

## CHLAMYDIA ANTIBODIES IN RHEUMATIC PATIENTS

Sofia Constantiniu<sup>1</sup>, Angela Romaniuc<sup>1</sup>, Cecilia Durnea<sup>1</sup>, Elena Rezuș<sup>2</sup>  
Rodica Chiriac<sup>2</sup>, C.Berea<sup>2</sup>

1. Institute of Public Health Iasi
2. Rehabilitation Clinical Hospital

**Abstract.** 93 patients with recent inflammatory joint disease were investigated for the presence of anti- *Chlamydia* IgM antibodies. The specific antibodies have been found in 17 patients (18.2%) by ELISA *Chlamydia* TRUE- IgM commercial kit. 58.8% of positive patients had clinical diagnosis of reactive arthritis; 29.4% of ankylosing spondylitis and 5.9% of sacroiliitis and Reiter's syndrome too. Serological investigation of patients with arthritis appeared as necessary for a better understanding the germ etiology of seronegative arthritis.

**Key words:** reactive arthritis, ankylosing spondilitis, IgM antibodies, *Chlamydia*, ELISA

**Rezumat.** 93 seruri de la pacienți cu afecțiuni reumatismale recente au fost investigate pentru prezența anticorpilor IgM anti-*Chlamydia*. Utilizând kituri ELISA *Chlamydia* TRUE-IgM, la 17 pacienți (18,2%) au fost evidențiați anticorpi IgM anti- *Chlamydia*. Formele clinice ale cazurilor confirmate au fost: artrită reactivă (58,8%), spondilită anchilozantă (29,4%), sacroileită și sindrom Reiter câte 5,9%. Se subliniază importanța examenului serologic pentru stabilirea agenților responsabili în declanșarea artritelor reactive.

**Cuvinte cheie:** artrită reactivă, spondilită anchilozantă, anticorpi IgM, *Chlamydia*, ELISA

### INTRODUCTION

The original definition of reactive arthritis as a sterile joint inflammation following an infection elsewhere in the body was changed ten years ago when *Chlamydia* antigens and lipopolysaccharide (LPS) of *Yersinia enterocolitica* O:3 were demonstrated in the synovial fluid cells of patients with *Chlamydia* and *Yersinia* triggered reactive arthritis (1-5).

The clinical picture of reactive arthritis varies from mild arthralgia to a severe invaliding disease affecting different organs (2,4-6).

Usually, reactive arthritis is an acute complication after certain infections of both gastrointestinal and urogenital tract. But, reactive arthritis is not always

associated with clear gastrointestinal or urogenital symptoms. In most cases at the time of arthritic complications stool and samples collected for *Chlamydia* are negative and the diagnosis of the triggering infection must rely on serological results only.

The bacteria known to trigger reactive arthritis as well as many other organisms that have been postulated as potentially associated are:

- established association:
  - enteric: *Shigella* sp., *Salmonella* sp., *Campylobacter* sp., *Yersinia* sp., *Clostridium difficile*
  - urogenital: *Chlamydia trachomatis*
- no established association:
  - urogenital: *Neisseria gonorrhoea*, *Ureaplasma ureolyticum*

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- other: *Brucella* sp., *Borrelia burgdorferi*, *Leptospira* sp., *Mycobacterium* sp., *Streptococcus* sp., *Staphylococcus* sp., *C.psittaci* (1-4,6,7-9).

Although, microbial antigens or intact pathogens are important for the pathogenesis of reactive arthritis their role into development of polyarthritis has remained unsolved (1,8-9).

*Chlamydia trachomatis* is the only sexually transmitted infection that clearly triggers reactive arthritis. Convincing “proof” of causation is the discovery of synovial chlamydial antigens and genetic material by immunoperoxidase, direct immunofluorescent and polymerase chain reaction-PCR techniques. There is also evidence that *Ureaplasma ureolyticum*, another common agent in non-gonococcal urethritis, cervicitis, may induce reactive arthritis (4,10).

In order to evaluate the presence of specific antibodies against *Chlamydia*, a group of patients with a recent inflammatory joint disease was investigated.

