Use of a Peptide Based Enzyme Immunoassay in Diagnosis of Chlamydia trachomatis Triggered Reactive Arthritis

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ABSTRACT. ***Objective.*** To assess the presence of circulating IgA and IgG antibodies to *Chlamydia trachomatis* in sera of patients with reactive arthritis (ReA) and other arthritides.

Methods. A peptide based enzyme immunoassay (EIA) was used to study 132 patients divided into 5 groups: *C. trachomatis* triggered ReA, uroarthritis, enteroarthritis, oligoarthritis, and rheumatoid arthritis (RA). Followup sera were available from 19 patients.

Results. An increased prevalence of *C. trachomatis* antibodies was observed in patients with ReA triggered by *C. trachomatis*; 18/23 (78%) had IgA and 19/23 (83%) had IgG antibodies. In patient groups with uroarthritis (n = 12), enteroarthritis (n = 56), oligoarthritis (n = 16), and RA (n = 25), *C. trachomatis* IgA/IgG antibodies were detected in 58%/75%, 27%/21%, 25%/31%, and 20%/32% of patients, respectively. Both the IgA and IgG antibodies were positive in 74%, 50%, 16%, 25%, and 12% of the patients with *C. trachomatis* triggered ReA, uroarthritis, enteroarthritis, oligoarthritis, and RA, respectively. Based on positivity of both isotypes the sensitivity of the assay was 74% and specificity 84%. In the followup sera, an association between circulating *C. trachomatis*-specific antibody concentrations and clinical disease outcome of the arthritis was seen in patients with culture-positive *C. trachomatis* triggered ReA.

Conclusion. *C. trachomatis* species-specific peptide EIA correlates well with conventional diagnosis of primary *C. trachomatis* infection in patients with ReA. This assay may be a valuable contribution to the diagnosis of *C. trachomatis* triggered ReA. (J Rheumatol 2001;28:2487–93)

Key Indexing Terms:
REACTIVE ARTHRITIS ENZYME LINKED IMMUNOASSAY
CHLAMYDIA TRACHOMATIS ANTIBODIES

The definite microbiological diagnosis of reactive arthritis (ReA) is based on the isolation of pathogenic bacteria from the stool sample in enteroarthritis and detection of *Chlamydia trachomatis* from the genitourinary tract or urine in uroarthritis. Other possible methodologies include antigen detection of bacterial structures and nucleic acids from the site of joint inflammation, improved diagnosis of primary infection, and measurement of host cellular or humoral immune responses to the etiologic agent.

In ReA triggered by *Yersinia*[1], *Salmonella*[2], *Shigella*[3], and *C. trachomatis*[4], bacterial antigens may be detected at the site of arthritis, in the synovial fluid cells and synovial tissue, and even for extended periods of time in cells of peripheral blood[5]. However, these methodologies are laborious and the antibodies used in the assays are not commercially available.

In *C. trachomatis* triggered ReA and Reiter's syndrome the presence of *C. trachomatis*-specific nucleic acids at the site of joint inflammation has been reported by several groups[6-12]. On the other hand, there are studies where *C. trachomatis* DNA was not detectable in joint samples from patients with sexually acquired ReA[13,14]. To add controversy to the laboratory diagnosis of *C. trachomatis* triggered ReA, a lack of correlation between the detection of *C. trachomatis* DNA in synovial fluid from patients with arthritis and the presence of antichlamydial immune response was recently reported[15]. Further, by using nested polymerase chain reaction, *C. trachomatis* DNA can be detected in the synovium of asymptomatic subjects[16]. Therefore, due to the controver-

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sial findings described above, molecular DNA techniques are not used routinely in detection of *C. trachomatis* DNA from synovial samples for the laboratory diagnosis of uroarthritis. However, contrary to this, techniques based on molecular gene amplification are widely used in reliable diagnosis of primary *C. trachomatis* infection from genitourinary tract swabs and first-void urine[17].

Serological diagnosis of *C. trachomatis* infections has not been considered beneficial due to the scarce serological response seen in superficial infections[18] and to the genus-specific nature of many commercial tests. In particular, the high prevalence of *C. pneumoniae* antibodies in the population has led to misinterpretation of serological assays detecting antibodies against epitopes that are common to all chlamydial species. The differential diagnosis of *C. trachomatis* triggered ReA from other arthritides would be important due to findings that this subgroup of ReA patients might benefit from longterm antibiotic treatment[19-21].

