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# Attempt to Detect Mycoplasma and Chlamydia by Culture in the Synovial Fluid of Patients with Arthritis

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**Abstract**

The objective was to investigate the presence of *Mycoplasma* and *Chlamydia* in the synovial fluid of patients with rheumatoid arthritis (RA) and other chronic arthritides. Samples of synovial fluid (SF) were collected from all patients presenting with an articular effusion. Seventy SF samples were subjected to study for *Mycoplasma* and cultured on standard media for *Mycoplasma*. The 70 other SF samples were subjected to study for the presence of *Chlamydia* and cultivated on cell cultures specially MC coy cell lines. All standard cultures for *Mycoplasma* and *Chlamydia* remained negative, consistent with the fact that synovial fluid is sterile, despite of many investigations that have indicated DNA of some bacteria in the SF of patients with arthritis. However, as many other attempts to detect the presence of these fastidious organisms in the joints of patients with such arthritides have failed, the question of their possible roll in the pathogenesis of human rheumatic diseases remains controversial and needs to be re-examined.

***Keywords:*** Arthritis, Mycoplasma, Chlamydia, Culture

## Introduction

The hypothesis that rheumatoid arthritis ( RA ), like other 'autoimmune' diseases, could, in fact, be caused by an infectious agent, virus or slow-growing bacteria , has been widely discussed [11]. Among the infectious agents suggested, *Mycoplasma* and *Chlamydia* seem to be especially good candidates. They are known to be a major cause of acute and chronic arthritis in animals. They frequently induce an immune disturbance in the host organism [11, 12].

*Mycoplasma* is commonly found in the oral cavity and as symbiotic gut flora. Formerly, *Mycoplasma* was considered as relative benign microorganisms with a low pathogenic potential. When they penetrate into blood vessels and colonize major organs, certain species can, however, cause acute and chronic illnesses [3]. The possibility of *Mycoplasma* being isolated from synovial specimens of patients with various rheumatic disorders, including RA, was largely debated in the 1960s and the early 1970s but, as a matter of fact, very few teams reported such isolation, and these results were sometimes contested [11].

In arthritis triggered by *Chlamydia*, microbial components have been identified in the synovial membrane and the synovial fluid cells. Two early papers even report the culture of *Chlamydia* from the joint of five patients. There are several reports on the presence of *Chlamydia trachomatis*-specific nucleic acids at the site of inflammation [8]. Negative results have also been published, concerning Chlamydia isolation, as well as the presence of its DNA [4, 6, 9, 14].

As early as the 1970s, the first studies disclosed microscopic intracellular inclusions in synovial tissues which could correspond to *Chlamydia trachomatis*, the principle arthritogenic microbe involved in arthritis. Confirmation of this work was however, hindered for many years by the fact that most of these bacteria are very difficult or almost impossible to cultivate from synovial samples [12].

Since the beginning of the 1990s, the explosive development of new molecular biology techniques has led to the detection of *C.trachomatis* DNA in the articular cavity [1, 2, 5, 10, 13]. These first results immediately raised a large numbers of questions. Did they indicate the presence of viable bacteria in the joint, or were these simply genomic vestiges of bacteria passively transported into the joint by macrophages?

In this study, the aim is to determine the viability and activity of these two organisms as live organisms in the synovial fluid of patients with different types of arthritis.

## Materials and methods

In this study, 140 synovial fluids were collected from 140 patients diagnosed as having arthritis. The patients were selected from those who referred to Rheumatology Research Center, Shariati hospital, Tehran University of Medical Sciences, Tehran, Iran.

The method used in this study was culture, differently used for *Mycoplasma* and *Chlamydia*.

*Mycoplasma* culture conditions: Because *Mycoplasma* is common cell culture contaminants, the studies in which cell cultures are used to isolate *Mycoplasma* from synovial specimens were not reliable [11]. Each sample of SF was added to 3 ml of PPLO (Pleuro Pneumonia like Organism) broth medium, and immediately brought to the laboratory to be cultured on specific media for *Mycoplasma*. The mixture of SF and transport medium was inoculated on different specific media: 1ml in 2 ml PPLO broth medium supplemented with glucose, 1ml in 2ml PPLO broth medium supplemented with arginine, 1ml in 2 ml PPLO broth

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medium supplemented with urea for ureaplasma. These media were incubated for 1 month at 37ºC under a carbon dioxide atmosphere.

*Chlamydia* culture conditions: In this study Mc Coy cell lines with CMGA was used for detection of *Chlamydia*. One ml of each SF was inoculated in cell culture medium. Centrifugation was used to facilitate the contact of EBs of specimen with cells in culture medium. For this purpose 35ºC to 36ºC and 10000 rpm was used. After centrifugation, culture media were incubated in 37ºC for 2 hours. In order to exchange the media, CMGA (Culture Minimal Essential Medium supplemented with Glucose and Antibiotic) medium was used. Cultures were incubated in 37ºC for 3 days. After this period of time cells were stained with Gimsa method and examined with dark field and light microscope for the detection of intracytoplasmic inclusions of *Chlamydia*.

## Results

Positive controls were grown on the culture media and cell culture media prepared for *Mycoplasma* and *Chlamydia*, respectively, reflecting the fact that any live organism in the specimen will grow on the culture media and cell culture media prepared.

In this study, no viable *Mycoplasma* or *Chlamydia* was detected using culture methods.

## Discussion

Although we did not detect any viable *Mycoplasma* or *Chlamydia* and although the underlying causes of rheumatoid arthritis and other arthritic diseases are not known, RA and other autoimmune diseases could be triggered, at least in part, by infectious agents. The remarkable clinical and pathological similarities between certain infectious diseases in animal species and those of some human rheumatic illnesses have encouraged the search for a microbial etiology for these syndromes [3]. A long list of microorganisms, including aerobic and anaerobic intestinal bacteria, several viruses, *Mycoplasma* and *Chlamydia*, has been proposed as important in these illnesses [7]. Although several findings on many etiological agents are not corroborated by other studies, the concept of a microbial trigger for RA and other rheumatic diseases is attractive.

Bacterial antigens, whether produced by a viable organism sometimes existed in synovium, or brought into the arthicular cavity by monocytes, may have an immunostimulatory action. These antigens can most likely persist in the joint either “stuck” to the extracellular matrix or within antigen presenting cells and by the various mechanisms can trigger a lymphocyte reaction potentially responsible for arthritis. It remains to be explained why this antibacterial response can in certain subjects exceed its physiological protective role and induce synovitis [12].

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