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SPONDYLOARTHRITIS (MA KHAN, SECTION EDITOR)

Causality of Chlamydiae in Arthritis and Spondyloarthritis: a Plea for Increased Translational Research

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Abstract Current molecular genetic understanding of the metabolically active persistent infection state of *Chlamydia trachomatis* and *Chlamydia pneumoniae* in the synovium in patients with arthritis and spondyloarthritis favors a causal relationship. Here, we examine how adequately the accepted criteria for that etiologic relationship are fulfilled, emphasizing the situation in which these microorganisms cannot be cultivated by standard or other means. We suggest that this unusual situation of causality by chlamydiae in rheumatic disease requires establishment of a consensus regarding microorganism-specific terminology as well as the development of new diagnostic and classification criteria. Recent studies demonstrate the value of molecular testing for diagnosis of reactive arthritis, undifferentiated spondyloarthritis, and undifferentiated arthritis caused by *C. trachomatis* and *C. pneumoniae* in clinical practice. Data regarding combination antibiotic therapy is consistent with the causative role of chlamydiae for these diseases. Observations of multiple intra-articular coinfections require more research to understand the implications and to respond to them.

Keywords *Chlamydia* arthritis · *Chlamydia* spondyloarthritis · *Chlamydia trachomatis* · *Chlamydia pneumoniae* · Reactive arthritis · Spondyloarthritis · Slow bacterial infection · Coinfection · Antibiotic combination therapy

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Introduction

The knowledge of the relationship between microbes and humans has increased enormously in detail and complexity. Research has consistently provided evidence for the intimate, and often subtle and long-term, relationship we share with microorganisms [e.g., 1]. A critical corollary of the new molecular insights has been the realization that microbes cause not only acute diseases but they also can elicit chronic diseases. One of the latter that was elucidated relatively early on was of course tuberculosis, caused by *Mycobacterium tuberculosis*, an extremely slow-growing bacterium of low virulence which, over the many centuries of human history and before, has elicited disease with an extremely high mortality rate [e.g., 2].

Advances in understanding of the molecular biology of the unusual obligate intracellular eubacterial pathogens *Chlamydia trachomatis* and *Chlamydia pneumoniae* have provided a number of important and clinically relevant surprises concerning their disease-causing potential. While the former is a well-established causative agent in both blinding trachoma and genital infections and the latter is a more recently identified agent responsible for a large proportion of community-acquired pneumonia, both have been implicated in causation for acute and chronic reactive arthritis (ReA) and spondyloarthritis (SpA) [3, 4]. As reviewed below, studies from a number of groups have provided important insights into subtle and unexpected aspects of the pathobiology of these organisms in relation to elicitation and maintenance of joint disease [e.g., 5 for recent discussion of unexpected aspects of chlamydial biology].

While research expanding our understanding of chlamydial biology and genital/ocular and pulmonary pathogenesis continues to be aggressively pursued, the last several years have seen a major hiatus in applying newly acquired knowledge

concerning chlamydial biology to the problems of joint disease, as well as to diagnostic testing, and various aspects of translational research, and importantly, that hiatus includes a singular lack of interest concerning potential treatments for these clinical entities [6–8]. To our knowledge, 2013 saw only five relevant original articles [9•, 10•, 11••, 12•, 13]. Four reports appeared in 2014 [14••, 15•, 16••, 17•], and three relevant reports were published in 2015 by the end of September [18, 19•, 20••]. Interestingly, one additional paper, a review, published in 2015 expressed a number of caveats regarding the genesis of ReA and SpA as a function of chlamydial infection [21•] (see also below).

Most significantly, evidence-based guidelines for diagnosis and management of *Chlamydia*-induced ReA and other possible bacterially caused joint diseases are missing and that lack can engender misdiagnosis/underdiagnosis in clinical practice; in turn, this must impair the validity of any classification of seronegative rheumatoid arthritis (RA), undifferentiated arthritis, and SpA, in clinical trials [e.g., 22–25]. In this review, we discuss the advances in understanding the causality attributable to persistent low-level bacterial infection by chlamydiae in arthritis and SpA and we review the most recent data from relevant clinical studies, diagnostic investigations, and therapeutic trials. Finally, and importantly, we define the currently unmet needs of translational and clinical research regarding chlamydiae-induced joint disease.

Chronic Bacterial Infection and Causality in Joint Disease

For 150 years and more, Robert Koch's four postulates provided the intellectual and experimental foundation for confirmation of a postulated etiologic relationship between any particular microbial pathogen and a specific disease; this approach was adequate for determination of causality in most cases of acute disease but has proved to be problematic with regard to many pathogens, especially those associated with chronic diseases (see below). That inadequacy is a primary corollary of the realization that microbes and humans share a complex, long-term relationship, a realization which emerged largely from microbiome studies made possible by the advent of rapid, inexpensive DNA sequencing technologies and from molecular genetics-based screening methods. More than two decades ago, relatively early on in the process of the reorientation of our understanding, Rook and co-workers reviewed evidence in support of the hypothesis that RA, ReA, and a number of other idiopathic diseases, in addition to Lyme disease caused by *Borrelia burgdorferi* and Whipple's disease elicited by *Tropheryma whipplei* all result from long-term infection by slow-growing, and in some instances possibly non-culturable, microorganisms similar to mycobacteria; they concluded that the evidence available supported that hypothesis [26].

Recognizing the increasingly apparent shortcomings of Koch's postulates for etiologic definition, Fredricks and Relman later suggested molecular genetic guidelines for production of a convincing definition of disease causation in the absence of cultivated or purified microorganisms (Table 1) [27].

Importantly, these authors argued that strict adherence to each of these guidelines may not be required for a functional demonstration of disease causality and that the ability to fulfill some of the criteria should provide strong evidence of a clinically important host-pathogen relationship [27]. As discussed below, several but not all of these criteria have been fulfilled regarding chlamydiae as causal microbes in arthritis and SpA.

