Chlamydial Nucleic Acids in Synovium in Osteoarthritis: What Are the Implications?

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ABSTRACT. ***Objective.*** To study whether there is evidence of bacterial DNA in some osteoarthritic (OA) joint tissues, and the clinical implications of finding bacterial DNA in this relatively noninflammatory disease.

Methods. Polymerase chain reaction (PCR) was used to detect DNA of *Chlamydia trachomatis*, *Chlamydia pneumoniae*, and other bacteria using panbacterial primers in synovial membranes and other articular tissues of 32 consecutive patients undergoing surgery for hip and knee OA. Patients were interviewed and examined postoperatively. Operative reports were reviewed and followup examinations were accomplished on all patients.

Results. Nine of 32 patients with OA (28.1%) had evidence for bacterial DNA in joint tissues with at least one set of primers for Chlamydia: 7 for *C. trachomatis* (21.9%), 2 for *C. pneumoniae* (6.2%). Five of 32 (15.6%) patients had postoperative complications; 3 of these were in patients who showed amplified DNA of C. *trachomatis* in joints and one in a patient in whom we detected *Escherichia coli*.

Conclusion. *C. trachomatis* and *C. pneumoniae* nucleic acids can be present in joints in some cases of apparently classical OA. Whether chlamydial or other difficult to culture bacterial presence is associated with complications is suggested, but remains to be determined. Simple presence of *C. trachomatis* by PCR does not define a clinical syndrome or disease course. (J Rheumatol 2001;28:1874–80)

Key Indexing Terms:
POLYMERASE CHAIN REACTION *CHLAMYDIA TRACHOMATIS*
CHLAMYDIA PNEUMONIAE OSTEOARTHRITIS REACTIVE ARTHRITIS

The weight of evidence favoring a role for bacterial antigens in the initiation and perpetuation of reactive arthritis (ReA) is extensive. The presence of intraarticular bacterial nucleic acids or antigens has now been firmly established with the description of bacteria, bacterial fragments, DNA, RNA, and bacterial lipopolysaccharide in joints of patients with ReA[1-5]. Although it is not yet clear how these bacterial components might be acting as arthritogens, ReA stands out as a human model system from which clues to the pathogenesis of more common arthropathies may emerge[6].

Techniques based on polymerase chain reaction (PCR) and molecular hybridization appear to be the most sensitive and specific means of detecting bacterial DNA and RNA in the absence of positive cultures. Results from use of these techniques need further evaluation in studies that involve adequate numbers of control patients other than ReA[6]. PCR evidence of *Chlamydia trachomatis* DNA, although most often identified in ReA, has also been shown to occur in other inflammatory diseases including rheumatoid arthritis[7–10] and occasionally in apparently normal joints[11].

Because of lack of information about background levels of nucleic acids of infectious agents including *C. trachomatis* and *C. pneumoniae* in "noninflammatory" diseases, we have been collecting synovium from patients with osteoarthritis (OA) at the time of hip or knee arthroplasty in order to evaluate the prevalence of these agents in OA. If bacterial nucleic acids can occur in synovial tissue in OA, a prototype noninflammatory disease, we then ask whether chlamydial nucleic acid in these patients is associated with any distinctive features.

MATERIALS AND METHODS

Patients. We studied a series of synovial specimens from 32 consecutive patients undergoing surgery at the Philadelphia VAMC for hip and knee OA diagnosed according to American College of Rheumatology criteria[12]. Twenty-seven patients had knee arthroplasty and 5 patients had hip arthroplasty. All patients were male. Ages ranged from 46 to 86 years and the mean age was 64 years; 7 patients were African-American and 25 Caucasian. Synovial tissue and in some cases cartilage, bone, meniscus, and tendon were collected before placement of the prosthesis.

