The Role of *Chlamydia* and *Chlamydophila* Infections in Reactive Arthritis

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**Abstract**

*Chlamydia trachomatis* and *Chlamydophila pneumoniae* are human pathogens; the former being the etiologic agent for trachoma as well as a prevalent sexually transmitted bacterium, while *C. pneumoniae* is a respiratory pathogen responsible for community-acquired pneumonia. Patients with reactive arthritis show evidence of present or past Chlamydial infection. *Chlamydia spp.*, has been strongly implicated as a triggering factor for reactive arthritis. We describe the simultaneous occurrence of *C. pneumoniae* and *C. trachomatis* infections in a subject with reactive arthritis. We suggest treatment for a patient with *Chlamydia*-associated arthritis to define a means by which persistent organisms can be induced to return to the active developmental cycle, thereby making them more accessible to antibiotic activity.

**Key words:** infection, reactive arthritis, *Chlamydia trachomatis*, *Chlamydophila pneumoniae*  

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**Introduction**

Reactive arthritis (ReA) is characterized by a non-purulent joint inflammation that develops in response to infections, often with some latency, at joints distant from the site of the primary infection. Several bacteria, such as *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter* and *Chlamydia spp.*, have been strongly implicated as a triggering factor for reactive arthritis (1) and factors common to these microbes are that they are intracellular pathogens and Gram-negative bacteria (2).

Several studies have found that a large proportion of patients with ReA show evidence of present or past chlamydial infection (3, 4).

While *Chlamydia trachomatis* is sexually transmitted, *Chlamydophila pneumoniae* commonly causes respiratory tract infections (2) and is responsible for community-acquired pneumonia, acute bronchitis, acute sinusitis (5) and asymptomatic infections of the upper airways (6). Infection with *C. trachomatis* has emerged as a major causative agent of reactive arthritis (3, 5). In fact, while *C. trachomatis*-triggered ReA and the role of the infection as the triggering factor has been extensively described (5, 7), it is less well known whether *C. pneumoniae* can trigger inflammatory arthritis or chronic arthritis (8).

As there are no international standards for the tests, or indications of what specific clinical and laboratory investigations are appropriate, the techniques vary greatly (9). In addition, Chlamydial infections may be sub-clinical and, therefore, laboratory identification of the triggering agent is fundamental (10).

Recently, the role of infective agents such as *Chlamydiae* in the pathogenesis of ReA has been confirmed by molecular, cultural and serological examinations (6). In particular, diagnostic methods, such as Real Time Polymerase Chain Reaction (RT-PCR), have made it possible to establish a relationship between chlamydial infection and rheumatological diseases (11). The detection of a microbe or its components at the site of the primary infection or at the joint is optimal to confirm the infectious etiology of ReA. Here, we describe the simultaneous occurrence of *C. pneumoniae* and *C. trachomatis* infections in a subject with ReA. To assess the potential clinical implications of these chlamydial infections in the patient and to investigate the synovial presence, a culture method and combined species specific RT-PCR were em-

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employed to investigate the bacteria in the synovial fluid (SF), bronchoalveolar lavage (BAL) and urine specimens. In addition, the serological status of the patient and the effectiveness of antibiotic treatment were studied.

### Case Report

A 52-year-old man was admitted to our hospital in late October 2009. The patient related that he had a history of osteoarthritis of both knees not requiring any regular treatment. In January 2009, he had been diagnosed as having *C. trachomatis* urethritis after the detection of *C. trachomatis* DNA from the urine sediments. He had been successfully treated with levofloxacin and had completely recovered from urethritis. In July 2009, he had noticed upper respiratory symptoms (a non-productive but persistent cough and mild headache) and a simultaneous painful swelling of the second proximal interphalangeal joint of his left hand.

The patient was referred to our center for further investigation. On admission he was unable to put weight on his left leg, presented with marked synovitis of the left knee and had tenderness at the insertion of the left Achilles tendon.

Considering the clinical picture, the symptomatic respiratory and past urethral infections, a diagnosis of ReA was suggested and we investigated the possible role of *C. trachomatis* and/or *C. pneumoniae* as the trigger of the infection in this patient. This study was undertaken to determine whether *C. trachomatis* and *C. pneumoniae* can be simultaneously detected in reactive arthritis, or whether the detection of one of these bacteria excludes the presence of the other.