The continued use and acceptance of molecular screening methods led to increased identification of low numbers of bacteria in arthritic joints, e.g., identifiable spirochaetes within diseased joints in Lyme disease, mycoplasmas in arthritis in patients with hypogammaglobulinemia, chlamydiae in ReA patients, and others. A few years after publication of the Fredricks and Relman criteria, Taylor-Robinson and Keat, two well-known researchers with a long-term interest in the latter, asked by what means an etiologic role for bacteria in chronic inflammatory arthritides could be established or refuted [28]. They suggested several criteria similar to those of the earlier proposal, but with more specificity for judging whether any given bacteria function as

Table 1 Guidelines for production of a convincing definition of disease causation in the absence of cultivated or purified microorganisms [27]

- A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased, but not in those organs that lack pathology
- Fewer, or no, copies of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease
- With resolution of disease, the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the opposite should occur
- When sequence detection predates disease or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship
- The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms
- Tissue-sequence correlates should be sought at the cellular level: efforts should be made to demonstrate specific in situ hybridization of microbial sequence to areas of tissue pathology and to visible microorganisms or to areas where microorganisms are presumed to be located
- These sequence-based forms of evidence for microbial causation should be reproducible

Text reprinted from Fredricks and Relman [27, with permission from the American Society for Microbiology]

a causal agent in a particular form of arthritis. Their criteria specified that a causal microorganism should

- Be found by the use of a polymerase chain reaction (PCR) or other molecular techniques more often in specimens (synovial fluid and/or membrane) from patients with the particular arthritis than in those from controls
- Preferably be found in joint specimens using a culture method also
- Be found in more than one joint specimen, that is, in sequential specimens, from the same patient, particularly in chronic disease and preferably in more than one site if more than one is involved
- Be found specifically in joint specimens from patients with early disease
- Be found by other investigators studying different groups of patients with the same disease, preferably in another geographical location
- Stimulate humoral antibody more often and in higher titers, particularly in synovial fluid (SF), in people with arthritis than in those without
- Stimulate a specific cellular response in people with disease rather than in those without
- In addition, there should be clinical improvement after treatment with an antibiotic to which the microorganism is sensitive
- Disease should be prevented or improved by a vaccine made against the microorganism

Applying these criteria to *C. trachomatis* and *C. pneumoniae* in the possible causation of ReA, the authors concluded that further research was needed to establish causality unequivocally [28]. Table 2 summarizes our understanding of how well currently available data fulfills these criteria (see also below).

Interestingly and as mentioned above, these same authors recently reviewed again the evidence for and against a chlamydial etiology for ReA and concluded that it was still not sufficient to support it unequivocally [21•]. However, several of the criteria suggested in both the Fredricks/Relman and Taylor-Robinson/Keat publications clearly have been met for *C. trachomatis* by observations from continuing research over the last 15 years; data for *C. pneumoniae* is far less abundant in relation to causation of inflammatory arthritis at this point. The question remains, though, whether most or all of these criteria actually can be met for either organism, given the current understanding of chlamydial biology and pathobiology.

An issue that has become central to our understanding of chlamydial biology concerns an unusual form of infection which has been designated *persistence*. In relation to causation by *C. trachomatis* in ReA, the biologic details relating to persistent infection directly inform the ability to demonstrate the culture of the organism from relevant patient materials, the ability to demonstrate the organism in those materials at early stages of disease, and understanding how the organism in this infection state might elicit inflammation. The general understanding of persistence for both *C. trachomatis* and *C. pneumoniae* currently indicates that chlamydiae transition to it from a normal, active, antibiotic-sensitive infection state in response to the stresses of the intracellular milieu; in particular, this transition happens within monocytes or within epithelial or other cell types in the presence of IFN-γ or other cytokines. Persistence with slightly different genetic and metabolic characteristics can be elicited in these organisms undergoing normal active infection by the presence of certain antibiotics in the growth medium, and under conditions of iron deprivation [e.g., 29]. *C. trachomatis* nucleic acids can and frequently have been demonstrated in synovial tissue (ST) samples of patients with ReA using PCR-based assays, but these organisms are

Table 2 Extent of fulfillment of proposed criteria for determining whether chlamydiae are causative in reactive arthritis reviewed by Taylor-Robinson and Keat [28] in 2001 and updated through 2015

Criterion	*Chlamydia trachomatis*		*Chlamydia pneumoniae*	
	2001	Update 2015	2001	Update 2015
1. Detection using a molecular method	+++	+++	–	+++
2. Isolation by culture	–	–	–	–
3. Detection in sequential samples	?–	++	?–	++
4. Detection in early disease	+++	+++	?	?
5. Consensus among investigators	+	+++	?	+
6. Specific antibody response	+++	+++	?	++
7. Cellular proliferative response	++	+++	++	++
8. Response of arthritis to appropriate antibiotic treatment	+	++	?	++
9. Prevention or improvement of disease by appropriate vaccine	No vaccine available		No vaccine available	

–, +, ++, +++ = no, weak, moderate, and strong fulfillment of criterion, respectively; ? = still questionable because of little or no information; ?– = questionably negative because of few opportunities for sequential samples

universally in the persistent infection state, even upon arrival in the joint [3, 4, 30, 31 for review; see below].

Significantly for this discussion, persistently infecting chlamydiae, while metabolically active, are culture negative. Molecular genetic studies from our group and others have shown that this culture negativity results from arrest of the biphasic developmental cycle at a late stage, prior to the production of new infectious elementary bodies from de-differentiating reticulate bodies [5, 30, 31 for recent reviews]; the arrest is due to the severe attenuation of expression from several genes whose products are critical for cell division, and the attenuation of expression from these and other genes obtains in both *C. trachomatis* and *C. pneumoniae* during persistence [e.g., 32–35]. Differential expression of these and other gene sets may be a general characteristic of chlamydiae and other bacterial pathogens with the ability to enter that infection state [36].

Culture of chlamydiae of either species from synovial materials is thus highly unlikely if not impossible during established, chronic disease. A related issue is whether these obligate intracellular pathogens can be demonstrated in synovial materials from patients with early disease. With the caveat that the synovial material must be chosen properly, nucleic acids should be demonstrable in them by PCR or other molecular genetic methods. The critical question here centers on what synovial material is assessed by a molecular method for chlamydial nucleic acids. Our studies have indicated that the vehicle of *C. trachomatis* from the genital system to the joint is the monocyte, and in vitro studies have indicated clearly that this organism enters the persistent infection state extremely rapidly upon infecting these immune system cells [e.g., 3–5, 30, 31]. *C. trachomatis* is already in the persistent, non-culturable state upon arrival in the joint from the genital system; presumably, *C. pneumoniae* is similar in the persistent form upon arrival from the pulmonary system. From the arrival milieu, infected monocytes enter SF and ST, where they can remain for extended periods causing inflammation. Thus, molecular assessment of chlamydial nucleic acids in SF from patients in early stages of disease may be successful but ST is the material of choice for assessment in patients with chronic disease [30].

