*Journal of Medical Microbiology* (2004), **53**, 787–790 DOI 10.1099/jmm.0.45583-0

# Is there any relationship between *Chlamydophila pneumoniae* infection and juvenile idiopathic arthritis?

Sibel Altun, Ozgur Kasapcopur, Mustafa Aslan, Senay Karaarslan, Vedat Koksal, Suat Saribas, Sevgi Ergin, Nil Arisoy and Bekir Kocazeybek

Departments of Microbiology and Clinical Microbiology and Pediatric Rheumatology, Cerrahpaşa Medical School, Istanbul University, Turkey

Correspondence
Bekir Kocazeybek
bekirkcz@superonline.com

Received 7 January 2004
Accepted 14 April 2004

The role of *Chlamydophila pneumoniae* in the development and exacerbation of juvenile idiopathic arthritis (JIA) was investigated. Blood samples were taken from 60 JIA patients during an active disease period and for 4 weeks after. Synovial fluid samples were obtained from 20 of the 60 patients. In addition, 22 patients with familial Mediterranean fever (FMF) during the active period and 35 healthy children were included in the study as control groups. Synovial fluid samples were also obtained from three children with FMF. IgG, IgM and IgA levels against *C. pneumoniae* in serum samples were studied by immunofluorescence and IgG antibody and PCR studies were performed for *C. pneumoniae* DNA in synovial fluid samples. Twenty-nine (48·3 %) patients with JIA, 18 (81·8 %) patients with FMF and 22 (62·8 %) healthy children were found to be pre-infected with *C. pneumoniae*. Pre-infection with *C. pneumoniae* among FMF patients was found to be significantly higher than among those with JIA. We did not find a significant difference between JIA patients and healthy children. Chronic *C. pneumoniae* infection was observed only in six JIA patients, one FMF patient and two healthy children. Synovial fluid antibodies were found at higher than 1/512-fold dilution in one JIA patient and four times higher than normal serum in three JIA patients. *C. pneumoniae* DNA was not detected in any synovial fluid sample from FMF or JIA patients by PCR. In conclusion, *C. pneumoniae* infection does not have a triggering or a progressive effect on the clinical situation in JIA aetiopathogenesis, as a result of a multifactorial aetiology. New, extensive and serial studies (especially PCR studies of synovial tissue) are needed in order to confirm the indirect results.

## INTRODUCTION

*Chlamydophila pneumoniae* is an obligate intracellular bacterium with a distinct cycle of replication (Kuo *et al.*, 1995). Patients may be asymptomatic or mildly to moderately ill with a variety of respiratory tract diseases including pneumonia, acute bronchitis and prolonged cough. Although some investigators have suggested that *C. pneumoniae* infection may be associated with atherosclerotic cardiovascular disease, asthma, multiple sclerosis and other conditions, the supporting evidence has been limited so far (Kuo *et al.*, 1995; Campbell, 2002). One of the most studied diseases, rheumatoid arthritis is characterized by chronic inflammation of the synovium (Albert, 2000). Although *C. pneumoniae* has been known to trigger a strong inflammatory response, there is no evidence to indicate a relationship between inflammation of the synovium and *C. pneumoniae*. It is also not clear whether this micro-organism can trigger or exacerbate erosive joint damage (Inman *et al.*, 2000). Juvenile idiopathic arthritis (JIA), another inflammatory disease, has also been investigated. The aetiology of JIA remains unknown and microbiological studies to find a possible trigger have failed (Schneider & Passo, 2002). Taylor-Robinson *et al.* (1998) investigated the relationship between JIA and *C. pneumoniae* and suggested that larger studies with control groups were needed for more conclusive results.

The aim of this work was to determine whether a relationship exists between *C. pneumoniae* and the aetiology of JIA. We have investigated *C. pneumoniae* antibodies in serum and synovial fluid samples and *C. pneumoniae* DNA in synovium fluid samples which were obtained from patients with active disease.

