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Prevalence of *Yersinia* spp., *Chlamydia trachomatis*, *Chlamydophila pneumoniae* and *Borrelia burgdorferi* antibodies in healthy blood donors' sera

Występowanie przeciwciał przeciwko Yersinia spp., Chlamydia trachomatis, Chlamydophila pneumoniae i Borrelia burgdorferi w surowicach zdrowych dawców krwi

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Key words: antibacterial antibodies, healthy blood donors, undifferentiated arthritis.

Słowa kluczowe: przeciwciała antybakteryjne, zdrowi dawcy krwi, niesklasyfikowane zapalenia stawów.

Summary

Aim of the study: *Yersinia* spp., *Chlamydia trachomatis*, *Chlamydophila pneumoniae* and *Borrelia burgdorferi* are microorganisms from different taxonomic units. In the literature they are described as responsible for different types of arthritis of suspected bacterial origin, as well as concomitant other complications. The aim of the study was to estimate the prevalence of specific bacterial antibodies in a population of healthy people.

Material and methods: The study included 90 healthy blood donors' sera. Prevalence of antibacterial antibodies was determined by ELISA (enzyme-linked immunosorbent assay) technique.

Results: Detailed analysis showed that, regardless of the antibody class, the most frequently detected antibacterial antibodies in healthy blood donors' sera were anti-*Chlamydophila pneumoniae* (55.6%) and anti-*Yersinia* spp. (52.2%) antibodies. It may indicate a previous infection of this etiology. Much less often anti-*Borrelia burgdorferi* (12.2%) and anti-*Chlamydia trachomatis* antibodies (6.6%) were detected. The analysis of the correlation between prevalence of antibodies and donors' gender and their age did not show specific differences. We observed a high percentage of the co-occurrence of antibodies for 2-4 tested microorganisms in donors' sera (42.2%). What is more, there was a predominance of sera with antibodies concomitant to anti-*Yersinia* spp. + anti-*Chlamydophila pneumoniae* (27.8%). It may indicate cross reactivity or frequent contact with the microorganism and/or previous infection.

Conclusions: The obtained results may significantly reduce the diagnostic value of these serological tests in undifferentiated arthritis. The results clearly show that the serological diagnosis results of arthritis of suspected bacterial origin has to be always done in close correlation with carefully conducted medical history and the clinical picture.

Streszczenie

Cel pracy: *Yersinia* spp., *Chlamydia trachomatis*, *Chlamydophila pneumoniae* oraz Borrelia *burgdorferi* należą do różnych jednostek taksonomicznych. W piśmiennictwie drobnoustroje te opisywane są jako odpowiedzialne za m.in. różnego rodzaju stany zapalne stawów o ewentualnej etiologii zakaźnej, jak również współistniejące z nimi powikłania układowe. Celem pracy była ocena występowania przeciwciał swoistych dla badanych drobnoustrojów w surowicach zdrowych dawców krwi.

Materiał i metody: Do badań wykorzystano 90 surowic zdrowych dawców krwi. Badania na obecność przeciwciał antybakteryjnych wykonywane były metodą immunoenzymatyczną ELISA (*enzyme-linked immunosorbent assay*).

Wyniki: Szczegółowa analiza wykazała, że niezależnie od klasy badanych przeciwciał najczęściej wykrywano przeciwciała dla *Chlamydophila pneumoniae* (52,2% surowic) oraz *Yersinia* spp. (55,6% surowic), co może wskazywać na przebyte wcześniej zakażenie o tej etiologii. Zdecydowanie rzadziej wykrywano w surowicach dawców krwi przeciwciała przeciwko *Borrelia burgdorferi* (12,2% surowic) i *Chlamydia trachomatis* (6,6% surowic). Nie zaobserwowano znaczących zależności obecności przeciwciał dla badanych drobnoustrojów od wieku i płci badanych dawców. Zaobserwowano wysoki odsetek surowic dawców krwi (42,2%), w których wykazano współwystępowanie przeciwciał dla 2–4 badanych drobnoustrojów, w tym aż 27,8% surowic wykazywało obecność przeciwciał dla *Yersinia* spp. + *Chlamydophila pneumoniae* jednocześnie. Świadczyć to może o krzyżowej reaktywności albo częstym kontakcie z drobnoustrojem i/lub przebytej infekcji.