Other aspects of the proposed criteria in Table 2 that are either not well established or, at the time of this writing, not even on the horizon are (i) response of arthritis/eradication of chlamydiae from the joint in response to antibiotic treatment and (ii) prevention or improvement of joint disease in response to an appropriate vaccine. Regarding the former, many studies have shown that treatment of ReA in the standard clinical manner with a single antibiotic is ineffective [e.g., 6–8, 31, 37 for recent reviews]. Importantly, however, recent studies from this group have indicated that combination antibiotic therapy may be the strategy of choice [6–8, 38]. To our knowledge, this initial report has not yet been confirmed (or obviated) by additional studies and we thus contend that this is an area of immense clinical and basic science interest for future research. Regarding improvement and/or prevention of ReA as a function of use of an appropriate vaccine, this criterion is simply not available for assessment at this point. Over the last three decades, extensive resources have been expended to develop a usable and effective anti-*Chlamydia* vaccine but, at this point, no such vaccine is available or even in sight. One further issue which should be mentioned here concerns the report from our group that ocular, rather than genital, strains of *C. trachomatis* are present in ST samples from patients with ReA. We were of course surprised when these data emerged from our molecular genetic characterization of synovial chlamydiae, but we have argued that this observation, while initial and requiring confirmation by other laboratories, may well explain some aspects of the epidemiology of ReA due to *C. trachomatis* infection [39]. Nevertheless, an intriguing question is what are the differences, if there are any, between *C. trachomatis* that causes trachoma and the one that causes ReA. The isolates we made from ReA patients were characterized, as reported in our paper [39], at several loci which others had shown are characteristic for differentiating trachoma from genital strains. However, our isolates have not yet been fully sequenced. The isolates now are in the hands of an expert in Australia for full sequence determination. Thus, the answer to this question will come some time in the future. One may further ask if trachoma can itself trigger ReA in those children who have no genital infection with *C. trachomatis*. To the best of our knowledge, no study up today reported ReA in children with trachoma. Over the years, many researchers have been contacted who study trachoma. The question we asked multiple times to these researchers was: do you see ReA (or SpA) in populations with endemic trachoma? The answer we received universally from all these investigators was: these people have so many health issues that a painful knee or hip or foot would be the least of their problems. In other words, no one has ever looked to see if trachoma-endemic areas in Gambia or Tanzania or the outback in Australia or elsewhere also have demonstrable ReA.

While they are not the sole etiologic agents, our view is that causation of ReA, and very probably other spondyloarthritides, is well documented for *C. trachomatis*. The data for *C. pneumoniae* are sparse but suggestive, and more study of this interesting pathogen in joint disease is likely to provide clinically significant information.

The Time Has Come to Adapt Terminology and Develop Classification Criteria

The musculoskeletal manifestations of chlamydial infections are conventionally allocated to the group of diseases termed ReA and are regarded as a form of SpA [40, 41]. ReA has been defined historically as “an arthritis which develops soon

after or during an infection elsewhere in the body, but in which the microorganisms cannot be recovered from the joint" [42]. While certainly accurate in the case of *Chlamydia*-induced ReA given the persistent infection state of the organism in the synovium, this definition is at best minimal and we therefore suggest an expansion of it to include more useful and accurate terms.

The term *Chlamydia*-induced arthritis was first introduced to describe specifically the rheumatologic signs and symptoms following urogenital infections with *C. trachomatis* [43]. Subsequently, a number of other terms were used to describe the arthritis caused by genital chlamydial infections, e.g., *Chlamydia* arthritis, *Chlamydia*-induced reactive arthritis, *Chlamydia* reactive arthritis, and others [44–50]. Later, the *Chlamydia* species was specified, e.g., *C. trachomatis* sexually acquired reactive arthritis, *C. trachomatis* arthritis, *C. trachomatis* reactive arthritis, and others [51–57]. Fully accepting the etiologic role of *Chlamydia* and expanding the somewhat minimalist, and thus somewhat misleading, concept of ReA as a disease characterized by the absence of bacteria in the joint, we contend that it would be most appropriate to designate the causative agent and the prevailing clinical manifestation together: *C. trachomatis* arthritis, *C. trachomatis* SpA, *C. pneumoniae* arthritis, and *C. pneumoniae* SpA. Such an increase in the precision of terminology also will be relevant for future coding. Current ICD coding is not up to date; both in the ICD-9-CM code 099.3 and the replacement by the ICD-10-CM code M02.3 to be used in the USA by October 1, 2015, it still employs the outdated term Reiter's disease applicable to ReA defined as "an aseptic, inflammatory arthritis developing secondary to a primary extra-articular infection, most typically of the gastrointestinal tract or urogenital system" (http://www.icd10data.com/ICD10CM/Codes/M00-M99/M00-M02/M02-/M02.3). Morris and Inman argued in a recent review that ReA, in general, and *Chlamydia* arthritis, in particular, are variants of septic arthritis in which the pathogen cannot be cultured [58]. Therefore, it would be more appropriate to code *Chlamydia* arthritis within M01, which covers direct infections of the joint in infectious and parasitic diseases classified elsewhere.

At present, no specific criteria are available to classify ReA and *Chlamydia* arthritis. Chlamydial ReA is part of the concept of SpA, which groups together related diseases with common features encompassing ankylosing spondylitis (AS), psoriatic arthritis (PSA), inflammatory bowel disease-related arthritis, ReA, and undifferentiated SpA (uSpA) [7, 8, 59, 60]. The Amor criteria and the ESSG criteria include all forms of SpA and together are considered as one criterion related to ReA preceding urogenital and enteric infections. However, neither *C. trachomatis* nor *C. pneumoniae* infection are specifically noted. Also, the most recent ASAS classification criteria for axial and peripheral SpA, developed to advance present clinical trials, do not take into account the advanced knowledge of the etiology of *Chlamydia* in arthritis and SpA. Importantly, without laboratory testing for chlamydial infections, patients with asymptomatic or undiagnosed symptomatic chlamydial infections may be misclassified as non-radiographic SpA in cases of prominent axial manifestation. The fundamental studies of Carter et al. and others reported inflammatory back pain in 73 % of undifferentiated SpA cases and in up to 80 % in *Chlamydia*-induced ReA [38, 61]. Thus, progress in establishing the causality of *Chlamydia* in rheumatic conditions calls for microorganism-specific terminology as well as the development of specific and sensitive classification criteria.

Challenges in Diagnosis

The inclusion of the causative role of chlamydiae in diagnostic practice has been hindered by several considerations. First, universally validated diagnostic criteria are not available and no international recommendations exist concerning which specific clinical and laboratory investigations are indicated and appropriate [7, 58]. In addition, chlamydial infection is frequently subclinical and laboratory testing is therefore fundamental to identify the causative agent. Unfortunately, the most easily available commercially available serologic test has several limitations: the prevalence of antibodies against *C. trachomatis* and/or *C. pneumoniae* increases with age in the healthy population, both sensitivity and specificity without clinical symptoms are poor, and the diagnostic value is further limited in cases of simultaneous or consecutive exposure to both chlamydial species, given cross-reactivity between *C. trachomatis* and *C. pneumoniae* and the nonspecific stimulation of anti-chlamydial antibodies. Likewise, positive testing for *Chlamydia* at the urogenital or respiratory entry site of infection, although highly suspicious, does not prove causality. Consequently, identification of *Chlamydia* or its components in the joint and/or in blood samples using molecular testing methods has evolved as the most specific diagnostic approach available to date [38, 39, 58, 61–66].