Abbreviations: FMF, familial Mediterranean fever; JIA, juvenile idiopathic arthritis.

## METHODS

### Patient and control groups

The study covered a period from February 2002 to June 2003. This cohort study was conducted using three different groups.

**JIA group.** Sixty children with JIA, all with active disease fulfilling 1997 International League Against Rheumatism (ILAR) criteria, were included in the study (Petty *et al.*, 1998; Giannini *et al.*, 1997). All children underwent a physical examination; patients with upper respiratory tract disease were noted, but all children with lower respiratory tract disease and a history of antibiotic use during the previous month were excluded from the study. Children with enthesitis-related arthritis, a subtype of JIA related to reactive arthritis, and post-infectious arthritis were not evaluated here (Schneider & Passo, 2002; Petty *et al.*, 1998; Burgos-Vargas, 2002).

**Familial Mediterranean fever (FMF) group.** Twenty-two children with FMF were selected as the diseased control group. All of the children had active disease with fever above 38·5 °C on admission.

**Healthy children group.** Thirty-five age-matched healthy children were included as a healthy control group. None of the children had a familial joint disease history and none had respiratory tract disease during the previous 2 months or history of antibiotic use during the previous month. The demographic characteristics of the study groups are summarized in Table 1.

**Collection of blood samples and laboratory methods.** Blood samples (5 ml venous blood) were obtained from all children at the time of admission and during the active disease period. All blood samples were centrifuged at 3000 ***g*** for 5 min and stored at −70 °C for later analysis of *C. pneumoniae* antibody levels. Synovial fluid samples were obtained during diagnostic or therapeutic arthrocentesis from 20 JIA patients and three FMF patients who had active large joint arthritis. Synovial tissue samples were not obtained from these patients, because there was no indication.

Synovial fluid samples were stored at −70 °C for later analysis of *C. pneumoniae* antibodies and bacterial DNA. Between 4 and 8 weeks following the withdrawal of the first serum sample, the second sample was obtained from children with JIA and FMF in order to evaluate seroconversion. Similarly, the second blood sample was also stored at −70 °C for later analysis of *C. pneumoniae* antibodies.

**Serological methods.** Levels of IgG, IgM and IgA antibodies against *C. pneumoniae* in serum and IgG antibodies in synovial fluid were analysed using a microimmunofluorescence method (Euroimmun). Elementary body antigen was used in solid form.

A doubling dilution method starting from 1/32 to 1/512 for IgG, 1/16 for IgM and 1/40 for IgA were employed for antibody detection. Prior to measuring IgM antibody, an immunoadsorption step was performed by incubating 100 µl serum with 100 µl Eurosorb (Euroimmun) for 20 min, followed by centrifugation for 5 min and finally staining using the microimmunofluorescence method. In synovium fluid samples only the IgG antibody titre was determined.

Microimmunofluorescence staining was performed according to the manufacturer's guidelines. All the slides were evaluated using a fluorescence microscope (Zeiss-Axioskop 40).

**Evaluating the results of *C. pneumoniae* antibody titres.** Seropositivity criteria for diagnosis of chronic *C. pneumoniae* infection were set to IgG ⩾1/512 and IgA ⩾1/40 and for the community population titres were set to IgG ⩾1/32, based on the criterion established for previous infection. The highest result among the first or second blood samples obtained from both patients and the control groups was accepted as the final titre (Hannu *et al.*, 1999; Kocazeybek, 2003).

**Nucleic acid studies and *C. pneumoniae* DNA purification.** Frozen synovial fluid samples were thawed at room temperature and centrifuged for 10 min at 3000 ***g***. DNA extraction was performed with a NucleoSpin Blood kit (Macherey-Nagel) using the manufacturer's instructions. The supernatant was used to measure *C. pneumoniae* antibody levels.