Wnioski: Otrzymane wyniki w znacznym stopniu mogą obniżyć wartość diagnostyczną badań serologicznych w niesklasyfikowanych zapaleniach stawów. Uzyskane dane jednoznacznie pokazują, że wyniki

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diagnostyki serologicznej w kierunku ewentualnych zapaleń stawów o podejrzewanej etiologii bakteryjnej muszą zawsze przebiegać w ścisłej korelacji z dokładnie przeprowadzonym wywiadem lekarskim oraz obrazem klinicznym.

Introduction

Yersinia spp., *Chlamydia trachomatis*, *Chlamydophila pneumoniae* and *Borrelia burgdorferi* are microorganisms from different taxonomic units. In the literature they are described as responsible for different types of arthritis of suspected bacterial origin, as well as for other concomitant complications [1–5].

Basic laboratory diagnosis of these arthritis is serodiagnosis. It is based on determination of prevalence of specific antibodies in sera using immunoenzymatic methods such as ELISA (*enzyme-linked immunosorbent assay*) and confirmation of positive results by Western blot with specific recombinant antigens. Using this two-step diagnosis in close correlation with the clinical picture may suggest the bacterial origin of the disease. The interpretation of the results may be made difficult by the same and/or similar clinical symptoms of different microbial infections, previous, often asymptomatic contact with a pathogen, and the microorganisms cross reactivity [6–12].

We have studied the prevalence of antibodies for *Yersinia* spp., *Chlamydia trachomatis*, *Chlamydophila pneumoniae* and *Borrelia burgdorferi* suspected of bacterial origin arthritis in healthy blood donors' sera. The aim of the study was to estimate the prevalence of specific bacterial antibodies in a population of healthy people and to compare them with previously obtained results in a group of patients with undifferentiated arthritis.

Material and methods

Sera from 90 healthy blood donors were tested. The blood was supplied by the Regional Centre for Blood Donation and Blood Treatment in Warsaw from women and men aged 18–70 years. Donors meet the requirements of the Regional Blood stations and agree to collect blood for research purposes. Sera were divided into four age groups (Table I).

Prevalence of antibacterial antibodies was determined by ELISA technique, using the following tests:

- IgA- and IgG-class antibodies against *Yersinia* spp. – recomWell Yersinia (Microgen Diagnostic). The recombinant antigens used in these tests are YOPs (*Yersinia* outer membrane proteins).
- IgA- and IgG-class antibodies against *Chlamydia trachomatis* – Novalisa *Chlamydia trachomatis* (NovaTec Immunodiagnostica GmbH). The antigens used in these tests are highly purified *Chlamydia trachomatis* LGV type II strain 434 antigens.
- IgA- and IgG-class antibodies against *Chlamydophila pneumoniae* – *Chlamydophila pneumoniae* IgA-IgG EIA (AnilabSystems). Plates are coated with *Chlamydophila pneumoniae* elementary bodies.
- IgG- and IgM-class antibodies against *Borrelia burgdorferi* – Borrelia IgG/IgM Recombinant (ELISA) (Biomedica). The recombinant antigens used in these tests are p21, p18, p100 and VlsE.

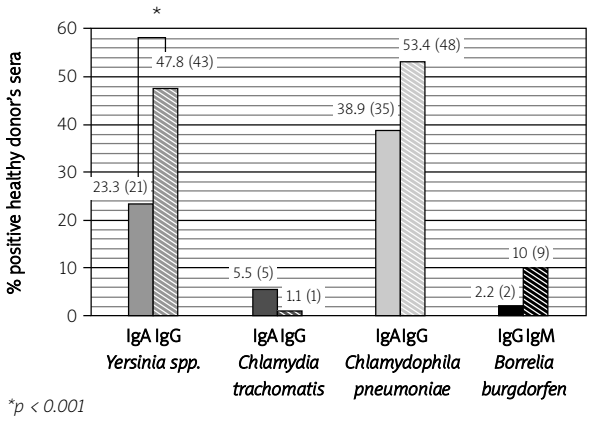
The occurrence of antibodies in different groups was compared with the χ^2 test or χ^2 test with Yates' correction. The significance level was assumed at $p < 0.05$.

