INTRODUCTION

Campylobacter jejuni (C. jejuni), and Campylobacter coli (C. coli), thermophilic campylobacters, represent the main cause of bacterial diarrhea in developed countries (1) and one of the most frequent causes of enterocolitis in developing countries (2) especially in early childhood (3). Clinical manifestations of enterocolitis caused by Campylobacter species are diarrhea, fever, abdominal pain, and in some patients, fecal blood (4). Symptom severity differs in developing and developed countries. C. jejuni is the most often isolated agent among thermophilic Campylobacters (2). After C. jejuni infection, severe chronic squeals may occur, such as reactive arthritis (5), postinfective neuropathy, Guillain-Barré syndrome (GBS) and Miller-Fisher Syndrome (MFS).

SUMMARY

The aim of the paper was to investigate the occurrence of musculoskeletal disorders in patients following enterocolitis caused by C. jejuni and the role of C. jejuni in triggering the seronegative spondyloarthropathies detecting specific antibodies in patient sera. We investigated 146 patients with C. jejuni diarrhea and 30 patients with seronegative spondyloarthropathies. Stool samples were submitted to the Center for Microbiology at the Public Health Institute for C. jejuni detection. In each patient with seronegative spondyloarthropathies, one serum sample was examined for antibodies against C. jejuni using complement fixation test. Rheumatic manifestations seen in 146 (11%) patients with enterocolitis were arthralgia, myalgia, enthesitis and reactive arthritis. The cases of reactive arthritis caused by C. jejuni are the first reported cases in Serbia and Montenegro. In the group of patients (30 pts) with seronegative spondyloarthropathies, (Reiter’s syndrome, Morbus Bechterew, enteropathic arthritis, psoriatic arthritis, reactive arthritis, oligoarthritis) antibodies against Campylobacter jejuni were noticed in 26.7%. There were statistically significant differences between the presence of antibodies in the group of patients and the control group (p<0.05) No C. jejuni were discovered in the stools of these patients. In conclusion, based on the data obtained, C. jejuni may be an important factor in triggering the reactive arthritis, but for the detection of its role in other seronegative spondyloarthropathies, long-term follow-up studies of patients with C. jejuni enterocolitis are necessary. The prevention of infective disease can forestall chronic sequels, too.

Key words: Campylobacter jejuni, postinfective sequels, musculoskeletal system
Seronegative spondyloarthropathies comprise ankylosing spondylitis (AS), reactive arthritis/Reiter syndrome, spondyloarthritis associated with psoriasis and chronic inflammatory bowel diseases, and a form of juvenile chronic arthritis (pauciarticular, late onset type). All forms of spondyloarthropathies are associated with the histocompatibility antigen HLA-B27, although the strength of this association varies markedly not only among the various disease forms but also among the various ethnic and racial groups worldwide (6). These diseases tend to occur more often among males who are in their late teens and early twenties, and may start with features such as enthesitis (inflammatory lesions of the entheses, i.e. sites of ligamentous or tendinous attachment to bone), dactylitis or oligoarthritis, and in some cases may progress to sacroiliitis and spondylitis, with or without extra-articular features such as acute anterior uveitis or muco-cutaneous lesions. On the other hand, it is considered that among other bacteria, Campylobacter jejuni plays a role in the pathogenesis of seronegative spondyloarthropathies (7).

Reactive arthritis still remains not quite explained, but determined with certain HLA types: HLA-B27 (5).

We have evaluated the prevalence musculoskeletal manifestations (ReA) of C.jejuni triggered by postinfective sequels in patients with C.jejuni diarrhea, and patients with seronegative spondyloarthropathies.

**MATERIAL AND METHODS**

Two groups of patients were investigated: 146 patients with C.jejuni diarrhea, 30 patients with seronegative spondyloarthropathies. In addition, one control group for serological investigation was included: 30 sex- and age-matched healthy subjects for the group of patients with seronegative spondyloarthropathies. Control groups have been selected during a systematic check-up.

Stool samples obtained from either outpatient or hospitalized patients with diarrhea were submitted to the Center for Microbiology at the Public Health Institute for C.jejuni detection. Data about clinical characteristics of disease were obtained by questionnaire and patients were also interviewed personally or by telephone. Patients who complained about joint symptoms were directed to rheumatologist.

Patients with seronegative spondyloarthropathies were selected and rheumatologic investigations were conducted at the Institute of Rheumatology and Cardiology, “Niska Banja”.

In patients with seronegative spondyloarthropathies, single stool sample was investigated for the presence of C.jejuni and one serum sample for antibodies against C.jejuni.