#### PATIENTS AND METHODS

93 patients have been admitted, during 2000-2001, in the Rheumatology Department of Iasi Recovery Districtal Hospital for a recent inflammatory joint condition: 64 patients (68.9%) with reactive arthritis; 15 patients (16.1%) with ankylosing spondylitis; 8 patients (8.6%) with Reiter’s syndrome; 3 patients (3.2%) with sacroilitis and 3 subjects (3.2%) with collagen disease and intervertebral disc protrusion.

The presence of anti- *Chlamydia* antibodies was detected by “Sero

ELISA™ *Chlamydia* TRUE-IgM™ commercial kit (Savyon Diagnostics LTD). The technical procedure followed the general one required by the producer. The determination was done in the Automatic Microplate Analyzer produced by Biochemical Immunossystems SPA Italy. A test – run was valid if positive control absorbance was > 0.8 and negative control absorbance was < 0.15 , both at 450 nm.

The cut-off value (COV) was calculated according to the formula:

$$\text{COV} = 0.24 \times (\text{Pc} - \text{Nc}) + \text{Nc}$$

Pc = absorbance of positive control at 450nm

Nc = absorbance of negative control at 450nm

Above COV + 0.03 = positive for anti-*Chlamydia* IgM antibody. The producer positive sera do not need to be titrated and the results can be reported in terms of presence or absence of immune IgM antibodies.

Sero ELISA™ *Chlamydia* test employes the L<sub>2</sub> serovar broadly reacting antigen of *Chlamydia trachomatis*. It will detect *C. trachomatis*, *C. psittaci* and *C. pneumoniae* antibodies.

#### RESULTS AND DISCUSSION

Clinical forms of the investigated patients are presented in figure 1.

17 (18.2%) out of the 93 tested patients presented anti -*Chlamydia* IgM antibodies as table 1 data shows.

From all positive cases 58.8% had clinical diagnosis of reactive arthritis, 29.4% of ankylosing spondylitis and 5.9% of sacroilitis and Reiter’s syndrome respectively (figure 2).

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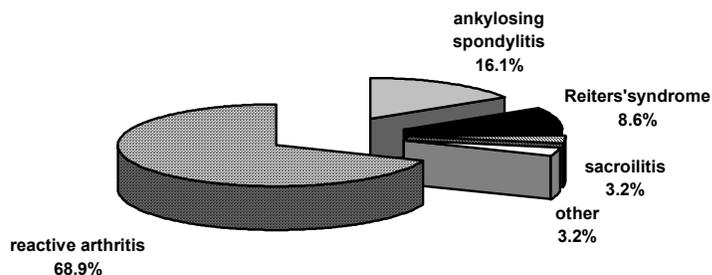


Fig. 1 Patients' distribution by rheumatic diseases

Table 1. Distribution by clinical forms, age and sex of positive serology for *Chlamydia* cases

Age of group	Total investigations	Total positive	Sex		Reiter's syndrome		reactive arthritis		ankylosing spondylitis		Sacroiliitis	
			M	F	M	F	M	F	M	F	M	F
years												
20-29	25	2	2	0	1	0	0	0	1	0	0	0
30-39	23	4	1	3	0	0	1	0	0	2	0	1
40-49	18	9	6	3	0	0	6	1	0	2	0	0
50-59	10	2	1	1	0	0	1	1	0	0	0	0
60-69	7	0	0	0	0	0	0	0	0	0	0	0
<b>Total</b>	<b>93</b>	<b>17</b>	<b>10</b>	<b>7</b>	<b>1</b>	<b>0</b>	<b>8</b>	<b>2</b>	<b>1</b>	<b>4</b>	<b>0</b>	<b>1</b>