Results

Detailed analysis for the prevalence of antibacterial antibodies in healthy blood donors' sera was performed. Antibodies against *Chlamydophila pneumonia*e [38.9% (35 sera) in IgA class and 53.4% (48) in IgG class] and against *Yersinia* spp. [23.3% (21) in IgA and 47.8% (43) in IgG] were the most frequently detected. Antibodies against *Borrelia*

Table I. Characteristics of healthy blood donors

Age groups	Number of healthy blood donors' sera		
	men	women	all
group I (18–30 years)	21	11	**32**
group II (31–45 years)	31	0	**31**
group III (46–60 years)	14	5	**19**
group IV (> 60 years)	0	8	**8**
all	**66**	**24**	**90**



*$p < 0.001$

Fig. 1. Percentages of healthy blood donors' positive sera of *Yersinia* spp., *Chlamydia trachomatis*, *Chlamydophila pneumoniae* and *Borrelia burgdorferi* in particular antibody classes, detected by ELISA.

burgdorferi [2.2% (2) in IgG and 10% (9) in IgM] and against *Chlamydia trachomatis* [5.5% (5) in IgA and 1.1% (1) in IgG] (Fig. 1) were rarely detected. These results confirm a general statement of the percentage of positive sera of antibacterial antibodies, without regard to immunoglobulin class. The anti-*Yersinia* spp. antibodies were detected in 52.2% (47) of sera, anti-*Chlamydophila pneumoniae* antibodies in 55.6% (50), whereas anti-*B. burgdorferi* antibodies were determined in 12.2% (11) of sera and anti-*Chlamydia trachomatis* antibodies in only 6.6% (6).

It was interesting to investigate the combinations of antibody classes against particular microorganisms in tested sera. In the case of *Yersinia* spp. usually only IgG class antibodies [28.9% (26)] and both IgA and IgG antibodies [18.9% (17)] were detected. The presence of only IgA class antibodies was observed in just 4.4% (4) of sera (Fig. 2A).

Antibodies against *Chlamydia trachomatis* were most often observed only in IgA [5.5% (5) sera] and only in IgG class [1.1% (1)]. No positive results were obtained the presence of both tested antibody classes (Fig. 2B). Concerning *Chlamydophila pneumoniae*, it was observed that the most detected antibodies were in both antibody classes simultaneously [IgA + IgG – 36.7% (33)]. Definitely rarely only IgA [2.2% (2)] and only IgG class [16.7% (15)] antibodies were determined (Fig. 2C). In the case of *B. burgdorferi*, there definitely predominated antibodies in only IgM class [10% (9) sera] and only IgG class antibodies were detected in slightly more than 2.2% (2) of sera. No antibodies were detected in both classes simultaneously (Fig. 2D).

A detailed analysis was performed to estimate the prevalence of antibodies against selected microorganisms in different age groups (I-IV) and by gender.

Both IgA [31.6% (6 sera)] and IgG class antibodies [73.7% (14)] against *Yersinia* spp. were most frequently detected in group III. The lowest percentage of IgA-positive sera was observed in group IV [12.5% (1)] and in group II for IgG antibodies [35.5% (11)] (Fig. 3).

The most often detected were antibodies (IgA, IgG) against *Chlamydophila pneumoniae* in sera of group III [IgA – 63.2% (12), IgG – 78.9% (15)] and group IV [IgA – 75% (6), IgG – 62.5% (5)] (Fig. 4).

Anti-*Chlamydia trachomatis* IgA class antibodies were detected most in group IV [37.5% (3)] and in group I and II (in 3.1% of sera). Immunoglobulin G antibodies were determined only in group II.

Significant differences in the percentages of positive sera for the prevalence of anti-*B. burgdorferi* antibodies in particular age groups were not observed. However, the absence of IgG class antibodies in the youngest (gr. I) and the oldest (gr. IV) healthy donor age groups was noticed.

The analysis of the correlation between prevalence of antibodies and donors' gender did not show specific differences. According to gender, the percentages of positive sera in different classes remained at the same level. A significantly higher percentage of IgA class antibodies in women was observed only in the case of *Chlamydia trachomatis* [16.6% (4) in women vs. 1.5% (1) in men].