For the detection of thermophilic Campylobacters the Columbia agar base was supplemented with 5% sheep blood and antibiotics (cefoperazon, 1.5g/L, colistin 10³ U, vancomycin 1g/L, amphotericin B 0.2g/L), (bioMérieux, Marcy l’Etoile, France). Inoculated plates were incubated at 42°C for 48 hours in a microaerophilic atmosphere (gas generating system “Torlak”, Belgrade, RS).

Colonies of Campylobacter were presumptively identified by staining (carbolufxin 1%), S- and spiral shaped bacteria having a “gull wing” morphology were observed. Colonies also tested positive for oxidase and catalase production. Strains presumptively identified as Campylobacters were differentiated to the species level by the API Campy (bioMérieux, Marcy l’Etoile, France).

Data about clinical characteristics of disease were obtained by questionnaire and patients were also interviewed personally or by telephone.

CFT was performed in 96 microtitration wells plate using the C.jejuni antigen, hemolytic system and complement (Serion Diagnostica, Wurtzburg, Germany) and serum dilution from 1:8 to 1:128. Positive results were considered to be 1:16 and higher dilutions.

Statistical analysis was performed using the Fisher’s exact test and program for statistical analysis, EpiInfo 6.1.

**RESULTS**

In patients with diarrhea, C.jejuni infection were proven in 211 out of 14264 stool cultures (1.48%). Out of 211 patients, 146 gave data related to the illness. Postinfection sequels included joint affections in 16 (11%) patients: 9 females and 7 male patients, with mean age of 29.55 ±SD24.06 years, and 18.14 ±SD14.96 years, respectively. The mean age for that group was 24.56± SD 25.56 years.

Musculoskeletal disorders were represented as arthralgia, myalgia, enthesopathy and arthritis reactiva (Table 1).

<table>
<thead>
<tr>
<th>Musculoskeletal manifestations</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthralgia</td>
<td>13 (8.9)</td>
</tr>
<tr>
<td>Arthritis reactiva</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Enthesopathy</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>No manifestations</td>
<td>130 (89)</td>
</tr>
<tr>
<td>Total</td>
<td>146 (100)</td>
</tr>
</tbody>
</table>
In three patients, arthralgia was seen in a radiocarpal joint; in three patients carpometacarpal joints were affected, one of which was associated with myalgia; in two patients arthralgia occurred in spinal joints, in one patient arthralgia was associated with sternocostal joints and with myalgia and body weight loss; in one patient arthralgia was associated with elbow joint, in one patient with the hip joint; in one patient, arthralgia was associated with sternocostal joints and with myalgia and weight loss; one patient had a knee joint affected. In one patient, arthralgia was seen in the ankle joint and carpometacarpal joints. Arthritis reactiva associated with restricted movements in involved proximal interphalangeal joints was seen in a patient with underlying connective tissue disease, scleroderma, and in one patient with suspected connective tissue disease. To our knowledge, the cases of reactive arthritis caused by C. jejuni are the first reported in Serbia. The patient suffering from entesopathy had involvement with the Achilles' tendons.

Of 30 patients with seronegative spondyloarthropathies, there were 19 males (mean age 38.47 ± SD 16.65) and 11 females (mean age 29.82 ± SD 10.22) with the mean age value for the group of 35.3 ± SD 15.0. The most frequent clinical diagnosis was arthritis reactiva (Table 1).

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C. jejuni was not discovered in any stool sample of these patients. Antibodies against C. jejuni were proven in eight patients with arthritis reactiva, oligoarthritis, Morbus Bechterew and spondyloarthropathies (Table 2).

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Number of patients (%)</th>
<th>Number of positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis reactiva</td>
<td>8 (26.7)</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Reiter's syndrome</td>
<td>5 (16.7)</td>
<td>0</td>
</tr>
<tr>
<td>Spondyloarthropathies</td>
<td>6 (20)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Morbus Bechterew</td>
<td>2 (6.7)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Arthritis psoriatica</td>
<td>4 (13.3)</td>
<td>0</td>
</tr>
<tr>
<td>Oligoarthritis</td>
<td>5 (16.7)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>Total</td>
<td>30 (100)</td>
<td>8 (26.7)</td>
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</tbody>
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In the control group, antibodies against C. jejuni were detected in one serum sample. Fisher’s exact test revealed significant statistical difference between patients and control group (p=0.026; p<0.05).

DISCUSSION

C. jejuni may trigger two types of post-infective sequels: neurological, whose manifestations are GBS, and musculoskeletal, which can be classified as arthritis reactiva (ReA) or tendonitis, enthesopathy and bursitis.

