Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2019, Vol. 47(2) 635–640

DOI: 10.1177/0300060518807062
journals.sagepub.com/home/imr
SAGE

No evidence of *Chlamydia pneumoniae* in the synovia of patients with osteoarthritis

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Abstract

Objective: Osteoarthritis (OA) is a common cause of disability affecting millions of people of all ages worldwide. The pathogenesis involves an inflammatory component, but the cause of the inflammation remains incompletely understood. The intracellular bacteria *Chlamydia trachomatis* and *C. pneumoniae* have been demonstrated in patients with reactive arthritis. Both of these microorganisms can cause chronic and persistent infections, with *C. trachomatis* being the most common cause of reactive arthritis. This study was performed to investigate the presence of *C. pneumoniae* in a large number of patients with primary OA.

Methods: The study included 75 patients who underwent total knee arthroplasty. During surgery, a synovial biopsy was performed and synovial fluid drawn. Real-time polymerase chain reaction (PCR) of *C. pneumoniae* was run on all patients, and real-time PCR of bacterial 16S rDNA was conducted on 30 of the 75 patients to screen for the presence of other bacteria.

Results: Real-time PCR showed no evidence of the presence of *C. pneumoniae* in the patients' specimens, nor were other bacteria detected.

Conclusions: Although an inflammatory component is part of the pathogenesis of OA, we found no evidence indicating that *C. pneumoniae* is a stimulator of that inflammation.

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Keywords
Osteoarthritis, *Chlamydia pneumoniae*, synovial membrane, inflammation, polymerase chain reaction, knee arthroplasty

Date received: 25 June 2018; accepted: 20 September 2018

Introduction

Osteoarthritis (OA) is the most common joint disorder and affects approximately 15% of the world's population.[1] Primary OA is a degenerative process in the synovial joints that is not caused by previous trauma, rheumatic arthritis, or septic arthritis. Known risk factors influencing the onset of OA are extrinsic factors (such as occupation, repetitive movement, and injuries), systemic factors (such as age, sex, race, genes, and hormones), and joint-related factors (such as overload, instability, deformity, and cartilage injury). The combination of these factors alters the structure and biochemistry in the joint, resulting in the development of OA. In the end stage of the disease, patients develop severe pain and disability. The disability is caused by articular cartilage ulceration with joint space obliteration and bone attrition (Ahlbäck classification), changes in the extracellular matrix, and diminished range of motion of the affected joint. Treatment mainly focuses on symptom relief and slowing the disease progression with joint arthroplasty as the end-stage treatment.[2]

The pathogenesis of OA is thought to be multifactorial with a complex etiology. Since the 1950s, a strong genetic component has been established on the basis of evidence that the incidence of OA is higher in certain families.[3] Several studies have also demonstrated that different genes are involved in OA and that the expression of certain genes is altered in OA.[3] Furthermore, different chemical mediators have been associated with the development of OA, including adipokines. Adipokines are related to obesity, which is a known risk factor for the development of OA.[1,3,4] OA was previously classified as a noninflammatory disease, but later studies showed that inflammatory components are involved in the pathogenesis of OA.[4] Compared with rheumatoid arthritis, however, the inflammation in OA is considered a low-grade chronic joint inflammation.

Chlamydia trachomatis is the most common cause of reactive arthritis (ReA).[5] Another species from the same genus, *C. pneumoniae*, has also been implicated as a causative agent of ReA.[6] *Chlamydia pneumoniae* is a respiratory pathogen estimated to cause 10% of all cases of community-acquired pneumonia. The prevalence of antibodies to *C. pneumoniae* increases with age, reaching 70% to 80% in patients of advanced age.[7] This obligate intracellular bacterium may cause chronic and persistent infections that are resistant to antibiotics.[7] However, asymptomatic infections are also known to occur.[8] As with other members of the family Chlamydiaceae, the presence of bacteria in tissue or body fluid is mainly established by polymerase chain reaction (PCR) because these bacteria are slow-growing and require growth in cell cultures.[7]

The inflammatory pathogenesis of OA is unknown. However, as in ReA, the pathogenesis may involve microorganisms as stimulating factors. The presence of viable and metabolically active *C. pneumoniae* has

been demonstrated in synovial tissue from a few patients with ReA and from a few patients with other forms of arthritides.[9] Furthermore, ReA caused by *C. trachomatis* and *C. pneumoniae* was improved by combination antibiotic therapy.[10] The purpose of this study was to investigate the presence of *C. pneumoniae* in synovia from patients undergoing knee replacement for treatment of symptomatic OA. If *C. pneumoniae* is involved in OA, its treatment with a combination of antibiotics might reduce the symptoms caused by the inflammation.

