal mucosa results. In the age group 11–18 years the difference in eosinophilia of nasal mucosa was highly significant with the same median but smaller variation of eosinophil count (Table 3).

Detection of eosinophilia of  $\geq 13\%$  in the nasal and/or bronchial mucosa swab, regarded as a potential respiratory allergy, was significantly less frequent in patients with RCPI. PCR weak positives were also found to exhibit a mild eosinophilic inflammation of the respiratory mucosa, which was comparable to PCR strong positives.

Table 2. Parameters for atopy and allergic inflammation, according to polymerase chain reaction (PCR) analysis, for *Chlamydia pneumoniae* in all children studied

|                                         | PCR positive |                       | PCR negative (n=51) | p-value† |
|-----------------------------------------|--------------|-----------------------|---------------------|----------|
|                                         | All (n=55)   | Weak/strong (n=14/41) |                     |          |
| Eosinophils (%)*                        |              |                       |                     |          |
| Nasal                                   | 0.0          | (0.0/0.0)             | 3.0                 | 0.04     |
| Bronchial                               | 0.0          | (0.0/0.0)             | 0.0                 | 0.35     |
| Eosinophils $\geq 13\%$ (%)**           |              |                       |                     |          |
| Nasal and/or bronchial                  | 7.3          | (7.1/7.3)             | 21.6                | 0.03     |
| Nasal                                   | 7.3          | (7.1/7.3)             | 17.6                | 0.14     |
| Bronchial                               | 5.5          | (0.0/7.3)             | 13.7                | 0.19     |
| ECP*                                    |              |                       |                     |          |
| BAL (Quotient ECP/protein; $10^{-3}$ %) | 2.0          | (3.0/2.0)             | 2.5                 | 0.90     |
| Serum ( $\mu\text{g/l}$ )               | 13.8         | (13.6/13.8)           | 15.2                | 0.70     |
| Total IgE (IU/ml)*                      | 13.3         | (50.0/13.3)           | 56.6                | 0.03     |
| Frequency of:**                         |              |                       |                     |          |
| Elevated total IgE (%)                  | 25.5         | (42.9/19.5)           | 33.3                | 0.40     |
| RAST class $\geq 3$ (%)                 | 16.4         | (28.6/12.2)           | 23.5                | 0.47     |
| RAST class $>0$ (%)                     | 25.5         | (42.9/19.5)           | 35.3                | 0.30     |

\*Median values are expressed, calculated by using the Mann-Whitney-*U*-test (all positives/negatives).

\*\*Chi-squared test (all positives/negatives).

†All positives/negatives.

BAL, bronchoalveolar lavage; ECP, eosinophil cationic protein; IgE, immunoglobulin E; RAST, radioallergosorbent test.

The ECP content in BAL (based on total protein in BAL) and serum was almost identical in all groups.

The frequency of a local or systemic glucocorticoid therapy was 29.4% (15/51) in the group without RCPI and 34.5% (19/55) in all positives, including PCR weak positives (42.9%, 6/14) and strong positives (31.7%, 13/41).

#### Development of allergies

Total IgE was lower in all children with RCPI ( $p = 0.032$ , Table 2). The detection rate of specific IgE no less than RAST class 3, regarded as a relevant allergy, against at least one of the six allergens tested was lower in the *C. pneumoniae* PCR-positive group also. With the belief that a potential allergy development initiated by *C. pneumoniae* infection was already in progress, specific IgE rates starting at RAST class 1 were additionally considered, with similar results obtained. In PCR weak positives, however, the frequencies of pathologic total and specific IgE rates were much greater than RCPI negatives (Table 2), separating this group from strong positives. Beside weak positivity, age was found to be a second risk factor. In 11–18-year-old children (Table 3) differences were much more obvious but insignificant because of low numbers.

#### Lung function tests

Lung function tests were performed in 51 children (Table 4). Flow-volume parameters and whole body plethysmography was measured in 46 children; in a further five children, because of

sub-optimal co-operation only flow-volume curves were determined.