The initial laboratory results obtained revealed a normal peripheral leukocyte count of 7,600 cells/mm^3 (61.2% neutrophils, 29.5% lymphocytes, 6.2% monocytes, 2.8% eosinophils, 0.3% basophils), a hemoglobin level of 12.8 g/dL and platelet count of 34.5×10^4/mm^3. The liver and renal functions were normal and the urate level was 180 μmol/L; rheumatoid factor and anti-nuclear antibody were negative. C-reactive protein (CRP) was elevated to 38.3 mg/dL and the results of the laboratory investigation showed an increased erythrocyte sedimentation rate (ESR) at 47 mm/h. Antibody titers to most pathogens (e.g., bartonella, borrelia, cytomegalovirus, adenovirus, mumps, measles and Parvovirus B19) were within the normal range and remained unchanged. Serotyping of the human leukocyte antigen (HLA) class I was positive for B27. The selected laboratory test results are shown in Table 1. The X-rays of the left wrist and right knee exhibited by the patient did not show any juxta-articular erosion, loss of cartilage or any enteopathetic lesion, but showed only soft tissue swelling. No cardiac murmurs were detected (data not shown).

With the written consent of the patient SF and BAL specimens were sent to our laboratory; the methods employed to investigate chlamydial DNA and viable bacteria in SF, BAL and urine specimens were the culture method combined with RT-PCR. The samples were cultured on HEP-2 and HeLa cell monolayers for evaluate the presence of *C. pneumoniae* and *C. trachomatis*, respectively. The specimens were thawed and vortexed; aliquots (100 μl) of each sample were inoculated in duplicate onto cells grown in two 96-well culture plates (Corning Costar, Bodenheim, Germany) (12). Total bacterial RNA was extracted from cultures and specimens using RNasy Mini Kit (Qiagen) according to the manufacturer’s instructions for bacterial cells. The presence of *C. pneumoniae* and *C. trachomatis* were assessed by RT-PCR with primers specific for 16S rRNA, respectively (13, 14). Serum was analyzed for antibodies to *C. pneumoniae* and *C. trachomatis*. The synovial fluid was slightly hazy and analysis revealed 62,500 leukocytes (91% neutrophils), 50 RBC and no crystals. The Gram stain was negative. Acid-fast bacilli were not revealed on the Ziehl-Neelsen stain. Blood and urine cultures were negative for bacterial growth. The molecular results of the RT-PCR of *C. trachomatis* and *C. pneumoniae* are shown in Fig. 1 and Table 2.

In particular, we observed the simultaneous presence in the synovial fluid from inflamed joints of DNA from *C. pneumoniae* and *C. trachomatis*, two bacterial species that have been typically associated with the pathogenetic process leading to arthritis. Surprisingly, the patient was found to have *C. pneumoniae* and *C. trachomatis* viable bacteria in the culture isolation of SF. Table 3 shows the immunological tests, which confirmed an ongoing *C. pneumoniae* infection.

### Table 1. Selected Laboratory Test Results

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WhiteCell Count (mm³)</td>
<td>7600</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.8</td>
</tr>
<tr>
<td>Platelets (mm³)</td>
<td>34.5×10⁴</td>
</tr>
<tr>
<td>Urate (μmol/L)</td>
<td>180</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>38.3</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>47</td>
</tr>
<tr>
<td>HLA-B27</td>
<td>+</td>
</tr>
</tbody>
</table>

### Figure 1. Representative agarose gel electrophoresis of RT-PCR for 16S rRNA genes of the specimens (A): Line 1, Negative Control; Line 2, Positive Control (*C. pneumoniae*); Lines 3-6, SF, BAL, SF culture and BAL culture, respectively. (B): Line 1, Negative Control; Line 2, Positive Control (*C. trachomatis*); Lines 3-5, SF, Urine, SF culture and BAL culture, respectively.

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**Note:** The table and figure represent the data from the study, which involves the laboratory analysis of the patient's samples to determine the presence of *C. pneumoniae* and *C. trachomatis*. The analysis includes the detection of DNA from these bacteria using RT-PCR and the confirmation of the presence of viable bacteria in the synovial fluid, urine, and bronchoalveolar lavage specimens. The results are consistent with the diagnosis of reactive arthritis associated with these bacterial infections.
[positive immunoglobulin (Ig)G and IgM, 1.9 and 2.7, respectively] and a past and persistent positive immunoglobulin (Ig)G and IgM, 1.9 and 2.7, respectively) by enzyme-linked immunosorbent assay (ELISA, Eurospital S.p.A. Trieste, Italia).

Based on these findings, the chronic C. trachomatis infection and, to avoid chronicity, the acute C. pneumoniae infection were treated with a combination of azithromycin and rifampin. In-vitro studies suggest a synergistic eradication of a persistent chlamydial infection with a combination of azithromycin and rifampin (15). A recent study proposed that prolonged treatment with a combination of antibiotics significantly improves symptoms of chronic undifferentiated spondylarthritis, with a special focus on Chlamydia (16). Rifampin has excellent tissue penetration, which is mandatory when treating obligate intracellular pathogens such as Chlamydia, and azithromycin blocks chlamydial protein synthesis.

The patient was treated for 3 months and, after discontinuing the medications for one month, was treated for another 2 months. The purpose of discontinuing these medications was to induce the persistent organisms to return to their active developmental cycle. The symptoms began to regress after the first 3 months of therapy and by the sixth month, the patient’s joint movement had completely recovered.