The development of specific serological assays for *Salmonella*[22] and *Yersinia*[23] has considerably improved the laboratory diagnosis of enteroarthritides. At the onset of arthritis the gastroenteritis may have subsided and the stool cultures for these pathogens are often negative; in addition, the primary infection may also be asymptomatic. The same may apply in *C. trachomatis* triggered ReA and detection of *C. trachomatis* from the site of the primary infection. ReA may follow silent as well as symptomatic primary *C. trachomatis* infection[24]. Particularly in chronic and recurrent *C. trachomatis* infections, the isolation or detection of *C. trachomatis* at the site of primary infection can prove difficult, because the organism has often disappeared from the primary site of infection or because of lack of assay sensitivity.

To determine the usefulness of a synthetic peptide based species-specific enzyme immunoassay (EIA), we applied the technique to detect circulating IgA and IgG antibodies to *C. trachomatis* in 132 patients with arthritis. The peptides are derived from the major outer membrane protein (MOMP) of *C. trachomatis* and they do not share sequence homology with *C. pneumoniae* MOMP[25].

MATERIALS AND METHODS

Patients. C. trachomatis triggered ReA group. The diagnosis of 23 patients with *C. trachomatis* triggered ReA (18 men, 5 women; mean age 28, range 16–48 yrs; duration of arthritis 7 weeks, range one week to 7 mo) (Table 1) was based on clinical examination by a rheumatologist and isolation of *C. trachomatis* (Patients 1–13) or detection of *C. trachomatis* antigen (Patients 14–23) from the urethral or cervical swab. Six of the patients were positive for HLA-B27 antigen, 5 were negative, and HLA-B27 status was not determined in 12 patients. The patients did not have significant circulating IgM, IgA, or IgG class antibodies against *Yersinia*, *Salmonella*, or *Campylobacter*.

Uroarthritis group. The 12 patients included in the uroarthritis group did not have laboratory confirmation of primary *C. trachomatis* infection (11 men, one woman; mean age 32, range 21–47 yrs; duration of arthritis 14 weeks, range one week – 16 mo) (Table 2). HLA-B27 was positive in 4, negative in 2, and not known in 6 of these patients. Four patients (Patients 1–4) had Reiter's disease and 7 had urethritis in addition to arthritis

Table 1. Clinical characteristics of patients with *C. trachomatis* triggered reactive arthritis.

Patient	Age	Sex	HLA-B27	Time from Onset of Arthritis	Urethral or Endocervical *C. trachomatis* Testing	*C. trachomatis*-specific Antibodies IgG	IgA
1	20	M	–	10 days	Culture +	+	+
2	25	M	+	1 wk	Culture +	+	+
3	34	M	+	6 wks	Culture +	+	+
4	42	M	+	3 wks	Culture +	+	+
5	23	M	+	2 mo	Culture +	+	+
6	37	FM	ND	5 wks	Culture +	+	+
7	21	M	ND	3 mo	Culture +	+	+
8	19	M	ND	2 wks	Culture +	–	–
9	37	FM	ND	5 wks	Culture +	+	+
10	21	M	ND	3 mo	Culture +	+	+
11	21	FM	ND	5 mo	Culture +	+	+
12	35	M	ND	7 mo	Culture +	+	+
13	41	FM	ND	4 wks	Culture +	+	–
14	28	M	+	3 wks	Antigen +	+	+
15	25	FM	–	4 wks	Antigen +	–	–
16	21	M	ND	3 mo	Antigen +	+	–
17	48	M	ND	3 wks	Antigen +	–	+
18	22	M	ND	4 wks	Antigen +	+	+
19	37	M	–	3 wks	Antigen +	–	–
20	16	M	ND	4 wks	Antigen +	+	+
21	21	M	+	6 wks	Antigen +	+	+
22	21	M	–	3 wks	Antigen +	+	+
23	31	M	–	5 wks	Antigen +	+	+

ND: not done.

Table 2. Clinical characteristics of patients with uroarthritis.