VC characterizing lung volume was reduced in children with RCPI ( $p = 0.045$ ). In the 11–18-year age-group, reduction in VC was more pronounced (83% vs. 103%,  $p = 0.002$ ), which also affected FEV<sub>1</sub> (88% vs. 105%,  $p = 0.004$ ), with normal FEV<sub>1</sub> % FVC (restrictive disturbance). All parameters for the detection of obstructive ventilation disturbances (MEF<sub>50</sub> and <sub>25</sub>, MMEF, specific resistance, FEV<sub>1</sub> % FVC) were, in contrast, slightly higher in children with RCPI.

The group of PCR weak positives contained the patients with the most striking impairment of lung function. VC was significantly lower compared with PCR negatives ( $p = 0.004$ ) but also strong positives ( $p = 0.02$ ). Reduced VC and FEV<sub>1</sub>, but normal FEV<sub>1</sub> % FVC, characterized a restrictive disturbance. Elevated specific resistance and reduced MEF<sub>50</sub> is indicative of obstructive disturbance. As weak positivity occurred mostly in preschool children who showed minimal co-operation, the number of lung function tests was unfortunately small.

In PCR positives, adequate antibiotic therapy for at least 14 days was employed mainly in children with impaired lung function tests, resulting in improvement to normal levels. In children without treatment, VC and TLC deteriorated somewhat from initially higher values (Table 5).

#### Discussion

It was hypothesized that children with recurrent or chronic respiratory diseases exhibit a higher

Table 3. Parameters for atopy and allergic inflammation, according to polymerase chain reaction (PCR) analysis, for *Chlamydia pneumoniae* in children aged 11–18 years only

|                                                | PCR positive |                      | PCR negative (n=11) | p-value† |
|------------------------------------------------|--------------|----------------------|---------------------|----------|
|                                                | All (n=13)   | Weak/strong (n=3/10) |                     |          |
| Eosinophils (%)*                               |              |                      |                     |          |
| Nasal                                          | 0.0          | (0.0/0.0)            | 3.0                 | 0.01     |
| Bronchial                                      | 0.0          | (0.0/0.0)            | 0.0                 | 0.57     |
| Eosinophils ≥ 13% (%)**                        |              |                      |                     |          |
| Nasal and/or bronchial                         | 7.7          | (0.0/10.0)           | 27.3                | 0.30     |
| Nasal                                          | 7.7          | (0.0/10.0)           | 9.1                 | 1.0      |
| Bronchial                                      | 7.7          | (0.0/10.0)           | 27.3                | 0.30     |
| ECP*                                           |              |                      |                     |          |
| BAL (Quotient ECP/protein; 10 <sup>-3</sup> %) | 1.7          | (4.8/3.0)            | 0.0                 | 0.09     |
| Serum (µg/l)                                   | 17.9         | (8.3/16.2)           | 20.4                | 0.49     |
| Total IgE (IU/ml)*                             | 68.7         | (373.0/20.4)         | 116                 | 0.07     |
| Frequency of:**                                |              |                      |                     |          |
| Elevated total IgE (%)                         | 30.8         | (66.7/20.0)          | 18.2                | 0.27     |
| RAST class ≥ 3 (%)                             | 15.4         | (33.3/10.0)          | 27.3                | 0.63     |
| RAST class >0 (%)                              | 30.8         | (66.7/20.0)          | 63.6                | 0.22     |

\*Median values are expressed, calculated by using the Mann–Whitney-*U*-test (all positives/negatives).

\*\*Chi-squared test (all positives/negatives).

†All positives/negatives.

prevalence of *C. pneumoniae* infection as this pathogen can cause persistent infections. This hypothesis was proven to be true in the present study.

Those children who are often ill are at higher risk of suffering bronchial asthma (2,3). Children with relapsing or chronic respiratory diseases and a higher prevalence of *C. pneumoniae* infection (hypothesis 1) could therefore exhibit an eosinophilic inflammation of the respiratory mucosa or a sensitization against common allergens, or present with obstructive ventilation disturbances typical for asthma. Confirmation of this second hypothesis proved to be more complicated. Weak positives only had the highest allergic sensitization, reduction of lung volume, and obstructive disturbance, but low eosinophilic allergic inflammation of the respiratory mucosa.