DNA extraction. Tissue was removed under sterile conditions, transported

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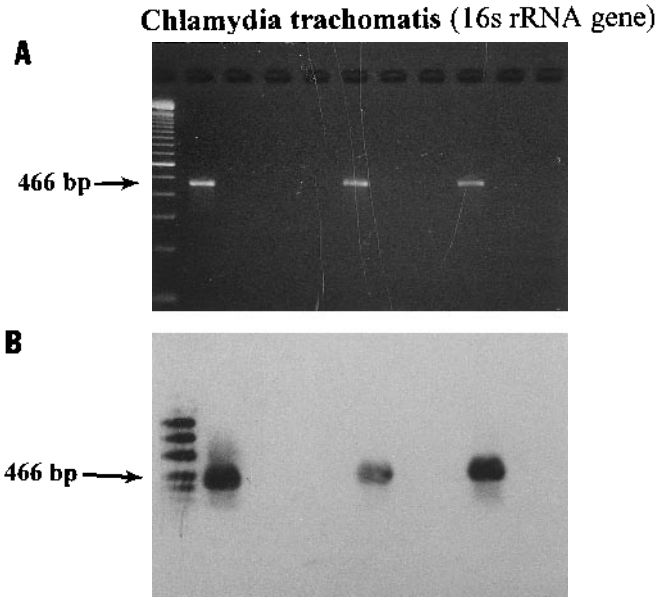
promptly in a sterile container on dry ice to our laboratory in the same building, and stored at –70°C until DNA extraction and analysis by PCR. Hyaluronidase enzymatic digestion buffer was added and the samples were incubated overnight at 50°C, extracted with phenol-chloroform and precipitated in cold ethanol, washed in 70% ethanol, centrifuged, dried, and stored in 50 mM Tris-HCL, 1 mM EDTA at 4°C. The DNA concentration was determined by ultraviolet absorption with thymus DNA as standard and adjusted to 2 μg/ml for use in a 50 μl PCR reaction mixture.

PCR conditions. All specimens were tested using established primers for *C. trachomatis* 16srRNA[13], 466 base pair major outer membrane protein (MOMP)[14], and plasmid[10]. All specimens were also tested for *C. pneumoniae* 16srRNA and MOMP[15] and with panbacterial 16srRNA primers[16]. The latter used a non-nested assay system employing 2 primers: 5´-GCGTTAATCGGAATTACTGGGCGTAAG-3´ and 5´-GGTTGCGCTCGTTGCGGGACTTAACC-3´. Cycling conditions were: 4 min/95°C, then 35 cycles of 1 min/95°C, 1 min/52°C, 1 min/72°C, then 10 min/72°C. We chose a non-nested system to limit the sensitivity of assays, since our overall intention was to identify non-chlamydial species present in synovial samples at some reasonable titer. This primer system is referred to as panbacterial, since it amplifies 16S ribosomal RNA gene sequences from a number of bacterial species. As designed, the system amplifies a DNA fragment of roughly 577 bp (depending on the organism), spanning the region from nucleotides 500 to 1077 in the standard (*Escherichia coli*) 16srRNA gene. As with other similar systems, the rationale for design of this broad-range primer system resides in the observation that prokaryotic 16srRNA gene sequences have been conserved over evolution and those sequences have been especially well conserved in particular regions that function importantly in the ribosome. All studies had positive controls from *C. trachomatis* L strain elementary bodies or *C. pneumoniae* TWAR and interspersed negative controls without DNA. Thermocycling was done as follows in a separate room: Initial melting was done at 95°C for 3 min. This was followed by 35 cycles of denaturing at 95°C for 1 min, annealing at 52°C for 1 min, and extending at 72°C for 1 min. Final extension was at 72°C for 10 min. Amplification products were detected by gel electrophoresis on 2% agarose gel that contained ethidium bromide. Agarose gels were transferred to Hy-Bond (Amersham, Arlington Heights, IL, USA) positively charged nylon membranes. All positive amplifications were confirmed by hybridization with a DIG DNA labeling kit (Boehringer Mannheim, Indianapolis, IN, USA) oligonucleotide probe, followed by immunological detection. Only those confirmed by hybridization are reported. Figure 1 shows detection of *C.trachomatis* 16srRNA by DNA amplification and hybridization.

Blood monocytes, urine, and synovial fluid (SF) of 5 patients who had amplification of bacterial DNA from joints were also studied. Samples of first morning urine were collected in 50 ml sterile tubes and frozen until used. The samples were thawed and centrifuged at 10,000 g for 20 min. The pellets were resuspended in 1 time diluted SSC [20×: sodium citrate (88 g/l), 3 M NaCl] and washed again in the same buffer[18]. SF was studied as described[17]. Blood was collected in vacutainer cell preparation tubes with sodium citrate (Becton Dickinson vacutainer systems, Franklin Lakes, NJ, USA). The tube was immediately inverted 10 times to mix anticoagulant additive with blood and kept at room temperature. The tube was centrifuged for 20 min at room temperature in a horizontal rotor (swing-out head) within 24 h (usually within a few hours of collection): roughly 1650 g measured from the axis of rotation to the bottom of the vacutainer tube in horizontal position. The peripheral blood monocyte cell (PBMC) band above the gel was transferred to an Eppendorf tube using a disposable pipette and stored at –70°C until DNA extraction and PCR[19].