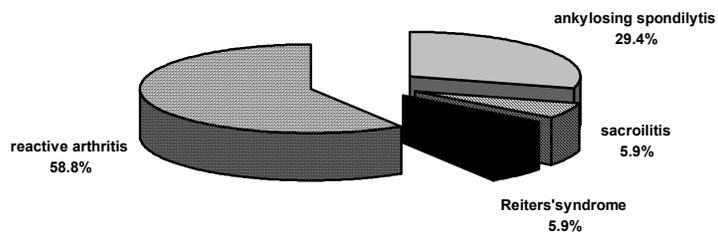
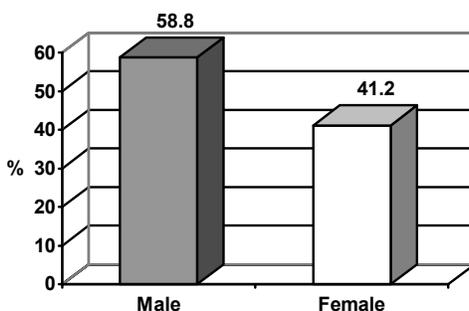


Fig. 2 The distribution of patients with positive serology by rheumatic disease

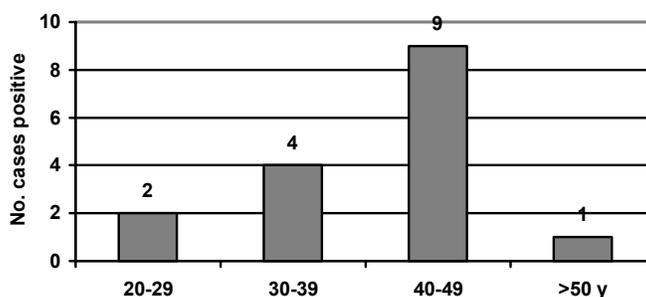
More than half of positive patients were males (58.8%) as figure 3 shows. Generally enteric reactive arthritis is equally distributed between the sexes but sexually acquired reactive arthritis

is reported 20 times more common in males than females (4,10).

The age of the positive patients was of 22-55 years. 76.4% of cases were middle-aged people (30-49)–figure 4.



**Fig. 3 Sex distribution of patients with positive serology by rheumatic disease**



**Fig. 4 Distribution of positive serology cases by age groups**

Reactive arthritis by *Chlamydia* - triggered was more frequently in males ( $p < 0.005$ ) of 40-49 age group (6/8). Ankylosing spondilitis was more frequently in females of 30-49 years of age ( $p < 0.05$ ).

Our data represents a serological survey of the chlamydial antibodies in a population's group. There are few data concerning the presence of

*Chlamydia* antibodies in the different categories of Romanian population. Botez et al. pointed out that the cases with Reiter's syndrome were confirmed by isolation of the *Chlamydia* and by positive serological tests (11).

Reactive arthritis has only a clinical diagnosis; there are no specific diagnosis laboratory tests. However, detecting a current or prior infection

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with specific causative organisms is a strong supportive evidence for the disease. Reactive arthritis usually develops 2–4 weeks after a genitourinary or gastrointestinal infection. Stool cultures may reveal infection with one of the causative enteric organisms even in a patient without bowel symptoms. Lozada et al. (4) pointed out that about 10% of patients do not have a previous symptomatic infection. Whereas *Chlamydia* can be demonstrated in urogenital specimens in at least one-third of patients with *Chlamydia* induced acute reactive arthritis, the triggering bacteria are usually no longer detectable in post-enteric reactive arthritis (4).

Since culturing *Chlamydia* is difficult in a routine clinical setting, non-culture techniques are more readily used. Cervical and urethral swabs analysis by direct fluorescent antibody and enzyme immunoassay provide 90% sensitivity and 98 % specificity for *Chlamydia* infection (4,5,12). Newer DNA probes to *Chlamydia* major outer membrane protein (MOMP) gene are highly sensitive and specific (5,10,12). Recent infection with *Chlamydia*, *Salmonella*, *Shigella*, *Yersinia* and *Campylobacter* can be confirmed by positive organism specific antibody serology's ( positive IgM, fourfold rise in IgG titer, or IgG titer greater than 128) (10,12).

The most widely used serological test for diagnosis of chlamydial infection was the complement fixation (CF) test. The genus – specific and relatively insensitive CF test is not particularly useful in diagnosis of trachoma, inclusion conjunctivitis or the related

genital tract infections and it plays no role in diagnosis of neonatal chlamydial infections.