It was important to estimate the co-occurrence of tested antibacterial antibodies in the sera from healthy blood donors. It was found that up to 42.2% (38) of the donors' sera were positive in ELISA test for 2-4 tested microbes (Fig. 5). By far the most often [27.8% (25) of sera] anti-*Yersinia* spp. and anti-*Chlamydophila pneumoniae* antibodies coexisted at the same time. In 4.4% (4) of the sera the simultaneous presence of antibodies against *B. burgdorferi* + *Chlamydophila pneumoniae* was observed. Other combinations of co-occurring antibodies to various microorganisms were found in small percentages of sera [1.1-2.2% (1-2)] and among them there was one with positive results for all four microorganisms.

Analysis of positive sera of *Yersinia* spp. + *Chlamydophila pneumoniae* was done in 25 sera and the co-occurrence of *anti-Yersinia* spp. + anti-*Chlamydophila pneumoniae* antibodies was tested. The most often detected antibodies were only in IgG class for both microorganisms [68% (17) of sera] and in 20% (5) of sera antibodies for both classes (IgA + IgG) were found. Antibodies of different classes, IgA for the first microorganism and IgG for the second one, and vice versa, occurred in 12% (3) of tested sera.

Discussion

The rule of serological diagnosis is comparison of positive patients' results to the control group, most often healthy blood donors. It was already repeatedly emphasized that the serological diagnosis of antibacterial antibodies always has to be performed in close correlation with the clinical picture, due to the fact that prevalence of these anti-

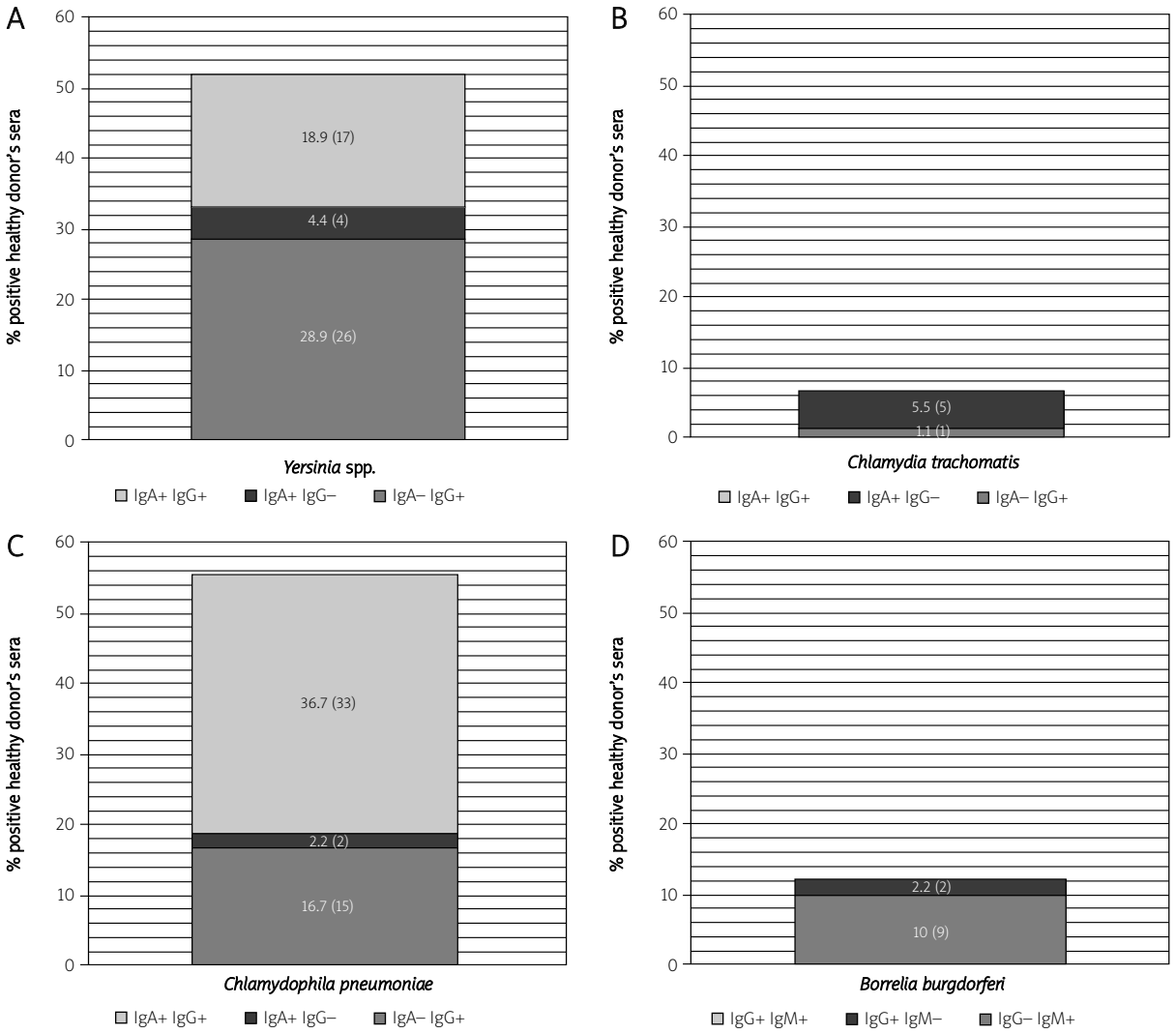
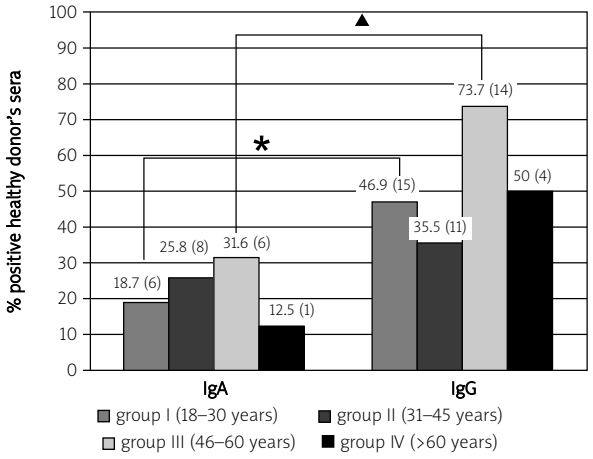