Patients were interviewed and examined postoperatively in all cases, operative reports were reviewed, and followup examinations were accomplished at clinic visits with all patients. Details of any preoperative clues to systemic inflammatory diseases, infectious agent exposures, and postoperative complications were recorded.

RESULTS

Table 1 shows the PCR results. Nine of 32 (28.1%) patients with OA were found to be positive for bacterial DNA in synovium or other joint tissues with at least one set of primers for chlamydia: 7 for *C. trachomatis* (21.9%) and 2 for *C. pneumoniae* (6.2%). Of the 7 patients positive for *C. trachomatis,* one was positive for 16srRNA, 5 for plasmid, and one for both. None had amplification of DNA for *C. trachomatis* MOMP. Of 2 patients positive for *C. pneumo-*

Table 1. PCR product hybridization results.

	PCR Product Hybridization					
	C. trachomatis			*C. pneumoniae*		Panbacteria Only
Amplified product	16srRNA	Plasmid	MOMP	16srRNA	MOMP	16srRNA
No. of positive patients	2	6	0	0	2	1
Positive/total	7/32 (1 for both)			2/32		1/32

Table 2. Characteristics of PCR positive patients with multiple samples from different tissues in the same joint at total knee arthroplasty.

Patient	Pos Sample	Neg Sample	Agent	Amplified Product
20	Ten	Syn, Car	PB	16srRNA *E. coli* (identified by sequencing)
24	Syn + Men	Bone Car	CP	MOMP
25	Bone	Syn, Car	CP	MOMP
27	Bone	Syn	CT	Plasmid

Syn: synovium, Men: meniscus, Ten: tendon, Car: cartilage, PB: panbacteria, CT: *C. trachomatis,* CP: *C. pneumoniae*.

niae, both were positive for MOMP. One patient was negative for Chlamydia and only positive with the panbacteria 16srRNA screen. Sequence analysis in this patient, who had clinically evident postoperative infection that required removal of the prosthesis, showed 100% homology with *E. coli*. Three of the samples were negative for amplification of bacterial DNA in synovium, but positive in bone or tendon. Table 2 shows the characteristics of patients who were positive for PCR in different samples from individual joints.

We had inquired from all patients whether they had knee swelling and typical OA pain on use and whether joints had been aspirated. Thirteen gave a history of swelling and/or previous aspiration of the replaced joint. Five of these 13 (38.5%) patients showed PCR evidence of organisms. Six patients had histories of fracture adjacent to or at the joint studied. Three of these patients had internal fixation of the fracture in the replaced joint. Three of 6 patients (50%) with history of fracture as a cause of OA showed amplification of a bacterial DNA. One had amplification of *C. trachomatis* plasmid, one both *C. trachomatis* 16srRNA and plasmid, and one *C. pneumoniae* MOMP. No patient had history of genital infections, sexually transmitted disease, or treatment for sexually transmitted disease in their partners.

Patients were followed for postoperative complications. Table 3 shows the postoperative complications in 5 of 32 patients. Three of the 5 postoperative complications were in patients with chlamydial nucleic acids detected in the joints, one with panbacteria 16srRNA, and one was negative for bacterial DNA. Two of the 32 patients needed removal of their prostheses; one was positive for panbacteria 16srRNA shown to be *E. coli* and the other for *C. trachomatis* plasmid. Factors involved in complications were often complex, as shown in the following cases.

Patient 9, who had *C. trachomatis* plasmid DNA, had a clinically suspected infection of the prosthetic joint with continuous swelling, and had a wound dehiscence after a fall 4 weeks postoperatively. Within 4 h after the fall his wound was irrigated and debrided and he was given antibiotics (vancomycin). Before stopping his antibiotic, he developed a clinically suspected right knee infection and his prosthetic components were removed. We do not have culture results from before the initial operation. Cultures were repeatedly negative before the revision operation, but he was taking one or another antibiotic throughout this period. At surgery to remove the prosthesis *Staphylococcus aureus* was cultured. The SF had a leukocyte count of 64,890/mm^3 with 95% segmented neutrophils. We obtained synovial tissue during the revision operation and again DNA amplification yielded *C. trachomatis* plasmid.