Patient	Age	Sex	HLA-B27	Time from Onset of Arthritis	Urethral or Endocervical *C. trachomatis* Testing	*C. trachomatis*-specific Antibodies IgG	IgA
1	31	M	–	1 mo	Culture –	+	–
2	40	M	+	2 wks	Culture –	+	+
3	41	M	ND	1 wk	Antigen –	+	+
4	43	M	ND	11 mo	ND	–	–
5	27	FM	ND	16 mo	ND	+	+
6	23	M	ND	6 wks	ND	+	+
7	30	M	ND	2 mo	ND	+	+
8	27	M	–	2 wks	Antigen –	–	–
9	21	M	+	1 mo	ND	+	–
10	47	M	+	3.5 mo	Culture –	+	–
11	25	M	+	10 wks	Culture –	+	+
12	29	M	ND	2 mo	ND	–	+

ND: not done.

(Patients 5–11). As in the previous group, these patients did not have circulating IgM, IgA, or IgG class antibodies against *Yersinia*, *Salmonella*, or *Campylobacter* suggestive of recent enteroarthritis.

Enteroarthritis group. Enteroarthritis in 56 patients (34 men, 22 women; mean age 36, range 16–64 yrs; duration of arthritis 3.4 weeks, range 2 days – 4 mo) was verified by stool culture and/or serologic analysis. Circulating antibodies against *Yersinia*, *Salmonella*, and *Campylobacter* were measured from all patients. The etiologic agent was isolated from stool culture from 28 patients (*Salmonella*, *Yersinia,* and *Clostridium difficile* in 20, 5, and 3 patients, respectively). Thirty patients had *Salmonella* triggered ReA. The triggering infection was *Yersinia* and *Campylobacter* in 9 and 3 patients, respectively. Five patients had antibiotic treatment related diarrhea preceding the arthritis. In 9 patients the microbial etiology of enteroarthritis remained unknown. HLA-B27 was positive in 36, negative in 3, and not known in 17 patients with enteroarthritis.

Oligoarthritis group. Sixteen patients had oligoarthritis (9 men, 7 women; mean age 49, range 19–88 yrs; duration of arthritis 5 mo, range 3 wks – 11 mo). Two of the patients were rheumatoid factor (RF) positive, 14 RF negative. They had no other rheumatic disease and their arthritis was not preceded by any known infection by history. These patients did not have significant antibodies against *Yersinia*, *Salmonella*, or *Campylobacter*.

Rheumatoid arthritis group (RA). All 25 patients with RA (7 men, 18 women; mean age 54, range 23–77 yrs; duration of disease 12 yrs, range 4 mo – 34 yrs) met American College of Rheumatology criteria for RA[26].

A total of 156 followup sera were studied from 5, 3, one, and 10 patients with *Chlamydia* triggered ReA, uroarthritis, oligoarthritis, and enteroarthritis, respectively.

The sera for this study were collected during 1987–96. All samples were coded and analyzed blindly for *C. trachomatis* IgA and IgG antibodies; the examiner was not aware of the patient data. The *C. trachomatis* serology results did not influence the diagnosis of the patients. From some patients *Chlamydia* serology had been initially performed by other methods, but these results were not used when the diagnosis of the patients was established for this study.

C. trachomatis enzyme immunoassay. The species-specific *C. trachomatis* indirect enzyme immunoassay (EIA; Labsystems, Helsinki, Finland) method was used as described[25]. Synthetic peptides deriving from the variable domain IV of 3 different *C. trachomatis* serovars (C, E, G) were used as antigen in the solid phase. As secondary antibodies anti-human-IgG or IgA-horseradish peroxidase conjugates were used. The results were expressed as optical density (OD) values at 450 nm obtained by a spectrophotometer. A specimen was interpreted to be positive if the signal/cutoff value (S/C) was > 1 or negative if S/C was < 1. A known positive serum sample was used as positive control. The cutoff value was defined as 0.3 × absorbance value of the positive control.

Statistical analysis. A sample was considered truly positive if it was from a patient in the *C. trachomatis* triggered ReA group and the S/C was > 1. A sample from a patient in the enteroarthritis group with S/C < 1 was considered truly negative. Thus assay sensitivity in diagnosis of *C. trachomatis* triggered ReA was calculated as true positive samples/all samples in the *C. trachomatis* triggered ReA group; and specificity as true negatives/all samples in the enteroarthritis group.