### Discussion

Reactive arthritis is inflammatory arthritis that occurs as a consequence of infection at a remote site. It should be suspected when there is a recent history of diarrhea, cystitis, conjunctivitis, unexplained genitourinary symptoms, prostatitis or chlamydial infection (17). Some studies have raised the possibility that C. pneumoniae, like C. trachomatis, may be a causative agent in ReA (18-20) and as both are often asymptomatic (21, 22), the causative trigger is less clinically apparent in many cases (23). Genetic factors and infections have a very strong correlation with the development of ReA (24). The HLA-B27 antigen was found in 65-80% of patients with ReA, and in 40-50% of cases of chlamydial infection the HLA-B27 antigen was present (25). Bacterial degradation products and even DNA of several microorganisms have been detected in joint fluid/tissue from patients with ReA (26). It is clear that bacteria persist in patients with ReA but the role of such disseminated microbial elements in the cause or maintenance of arthritis remains unclear. Some evidence suggests that Chlamydia may enter the articular cavity during bacteremia or within monocytes and can survive, probably with intermittent periods of replication sustained by as yet unknown phenomena (27, 28). The life-cycle of an infectious chlamydial organism probably includes at some stage an arrest in its development that makes it viable but non-culturable.

Chlamydial DNA and intact cells have been found in the synovia from patients with various arthritides (7, 20, 29-31) and in those with chronic juvenile arthritis (32). The precise mechanism by which chlamydial infection leads to ReA, and especially to chronicity and recurrence, is still unknown.

We describe for the first time the simultaneous detection of C. pneumoniae and C. trachomatis in the synovial fluid from inflamed joints. These two bacterial species have been typically associated with the pathogenetic process leading to arthritis.

Previous RT-PCR-based studies demonstrated that DNA from these organisms is often present in synovial tissue in ReA patients (33), but the simple presence of chlamydial DNA by PCR does not define a clinical syndrome or disease course and it does not provide insight into bacterial viability or metabolic activity (34). Therefore, the association of a culture and the molecular evidence that these organisms or their molecular components are found in the synovia of affected individuals is important (30). The high molecular positivity rates especially after culture of SF and BAL samples indicate that C. pneumoniae and C. trachomatis can grow and survive in an infective stage. Although serology alone is seldom useful in the final diagnosis, increased IgA antibodies are considered one of the most clinically significant markers of persistent chlamydial infection or reactivation (35, 36).

This case provides the first documented evidence of the simultaneous occurrence of Chlamydia and Chlamydia infections in a subject with reactive arthritis. This diagnosis was based on serological data and on a direct demonstration of these organisms or their molecular components in the synovial material. Numerous examples of co-infection, either bacterial or viral, are known in medicine (37). The detection of two known arthritis-causing microorganisms in one inflamed joint sets the scene for several possible pathogenetic models. Either both organisms cause inflammation, or one organism triggers the disease and the second is an innocent bystander disseminated into the inflamed joint by increased vascularization. However, a casual co-existence cannot be excluded, especially when the bacteria involved are frequent in the population (10), but certainly, the finding of two pathogenic organisms at the disease site does not indicate that they are both innocent bystanders.
Although the concept of persistent chlamydial infection in reactive arthritis and the factors that lead to its persistence in the synovium have not yet been established, the present case suggests that reactive arthritis may occur not only after the long-term duration of a chlamydial infection but also in response to a second chlamydial species. The persistence of chlamydial infection (38, 39) may amplify joint inflammation and delay the clearance of the pathogen (34). The atypical reticulocline bodies were postulated to explain why this infection escapes detection by conventional methods (40) and escapes the immune surveillance (41). The role of antibiotic treatment in ameliorating the natural course of ReA has been explored in several later studies, but solid evidence in its favor is still lacking (42-44). Persistent *Chlamydia* is largely responsible for eliciting synovial inflammation in both the acute and chronic disease. We therefore argue that when treating patients with chronic *Chlamydia*-associated arthritis, and probably also those with an acute disease, we must contend with persistent organisms rather than actively growing organisms, and with the altered biology that characterizes the persistent state. Indeed, persistence should be considered the usual state of the organism in *Chlamydia*-associated arthritis (7). Importantly, we do not know whether after antibiotic treatment these bacteria are fully cleared from the synovium in the patients who do not progress to a chronic disease, or whether the organisms remain at that site in a persistent but sub-clinical state. The reason for stopping therapy for one month was to allow the organisms to return to the active developmental cycle, thereby making them more accessible to the second cycle of antibiotic therapy. Further study may clarify this interpretation in order to obtain the most efficacious combination of antimicrobial therapy, including dosing and duration, as a potential cure for *Chlamydia*-induced ReA.

The authors state that they have no Conflict of Interest (COI).

References