Patient 10, who had *C. trachomatis* 16srRNA and plasmid, had consistent swelling and warmth of the replaced joint that lasted 6 mo. No infection was documented or treated. This patient had a history of an infected tibial fracture (1971) followed by malunion. Bone scan in 1994 showed no evidence of osteomyelitis involving the tibia, but revealed increased activity in all 3 phases at the lateral aspect of the left knee. On indium scanning there was no corresponding abnormal white cell accumulation.

Patient 14, with tissue showing DNA amplification for *C. trachomatis* 16srRNA, had slow wound healing, serosanginous drainage, and hemarthrosis that lasted 2 mo, but which then resolved with cephalexin therapy that was used despite repeatedly negative SF cultures. He also had a history of

Table 3. Complications in 5 of the 32 patients followed after surgery for OA of the knee or hip.

Patient	PCR Results	Complication
9	CT plasmid	Removal of prosthesis because of suspected infection Cultures done only at the removal operation revealed *S. aureus*.
10	CT 16srRNA and plasmid	Persistent joint swelling for 6 mo after operation History of infected tibial fracture and malunion
14	CT 16srRNA	Slow wound healing, serosanginous drainage, hemarthrosis, history of osteochondromatosis
19	Negative	Infection of prosthesis 2 mo after operation Culture positive for *Klebsiella oxytoca:* controlled with antibodies Did not need removal of prosthesis
20	Panbacterial 16srRNA sequence analysis showed 100% homology with *E. coli*	Infected arthroplasty and removal of prosthesis Culture later positive for *P. acnes* and *S. magnus*. One month after removal of prosthesis a new culture grew *S. aureus*

osteochondromatosis found before the operation and persisting after the operation.

Only one of the patients who revealed no bacterial DNA (case 19) had a postoperative complication (infection), which was diagnosed 2 mo after the operation. He had no immediate postoperative signs of infection, then developed swelling and warmth in the prosthetic joint. The culture was positive for *Klebsiella oxytoca*. His SF had a white blood cell (WBC) count of 48,000/mm^3 with 92% segmented neutrophils. His sedimentation rate was 75 mm/h. He continued antibiotics (ofloxacin) with persistent swelling and warmth, although the SF culture is now negative. PCR has not yet been repeated.

Patient 20, who had amplified DNA for *E. coli* from tendon tissue collected at surgery, had persistent pain and swelling, and the prosthesis was removed after the initial surgery. One month after his knee arthroplasty his SF had a WBC count of 56,300/mm^3 with 96% segmented neutrophils. A culture was positive for *Propionibacterium acnes*; at the time of the removal operation cultures were positive for *Peptostreptococcus magnus*. Both organisms were considered as likely contaminants. Repeated PCR was not done. One month after the removal operation culture was positive for *S. aureus*. A series of antibiotics (vancomycin, trimethoprim/sulfamethoxazol, cephalexin) were used, but pain and swelling persisted. Additional debridement has not yet been done.

One of the 2 patients with bacterial DNA amplified from joints from whom we collected blood and urine samples had *C. trachomatis* plasmid DNA in synovial tissue taken during the operation and also in the blood monocyte sample. The other patient had no *C. trachomatis* DNA in blood or urine.

DISCUSSION

We investigated whether there is PCR evidence for Chlamydia or other bacteria in a generally noninflammatory disease, osteoarthritis, and the clinical implications of bacterial DNA in OA.