Recent studies demonstrate the diagnostic value of molecular testing for *Chlamydia*. Kumar et al. screened 76 arthritis patients with ReA ($n = 16$), uSpA ($n = 22$), and RA ($n = 38$) for the presence of *C. trachomatis* DNA in the SF by semi-nested PCR (snPCR) and nested PCR (nPCR); these assays targeted two different genes of *C. trachomatis*: the major outer membrane protein and a gene on the common plasmid [16••]. SF from 9/38 (23.6 %) patients (5 with ReA and 4 with uSpA) was positive for at least one *C. trachomatis* DNA sequence by snPCR or nPCR, in comparison to RA (1/38; 2.6 %). There was no correlation between the snPCR or nPCR and the presence of serum or SF immunoglobulin IgG and IgA antibodies against *C. trachomatis* as assessed using commercial enzyme-linked immunosorbent assay kits. The same group screened

SF samples from patients with ReA and uSpA ($n=20$) attending a major city hospital in New Delhi for chlamydial elementary bodies (EBs), using a commercial kit for performing direct fluorescence assay (DFA) [17•]. *C. trachomatis* EBs were detected in 33.3 % (4/12) ReA patient samples and in 25 % (2/8) uSpA samples, compared to negative results in control patients with RA or osteoarthritis (OA) ($n=20$). From these data, it was concluded that the prevalence of *C. trachomatis*-induced arthritis is underestimated and that DFA can be used as an initial diagnostic tool for screening followed by nuclear acid amplification techniques for validation. Another recent study examined the performance of two optimized molecular biology methods to determine which is best suited for detecting *C. trachomatis* in SF clinical samples from a total of 329 outpatients seen by rheumatologists in Germany with the following diagnoses: ReA ($n=10$, 4 had posturethritic ReA), undifferentiated arthritis (UA) ($n=66$), RA ($n=169$), PSA ($n=12$), and OA ($n=72$) [20••]. Alkaline lysis in combination with *C. trachomatis-omp1*-directed 152-bp PCR emerged as the most sensitive approach for identification of this organism in clinical SF samples. With this method, 3/10 (30 %) ReA patients (all with posturethritic ReA) and 20/66 (38 %) UA patients were positive, compared to negative test results from all samples from patients with OA and RA. Moreover, 2/12 (17 %) SF samples from PSA patients tested positive with this method. These frequencies are comparable to an earlier study of a group of patients with SpA, which obtained positive urogenital cultures for *C. trachomatis* in 39.4 % of patients with Reiter's syndrome, in 22.2 % of patients with PSA, and of note, in 20 % of patients with AS [45]. All three recent case-controlled studies prove the value of molecular biology testing for the diagnosis of ReA, uSpA, and UA caused by *C. trachomatis* in clinical practice. This level of clear laboratory evidence also will be required to establish *C. pneumoniae* in arthritis patient samples, to translate the present etiological knowledge into the diagnostic approach of arthritis and SpA.

Future diagnostic testing for *Chlamydia* must take into account coinfection with *C. trachomatis* and *C. pneumoniae*, as has been described occasionally in ST, SF, and peripheral blood mononuclear cells (PBMCs) of patients with uSpA and ReA [38, 61, 67]. To complicate matters further, multiple coinfections of chlamydial species and other microorganisms implicated in ReA were reported in a case study of postvenereal ReA ($n=22$), which assessed the presence of *C. trachomatis*, *C. pneumoniae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* in the samples of ST, SF, and PBMC at the time of synovectomy and after 4-month antibiotic combination therapy [14••]. Coinfection with two or three different bacteria was detected in 16/22 (72.7 %) patients, most frequently in ST (8/17; 47.1 %) and PBMC (10/22; 45.5 %) samples. Rheumatologists must also be aware of sexually acquired reactive arthritis caused by lymphogranuloma venereum serovars of *C. trachomatis*, given the increasing incidence of this infection in men who have sex with men [18]. Future research must address the frequency and clinical implication of coinfections and the intriguing recent and as yet unconfirmed observation that patients with *Chlamydia*-induced arthritis have ocular (trachoma), not genital, serovars of *C. trachomatis* in ST [39].

Combination Antibiotic Use: the Promise of Cure!

Until recently, antibiotic therapy was recommended only for acute or persistent urogenital *C. trachomatis* infection to prevent reinfection and complications in patients and partners [68]. Trials using antibiotic monotherapy to eliminate the pathogen from the joint and change the course of the disease were unsuccessful or equivocal at best [41, 69•]. Standard antibiotic therapy therefore was not justified for treatment of *Chlamydia*-induced arthritis until the first open-label pilot study demonstrated a therapeutic benefit in chronic uSpA, using the combination doxycycline plus rifampin for 6 months [70]. To prove the concept of antibiotic combination therapy, Carter et al. undertook a double-blind, placebo-controlled, prospective trial with a 6-month course of rifampin (300 mg/day) plus doxycycline (200 mg/day) or plus azithromycin (500 mg/day followed by 5 days of 2–500 mg once/week) in patients with chronic *Chlamydia*-induced ReA with PCR-positive testing either in blood or joint fluid for *C. trachomatis* or *C. pneumoniae* [38]. A response was observed in 63 % of patients undergoing active treatment compared to 20 % in placebo; 22 % of the patients under antibiotic treatment went into complete remission compared to none in the placebo arm. Five of the 6 subjects who achieved remission were randomized to the azithromycin and rifampin group suggesting this combination as most effective, although the study was not powered to determine which combination of antibiotics is superior. Most importantly, 16/23 subjects (70 %) receiving combination antibiotics and 3/11 subjects (27 %) receiving placebo became negative for *C. trachomatis* or *C. pneumoniae* at month 6 when data from PCR from PBMCs and available ST were included. These observations are a major step toward etiological management and curative treatment of *Chlamydia*-induced arthritis and *Chlamydia* SpA. However, several issues need to be resolved to encourage the implementation in clinical practice, such as already partly addressed by Carter et al. and in an editorial accompanying their report [38, 71]:

1. The number of patients ($n=42$) included in the trial was small; thus, studies with larger numbers of patients are required to confirm the initial positive findings.
2. Further studies should determine which combination of antibiotics is most effective, since this trial was not powered to compare the two different antibiotic regimens.