RESULTS

In patients with ReA triggered by *C. trachomatis*, specific IgA antibodies were observed in 18/23 (78%) and IgG antibodies in 19/23 (83%); 17/23 (74%) patients had anti-*Chlamydia* antibodies of both IgA and IgG isotypes. Three patients (13%) had neither IgA nor IgG anti-*Chlamydia* antibodies (Table 1). The microbiological diagnosis of one of these patients (Patient 8) was based on *Chlamydia* culture from the urethral swab; in 2 patients the diagnosis was based on detection of *Chlamydia* antigens from the urethral or endocervical swab (Patients 15 and 19). Clinically these patients did not differ from the patients with a positive anti-*Chlamydia* antibody result.

In the uroarthritis group *Chlamydia*-specific IgA and IgG antibodies were detected in 7/12 (58%) and 9/12 (75%) patients, respectively (Tables 2 and 3). In enteroarthritis, oligoarthritis, and RA, *C. trachomatis* IgA or IgG antibodies were detected in 15/56 (27%) and 12/56 (21%), 4/16 (25%) and 5/16 (31%), and 5/25 (20%) and 8/25 (32%) of patients, respectively (Table 3).

Anti-*Chlamydia* antibodies of both isotypes were concurrently detectable in 17/23 (74%), 6/12 (50%), 9/56 (16%), 4/16 (25%), and 3/25 (12%) of the patients with *C. trachomatis* triggered ReA, uroarthritis, enteroarthritis, oligoarthritis, and RA, respectively (Table 3). In the 13 patients (Table 1, Patients 1–13) from whom *C. trachomatis* had been isolated from the endocervix or urethra, specific IgA and IgG antibodies were detected in 11/13 (85%) and

Table 3. C. trachomatis serum antibody prevalence in the study groups.

Group	Total	Female/Male (%)	Age, mean (range)	Duration of Arthritis, mean (range)	Serum Antibodies to *C. trachomatis* (%) IgA	IgG	IgA and IgG
C. trachomatis triggered ReA	23	5/18 (22)	28 (16–48)	1.8 mo (1 wk–7 mo)	78	83	74
Uroarthritis	12	1/11 (8)	32 (21–47)	3.5 mo (1 wk–16 mo)	58	75	50
Enteroarthritis	56	22/34 (39)	36 (16–64)	0.9 mo (2 days–4 mo)	27	21	16
Oligoarthritis	16	7/9 (44)	49 (19–88)	5.0 mo (3 wks–11 mo)	25	31	25
RA	25	18/7 (72)	54 (23–77)	12 yrs (4 mo–34 yrs)	20	32	12

12/13 (92%), respectively. In this group antibodies of both isotypes were detected in 11/13 (85%).

The S/C values of *C. trachomatis* IgA and IgG antibodies measured from the first serum sample obtained from the patients with arthritis are illustrated in Figure 1. The median values of 3.1/8.1 (IgA/IgG) for *C. trachomatis* triggered ReA and 1.6/3.5 (IgA/IgG) for uroarthritis are significantly higher than for the enteroarthritis 0.52/0.40 (IgA/IgG), oligoarthritis 0.39/0.47 (IgA/IgG), and RA 0.43/0.41 (IgA/IgG) groups.

Based on presence of both isotypes the sensitivity of the assay in diagnosis of *C. trachomatis* triggered ReA is 74% (17/23) and specificity 84% (47/56). Similarly, if patient samples with detectable antibodies of only one (IgA or IgG)