Materials and methods

Patients and specimens

This study involved patients undergoing total knee arthroplasty for treatment of primary OA of the knee. Patients with known posttraumatic OA, rheumatic disease, or chronic infections were excluded. The clinical data of the patients are presented in Table 1. Synovial fluid was aseptically collected by means of needle aspiration before the joint was surgically opened, and the fluid was immediately frozen at −70°C until analysis. Synovial biopsies were collected at the beginning of each surgery, directly after arthrotomy. A 5-mm × 5-mm biopsy of the synovial membrane was excised at a random and easily accessible part of the synovial membrane and immediately frozen at −70°C until processing.

Table 1. Clinical data of patients undergoing total knee arthroplasty for treatment of primary osteoarthritis

Number of patients	n = 75
Arterial hypertension	8/75 (11%)
Type 2 diabetes mellitus	7/75 (9%)
Previous myocardial infarction	1/75 (1%)
Hyperlipidemia	4/75 (5%)
Steroid treatment for inflammatory disease	2/75 (3%)

For technical reasons, synovial biopsies were not taken from two patients and synovial fluid was not collected from two other patients.

DNA extraction

DNA was extracted from approximately 20 mg of tissue and 0.5 to 4.0 mL of synovial fluid using the QiaAmp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Biopsy samples were run in a TissueLyser (Qiagen) for 2 × 20 s at 30 Hz and then 1 × 20 s at 15 Hz in Buffer ATL (Qiagen). DNA was eluted from the columns in 55 μL of Buffer AE (Qiagen). In every round of extraction, a negative (no template) control was processed in the same way as the samples.

Real-time PCR

DNA samples were subjected to real-time PCR. For detection of *C. pneumoniae*, a fragment of the *ompA* gene[11] was amplified on all DNA samples as previously described.[12] To screen for the presence of other bacteria in the material, real-time PCR of 16S bacterial DNA was run on both synovial biopsy tissue and fluid of 30 patients. The primers were as follows: forward 5′-TTG GAG AGT TTG ATC MTG GCT C-3′[13] and reverse 5′-GTA TTA CCG CGG CTG CTG-3′.[14] The PCR mix consisted of 15 μL with 1 × LightCycler 480 SYBR Green I Master (Roche, Basel, Switzerland), 670 nM forward primer (Eurogentec, Liège, Belgium), 670 nM reverse primer (Eurogentec), and a 5-μL sample and was run with the following program: 95° for 10 minutes followed by 35 cycles at 95° for 10 s, 64° for 10 s, and 72° for 30 s, with a following melt curve from 65° to 97°. All runs included positive and negative controls. To verify that DNA extraction had been successful, PCR of the

human beta-actin gene was run on all DNA samples.[15]

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Uppsala University, Uppsala (Dnr 2008/045). Written informed consent was obtained from all patients before the study, and the investigation conformed to the principles outlined in the Declaration of Helsinki.

Results

In total, 75 patients (38 men, 37 women; mean age, 63 years; age range, 49–72) were evaluated. All patients tested negative for *C. pneumoniae* DNA in both the synovial tissue biopsy and synovial fluid. All samples tested for the presence of bacterial 16S DNA were also negative. All samples were positive for human beta-actin, indicating successful DNA extraction.

Discussion

The pathogenesis of OA is not fully understood but involves an inflammatory component. In this study, the presence of the intracellular bacterium *C. pneumoniae* in patients with knee OA was investigated. None of the investigated synovial biopsies or synovial fluid samples were positive for *C. pneumoniae* when tested with real-time PCR, suggesting that *C. pneumoniae* is not part of the pathogenesis of OA.

To our knowledge, the presence of *C. pneumoniae* in patients with OA has been investigated in only one previous study. In that study, *C. pneumoniae* was demonstrated in 2 of 32 male patients undergoing surgery for knee or hip arthroplasty.[16] However, other studies in which patients with OA were used as controls for ReA or osteoporosis did not demonstrate *C. pneumoniae* in any of the patients investigated.[17–19] This latter finding agrees with our results, in which *C. pneumoniae* was not detected by PCR in any of the 75 patients studied.