3. The most appropriate dosing and the best duration of therapy for long-term administration and cure remain to be established.
4. The efficacy of antibiotic combination in recent-onset *Chlamydia*-induced arthritis is not known.
5. The long-term administration of antibiotics, especially rifampin, poses the risk of bacterial resistance.
6. Because of the trial design, the efficacy of antibiotic combination has only been shown in patients positive for *Chlamydia* on PCR testing of ST biopsy samples or PBMCs, a diagnostic tool available in only a few research laboratories. No data are available for patients diagnosed by positive PCR testing of SF samples. We proposed an algorithm for the diagnosis of *Chlamydia*-induced arthritis using symptoms of clinical infection, serology, and direct detection of *Chlamydia* at the portal of entry to overcome the present diagnostic limitation [7]. Therefore, studies are needed in patients identified according to this diagnostic approach to facilitate the implementation of antibiotic combination therapy into clinical practice as long as commercially tests are not available to identify *Chlamydia* in synovial samples and blood.

Regardless, the antibiotic combinations tested by Carter et al. remain the most promising to advance the translation of growing knowledge of the causative role of *Chlamydia* in arthritis and SpA in the clinic. Several arguments are in favor of these strategies: "Rifampin has excellent tissue penetration, which is mandatory when treating obligate intracellular pathogens such as *Chlamydia*. Rifampin also has been shown to attenuate chlamydial gene transcription, including the heat-shock proteins (HSPs). The HSPs may prime the infected cell for eradication, allow for proper apoptosis, and/or eliminate the immunogenic source. Combining this effect with antibiotics that block chlamydial protein synthesis (e.g., doxycycline or azithromycin) may allow for successful eradication of the cell harboring persistently infecting intracellular organisms" [38]. In particular, the potential to eradicate persistent chlamydial infections is further supported by studies with the combination of azithromycin and rifampin in animal models of *C. pneumoniae* pneumonitis in mice and in an in vitro model of HEP-2 cells infected with *C. trachomatis* [71–73]. Hence, not surprisingly, an extremely recent randomized, double-blind, placebo-controlled study showed no advantage over placebo of a 3-month treatment with the combination of ofloxacin and roxithromycin in recent-onset ReA ($n = 56$), including 9 patients with uroarthritis [12•]. Finally, two uncontrolled studies of patients with chronic postvenereal ReA performed at the same institution did not show remission with 3-month triple alternating antibiotic (ciprofloxacin, tetracycline, and roxithromycin) treatment (63 %) following arthroscopic synovectomy; this result was similar to treatment using 3-month azithromycin alone after arthroscopic synovectomy (77 %) [14••, 15•].

Conclusions and Proposals

The understanding of causality given by recent studies of *Chlamydia* in arthritis and SpA has not been adequately translated into clinical practice. Compelling evidence suggests that *Chlamydia* arthritis is frequently underdiagnosed, primarily because of the high remission rate before the patient is diagnosed, the frequency of asymptomatic chlamydial infections, insufficient awareness of *C. pneumoniae* infection, and the lack of specific diagnostic criteria [74]. Patients with seronegative arthritis, UA, uSpA, and even AS are all candidates for a search for causative chlamydial infection [25, 61, 75]. Unfortunately, reliable optimized molecular testing for the presence of *Chlamydia* in the ST, SF, and peripheral blood is not available commercially. This is the reason that today, outside research facilities, diagnosis still relies in clinical practice on medical history, direct detection of *Chlamydia* at the portal of entry, and serological testing [7]. This and other reasons discussed above impede the translation of the promising combination antibiotic treatment for rheumatic conditions caused by chlamydial infections.

For purposes of focusing future research, a major, currently unmet, need centers on the development of classification and diagnostic criteria which cover the broad spectrum of musculoskeletal and related extra-articular manifestations caused by chlamydial infections, and which are accompanied by consensus on terminology. The most recent observation of intra-articular multiple coinfections needs more study to understand the pathogenetic, clinical, diagnostic, and therapeutic implications [14••, 15•]. It is important to reproduce the results of the efficacy of antibiotic combination in larger trials; to extend the new therapeutic strategy to patients with early chlamydial arthritis and SpA; to test modifications in dosing, duration, and combinations; to investigate potential bacterial resistance during long-term or repeated application; and to address the issue of utilization in patients only diagnosed by serology and/or direct detection of *Chlamydia* at the portal of entry. Finally, basic research must elucidate in detail the means by which persistent infection by chlamydiae maintains and perpetuates the disease and how genetic and other factors of the host-microorganism interaction contribute to the etiopathogenesis. Other priorities include mechanisms of protective immunity and immunopathology as well as vaccine development [76•].