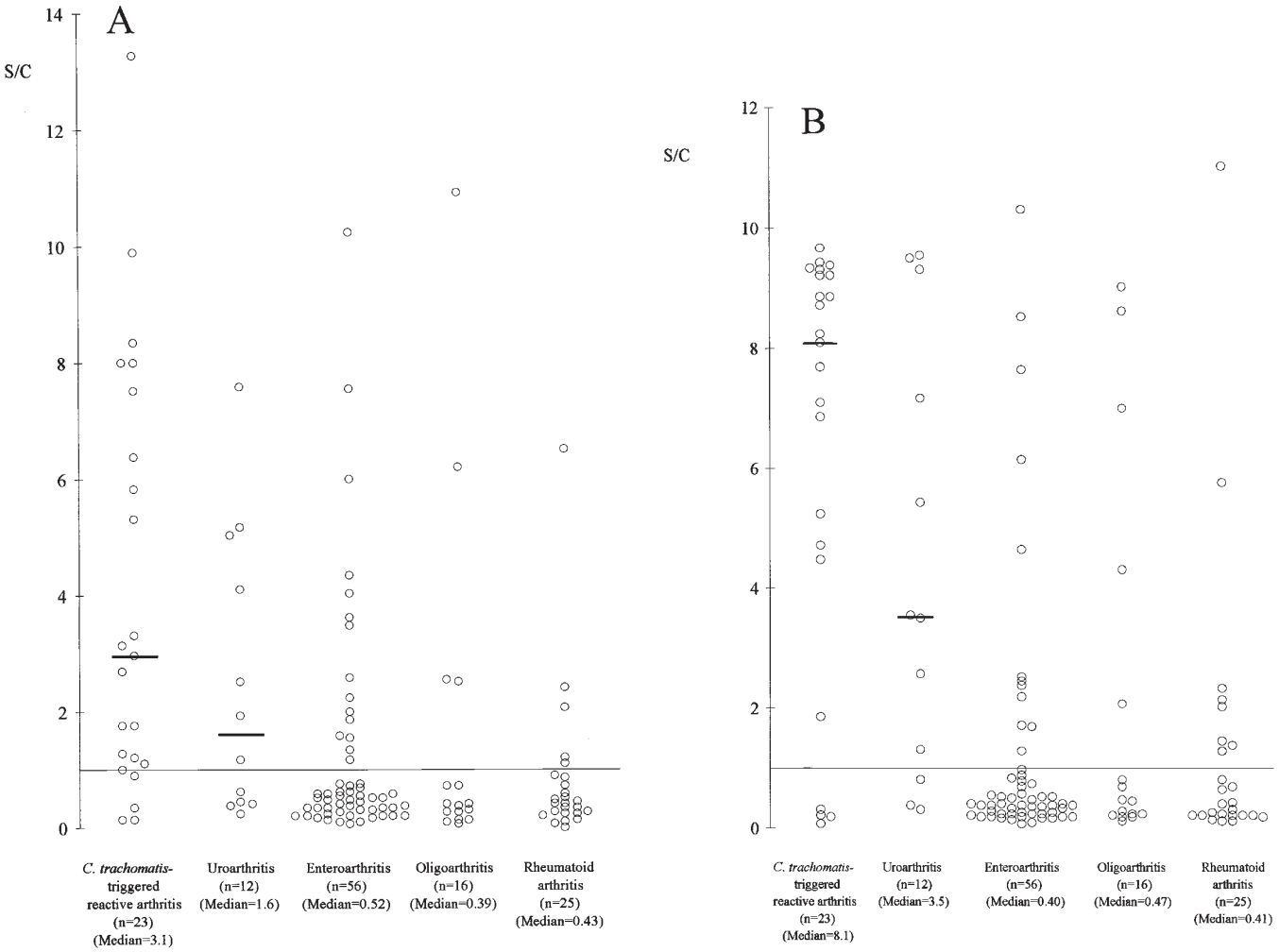

Figure 1. Signal/cutoff (S/C) values of *C. trachomatis* IgA (A) and IgG (B) antibodies measured from the first available serum sample from patients with arthritis. Median values are marked with a horizontal line for the *C. trachomatis* triggered ReA and uroarthritis groups. In the other groups this value was below the cutoff level. The S/C cutoff value for a positive result is 1.