Fig. 2. Percentages of healthy blood donors' positive sera of *Yersinia* spp. (A), *Chlamydia trachomatis* (B), *Chlamydophila pneumoniae* (C) and *Borrelia burgdorferi* (D) detected by ELISA.

bodies may result from antibody cross reactivity, polymorphic B-cell induction, additional infection or previous contact with a pathogen [8–11]. Therefore, the administration of antibiotic therapy is often not justified and the expected results are not achieved. Moreover, the high percentages of antibacterial antibodies detected in healthy blood donors' sera clearly show that the interpretation of serological test results has to be strictly considered with the patient's clinical status.

The presented study deliberately focused on prevalence of antibodies to microorganisms with taxonomic diversity and different transmission routes. All of the tested microorganisms may induce arthritis. Data available from the literature, as well as our own long-term research conducted on sera of patients hospitalized in the Institute of Rheumatology in Warsaw [1, 2, 5–8, 11], show that prevalence of antibodies to *Yersinia* spp., *Borrelia burgdorferi*, *Chlamydia trachomatis* and *Chlamydophila pneumoniae* was determined and analyzed in detail. The prevalence of these antibodies in healthy blood donors' sera has not been studied comprehensively yet.

The results were somewhat surprising. Regardless of the antibody class, we detected a very high percentage (52.2%) of positive results for the prevalence of anti-*Yersinia* spp. (*Y. enterocolitica* and *Y. pseudotuberculosis*) and anti-*Chlamydophila pneumoniae* (55.6%) antibodies in healthy blood donors' sera. These results are higher than in other studies showing that 20.5%, 30% and 32% of the population has antibodies against *Yersinia* spp. [12–14]. It may indicate a previous infection of this etiology. In the general population both *Yersinia* spp. and *Chlamydophila pneumoniae* cause frequent infections with different clinical



*$p < 0.05$, ▲$p < 0.05$

Fig. 3. Percentages of healthy blood donors' positive sera of *Yersinia* spp. (IgA and IgG class) in four age groups.