Eosinophilic granulocytes play a very important pathogenetic role in an allergic inflammation normally seen in bronchial asthma. Eosinophilic granulocytes are typically found in the respiratory mucosa of asthma patients. A frequency of at least 13% in the nasal and/or bronchial swab is considered a marker for respiratory allergy (15). In the RCPI group, respiratory eosinophilic inflammation was significantly more infrequent in the population examined. The secretion product of eosinophils (ECP), however, showed the same concentration in BAL and serum in both groups. This could be explained by a higher percentage of activated eosinophils in children with RCPI. Their detection is, unfortunately, impossible because of the very low content of eosinophils in BAL (17).

Eosinophilic granulocytes are influenced by a series of factors. Blood eosinophils have a diurnal rhythm in which the highest levels are recorded during the night (04.00 h) and the lowest levels in the morning, reciprocal to the steroid hormone production (18). These changes have not been studied in eosinophils of the respiratory mucosa. In order to avoid possible diurnal changes of the eosinophils in the respiratory mucosa, we always performed bronchoscopy in the morning within a time limit of 3 h. The blood eosinophils are at their minimum during this time.

Eosinophilia has not only been described in atopy, but also in parasitic infections, autoimmune diseases, cancer, and drug-associated reactions (19). In the present study, these diagnoses were criteria for exclusion. A possible influence on the eosinophils in respiratory tract secretions by gluco-corticoids has been reported. Dexamethasone, for example, is believed to induce eosinophilic granulocyte apoptosis but inhibits neutrophil apoptosis (20). As topically applied therapeutic gluco-corticoids are often indicated in obstructive respiratory diseases, any influence on the count of eosinophils was excluded here: the percentage of children who received topical or systemic glucocorticoids within 7 days before bronchoscopy was the same in children with or without RCPI.

Determination of total IgE and specific IgE against six of most common allergens was used to assess development of allergies. Contrary to our second hypothesis, children with RCPI had lower levels of total IgE. Specific IgE disclosed the tendency towards a lower rate of sensitization in

Table 4. Lung function of children with or without respiratory *Chlamydia pneumoniae* infection (RCPI)

|                        | PCR positive   |                             | PCR negative (n=26/23)* | p-value† |
|------------------------|----------------|-----------------------------|-------------------------|----------|
|                        | All (n=25/23)* | Weak/strong (n=5/4; 20/19)* |                         |          |
| VC                     | 87.5           | (75.3/90.5)                 | 95.3                    | 0.04     |
| TLC                    | 102.6          | (94.7/104.3)                | 106.8                   | 0.26     |
| ITGV                   | 104.6          | (98.3/106.0)                | 107.6                   | 0.68     |
| SR                     | 169.3          | (200.8/162.7)               | 171.8                   | 0.89     |
| FEV <sub>1</sub>       | 96.0           | (81.5/99.6)                 | 100.6                   | 0.38     |
| FEV <sub>1</sub> % FVC | 108.2          | (107.3/108.5)               | 103.5                   | 0.13     |
| MEF <sub>50</sub>      | 87.6           | (72.7/91.4)                 | 82.4                    | 0.47     |
| MEF <sub>25</sub>      | 95.8           | (87.1/97.3)                 | 91.2                    | 0.66     |
| MMEF <sub>75-25</sub>  | 93.2           | (78.5/96.9)                 | 87.8                    | 0.52     |

Values are expressed as percentage of predicted value (*t*-test).

\*The first number represents flow-volume parameters (VC, FEV<sub>1</sub>, FEV<sub>1</sub> % FVC, MEF<sub>50</sub>, 25, 75–25); the second number represents parameters of body plethysmography (TLC, ITGV, SR).

†All positives/negatives.

FEV<sub>1</sub>, forced expiratory volume in 1 s; FEV<sub>1</sub> % FVC, FEV<sub>1</sub> in relation to forced vital capacity; ITGV, intrathoracic gas volume; MEF<sub>50</sub>, MEF<sub>25</sub>, maximum expiratory flow at 50% or 25% of vital capacity; MMEF<sub>75-25</sub>, maximum mid-expiratory flow between 75 and 25% of vital capacity; SR, specific resistance; TLC, total lung capacity; VC, vital capacity.

the RCPI group in both clinically relevant and not yet relevant allergy.