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

Human and Animal Rights and Informed Consent With regard to the authors' research cited in this paper, all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Batut J, Andersson SG, O'Calaghan D. The evolution of chronic infection strategies in the alpha-proteobacteria. Nat Rev Microbiol. 2004;2(12):933–45.
2. Perrin P. Human and tuberculosis co-evolution: an integrative view. Tuberculosis (Edinb). 2015;95 suppl 1:112–6.
3. Gérard HC, Whittum-Hudson JA, Carter JD, Hudson AP. The pathogenic role of *Chlamydia* in spondyloarthritis. Curr Opin Rheumatol. 2010;22(4):363–7.
4. Carter JD, Hudson AP. Spondyloarthritis—reactive arthritis. Encyclopedia of medical immunology—autoimmune diseases. IR Mackay, NR Rose (eds); Springer Science and Business Media, New York. 2014; pp. 1115–22.
5. Borel N, Pospischil A, Hudson AP, Rupp J, Schoborg RV. The role of viable but non-infectious developmental forms in chlamydial biology. Front Cell Infect Microbiol. 2014;4:97.
6. Carter JD, Gérard HC, Whittum-Hudson JA, Hudson AP. Combination antibiotic treatment for *Chlamydia*-induced arthritis: is a cure in sight? Int J Clin Rheumatol. 2011;6(3):333–45.
7. Zeidler H, Hudson AP. New insights into *Chlamydia* and arthritis: promise of a cure? Ann Rheum Dis. 2014;73(4):637–44.
8. Zeidler H, Hudson AP. *Chlamydia*-induced arthritis: new insights and a possible cure. Rheumatol Network. 2014.
9. • Alvarez-Navarro C, Cragnolini JJ, Dos Santos HG, Barnea E, Admon A, Morreale A, et al. Novel HLA-B27-restricted epitopes from *Chlamydia trachomatis* generated upon endogenous processing of bacterial proteins suggest a role of molecular mimicry in reactive arthritis. J Biol Chem. 2013;288(36):25810–25. **The in vitro study shows high homology of three *Chlamydia trachomatis* peptides with human sequences containing the binding motif of HLA-B27. The results suggest that the molecular mimicry between chlamydial and self-derived HLA-B27 ligands may contribute to the pathology of reactive arthritis**.
10. • Carter JD, Rehman A, Guthrie JP, Gerard HC, Stanich J, Hudson AP. Attack rate of *Chlamydia*-induced reactive arthritis and effect of the CCR5-Delta-32 mutation: a prospective analysis. J Rheumatol. 2013;40(9):1578–82. **The first study which investigated the hypothesized genetic factor chemokine receptor-5-delta-32 allele that may predispose patients to *Chlamydia*-induced reactive arthritis when tested positive for *Chlamydia trachomatis* infection. The attack rate of arthritis was higher than that previously reported, but the CCR5-delta-32 mutation does not seem to play a role in disease susceptibility**.
11. •• Gérard HC, Carter JD, Hudson AP. *Chlamydia trachomatis* is present and metabolically active during the remitting phase in synovial tissues from patients with chronic *Chlamydia*-induced reactive arthritis. Am J Med Sci. 2013;346(1):22–5. **The study show for the first time that the bacterial load in synovial tissue of patients with remitting chronic *Chlamydia*-induced reactive arthritis is lower than that of active disease, but messengers encoding pro-inflammatory proteins are equal to/higher than those of active disease**.
12. • Kuuliala A, Julkunen H, Paimela L, et al. Double-blind, randomized, placebo-controlled study of three-month treatment with the combination of ofloxacin and roxithromycin in recent-onset reactive arthritis. Rheumatol Int. 2013;33(11):2723–9. **Three-month treatment with the combination of ofloxacin and roxithromycin has no advantage over placebo in patients with recent-onset reactive arthritis**.
13. Chen YJ, Li RN, Lin CH, Yen JH. The role of monocytes and SLC11A1 polymorphisms in the pathogenesis of *Chlamydia*-induced reactive arthritis. Scand J Rheumatol. 2013;42(2):146–9.
14. •• Strelić N, Bojović J, Pavlica L, Cikota-Aleksić B, Miličić B, Magić Z. Detection of bacteria and analyses of *Chlamydia trachomatis* viability in patients with postvenereal reactive arthritis. Intern Med J. 2014;44(12a):1247–51. **The study reports the results of a small open trial in patient positive for *Chlamydia trachomatis* with postvenereal reactive arthritis which underwent synovectomy before a 4-month course of antibiotics (ciprofloxacin, tetracycline, and roxithromycin). Clinical remission was achieved in approximately 70 % patients. Coinfection with *Chlamydia pneumoniae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* were seen in synovial tissue, synovial fluid, and peripheral blood mononuclear cells**.
15. • Bojović J, Strelić N, Pavlica L. Reiter's syndrome—disease of young men—analysis of 312 patients. Med Pregl. 2014;67(7–8):222–30. **The large case study of 312 patients with arthritis and/or sacroiliitis, which developed 1 to 4 weeks after the previous enteric or urogenital infection, is important because of the frequent identified coinfection with *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* in synovial tissue, synovial fluid, and peripheral blood mononuclear cells in the subgroup of patients with posturethritis reactive arthritis**.
16. •• Kumar P, Bhakuni DS, Rastogi S. Diagnosis of *Chlamydia trachomatis* in patients with reactive arthritis and undifferentiated spondyloarthropathy. J Infect Dev Ctries. 2014;8(5):648–54. **A very important case-control study which investigated the presence of *Chlamydia trachomatis* DNA by semi-nested PCR (snPCR) and nested PCR (nPCR) targeting two different genes of *C. trachomatis* in reactive arthritis, undifferentiated spondyloarthritis, and rheumatoid arthritis. There was no correlation between the snPCR or nPCR and the serological results of patients with reactive arthritis or undifferentiated spondyloarthritis**.
17. • Kumar P, Khanna G, Batra S, Sharma VK, Rastogi S. *Chlamydia trachomatis* elementary bodies in synovial fluid of patients with reactive arthritis and undifferentiated spondyloarthropathy in India. Int J Rheum Dis. 2014. doi:10.1111/1756-185X.12364. **The first study from India reporting the presence of *Chlamydia trachomatis* elementary bodies in the synovial fluid of spondyloarthritis patients, namely reactive arthritis and undifferentiated spondyloarthritis. A commercial kit (MicroTrak C. trachomatis Direct Specimen Test; Trinity Biotech, USA) was used for performing direct fluorescence assay. It is suggested that this test can be used as an initial diagnostic tool for screening**.
18. Perry ME, White JA. Three cases of reactive arthritis secondary to lymphogranuloma venereum. J Clin Rheumatol. 2015;21(1):33–4.
19. • Baillet AC, Rehaume LM, Benham H, O'Meara CP, Armitage CW, Ruscher R, et al. High *Chlamydia* burden promotes tumor necrosis factor-dependent reactive arthritis in SKG mice. Arthritis Rheumatol. 2015;67(6):1535–47. **The first animal model which shows that *Chlamydia muridarum* induced in mice arthritis, spondylitis, and psoriasiform dermatitis. The arthritis develops as a result of deficient intracellular pathogen control, with antigen-specific TNF production upon dissemination of antigen, and TNF-dependent inflammatory disease**.
20. •• Freise J, Bernau I, Meier S, Zeidler H, Kuipers JG. Optimized testing for C. trachomatis DNA in synovial fluid samples in clinical practice. Z Rheumatol. 2015;74:824–8. **This case-control study tested the performance of two optimized molecular biology methods, to determine which is best suited for detecting *Chlamydia trachomatis* in synovial fluid samples from patients with reactive arthritis, undifferentiated arthritis, rheumatoid**

arthritis, psoriatic arthritis, and osteoarthritis collected from various rheumatologist in routine clinical practise. Alkaline lysis in combination with *C. trachomatis-omp1*-152-bp PCR emerged as the most sensitive and specific method for the identification of *C. trachomatis*.

21.• Taylor-Robinson D, Keat A. Observations on *Chlamydia trachomatis* and other microbes in reactive arthritis. Int J STD AIDS. 2015;26(3):139–44. **The review discusses problems in attributing causality of *Chlamydia trachomatis* and other urogenital microorganism implicated in arthritis. The authors argue that there is much in favor of a causal relationship for *C. trachomatis* in sexually acquired in reactive arthritis. Nevertheless, important caveats are formulated to be explored before it is possible to say unreservedly that *C. trachomatis* plays a causative role. However, the coincidence of eradication of the microorganism with clinical recovery would be a justifiable reason for recognising causality**.

22. Zeidler H. The need to better classify and diagnose early and very early rheumatoid arthritis. J Rheumatol. 2012;39(2):212–7.

23. Zeidler H. How can misclassification be prevented when using the 2010 American College of Rheumatology/European League Against Rheumatism rheumatoid arthritis classification criteria? Comment on the article by van der Linden et al. Arthritis Rheum. 2011;63(8):2544–6.