Musculoskeletal disorders associated with C. jejuni diarrhea have been described in early studies (8). Despite that fact, their pathogenesis still remains insufficiently understood. In the pathogenesis of a similar disease, caused by other enteric bacteria (Yersinia enterocolitica), the host and bacterial factors are important. In this study, musculoskeletal disorders were recorded in 11% of patients with diarrhea caused by C. jejuni: arthralgia in 8.9%, ReA and myalgia in 1.4%, enthesopathy in 0.7% of patients. Hannu et al. (9) have proven musculoskeletal disorders in 9% of patients with C. jejuni coli diarrhea: in 8% ReA, and in 1% tendonitis, enthesopathy or bursitis. According to Locht and Krogefert, 16% of patients with C. jejuni/C. coli diarrhea developed musculoskeletal disorders (10). In the outbreak caused by thermophilic campylobacters, joint affection occurred in 21% of patients (11). According to results of Skirrow and Blaser (1), 1% of patients with C. jejuni diarrhea suffer from ReA.

In a long-term follow-up study of patients with musculoskeletal disorders after an outbreak of C. jejuni diarrhea, in 4/9 patients rheumatic disorder persisted after five and a half years, and in one patient, the relapse of reactive arthritis occurred after seven years (12).

In this study, C. jejuni is possibly related to seronegative spondyloarthropathies in 26.7% patients. Most of them (4 pts) had the diagnosis of reactive arthritis. Locht et al. (13) detected C. jejuni as a triggering bacterium in 6/22 patients with ReA. In the study involving patients from Papua New Guinea, of 23 patients with oligoarthritis, antibodies against C. jejuni/C. coli were found in the sera of two patients as the proof of recent preceding infection (14). One research revealed C. jejuni DNA in the synovial fluid in two of 12 patients with oligoarthritis (15). Investigation on early arthritis in southern Sweden revealed the Campylobacter as preceding bacteria in the majority of cases of arthritis reactiva (16). In addition, our study revealed the presence of antibodies against C. jejuni in the patients with
arthritis reactiva, whereas these antibodies were rarely found in patients with other diagnoses. Despite the presented data, there are studies which have not proved the role of *C. jejuni* in etiology of seronegative spondyloarthropathies (17, 18).

### CONCLUSION

In conclusion, based on the data obtained, *C. jejuni* may be an important factor in triggering the reactive arthritis, but for the detection of its role in other seronegative spondyloarthropathies, long-term follow-up studies of patients with *C. jejuni* enterocolitis are necessary. Considering the fact that different studies represented diversity in prevalence of some musculoskeletal manifestations, further researches that would involve population-based studies are necessary to obtain more precise results of chronic postinfection sequels after *C. jejuni* diarrhea.

### ACKNOWLEDGMENTS

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### REFERENCES

Campylobacter jejuni i postinfektivne hronične sekvele: manifestacije na zglobovima

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**SAŽETAK**

Ovim istraživanjem ispitivana je pojava mišićnoskeletnih poremećaja kod bolesnika nakon enterokolitisa izazvanog *Campylobacter jejuni* (C. jejuni), a uloga C. jejuni u započinjanju seronegativnih spondiloartropatija praćena je detekcijom specifičnih antitela u serumu obolelih. Ispitivano je 146 bolesnika sa dijarejom izazvanom C. jejuni i 30 bolesnika sa seronegativnim spondiloartropatijama. Bakteriološki pregled stolice na prisustvo C. jejuni vršen je u Centru za mikrobiologiju Instituta za javno zdravlje u Nišu. Pored toga, kod bolesnika sa seronegativnim spondiloartropatijama ispitivan je i jedan uzorak seruma na antitela prema reakcijom vezivanja komplementa. Od 146 obolelih od enterokolitisa izazvanog C. jejuni, reumatske manifestacije, u vidu artralgija, mijalgija, entesitis i reaktivnog artritisa, javile su se kod 11% bolesnika. Prema našim saznanjima, ovo su, do sada, prvi opisani slučajevi reaktivnog artritisa izazvanog C. jejuni u Srbiji. Utvrđeno je da postoji statistički značajna razlika između prisustva antitela u grupi obolelih i u kontrolnoj grupi (p<0.05). U grupi obolelih od seronegativnih spondiloartropatija nije otkriveno prisustvo C. jejuni u stolici. Na osnovu dobijenih rezultata, zaključuje se da C. jejuni može biti značajan faktor u započinjanju reaktivnog artritisa, ali su neophodne dugotrajne studije koje bi pratile bolesnike sa enterokolitisom izazvanim C. jejuni da bi se rasvetoila njegova uloga u nastanku drugih seronegativnih spondiloartropatija. Pritom, prevencija ovog infektivnog procesa može da spreči prevenciju hroničnih postinfektivnih sekvela.

*Ključne reči: Campylobacter jejuni, postinfektivne sekvele, mišićnoskeletni sistem*