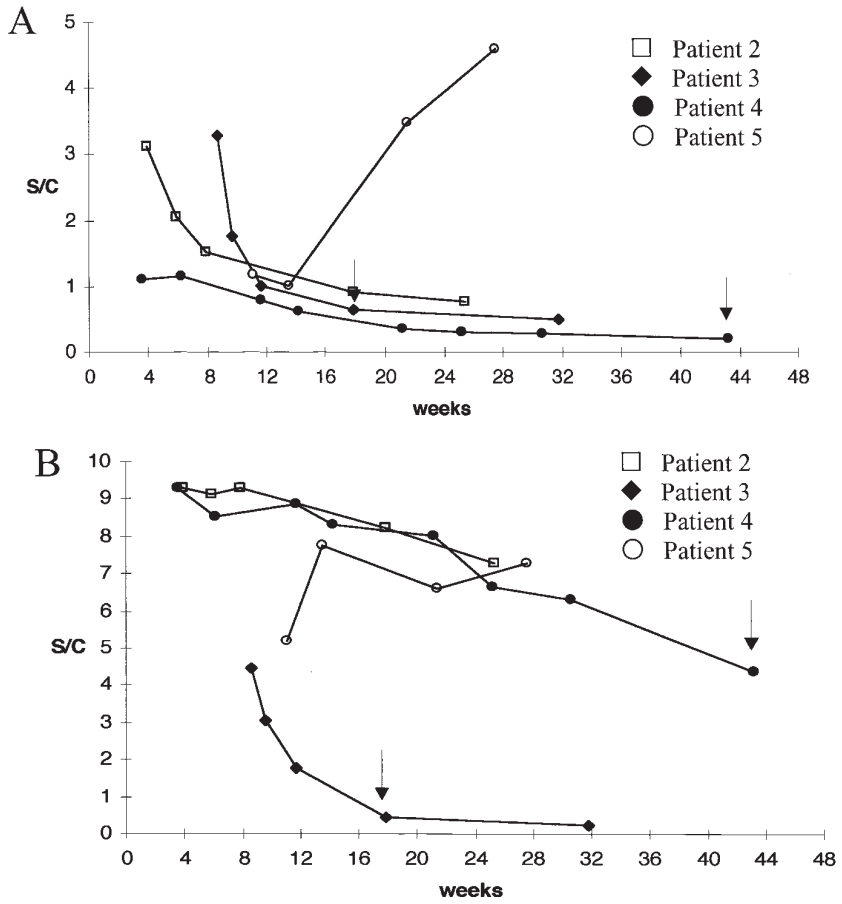

Figure 2. C. trachomatis-specific IgA (A) and IgG (B) antibodies in followup samples, starting from the onset of primary infection, from 4 patients with *C. trachomatis* triggered ReA. Patient numbers refer to Table 1. Clinical remission is marked with an arrow. In Patients 3 and 4 remission of arthritis occurred at Weeks 18 and 43, respectively (arrows). Patient 2 was lost to followup after Week 25. Patient 5 had persistent arthritis for at least 4 months after the last serum sample studied at Week 27. After this he was lost to followup.

isotype are also considered positive the sensitivity is higher (87%, 20/23), but the specificity decreases to 68% (38/56).

The results from the followup sera of 4 patients with culture positive *C. trachomatis* ReA are shown in Figure 2. In one of these patients (Patient 5) an increase in *C. trachomatis* antibody S/C values of both IgA and IgG isotypes was seen. This patient had prolonged arthritis during the followup period. He, as well as Patients 2, 3, and 4 in Figure 2, had received a short 2 week oral lymecycline treatment in the beginning of the study period. From these results it seems that in patients with culture positive *C. trachomatis* triggered ReA a decline in antibody concentrations is associated with remission of arthritis (Figure 2).

DISCUSSION

Specific serological assays developed for the detection of *Salmonella* and *Yersinia* have considerably improved the diagnosis of enteroarthritides. In contrast, serological diagnosis of *C. trachomatis* triggered ReA has been considered of limited value[27-29]. We used an EIA method based on synthetic peptides specific for *C. trachomatis* to evaluate its usefulness in diagnosis of ReA triggered by *C. trachomatis*[25].

We found that a significantly high number of ReA patients with microbiologically proven *C. trachomatis* primary infection had circulating antibodies compared to the controls with other arthritides. Antibodies of both IgA and IgG isotypes were detected in 74% of patients with *C. trachomatis* triggered ReA and in 50%, 16%, 25%, and 12% of patients with uroarthritis, enteroarthritis, oligoarthritis without preceding history of infection, and RA, respectively. In the 13 patients with culture positive *C. trachomatis* triggered ReA, antibodies of both isotypes were detectable in 85%.

The microbiological diagnosis of *C. trachomatis* triggered ReA is based on the detection of *C. trachomatis* at the site of the primary infection. The "gold standard" for diagnosis of *C. trachomatis* infections has been *Chlamydia* culture[30]. However, the sensitivity of *C. trachomatis* culture has been estimated to be only 70–80% for women with

cervical infection or even less. This has led to the suspicion of inapparent (i.e., nonculturable) chlamydial infections in humans[31] and the need for better diagnostic tools in differential diagnosis of *C. trachomatis* triggered ReA.