24. Zeidler H, Amor B. The Assessment in Spondyloarthritis International Society (ASAS) classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general: the spondyloarthritis concept in progress. Ann Rheum Dis. 2011;70(1):1–3.

25. Zeidler H, Rihl M. Time to prove the infective etiology of ankylosing spondylitis and related spondylarthritides: comment on the article by Carter et al. Arthritis Rheum. 2009;60(11):3519–20.

26. Rook GA, Lydyard PM, Stanford JL. A reappraisal of the evidence that rheumatoid arthritis and several other idiopathic diseases are slow bacterial infections. Ann Rheum Dis. 1993;52 Suppl 1:S30–8.

27. Fredricks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clin Microbiol Rev. 1996;9(1):18–33.

28. Taylor-Robinson D, Keat A. How can a causal role for small bacteria in chronic inflammatory arthritides be established or refuted? Ann Rheum Dis. 2001;60(3):177–84. **Criteria reprinted by permission from BMJ Publishing Group Limited**.

29. Klos A, Thalmann J, Peters J, Gérard HC, Hudson AP. The transcript profile of persistent *C. pneumoniae in vitro* depends on the means by which persistence is induced. FEMS Microbiol Lett. 2009;291(1):120–6.

30. Carter JD, Gerard HC, Whittum-Hudson JA, Hudson AP. The molecular basis for disease phenotype in chronic *Chlamydia*-induced arthritis. Int J Clin Rheumatol. 2012;7(6):627–40.

31. Carter JD, Inman RD, Hudson AP. *Chlamydia* and chronic arthritis. Ann Med. 2012;44(8):784–92.

32. Gérard HC, Krauß-Opatz B, Rudy D, Rao JP, Zeidler H, Schumacher HR, et al. Expression of *Chlamydia trachomatis* genes required for DNA synthesis and cell division in active *vs.* persistent infection. Mol Microbiol. 2001;41(3):731–41.

33. Byrne GI, Ouellette SP, Wang Z, Rao JP, Lu L, Beatty WL, et al. *Chlamydia pneumoniae* expresses genes required for DNA replication but not cytokinesis during persistent infection of HEp-2 cells. Infect Immun. 2001;69(9):5423–9.

34. Gérard HC, Freise J, Rudy D, Krauß-Opatz B, Köhler L, Zeidler H, et al. *Chlamydia trachomatis* genes whose products are related to energy metabolism are expressed differentially in active *vs* persistent infection. Microb Infect. 2002;4(1):13–22.

35. Gérard HC, Whittum-Hudson JA, Schumacher HR, Hudson AP. Differential expression of the three *Chlamydia trachomatis* hsp60-encoding genes in active *vs* persistent infection. Microb Pathog. 2004;36(1):35–9.

36. Gérard HC, Whittum-Hudson JA, Schumacher HR, Hudson AP. Synovial *Chlamydia trachomatis* up-regulates expression of a panel of genes similar to that transcribed by *Mycobacterium tuberculosis* during persistent infection. Ann Rheum Dis. 2006;65(3):321–7.

37. Carter JD, Hudson AP. Reactive arthritis: clinical aspects and medical management. In: Espinoza L, editor. Rheumatic disease clinics of North America. 35th ed. Philadelphia: Elsevier/Saunders; 2009. p. 21–34.

38. Carter JD, Espinoza LR, Inman RD, Sneed KB, Ricca LR, Vasey FB, et al. Combination antibiotics as a treatment for chronic Chlamydia-induced reactive arthritis: a double-blind, placebo-controlled, prospective trial. Arthritis Rheum. 2010;62(5):1298–307.

39. Gerard HC, Stanich JA, Whittum-Hudson JA, Schumacher HR, Carter JD, Hudson AP. Patients with *Chlamydia*-induced arthritis have ocular (trachoma), not genital, serovars of *C trachomatis* in synovial tissue. Microb Pathog. 2010;48(2):62–8.

40. Hannu T, Inman R, Granfors K, Leirisalo-Repo M. Reactive arthritis or post-infectious arthritis? Best Pract Res Clin Rheumatol. 2006;20(3):419–33.

41. Hannu T. Reactive arthritis. Best Pract Res Clin Rheumatol. 2011;25(3):347–57.

42. Ahvonen P, Sievers K, Aho K. Arthritis associated with *Yersinia enterocolitica* infection. Acta Rheumatol Scand. 1969;15(3):232–53.

43. Wollenhaupt HJ, Schneider C, Zeidler H, Krech T, Kuntz BM. Clinical and serological characterization of *Chlamydia*-induced arthritis. Dtsch Med Wochenschr. 1989;114(50):1949–54.

44. Lauhio A, Leirisalo-Repo M, Lähdevirta J, Saikku P, Repo H. Double-blind, placebo-controlled study of three-month treatment with lymecycline in reactive arthritis, with special reference to *Chlamydia* arthritis. Arthritis Rheum. 1991;34(1):6–14.

45. Silveira LH, Gutiérrez F, Scopelitis E, Cuéllar ML, Citera G, Espinoza LR. *Chlamydia*-induced reactive arthritis. Rheum Dis Clin N Am. 1993;19(2):351–62.

46. Bas S, Cunningham T, Kvien TK, Glennås A, Melby K, Vischer TL. Synovial fluid and serum antibodies against *Chlamydia* in different forms of arthritis: intra-articular IgA production in *Chlamydia* sexually acquired reactive arthritis. Br J Rheumatol. 1996;35(6):548–52.

47. Gérard HC, Wang GF, Balin BJ, Schumacher HR, Hudson AP. Frequency of apolipoprotein E (APOE) allele types in patients with *Chlamydia*-associated arthritis and other arthritides. Microb Pathog. 1999;26(1):35–43.

48. Schumacher Jr HR. *Chlamydia*-associated reactive arthritis. Isr Med Assoc J. 2000;2(7):532–5.

49. Appel H, Kuon W, Kuhne M, Wu P, Kuhlmann S, Kollnberger S, et al. Use of HLA-B27 tetramers to identify low-frequency antigen-specific T cells in *Chlamydia*-triggered reactive arthritis. Arthritis Res Ther. 2004;6(6):R521–34.

50. Ozgül A, Dede I, Taskaynatan MA, Aydogan H, Kalyon TA. Clinical presentations of chlamydial and non-chlamydial reactive arthritis. Rheumatol Int. 2006;26(10):879–85.

51. Bas S, Cunningham T, Kvien TK, Glennås A, Melby K, Vischer TL. The value of isotype determination of serum antibodies against *Chlamydia* for the diagnosis of *Chlamydia* reactive arthritis. Br J Rheumatol. 1996;35(6):542–7.

