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ORIGINAL ARTICLE

The seroprevalence of *Bartonella* spp. in the blood of patients with musculoskeletal complaints and blood donors, Poland: a pilot study

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Abstract

Background *Bartonella* spp. can cause a variety of diseases, such as lymphadenopathies, cat scratch disease, and trench fever, but can also give rise to many non-specific symptoms. No data exists regarding the prevalence of *Bartonella* spp. in patients with musculoskeletal complaints, nor among blood donors in Poland.

Methods The presence of anti-*Bartonella* IgM and IgG in the serum of blood donors ($n = 65$) (Lodz, Poland) and in the patients of the Department of Rheumatology Clinic ($n = 40$) suffering from musculoskeletal symptoms was tested by immunofluorescence. Blood samples were cultured on enriched media. Epidemiological questionnaires were used to identify key potential risk factors, such as sex, age, contact with companion animals, and bites from insects or animals.

Results Altogether, 27 of the 105 tested subjects were seropositive for *Bartonella henselae* IgG (23%) and three for *Bartonella quintana* IgG (2.85%); IgMs against *B. henselae* were found in three individuals (2.85%), and IgMs against *B. quintana* were found in one (1.54%). No statistically significant difference was found between the prevalence of *B. henselae* in the blood of donors or patients and the presence of unexplained musculoskeletal complaints (23% vs 30%). Individuals who had kept or been scratched by cats were not more likely to be *B. henselae* seropositive ($p > 0.01$). Tick bites were more commonly reported in patients, but insignificantly ($p > 0.01$).

Conclusion This is the first report of a high seroprevalence of anti-*Bartonella* IgG in patients with musculoskeletal symptoms and in blood donors in Poland. The obtained results indicate that such seroprevalence may have a possible significance in the development of musculoskeletal symptoms, although it should be confirmed on a larger group of patients. Asymptomatic bacteremia might occur and pose a threat to recipients of blood from infected donors. Hence, there is a need for more detailed research, including molecular biology methods, to clarify the potential risk of *Bartonella* spp. being spread to immunocompromised individuals.

Key Points

- *This is the first study presenting high seroprevalence of Bartonella spp. in Poland.*
- *IgG and IgM antibodies against B. quintana were found in blood samples of blood donors.*

Keywords *Bartonella* spp. · Blood donors · Musculoskeletal symptoms

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Introduction

Although *Bartonella* spp. are endemic pathogens in some areas of the world, there is very limited awareness of the diseases associated with them in some countries [1]. *Bartonella* can infect a wide range of mammalian hosts, and 17 of the 35 species are recognized as human pathogens http://www.bacterio.net.bartonella.htm. They are fastidious

facultative intracellular microorganisms, able to persist in erythrocytes, avoid the immune response of the host, and establish asymptomatic infection [2], and as such, they can be transmitted via bloodsucking arthropods; a high prevalence of *Bartonella* bacteremia has been reported in populations of rodents, cats, and ruminants worldwide [3, 4].

The most common such disease is cat scratch disease (CSD), caused by *Bartonella henselae* [5–8]. CSD is characterized by chronic lymphadenopathy; it affects mostly children and young adults and is typically first noticed as an erythematous papule or pustule which develops into a benign lymphadenopathy, resolving naturally in up to 6 months [9]. Atypical symptoms include neuroretinitis, Parinaud's oculoglandular syndrome, encephalopathy, hepatosplenomegaly, glomerulonephritis or prolonged fevers, and infective and culture-negative endocarditis in both immunocompetent and immunocompromised patients [1, 10, 11]. *B. henselae* replicates and persists in the endothelium; if the immune system is impaired, resolution of CSD may be hindered and vascular endothelial proliferation induced by *B. henselae* may be observed [12]. Other species naturally cause hemotropic infection in their respective animal reservoir: *Bartonella elizabethae* in rats, *Bartonella grahamii* in mice [11, 13]. Among humans, *Bartonella bacilliformis* infection is fatal in as many as 80% of cases of acute disease, e.g., Oroya fever or Carrión's disease [1, 14]. In addition, *Bartonella quintana* causes trench fever for those living in poor conditions; bacillary angiomatosis, peliosis hepatis, and chronic lymphadenopathy in immunocompromised patients, such as those with HIV; and blood culture–negative (BCN) endocarditis in immunocompetent patients [15]. Regarding other species, *Bartonella koehlerae*, *B. elizabethae*, *Bartonella alsatica*, and *Bartonella vinsonii* (subsp. *berkhoffii* and subsp. *arupensis*) are associated with endocarditis; *Bartonella rochallimae* and *Bartonella tamiae* are implicated in febrile illness and bacteremia; *B. grahamii* causes neuroretinitis or cat scratch disease [11, 15, 16].