Our small series suggests that DNA of *C. trachomatis* and *C. pneumoniae* can be present in synovial tissue of patients with OA. Thus, bacterial DNA is present in joints not only in ReA and other inflammatory joint diseases, but also in some patients with a prototype noninflammatory disease, OA, including some patients with no clinical evidence suggesting subclinical Chlamydia infection. It is difficult to compare these findings with previous work from our laboratory as our previous investigations used primers only for 16srRNA and MOMP. Most of the positive results in this study were for *C. trachomatis* plasmid DNA. Our panbacterial testing was only for the 16srRNA, so may have missed some bacterial DNA present in very small amounts. The 2 patients with *C. trachomatis* DNA amplification for 16srRNA were also positive by the panbacterial primers. This is as in other series, which showed that *C. trachomatis* plasmid targeted PCR was more sensitive than a MOMP gene targeted PCR[10,20,21]. There are 10 times more copies of plasmid DNA than 16srRNA in the organisms. The biological and clinical significance of the presence of plasmid DNA versus 16srRNA or MOMP remains to be determined. There was no pattern of correlation of *C. trachomatis* primers with the postoperative complications.

Only one study[10] described detection of *C. trachomatis* DNA by PCR in a patient with OA. In that study one of 2 patients with OA among 16 controls had *C. trachomatis* DNA in a synovial fluid sample. This patient, a 47-year-old man whose OA was said to follow a fracture of the patella, had recurrent synovial effusions for 1.5 years after the fracture. The fluid had 3800 leukocytes/mm^3, more than expected for a mechanical arthropathy, which was the diagnosis at the time of examination.

Other studies examining PCR evidence of bacteria in inflammatory joint diseases and using patients with OA as a control group reported no evidence of bacteria[22,23]. In these studies the number of patients was small, the majority of specimens were SF, not tissue, and specimens were not obtained at surgery. Wilbrink, *et al*[22] applied the general bacterial screen for 16s ribosomal RNA PCR to detect bacterial DNA in SF and synovial tissue from inflamed joints. Six patients with OA and one with arthritis secondary to joint trauma were used as a negative control group. No bacterial DNA was detected in the SF and synovial tissue from these patients including those with OA. Wilkinson, *et al*[23] used PCR to study *C. trachomatis* plasmid and outer membrane protein 1 gene of *C. pneumoniae* in SF from 54 adult patients with a range of rheumatic diseases that included one with OA. The patient with OA was PCR negative.

C. trachomatis was found in our patients with OA despite the absence of history of genital infections. We previously detected Chlamydia-specific RNA in synovial biopsy specimens from a number of patients with nonreactive arthritis in whom there had been no evidence implicating Chlamydia[1]. The possibility of subclinical chlamydial infection must clearly be considered. Seventy percent to 80% of women and 50% of men infected with *C. trachomatis* experience no genitourinary symptoms[24-26]. This results in a large population of unrecognized, infected individuals who are capable of transmitting the infection to sexual partners. This may explain our positive results in the patients who had no history of venereal disease.

What are the implications of finding *C. trachomatis* in OA joints? First, this shows that *C. trachomatis* DNA by PCR is present in situations other than ReA. Based on our previous studies in early inflammatory arthritis it seems clear that *C. trachomatis* does not define a clinical syndrome or disease course. The precise mechanism by which infection with this organism leads to development of inflammatory arthritis is still poorly understood[27]. The full clinical

implications of finding bacterial DNA in arthritic joints are still unknown. Elucidation of the means by which Chlamydia contributes to or causes ReA is critical to the development of appropriate and effective therapies for the disease, and in addition may provide insight into the pathogenesis of other spondyloarthropathies, as well as mechanisms basic to all chronic inflammatory synovitis[28].

Is detection of these organisms related to the postoperative complications seen in our patients? Our small series suggests the possibility that patients with evidence of chlamydial DNA were more likely to develop postoperative complications, since 4 of 5 patients who had postoperative complications were positive for one of the primers studied (3 for *C. trachomatis*, one for panbacteria). Three out of 7 of our patients who had amplification of *C. trachomatis* DNA had postoperative complications and one required revision arthroplasty. Only one of 22 patients who had no bacterial DNA had postoperative complications. Some complications seem especially likely to be related to *C. trachomatis*. Chlamydia may contribute to the postoperative swelling and inflammation that persist despite the use of antibiotics (other than antichlamydial antibiotics) and repeated negative culture results. No complications were seen in patients who had evidence of *C. pneumoniae* DNA, suggesting, as observed previously, that presence of this organism has a less distinct link with disease[29].