Infectious diseases have an influence on the development of atopic diseases during childhood; however, the direction of effect is under discussion. A protective effect against the development of asthma or allergic conditions has been reported to exist in females who have had tuberculosis during childhood. In males, however, persistent asthma was more frequent after an active tuberculosis (21). Bronchial asthma or atopy was rare in patients with a positive tuberculin skin test (22). Measles, too, has been reported to be either protective (2) or non-protective (23). A protective effect of older (24) or younger siblings (2) has been explained by the higher rate of infectious diseases in early childhood. Early contact with a pathogen leads to a switch towards the T-helper 1 (Th1) response of the T-helper cells. This, in turn, leads to the suppression of the birth physiological, but later atopy-typical, T-helper 2 (Th2) immune response, which promotes IgE production and eosinophilic inflammation (25).

Recent studies report, in contrast, that repeated episodes of fever and antibiotic treatment in early life are strongly associated with the prevalence of asthma (2,3). On the other hand, only within the group of asthmatic children were the number of fever episodes and antibiotic treatment strongly inversely related to the prevalence of atopy and bronchial hyper-responsiveness (3). This second finding favors the Th1/Th2 theory cited above. It was concluded therefore that there is a sub-group, within asthmatic children, who are not atopic (3). Could this be the children with asthma as a consequence of prolonged infections?

According to our data, a RCPI seems to be no risk factor for a respiratory allergic eosinophilic

inflammation, manifestation of allergies or an obstructive ventilatory disturbance in risk-children studied. Findings correspond to the theory of a switch towards a preferred Th1 immune response in cases of repeated contact to pathogens with less frequent IgE production and eosinophils. However, we looked for risk factors for respiratory allergy beside the two groups of RCPI positives and negatives. The level of PCR positivity and age were shown to influence parameters.

PCR weak positives differed from strong positives regarding most frequent allergy development, high total IgE levels, and highest impairment in lung function tests (restrictive and obstructive disturbance). The same low allergic eosinophilic inflammation of respiratory mucosa in the weak and strong positives groups may also be explained by an increased rate of glucocorticoid therapy highest in the weak positives, suppressing eosinophilic inflammation. From PCR negatives weak positives can be separated by impaired lung function, forming an interesting subgroup for further studies about the development of asthma. PCR weak positivity indicates a low amount of antigen acting as a stimulus for the immunological system with, as a possible consequence, an observed lack of systemic antibodies. Weak positivity might rather be the result of low-grade chronic infection than early acute infection because of a higher percentage of past antibodies than in PCR strong positives and long anamnesis. These highlight the need to be more specific in case selection to assess the influence of infections to asthma.

The frequency of *Chlamydia* antibody detection in childhood is highest in the 11–18-year-old age-group (12). Direct identification of *C. pneumoniae*

Table 5. Mean lung function of polymerase chain reaction (PCR) positives, according to therapy with or without appropriate antibiotics

| With therapy (n=6/5*) |       |                        | Without therapy (n=7/7*) |       |
|-----------------------|-------|------------------------|--------------------------|-------|
| Before                | After | Parameters             | Before                   | After |
| 77.3                  | 90.8  | VC                     | 96.9                     | 90.5  |
| 99.3                  | 92.4  | TLC                    | 109.7                    | 98.3  |
| 106.7                 | 86.8  | ITGV                   | 106.6                    | 91.2  |
| 208.9                 | 157.4 | SR                     | 132.0                    | 131.0 |
| 80.2                  | 97.2  | FEV <sub>1</sub>       | 109.9                    | 104.1 |
| 103.4                 | 107.1 | FEV <sub>1</sub> % FVC | 112.2                    | 113.6 |
| 72.0                  | 92.4  | MEF <sub>50</sub>      | 103.7                    | 107.7 |
| 82.0                  | 107.5 | MEF <sub>25</sub>      | 116.0                    | 119.1 |
| 76.3                  | 101.3 | MMEF <sub>75-25</sub>  | 112.4                    | 116.0 |

Values are expressed as percentage of predicted value.