Chlamydia trachomatis is the most common cause of ReA but has also been found in the synovial tissue of patients with OA.[16] Both *C. pneumoniae* and *C. trachomatis* are disseminated from their primary infection sites by monocytes: *C. pneumoniae* from the respiratory tract[20] and *C. trachomatis* from the urogenital tract.[21] They then reach the joint, where they primarily appear to reside in the synovial tissue and not in the synovial fluid.[22,23] In the joint, the infection appears to remain in a more persistent state as demonstrated by mRNA expression profiles.[24] Antibiotic treatment of persistent Chlamydiaceae infection is difficult, and few studies have demonstrated a clinical effect of long-term antibiotic treatment. One study in which combination antibiotic therapy was used to target persistent *C. trachomatis*- or *C. pneumoniae*-induced ReA demonstrated both reduction of clinical symptoms and eradication of the bacterium from synovia in many of the patients.[10]

The presence of *C. pneumoniae* and *C. trachomatis* has been demonstrated in peripheral blood mononuclear cells (PBMCs), although a low correlation was found between the presence of bacteria in synovia and the presence of bacteria in PBMCs.[17] The same applies for serological evidence of *C. pneumoniae* infection; both we and others have reported the presence of the bacterium in tissue despite negative serology.[12,25] This circumstance makes *C. pneumoniae* serology very difficult to interpret, and because it is such a common infection, we expected that about 70% of the patients in our study had antibodies to *C. pneumonia*.[7] Hence, we chose not to investigate the presence of *C. pneumoniae* in PBMCs and not to perform serology for *C. pneumoniae* in our patients.

Because the pathogenesis of OA may involve an inflammatory component,

bacteria may be involved as stimulators. Other researchers have demonstrated periodontal pathogens in synovial fluid from OA-affected knees undergoing arthroplasty using 16S rDNA sequencing.[26,27] In the present study, 30 patients were analyzed for the presence of bacterial DNA in synovial fluid and tissue. Using 16S rDNA PCR, all patients were negative, indicating that bacteria were not present in the synovia.

Patients with OA have synovial inflammation in which inflammatory mediators (such as cytokines and chemokines) are present in the synovial fluid.[4] The innate immune system has an important role in the pathogenesis of OA and is activated by pattern recognition receptors called Toll-like receptors.[28] These receptors may respond to microbes as well as to tissue damage[29] not resulting from an infection.

In conclusion, the common respiratory bacterium *C. pneumoniae*, which is known to cause chronic infection, was not demonstrated in patients with OA as evidenced by the fact that none of the synovial biopsies or synovial fluid samples in this study were positive. This finding suggests that *C. pneumoniae* is not part of the pathogenesis in OA and that other factors are instead responsible for the inflammatory component in the pathogenesis.

Acknowledgment

We thank Ylva Molin for providing technical assistance with the PCR.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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References

1. Garner M, Alshameeri Z and Khanduja V. Osteoarthritis: genes nature-nurture interaction and the role of leptin. *Int Orthop* 2013; 37: 2499–2505.
2. Bennell KL, Hunter DJ and Hinman RS. Management of osteoarthritis of the knee. *BMJ* 2012; 345: e4934.
3. Fernandez-Moreno M, Rego I, Carreira-Garcia V, et al. Genetics in osteoarthritis. *Curr Genomics* 2008; 9: 542–547.
4. Sokolove J and Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther Adv Musculoskelet Dis* 2013; 5: 77–94.
5. Carter JD and Inman RD. Chlamydia-induced reactive arthritis: hidden in plain sight? *Best Pract Res Clin Rheumatol* 2011; 25: 359–374.
6. Braun J, Laitko S, Treharne J, et al. Chlamydia pneumoniae–a new causative agent of reactive arthritis and undifferentiated oligoarthritis. *Ann Rheum Dis* 1994; 53: 100–105.
7. Grayston JT. Background and current knowledge of Chlamydia pneumoniae and atherosclerosis. *J Infect Dis* 2000; 181(Suppl 3): S402–S410.
8. Hyman CL, Roblin PM, Gaydos CA, et al. Prevalence of asymptomatic nasopharyngeal carriage of Chlamydia pneumoniae in subjectively healthy adults: assessment by polymerase chain reaction-enzyme immunoassay and culture. *Clin Infect Dis* 1995; 20: 1174–1178.
9. Gerard HC, Schumacher HR, El-Gabalawy H, et al. Chlamydia pneumoniae present in the human synovium are viable and metabolically active. *Microb Pathog* 2000; 29: 17–24.
10. Carter JD, Espinoza LR, Inman RD, et al. Combination antibiotics as a treatment for chronic Chlamydia-induced reactive arthritis: a double-blind, placebo-controlled, prospective trial. *Arthritis Rheum* 2010; 62: 1298–1307.