In uncomplicated superficial *C. trachomatis* infections, antibodies may not develop, particularly in men[18]. On the other hand, most (87%) of our patients with *Chlamydia* triggered ReA did develop a specific seroresponse, although in 3 patients the response remained undetectable. The peptide assay may be unable to diagnose antibodies against all strains of *C. trachomatis* or those patients may have developed antibodies to chlamydial epitopes not detectable by this assay. Indeed, it has been suggested that different serovars of *C. trachomatis* may contribute to the development of ReA[32]. Therefore, according to our data, a negative seroresponse to *C. trachomatis* does not definitely rule out ReA triggered by this organism, as has been reported[33]. In that study, the authors evaluated the usefulness of *C. trachomatis* antibody determination from serum and synovial fluid in diagnosis of ReA by using 2 commercial EIA preparations. They reported a sensitivity of 77% and specificity of 80% when synovial fluid IgG antibodies were analyzed with the same peptide based MOMP-EIA as used in our study. Based on their comparative study, Bas and Vischer considered the peptide based EIA for IgG antibodies from synovial fluid as clinically the most appropriate for diagnosis of probable *C. trachomatis* triggered ReA[33].

To our knowledge, *C. trachomatis* antibodies have not been previously measured in consecutive serum samples from patients with arthritis. Although our data are limited, it seems that in patients with culture positive *C. trachomatis* triggered ReA a decline in antibody concentrations is associated with remission of arthritis (Figure 2). However, in the other followup samples IgG as well as IgA antibodies could persist for several months after antibiotic treatment, and despite clinical remission (data not shown). The persistence of antibodies detected by this methodology may partially explain the high prevalence (20–32%) of IgA or IgG antibodies in the other patient groups with no other clinical or laboratory evidence of recent *C. trachomatis* infection (Table 3). The slightly higher concurrent prevalence of both isotypes in the oligoarthritis group may be explained by clinically silent *C. trachomatis* infections and lack of efficient primary microbiological diagnostics — *C. trachomatis* is notorious for causing asymptomatic infections in women. Of note is the higher percentage (44%) of women in the oligoarthritis group compared to the striking male predominance in the *C. trachomatis* triggered ReA (22% female) and uroarthritis groups (8% female).

Detection of *C. trachomatis* at the site of primary infection is still the method of choice in laboratory diagnosis of uroarthritis, particularly as new noninvasive methods based on molecular amplification have become widely available. Our serological results are in accord with studies suggesting that in patients with complicated *C. trachomatis* infections, such as those with ReA, high titers of *Chlamydia* antibodies in a single serum sample are strongly suggestive for diagnosis (Figure 1)[28,30]. In routine serology to diagnose primary infection, testing of paired sera is recommended. However, in *C. trachomatis* triggered ReA the primary infection is usually at such a late phase that a diagnostic rise in antibody titers is seldom seen. In our study, a diagnostic rise in antibody concentrations was not seen in any of the 8 followup patients with suspected *C. trachomatis* infection. Thus, analysis of both IgA and IgG isotypes from one serum sample in a patient with ReA seems to be sufficient for the differential diagnosis, with a sensitivity and specificity of about 80%. Of note is that in one study *C. trachomatis* antibodies were detected in less than 2% of children under 15 years with this assay, despite abundant presence of antibodies to *C. pneumoniae*[25]. Therefore, it seems that decrease in specificity of this assay in differential diagnosis of *C. trachomatis* triggered ReA is caused by a high incidence of primary or uncomplicated *C. trachomatis* infections in our control group, and in the adult population in general.

According to our data, *C. trachomatis*-specific EIA is a valuable method in the diagnosis of ReA triggered by *C. trachomatis*. This may particularly hold true if specimens from urogenital tract cannot be tested reliably (e.g., due to recent antibiotic treatment). Further, our results suggest that in a subgroup of patients with culture positive *C. trachomatis* triggered ReA declining antibody levels predict clinical remission. This interesting finding needs to be confirmed with a larger patient population and by using modern noninvasive detection methods for diagnosis of primary *C. trachomatis* infection.

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