\*The first number represents flow-volume parameters (VC, FEV<sub>1</sub>, FEV<sub>1</sub> % FVC, MEF<sub>50</sub>, 25, 75-25); the second number represents parameters of body plethysmography (TLC, ITGV, SR).

FEV<sub>1</sub>, forced expiratory volume in 1 s; FEV<sub>1</sub> % FVC, FEV<sub>1</sub> in relation to forced vital capacity; ITGV, intrathoracic gas volume; MEF<sub>50</sub>, MEF<sub>25</sub>, maximum expiratory flow at 50% or 25% of vital capacity; MMEF<sub>75-25</sub>, maximum mid-expiratory flow between 75 and 25% of vital capacity; SR, specific resistance; TLC, total lung capacity; VC, vital capacity.

documented, however, that younger children have the same high RCPI frequency. Why younger children fail to produce antibodies, despite documented infection, remains unclear. It is possible that frequent re-infections and/or a mature immune system are responsible for detectable antibody synthesis in older children.

Our data show that *C. pneumoniae* is very frequent in chronic bronchitis and pneumonia. This is a result both of selection of children with chronic respiratory diseases and exclusion of other important respiratory infections. The possibility of false-positive detection as a result of contamination was excluded by working according to the principles of a good PCR laboratory practice. PCR enzyme immunoassay (EIA) is both highly specific and sensitive. The oligonucleotide hybridization improves specificity and ensures that the correct PCR product is effectively detected. Petitjean et al. (26) tested 25 viral and bacterial species in this PCR assay and no hybridization was seen.

Johnston (4) also frequently detected *C. pneumoniae* in children with asthma or wheezing. Forty-seven per cent of the children were PCR positive at some stage during his study; in patients older than 5 years this value was 58%. Thus, *C. pneumoniae* plays an important role in chronic bronchitis, including asthma and chronic pneumonia. The question is whether *C. pneumoniae* is only an innocent bystander. We do not think so because of the differences between these groups and also in lung function. Vital capacity in RCPI is, on average, just normal, but significantly lower. Differences were pronounced in weak positives and in 11-18-year-old children with RCPI. This indicates the functional relevance of the inflammatory process during RCPI and the necessity for early

and adequate therapy. Children treated in this way improved in lung function tests; in contrast, those not treated had a slight decline in lung function.

In conclusion, in children with recurrent or chronic bronchitis, as well as pneumonia, bronchial *C. pneumoniae* infection was frequently demonstrated. An association of RCPI with eosinophilic inflammation of the respiratory mucosa, typical for asthma and the manifestation of allergies, could not be confirmed. In contrast, PCR weak positives revealed the highest frequency of allergies, elevated total IgE, and impaired lung function (restrictive and obstructive disturbance), but a low frequency of eosinophilic inflammation. All findings were more pronounced in teenagers, identifying age as additional risk factor. We recommend early diagnosis and treatment of respiratory *C. pneumoniae* infection in children with recurrent or chronic bronchitis as well as pneumonia.

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### References

1. NATIONAL INSTITUTES OF HEALTH, NATIONAL HEART, LUNG and BLOOD INSTITUTE. Global Strategy for Asthma Management and Prevention. NHLBI/WHO Workshop Report, Publication no. 95-3659. Bethesda, USA: NHLBI/WHO, 1995.
2. BODNER C, GODDEN D, SEATON A. Family size, childhood infections and atopic diseases. The Aberdeen WHEASE Group. Thorax 1998; 53: 28-32.
3. VON MUTIUS E, ILLI S, HIRSCH T, et al. Frequency of infections and risk of asthma, atopy and airway hyper-responsiveness in children. Eur Respir J 1999; 14: 4-11.
4. JOHNSTON SL. The role of viral and atypical pathogens in