11. Kuoppa Y, Boman J, Scott L, et al. Quantitative detection of respiratory Chlamydia pneumoniae infection by real-time PCR. *J Clin Microbiol* 2002; 40: 2273–2274.
12. Nystrom-Rosander C, Edvinsson M, Thelin S, et al. Chlamydophila pneumonia: specific mRNA in aorta ascendens in patients undergoing coronary artery by-pass grafting. *Scand J Infect Dis* 2006; 38: 758–763.
13. Petti CA, Bosshard PP, Brandt ME, et al. *Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved guideline. MM18A*. Vol. 28. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute, 2008, p.12.
14. Bosshard PP, Zbinden R and Altwegg M. Turicibacter sanguinis gen. nov., sp. nov., a novel anaerobic, Gram-positive bacterium. *Int J Syst Evol Microbiol* 2002; 52: 1263–1266.
15. Valimaa L, Sjoroos M, Luhtala M, et al. Detection of HLA-B27 alleles by group-specific amplification and time-resolved fluorometry. *J Immunol Methods* 1998; 219: 131–137.
16. Olmez N, Wang GF, Li Y, et al. Chlamydial nucleic acids in synovium in osteoarthritis: what are the implications? *J Rheumatol* 2001; 28: 1874–1880.
17. Carter JD, Gerard HC, Espinoza LR, et al. Chlamydiae as etiologic agents in chronic undifferentiated spondyloarthritis. *Arthritis Rheum* 2009; 60: 1311–1316.
18. Contini C, Grilli A, Badia L, et al. Detection of Chlamydophila pneumoniae in patients with arthritis: significance and diagnostic value. *Rheumatol Int* 2011; 31: 1307–1313.
19. Di Pietro M, Schiavoni G, Sessa V, et al. Chlamydia pneumoniae and osteoporosis-associated bone loss: a new risk factor? *Osteoporos Int* 2013; 24: 1677–1682.
20. Moazed TC, Kuo CC, Grayston JT, et al. Evidence of systemic dissemination of Chlamydia pneumoniae via macrophages in the mouse. *J Infect Dis* 1998; 177: 1322–1325.
21. Inman RD, Whittum-Hudson JA, Schumacher HR, et al. Chlamydia and associated arthritis. *Curr Opin Rheumatol* 2000; 12: 254–262.
22. Schumacher HR, Jr., Gerard HC, Arayssi TK, et al. Lower prevalence of Chlamydia pneumoniae DNA compared with Chlamydia trachomatis DNA in synovial tissue of arthritis patients. *Arthritis Rheum* 1999; 42: 1889–1893.
23. Branigan PJ, Gerard HC, Hudson AP, et al. Comparison of synovial tissue and synovial fluid as the source of nucleic acids for detection of Chlamydia trachomatis by polymerase chain reaction. *Arthritis Rheum* 1996; 39: 1740–1746.
24. Villareal C, Whittum-Hudson JA, et al. Persistent Chlamydiae and chronic arthritis. *Arthritis Res* 2002; 4: 5–9.
25. Esposito G, Blasi F, Allegra L, et al. Demonstration of viable Chlamydia pneumoniae in atherosclerotic plaques of carotid arteries by reverse transcriptase polymerase chain reaction. *Ann Vasc Surg* 1999; 13: 421–425.
26. Ehrlich GD, Hu FZ, Sotereanos N, et al. What role do periodontal pathogens play in osteoarthritis and periprosthetic joint infections of the knee? *J Appl Biomater Funct Mater* 2014; 12: 13–20.
27. Temoin S, Chakaki A, Askari A, et al. Identification of oral bacterial DNA in synovial fluid of patients with arthritis with native and failed prosthetic joints. *J Clin Rheumatol* 2012; 18: 117–121.
28. Scanzello CR, Plaas A and Crow MK. Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? *Curr Opin Rheumatol* 2008; 20: 565–572.
29. Piccinini AM and Midwood KS. DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm* 2010; 2010: pii: 672395. doi: 10.1155/2010/672395.