52. Bas S, Vischer TL. Humoral immunity in *Chlamydia trachomatis* arthritis. Rev Rhum Engl Ed. 1999;66(1 Suppl):34S–5.

53. Bas S, Muzzin P, Fulpius T, Buchs N, Vischer TL. Indirect evidence of intra-articular immunoglobulin G synthesis in patients with *Chlamydia trachomatis* reactive arthritis. Rheumatology (Oxford). 2001;40(7):801–5.

54. Nikkari S, Puolakkainen M, Närvänen A, Aakre O, Toivanen P, Leirisalo-Repo M. Use of a peptide based enzyme immunoassay

in diagnosis of *Chlamydia trachomatis* triggered reactive arthritis. J Rheumatol. 2001;28(11):2487–93.

55. Ardeniz O, Gülbahar O, Mete N, Çiçek C, Basoğlu OK, Sin A, et al. *Chlamydia pneumoniae* arthritis in a patient with common variable immunodeficiency. Ann Allergy Asthma Immunol. 2005;94(4): 504–8.
56. Harrer T, Bäuerle M, Bergmann S, Eismann K, Harrer EG. Inhibition of HIV-1-specific T-cells and increase of viral load during immunosuppressive treatment in an HIV-1 infected patient with *Chlamydia trachomatis* induced arthritis. J Clin Virol. 2005;34(3): 224–30.
57. Savolainen E, Kettunen A, Närvänen A, Kautiainen H, Kärkkäinen U, Luosujärvi R, et al. Prevalence of antibodies against *Chlamydia trachomatis* and incidence of *C. trachomatis*-induced reactive arthritis in an early arthritis series in Finland in 2000. Scand J Rheumatol. 2009;38(5):353–6.
58. Morris D, Inman RD. Reactive arthritis: developments and challenges in diagnosis and treatment. Curr Rheumatol Rep. 2012;14(5):390–4.
59. Zeidler HK, Schumacher HR. *Chlamydia*-induced arthritis. In: Calin A, Taurog JD, editors. The spondyloarthritides. Oxford: Oxford University Press; 1998. p. 69–96.
60. Zeidler H, Calin A, Amor B. A historical perspective of the spondyloarthritis. Curr Opin Rheumatol. 2011;23(4):327–33.
61. Carter JD, Gérard HC, Espinoza LR, Ricca LR, Valeriano J, Snelgrove J, et al. Chlamydiae as etiologic agents in chronic undifferentiated spondyloarthritis. Arthritis Rheum. 2009;60(5):1311–6.
62. Schumacher HR, Gérard HC, Arassi TK, Pando JA, Branigan PJ, Saaibi DL, et al. Lower prevalence of *Chlamydia pneumoniae* DNA compared with *Chlamydia trachomatis* DNA in synovial tissue of arthritis patients. Arthritis Rheum. 1999;42(9):1889–93.
63. Gérard HC, Stanich JA, Whittum-Hudson JA, Schumacher HR, Carter JD, Hudson AP. Patients with *Chlamydia*-associated arthritis have ocular (trachoma), not genital, serovars of *C. trachomatis* in synovial tissue. Microb Pathog. 2010;48(2):62–8.
64. Freise J, Bernau I, Meier S, Zeidler H, Kuipers JG. Detection of *Chlamydia trachomatis*-DNA in synovial fluid: evaluation of the sensitivity of different DNA extraction methods and amplification systems. Arthritis Res Ther. 2009;11(6):R175.
65. Freise J, Gérard HC, Bunke T, Whittum-Hudson JA, Zeidler H, Köhler L, et al. Optimised sample DNA preparation for detection of *Chlamydia trachomatis* in synovial tissue by polymerase chain reaction and ligase chain reaction. Ann Rheum Dis. 2001;60(2): 140–5.
66. Sieper J, Rudwaleit M, Braun J, van der Heijde D. Diagnosing reactive arthritis: role of clinical setting in the value of serologic and microbiologic assays. Arthritis Rheum. 2002;46(2):319–27.
67. Rizzo A, Domenico MD, Carratelli CR, Paolillo R. The role of *Chlamydia* and *Chlamydophila infections* in reactive arthritis. Intern Med. 2012;51(1):113–7.
68. Bébéar C, de Barbeyrac B. Genital *Chlamydia trachomatis* infections. Clin Microbiol Infect. 2009;15(1):4–10.
69.• Barber CE, Kim J, Inman RD, Esdaile JM, James MT. Antibiotics for treatment of reactive arthritis: a systematic review and metaanalysis. J Rheumatol. 2013;40(6):916–28. **A systematic review and meta-analysis of randomized controlled trials of antibiotics for treatment of reactive arthritis which came to the conclusion that antibiotics should at present remain restricted to clinical trials. However, the authors also suggest that combination antibiotic therapy might be the strategy most warranting future evaluation in larger trials**.
70. Carter JD, Valeriano J, Vasey FB. Doxycycline versus doxycycline and rifampin in undifferentiated spondyloarthropathy, with special reference to *Chlamydia*-induced arthritis. A prospective, randomized 9-month comparison. J Rheumatol. 2004;31(10):1973–80.
71. Wolf K, Malinverni R. Effect of azithromycin plus rifampin versus that of azithromycin alone on the eradication of *Chlamydia pneumoniae* from lung tissue in experimental pneumonitis. Antimicrob Agents Chemother. 1999;43(6):1491–3.
72. Bin XX, Wolf K, Schaffner T, Malinverni R. Effect of azithromycin plus rifampin versus amoxicillin alone on eradication and inflammation in the chronic course of *Chlamydia pneumoniae* pneumonitis in mice. Antimicrob Agents Chemother. 2000;44(6):1761–4.
73. Dreses-Werringloer U, Padubrin I, Zeidler H, Kohler L. Effects of azithromycin and rifampin on *Chlamydia trachomatis* infection in vitro. Antimicrob Agents Chemother. 2001;45(11):3001–8.
74. Carter JD, Inman RD. *Chlamydia*-induced reactive arthritis: hidden in plain sight? Best Pract Res Clin Rheumatol. 2011;25(3):359–74.
75. Feng XG, Xu XJ, Ye S, Lin YY, Chen P, Zhang XJ, et al. Recent *Chlamydia pneumoniae* infection is highly associated with active ankylosing spondylitis in a Chinese cohort. Scand J Rheumatol. 2011;40(4):289–91.
76.• Leonard CA, Borel N. Chronic chlamydial diseases: from atherosclerosis to urogenital infections. Curr Clin Micro Rpt. 2014;1(3–4):61–72. doi:10.1007/s40588-014-0005-8. **The review addresses recent information on pathogenesis, immune response, diagnosis, and treatment of chronic diseases including atherosclerosis, trachoma, urogenital infections, and arthritis induced by *Chlamydia trachomatis* and *Chlamydia pneumoniae***.