We applied a nested PCR method using two sets of primers which were designed to detect the 16S rRNA gene fragment of *C. pneumoniae* as described by Campbell *et al.* (1992) and Ouchi *et al.* (1998). External primers were HL-1, 5′-GTTGTTCATGAAGGCCTACT-3′, and HR-1, 5′-GCATAACCTACGGTGTGTT-3′, and internal primers were ON-1, 5′-TTGAGCATATTCGTGAGG-3′, and ON-2, 5′-GTACAG TTTCTCCGTTAG-3′.

Five microlitres purified *C. pneumoniae* DNA was added to 50 µl PCR mixtures containing 10 mM Tris/HCl, pH 8, 1 mM dNTPs, 50 mM KCl, 1 mM each primer, 2 µl $MgCl_2$ (25 mM) and 0·25 U *Taq* polymerase (Invitrogen).

The first amplification was performed in a Perkin-Elmer Cutups Thermocycler for 40 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min (10 min for the last cycle). The second amplification was performed using 5 µl of the first amplified PCR product under the conditions described above.

Fifteen microlitres of samples, positive and negative controls and 100 bp ladder DNA standards (Promega) were run on 2 % agarose gels by electrophoresis (Hoefer Scientific Instruments). DNA was stained with ethidium bromide and visualized under UV light (model Tuv 20; Owl Scientific) to detect the amplified 474-bp gene product.

**Statistical analysis.** Statistical analysis was performed using SSPS version 10.0 for Windows. Student's *t* test and the chi-squared test were used for continuous and discrete variables, respectively.

## RESULTS AND DISCUSSION

The relationships between chronic synovitis and different bacteria, including mycobacteria, and viruses have been extensively studied by several investigators (Albert, 2000; Carty *et al.*, 2003). Recently, detection methods for genetic material originating from micro-organisms in joint fluids have become readily available. Thus, a hypothesis stating that chronic synovitis is an immunological response to bacterial toxins has become more popular (Albert, 2000; Schumacher *et al.*, 1999; Carty *et al.*, 2003). Villareal *et al.* (2002) focused

**Table 1.** Demographic characteristics of the study groups

| Study group | *n* | Female/male | Mean age at investigation (years) | Mean duration of disease (years) |
|---|---|---|---|---|
| JIA | 60 | 41/19 | 6·86 ± 3·81 (range 1·16–15·6) | 2·54 ± 1·48 (range 0·4–5·7) |
| FMF | 22 | 16/6 | 7·76 ± 4·36 (range 0·91–16) | 3·35 ± 2·4 (range 1–6) |
| Healthy children | 35 | 14/21 | 8·19 ± 4·16 (range 2·4–15·5) | |
| Total | 117 | 71/46 | | |

on the role of *C. pneumoniae*, which has a high prevalence amongst the general population, in the pathogenesis of chronic arthritis. It was shown that *C. pneumoniae* and *Chlamydia trachomatis* can spread to other anatomical locations by dissemination and cause chronic infections.

IgG, IgM and IgA antibody positivity for *C. pneumoniae* in patients and controls is shown in Table 2. Twenty-nine (48·3 %) patients with JIA, 18 (81·8 %) patients with FMF and 22 (62·8 %) healthy children were found to possess IgG antibodies against *C. pneumoniae*. Only one of the JIA patients had IgM antibodies. When the JIA patients were compared with the FMF patients, seropositivity for *C. pneumoniae* was found to be significantly elevated in FMF cases ($P < 0{\cdot}05$). However, there was no statistically significant difference between the children with JIA and the healthy control group ($P > 0{\cdot}05$).

Among 60 patients with active disease, only six had IgG antibody titres above 1/512 and these also had IgA antibody levels above 1/40. One of the children was thought to have subclinical acute respiratory tract disease due to *C. pneumoniae*, as evidenced by IgM positivity. Two other patients with oligoarticular JIA showed fourfold increases in IgG levels (seroconversion) and were also thought to have subclinical acute respiratory tract disease caused by this pathogen.