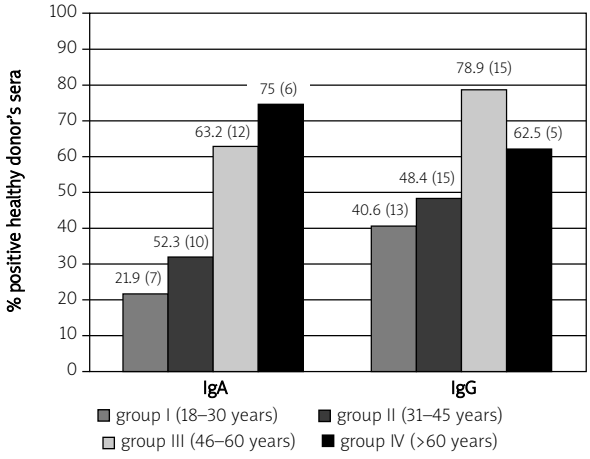


Fig. 4. Percentages of healthy blood donors' positive sera of *Chlamydophila pneumoniae* (IgA and IgG class) in four age groups.

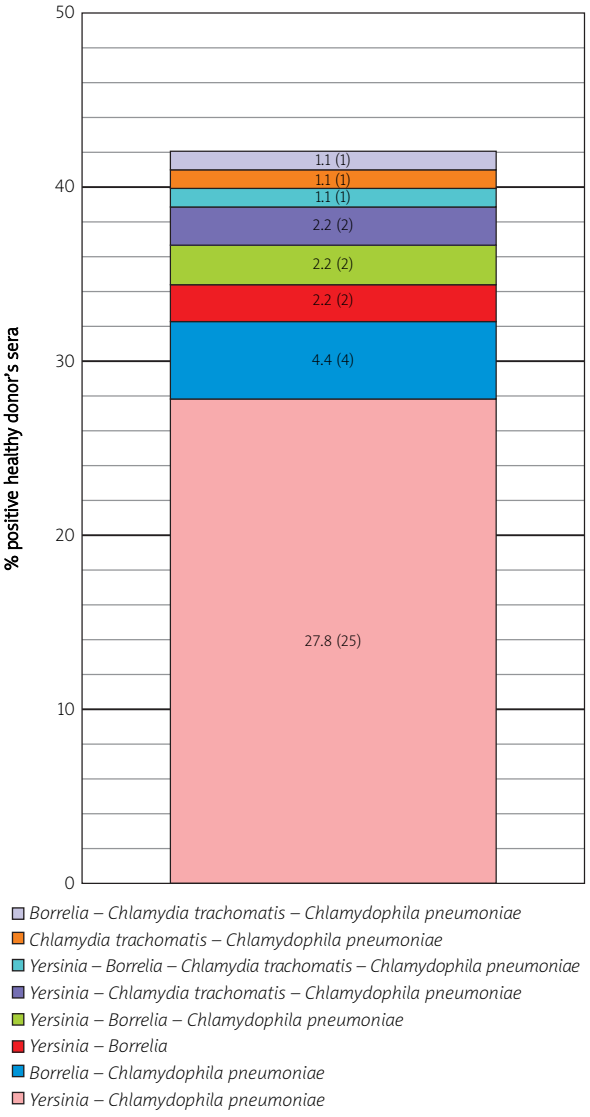


Fig. 5. Co-occurrence of antibacterial antibodies in healthy blood donors' positive sera.

symptoms. They can also cause infection with poorly marked systemic symptoms, often undiagnosed. Moreover, cross reactivity of antibodies to the same components of the cell wall of both microorganisms, such as YOP and OMP (outer membrane protein) proteins, may also occur. In this study, in the case of *Yersinia* spp. the percentage of positive healthy blood donors' sera was significantly higher than in the group of undiagnosed arthritis patients hospitalized in the Institute of Rheumatology (35.3%) [11]. Anti-*Chlamydophila pneumoniae* antibodies in patients' sera were detected in 47.6% (our unpublished data). So high prevalence of these antibodies may make correct diagnosis of patients with poorly recognized clinical symptoms difficult.

We detected much less often anti-*Borrelia burgdorferi* [(12.2% (11 sera)] and anti-*Chlamydia trachomatis* antibodies [6.6% (6)] in healthy blood donors' sera. Similar results were obtained in another Polish study, where the presence of anti-*B. burgdorferi* antibodies was detected in 12% of healthy blood donors' sera [15]. In Austria 7.7% of tested blood donors' sera were positive for anti-*B. burgdorferi* antibodies [16] and in Germany 5.5–7.2% of healthy blood donors' sera [17, 18]. This may indicate contact with an infecting agent and a possibility of asymptomatic course of the infection. In our previous study in patients with undifferentiated arthritis, these percentages were slightly higher, i.e. 14.7% for anti-*B. burgdor-*

feri and 10.6% for anti-*Chlamydia trachomatis* antibodies [11].