Puolakkainen M., made a study about clinical findings and diagnoses of 242 patients with diagnosis *Chlamydia* CF titers. The joint symptoms, mainly in the form of arthralgias and reactive arthritis, were recorded in 31 (13%) cases of which 19 were female and 12 male with 65% of these patients being under 40 years of age (13).

The micro – immunofluorescence ( IF) method is a much more sensitive procedure for measuring anti-chlamydial antibodies. Trachoma, inclusion conjunctivitis and genital tract infection may be diagnosed by micro-IF techniques of appropriately timed paired acute- and convalescent-phase sera can be obtained. However, it is often difficult to demonstrate rising antibody titers, particularly in sexually active people. Many of these individuals will be seen for chronic or repeat infections.

EIA techniques that measure anti-chlamydial antibodies have been described. The procedure may be of some use in selected instances and for serosurveys in laboratories where micro-IF techniques are not available (11,12).

About the serological tests in *Chlamydia* induced reactive arthritis Bas S. et al.- cited by Khan A.M. (7) showed that these patients had a pattern of reactivity that is compatible with infection by several serotypes to this bacterium might be involved in the development of reactive arthritis. She has also investigated whether determination of serum or synovial fluid IgG and IgA anti-*Chlamydia*

antibodies could be clinically helpful in detecting possible *Chlamydia trachomatis* infection. She found that the synovial fluid IgG antibody against the major outer membrane protein (MOMP) was the most appropriate determination with a sensitivity and a specificity equal to or close to 80%.

Clinical experience of some authors and recent studies indicate that IgM antibodies to *Chlamydia* may serve as a marker for acute and /or recent *Chlamydia* infection (4,7,10,14,15).

The mechanism of the interaction of the inciting organism with the host (of the HLA-B27 positive) leading to the development of reactive arthritis is not known. Synovial fluid cultures are negative for enteric organisms or *Chlamydia* species. However, a systemic and intrasynovial immune response to the organisms had been found with intra-articular antibody and bacterial reactive T cells. Furthermore, bacterial antigen has been found in the joints. Thus the elements exist for an immune-mediated synovitis.

Molecular evidence of bacterial DNA (using PCR) in synovial fluids has been found in *Chlamydia* -related reactive arthritis and one placebo controlled trial of a tetracycline derivative (Lymecycline) showed a reduction in the duration of acute *Chlamydia*-related but not enteric-related reactive arthritis. This suggests the persistent infection may play a role, at least in some cases of *Chlamydia* reactive arthritis (2,5).

The demonstration of chlamydial DNA and RNA in inflamed joints indicate that, at least in the early phase of *Chlamydia trachomatis* infection,

intact microorganisms have persist in dormant, unculturable form (1,2,4,15).

The antigens of the triggering infectious agents found in inflamed joints include processed forms of LPS of *Yersinia*, *Salmonella*, *Shigella* bacteria and outer membrane protein of *Chlamydia trachomatis*, YdA protein of *Yersinia enterocolitica* and 61 kD heat shock protein of *Yersinia* bacteria. These antigens are potential modulators of the immune system (1,3).

It has been speculated that the microbes or their components may enter the joints via blood vessels either as whole organisms within the cells or as a part of immune complex. It has been suggested that only in those patients who develop reactive arthritis do these antigens gain access to the joints (1,3,4).

By new sophisticated techniques, studies of humoral immune responses and studies demonstrating microbial antigens in the joints in patients with reactive arthritis triggered by various microbes will be undertaken. This will inevitably enhance the understanding of the pathogenesis of reactive arthritis.

#### CONCLUSIONS

- Anti-*Chlamydia* IgM antibodies have been detected in 18.2% of rheumatic patients.
- Clinical diagnosis of these cases was of reactive arthritis in 58.8%, ankylosing spondylitis in 29.4% and of 5.9% of sacroilitis and Reiter's syndrome too.
- The age of the patients was of 22-55 years. 76.4% of the cases were middle-aged people (30-49).

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- More than half of patients positive for anti-*Chlamydia* IgM antibodies were males (58.8%).

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