Hannu *et al.* (1999) evaluated 35 adults with reactive arthritis and demonstrated positive serology for *C. pneumoniae* infection in four patients. These investigators also demonstrated that three patients had recently recovered from a lower respiratory tract disease. With these findings, the authors reported that the reactive arthritis of these four patients had been triggered by *C. pneumoniae*, which could therefore be one of the triggering agents in the aetiology of reactive arthritis.

Although our patient group consisted of children with JIA, our serological findings, namely, six patients with IgG levels above 1/512 and IgA levels above 1/40, are similar to those reported by Hannu *et al.* (1999). In fact, these results have no statistical significance; however, they can be interpreted as an indicator for subclinical chronic *C. pneumoniae* infection.

A study investigating the relationship between JIA and *C. pneumoniae* in 19 children with JIA was reported by Taylor-Robinson *et al.* (1998). In this study, *C. pneumoniae* IgG antibodies were determined in ten patients, but *C. pneumoniae* DNA was found in only one child. The presence of very high antibody titres in synovial fluids from this patient, along with another child with negative results for *C. pneumoniae* DNA (1/2048 and 1/13 1072, respectively), suggested that the antibody may have been produced in the synovium.

**Table 2**. *C. pneumoniae*-specific antibodies in all groups

| Antibody titre | JIA ($n = 60$) | FMF ($n = 22$) | Healthy children ($n = 35$) |
|---|---|---|---|
| IgG ⩾1/32 | 29 | 18 | 22 |
| IgG ⩾1/512 | 7 | 1 | 2 |
| IgA ⩾1/40 | 15 | 3 | 5 |
| IgM ⩾1/16 | 1 | – | – |
| IgG ⩾1/512 and IgA ⩾1/40 | 6 | 1 | 2 |

In our study, synovial fluid samples were obtained from 20 JIA patients. One patient had a serum IgG level of ⩾1/512 and a synovial fluid antibody level of ⩾1/512. Three other patients with serum IgG levels of 1/16 demonstrated synovial fluid antibody levels of 1/128. All these patients had oligoarticular JIA. The remaining children with JIA had no measurable antibody levels in their synovial fluid. Among three patients with FMF, only one demonstrated a synovial fluid antibody level of 1/256.

Synovial fluids from 20 children with JIA and from three children with FMF failed to demonstrate *C. pneumoniae* DNA. In our study, the absence of *C. pneumoniae* DNA, high antibody levels in first and second samples from one patient with polyarthritis and higher IgG antibody levels observed in synovial fluid than those found in serum samples from three patients led us to believe that antibodies are produced within the joints.

It has been reported that *C. pneumoniae* can evoke a local inflammatory response when it is carried to the joint fluid (Gerard *et al.*, 2000). However, we were not able to demonstrate the presence of *C. pneumoniae* DNA in our patients' synovial fluids using the PCR method. Schumacher *et al.* (1999) evaluated 212 patients with arthritis and reported *C. pneumoniae* DNA was present in synovial tissues of 27 (13 %) patients, but it was found in synovial fluids of only five (4 %) patients. In another study with *Chlamydia trachomatis*, DNA levels in synovial tissue were shown to be higher than in synovial fluids and these values were statistically significant (Branigan *et al.*, 1996). Since we believe that it was ethically inappropriate to obtain synovial tissue samples from our patients, this may have contributed to negative findings for *C. pneumoniae* DNA in all subjects, including those with positive synovial antibodies.

In conclusion, our data indicate that the aetiopathogenesis of JIA does not include *C. pneumoniae* infection as a significant triggering or exacerbating factor. JIA is known to have a multifactorial aetiology. Case-oriented data from serum and synovial fluid antibody titres are far from explaining the hypothesized aetiopathogenic relationships. More studies with larger populations and longer follow-up durations, as well as direct detection of nucleic acids in the synovial tissues are needed for more conclusive results.

## ACKNOWLEDGEMENTS

This study was supported by the Research fund of the Istanbul University, project number T-247/06032003.