- asthma pathogenesis. *Pediatr Pulmonol Suppl.* 1999; 18: 141–3.
5. HAMMERSCHLAG MR, CHIRGWIN K, ROBLIN MR, et al. Persistent infection with *Chlamydia pneumoniae* following acute respiratory illness. *Clin Infect Dis* 1992; 14: 178–82.
6. HAHN DL, DODGE RW, GOLUBJATNIKOV R. Association of *Chlamydia pneumoniae* (Strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. *JAMA* 1991; 266: 225–30.
7. HAHN DL. Intracellular pathogens and their role in asthma: *Chlamydia pneumoniae* in adult patients. *Eur Respir Rev* 1996; 38: 224–30.
8. CUNNINGHAM AF, JOHNSTON SL, JULIOUS SA, LAMPE FC, WARD ME. Chronic *Chlamydia pneumoniae* infection and asthma exacerbations in children. *Eur Respir J* 1998; 11: 345–9.
9. HAHN DL. *Chlamydia pneumoniae*, asthma and COPD. What is the evidence? *Ann Allergy Asthma Immunol* 1999; 83: 271–92.
10. FALCK G, GNARPE J, GNARPE H. Prevalence of *Chlamydia pneumoniae* in healthy children and in children with respiratory tract infections. *Pediatr Infect Dis J* 1997; 16: 549–54.
11. EMRE U, ROBLIN PM, GELLING M, et al. The association of *Chlamydia pneumoniae* infection and reactive airway disease in children. *Arch Pediatr Adolesc Med* 1994; 148: 727–34.
12. SCHMIDT SM, HERRMANN H, WIERSBITZKY SKW. Prevalence of *Chlamydia*-antibodies in in-patient children with respiratory diseases. *Pädiatr Grenzgeb* 1999; 38: 293–304.
13. HAHN DL. Treatment of *Chlamydia pneumoniae* infection in adult asthma: a before–after trial. *J Fam Pract* 1995; 41: 345–51.
14. CAMPBELL LA, MELGOSA MP, HAMILTON DJ, KUO CC, GRAYSTON JT. Detection of *Chlamydia pneumoniae* by polymerase chain reaction. *J Clin Microbiol* 1992; 30: 434–9.
15. WIERSBITZKY S, BALLKE E-H, FÜGEMANN S, WASCHER S, BRUNS R. Die diagnostische Wertigkeit der Eosinophilie in respiratorischen Sekreten bei Kindern mit chronischen nicht spezifischen Erkrankungen der Respirationsorgane (CNSRD). *Z Ärztl Fortbild* 1988; 82: 217–20.
16. ZAPLETAL A. Lung function in children and adolescents. *Progress in Respiration Research*, Vol 22. Karger, 1987.
17. BOUSQUET J, CHANEZ P, LACOSTE JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323: 1033–9.
18. DAHL R, VENGE P, OLSSON I. Blood eosinophil leukocytes and eosinophil cationic protein. Diurnal variation in normal subjects and patients with bronchial asthma. *Scand J Resp Dis* 1978; 59: 323–4.
19. SMITH H, COOK RM. Immunopharmacology of Eosinophils. London: Academic Press, 1993.
20. MEAGHER LC, COUSIN JM, SECKL JR, HASLETT C. Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. *J Immunol* 1996; 156: 4422–8.
21. VON-HERTZEN L, KLAUKKA T, MATTILA H, HAAHTELA T. *Mycobacterium tuberculosis* infection and the subsequent development of asthma and allergic conditions. *J Allergy Clin Immunol* 1999; 104: 1211–4.
22. SHIRAKAWA T, ENOMOTO T, SHIMAZU S, HOPKIN JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997; 275: 77–9.
23. PAUNIO M, HEINONEN OP, VIRTANEN M, et al. Measles history and atopic diseases: a population-based cross-sectional study. *JAMA* 2000; 283: 343–6.
24. VON MUTIUS E, MARTINEZ FD, FRITZSCH C, et al. Skin test reactivity and number of siblings. *BMJ* 1994; 308: 692–5.
25. HOLT PG. A potential vaccine strategy for asthma and allied atopic diseases during early childhood. *Lancet* 1994; 344: 456–8.
26. PETITJEAN J, VINCENT F, FRETIGNY M, et al. Comparison of two serological methods and a polymerase chain reaction – enzyme immunoassay for the diagnosis of acute respiratory infections with *Chlamydia pneumoniae* in adults. *J Med Microbiol* 1998; 47: 615–21.