The comparison of the results of the group of healthy blood donors with positive results of the antibacterial antibodies in various classes with results of patients with undifferentiated arthritis is interesting and may be relevant for the future.

It is not surprising that the most often detected were only IgG class anti-*Yersinia* spp. antibodies (Fig. 2). However, a higher percentage of antibodies in the healthy blood donor group in this study than in patients in our previous study [11] (28.9% vs. 19.3%) can significantly reduce the diagnostic value of these serological tests in undifferentiated arthritis. A high level of antibodies, only in IgG class, can persist for months, years or even the whole life. It can indicate previous infection or a contact with this pathogen. However, it should be stressed that presence of high levels of only IgG class antibodies has little diagnostic significance in undifferentiated arthritis [6].

Detection of only IgA class anti-*Chlamydia trachomatis* antibodies in 5.5% (5) of healthy blood donors' sera should be interpreted similarly. The prevalence of only IgA class antibodies is found in infection of the mucous membranes and may indicate an active, recent, but also chronic infection process [7]. In the patients' sera, anti-*Chlamydia trachomatis* antibodies without regard to immunoglobulin class were detected in 10.6% of sera, of which in 6.3% only IgA class antibodies were detected. Notably, a cross-reaction of this pathogen with *Chlamydophila pneumoniae*, which infects mainly the mucosa of the respiratory system, often is possible. Slightly different results were observed by Podsiadły *et al*. [19]. They found that only 1.4% of sera were seropositive for *Chlamydia trachomatis* specific antibodies. Another study showed that anti-*Chlamydia trachomatis* antibodies were detected in 13% of sera in IgA and in 26% in IgG class antibodies [20].

We detected a high percentage of positive results for anti-*Chlamydophila pneumoniae* antibodies in healthy blood donors' sera, where 38.9% (35) of sera were IgA and 53.4% (48) were IgG class antibody positive. As mentioned above, this may indicate a previous infection. The dominance of the simultaneous prevalence of IgA and IgG class antibodies to *Chlamydophila pneumoniae* [36.7% (33)] may suggest frequent contact with this microorganism and consistent production of antibodies in healthy people [21]. A slightly lower percentage of IgG class anti-*Chlamydophila pneumoniae* antibodies in blood donors' sera was detected by Podsiadły *et al*. (35.1%) [19].

The analysis of the prevalence of antibodies by age and gender showed minor diagnostic significance. There were no significant dependences of the prevalence of antibodies to *Yersinia* spp., *Borrelia burgdorferi*, *Chlamydia trachomatis* and *Chlamydophila pneumoniae* analyzed by age and gender. However, the highest percentages of anti-*Yersinia* spp. and anti-*Chlamydophila pneumoniae* antibodies were detected mainly in the oldest age groups (group III and IV) (Fig. 3 and Fig. 4). These results confirm another study, where anti-*Chlamydophila pneumoniae* antibodies were detected more often in donors aged 50–59 [19]. With increasing age, older people could have contact with more diverse microorganisms [21].

Interestingly, we observed a significantly higher percentage of IgA class anti-*Chlamydia trachomatis* antibodies in women's sera.

Surprisingly, we found a high percentage of co-occurrence of antibodies for 2–4 tested microorganisms in donors' sera [42.2% (38)]. It may indicate cross reactivity or frequent contact with the microorganism and/or previous infection (especially when antibodies were mainly in IgG class) [11]. The consequence of the high percentages of Yersinia spp. [52.2% (47)] and *Chlamydophila pneumoniae* [55.6% (50)] positive sera was the dominance of sera with antibodies concomitant to anti-*Yersinia* spp. + anti-*Chlamydophila pneumoniae* [27.8% (25 sera)].

The above results showed that serological tests which detect antibodies against bacterial antigens *B. burgdorferi*, *Chlamydia trachomatis*, *Yersinia* spp. and *Chlamydophila pneumoniae* in the diagnosis of arthritis should be correlated with the clinical picture and previous medical history. The analysis must take into account the presence of these antibodies in the healthy population, which results both from prior contact with a microorganism and a history of infection, as well as cross-reactivity.

The authors declare no conflict of interest.

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