## REFERENCES


**Albert, L. J. (2000).** Infection and rheumatoid arthritis: guilt by association? *J Rheumatol* **37**, 564–566.

**Branigan, P. J., Gerard, H. C., Hudson, A. P., Schumacher, H. R., Jr & Pando, J. (1996).** Comparison of synovial tissue and synovial fluid as the source of nucleic acids for detection of *Chlamydia trachomatis* by polymerase chain reaction. *Arthritis Rheum* **39**, 1740–1746.

**Burgos-Vargas, R. (2002).** The juvenile-onset spondyloarthritides. *Rheum Dis Clin North Am* **28**, 531–560.

**Campbell, L. A. (2002).** *Chlamydia pneumoniae* pathogenesis. *J Med Microbiol* **51**, 623–625.

**Campbell, L. A., Perez Melgosa, M., Hamilton, D. J., Kuo, C. C. & Grayston, J. T. (1992).** Detection of *Chlamydia pneumoniae* by polymerase chain reaction. *J Clin Microbiol* **30**, 434–439.

**Carty, S. M., Snowden, N. & Silman, A. J. (2003).** Should infection still be considered as the most likely triggering factor for rheumatoid arthritis? *J Rheumatol* **30**, 425–429.

**Gerard, H. C., Schumacher, H. R., El-Gabalawy, H., Goldbach-Mansky, R. & Hudson, A. P. (2000).** *Chlamydia pneumoniae* present in the human synovium are viable and metabolically active. *Microb Pathog* **29**, 17–24.

**Giannini, E. H., Ruperto, N., Ravelli, A., Lovell, D. J., Felson, D. T. & Martini, A. (1997).** Preliminary definition of improvement in juvenile arthritis. *Arthritis Rheum* **40**, 1202–1209.

**Hannu, T., Puolakkainen, M. & Leirisalo-Repo, M. (1999).** *Chlamydia pneumoniae* as a triggering infection in reactive arthritis. *Rheumatology* **38**, 411–414.

**Inman, R. D., Whittum-Hudson, J. A., Schumacher, H. R. & Hudson, A. P. (2000).** *Chlamydia* and associated arthritis. *Curr Opin Rheumatol* **12**, 254–263.

**Kocazeybek, B. (2003).** Chronic *Chlamydophila pneumoniae* infection in lung cancer, a risk factor: a case–control study. *J Med Microbiol* **52**, 721–726.

**Kuo, C. C., Jackson, L. A., Campbell, L. A. & Grayston, J. T. (1995).** *Chlamydia pneumoniae* (TWAR). *Clin Microbiol Rev* **8**, 451–461.

**Ouchi, K., Fujii, B., Kanamato, Y., Karita, M., Shirai, M. & Nakazawa, T. (1998).** *Chlamydia pneumoniae* in coronary and iliac arteries of Japanese patients with atherosclerotic cardiovascular diseases. *J Med Microbiol* **47**, 907–913.

**Petty, R. E., Southwood, T., Baum, J. & 7 other authors (1998).** Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. *J Rheumatol* **25**, 1991–1994.

**Schneider, R. & Passo, M. H. (2002).** Juvenile rheumatoid arthritis. *Rheum Dis Clin North Am* **28**, 503–530.

**Schumacher, H. R., Jr, Gerard, H. C., Arayssi, T. K., Pando, J. A., Branigan, P. J., Saaibi, D. L. & Hudson, A. P. (1999).** Lower prevalence of *Chlamydia pneumoniae* DNA compared with *Chlamydia trachomatis* DNA in synovial tissue of arthritis patients. *Arthritis Rheum* **42**, 1889–1893.

**Taylor-Robinson, D., Thomas, B. & Rooney, M. (1998).** Association of *Chlamydia pneumoniae* with chronic juvenile arthritis. *Eur J Clin Microbiol Infect Dis* **17**, 211–212.

**Villareal, C., Whittum-Hudson, J. A. & Hudson, A. P. (2002).** Persistent *Chlamydiae* and chronic arthritis. *Arthritis Res* **4**, 5–9.