It would also be important to determine if the presence of bacterial DNA might identify a subset of patients with more erosive or more rapidly progressive OA. Unfortunately, with this small group it was difficult to establish correlations between duration and severity of disease and detection of the organisms. All our patients had advanced radiographic grade 3 or 4 OA that required joint replacement. Patients with bacterial DNA found by PCR were slightly more likely to have had swelling and/or arthrocentesis before surgery. Might this suggest a subset with more severe or complicated OA? Repeated arthrocentesis as well as the increased history of post-fracture associated OA might also suggest local introduction of bacteria in these patients.

When we began to observe *C. trachomatis* in these OA specimens we added studies of blood monocytes and urine in a few patients. One patient had *C. trachomatis* DNA in his circulating monocytes. Whether blood positivity will have clinical implications must still be determined. In future studies we and others may investigate whether Chlamydia is also present at other sites with no disease. We need to study more about the state of the organism. None of these specimens were studied by reverse transcription PCR for other genes to assess the biologic state of the organism, as we have done in inflammatory arthritis[30].

Can *C. trachomatis* nucleic acids be found in cartilage, tendon, bone, etc? To our knowledge these tissues have not been studied. We observed bacterial DNA in meniscus and tendon in one case each and in bone in 2 patients. Previous studies used SF and synovial tissue to look for PCR evidence for *C. trachomatis* DNA in joints. We reported higher PCR yields from synovial tissue than from synovial fluid[17]. There is one study[31] confirming the ability of *C. trachomatis* to reproduce in cartilage tissue. In that study human cartilage and fibroblasts were infected with Chlamydia CP-1 strain isolated from the patient's joint fluid and serially passaged in hen embryo yolk sacs. The inoculation results were assessed by direct staining with monoclonal and fluorescent antibodies. Further studies on all articular tissues will be of interest, as will attempts to culture organisms in these and other types of subjects with PCR evidence for bacterial DNA. Chlamydia in joints may exist in an altered form, lacking new elementary bodies and making the organism difficult to culture[32].

C. pneumoniae is widely recognized as a common cause of respiratory infection, with some 50% of adults showing serologic evidence of previous infection[33]. The role of *C. pneumoniae* in ReA and other arthropathies is not studied extensively. There are case reports[34,35] suggesting a role for *C. pneumoniae* as a factor in ReA based on serum antibodies. However, the prevalence of *C. pneumoniae* antibodies is high in the general population and it is difficult to establish whether the agent is associated with ReA or other type of arthritis. We reported *C. pneumoniae* in synovium in 13 of 118 (11%) patients with inflammatory arthritis; these covered a range of rheumatic diseases without any clear association with ReA[29].

The prevalence of the organisms detected using the PCR method varies widely in different series. Even in patients with sexually acquired ReA in whom presence of *C. trachomatis* was established the prevalence of *C. trachomatis* has ranged from 25%[5] to 80%[7,10]. The discrepancy in the results may have arisen for several reasons. Differences in the sensitivity of the PCR method (differences in primers used for detection of bacteria or use of nested techniques), the various types of arthritic conditions studied, or the disease duration may all cause conflicting results. Although technical variations, nature of specimens, bacteria present, quality of clinical samples, optimized sample preparation, stage of the disease, case selection, and even contamination of clinical samples may account for some conflicting data, cumulative evidence seems convincing that bacterial DNA can be present in ReA in other inflamed joints, and also, as we show, in some primarily noninflammatory joints.

The most recent guidelines from the Centers for Disease Control (CDC) on prevention and management of *C. trachomatis* infections recommend that all positive results of nonculture tests performed on low prevalence or low risk groups have to be considered for confirmation[36]. Confirmation of positive results increases the specificity and the positive predictive value of nonculture tests. Confirmation of nonculture tests is required for a definitive

diagnosis of *C. trachomatis* infection. At present, experience with PCR testing for diagnosis of *C. trachomatis* infection has been limited primarily to research laboratories and no method of confirmation for PCR has been approved for use. Recommendations from the CDC regarding a need for confirmation of DNA amplification tests could develop as further data become available[37]. In our studies with ReA confirmations were obtained with electron microscopy (EM), immuno-EM, and *in situ* hybridization[38-40].

C. trachomatis nucleic acid can be present in some classical OA synovial tissue. Whether presence of Chlamydia is associated with complications is suggested, but remains to be confirmed. Simple presence of *C. trachomatis* by PCR DNA amplification occurs in situations other than reactive arthritis and does not define a clinical syndrome or disease course.

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