The diagnosis of *Bartonella* spp. infection is difficult. Even though these bacteria can be grown in enriched media, the sensitivity is typically low. Therefore, serological tests are typically used in microbiological laboratories; however, the results can be influenced by cross-reactions between *Bartonella* spp. [17]. Improved results may be achieved through a combination of culture and molecular methods, such as PCR and DNA sequencing, but this is not routinely implemented [18] and there is a growing need for more effective diagnostic tools.

The wide geographical spread, broad spectrum of reservoirs, and range of possible vectors demand further studies on the epidemiology of *Bartonella* spp. Worldwide, most diseases caused by *Bartonella* spp. are not reportable, and hence, data on their incidence is limited. The few studies that exist suggest that the frequency of infections may be high: 22,000 cases of cat scratch disease appeared in 1 year in the USA, and 10% of them required hospitalization [19]. Interestingly, no data exists on the seroprevalence of *Bartonella* spp. among individuals hospitalized due to musculoskeletal complaints, even though such an association has been proposed [20–22].

Therefore, the aim of the present study was to determine the seroprevalence of *Bartonella* spp. among patients with unexplained musculoskeletal complaints attending the Rheumatology Clinic and identify possible routes of transmission. A group of healthy blood donors without musculoskeletal symptoms was included as a reference group. *Bartonella* can avoid the immune response of the host [23], with subclinical, asymptomatic bloodstream infections in humans being reported [24]; this may pose a hazard for blood recipients because donors are not typically screened for *Bartonella* infections.

Materials and methods

Bacterial reference strains and DNA

B. henselae–type strain (ATCC 49882, Houston 1), *B. quintana* (ATCC 51694, strain 90-268), *B. koehlerae* (ATCC 700693), *Bartonella clarridgeiae* (ATCC-700095, strain NCSU 94-F40), and *B. bacilliformis* (ATCC-35685D-5, strain K0583; genomic DNA) were purchased from ATCC. All the reference strains were stored in microbanks (Technical Service Consultants Ltd., Heywood, UK) with an addition of glycerol at − 70 °C for further use.

Subjects

The study was approved by the Ethics Committee of the Medical University of Lodz, Poland (no. RNN227/16KE; KE/1204/18). The blood donors from the Regional Centre of Blood Donation and Treatment in Lodz were enrolled into the reference group. They could join the group only after being accepted for donation: with the acceptance criterion comprising negative tests for infections caused by HIV, HBV, HCV, and *Treponema pallidum*, with no presence of musculoskeletal symptoms lasting for longer than 1 week. No subjects had received antibiotic therapy for 6 months before sampling. Pregnant women were excluded. All blood donors signed an informed consent form before taking part in the sampling procedure.

The test group included patients of the Rheumatology Clinic, University Hospital WAM, Lodz, Poland, with unexplained musculoskeletal complaints. None of the patients included in the study clearly fulfilled the diagnostic criteria for any rheumatic disorder, such as rheumatoid arthritis, collagen tissue disorder, crystalopathy, or vasculitis. Before enrolment, all patients signed an informed consent form.

All participants completed a questionnaire with the following information: sex; age; past diseases; occupational animal exposure; raising or current contact with cats, dogs, or other animals; contact with livestock; previous contacts with animals; bites from dogs, cats, or other animals; tick bites; the appearance of *erythema migrans*; and signs and symptoms which may be related to *Bartonella* sp. infections. A physician was available to help with the completion if necessary.

Blood sample collection

Blood samples (9 mL) from blood donors ($n = 65$) and patients ($n = 40$) were collected in plastic EDTA tubes (Sarstedt, Germany). At the same time, serum samples (9 mL) were collected into serum-separating tubes (Sarstedt, Germany) to examine the presence of antibodies against *B. henselae* and *B. quintana*. Aseptic technique was used; an experienced nurse performed venipuncture. Samples were transported to the laboratory within 2 h and processed immediately. The remaining blood samples, in EDTA tubes, and serum, distributed in 1.5-mL Eppendorf tubes, were stored at − 70 °C for further use.

Blood culture

Blood samples (0.5 mL) were cultured on fresh Chocolate Agar with Vitox (Argenta, Poland). The plates in duplicate were incubated at 35 °C in 5% CO_2 (GeneBag, bioMerieux, France) for 4 weeks, and the cultures were examined for bacterial growth once a week.

Immunofluorescence antibody test

The antibody titers against *B. henselae* and *B. quintana* in donor serum and patient blood were evaluated with the immunofluorescence antibody (IFA) test (Euroimmun, Germany) according to the manufacturer's protocol. Briefly, the serum samples were centrifuged for 15 min at 2500 rpm at 4 °C and transferred into sterile 1.5-mL Eppendorf tubes. Following this, 10 μL of serum was mixed with 90 μL of Eurosorb reagent to remove non-specific antibodies; then, a final 1:100 dilution was prepared to check the concentration of the IgM antibodies. To assay the IgG antibodies, the serum was diluted 1:320 in PBS with glycerol, with all other steps were performed according to the protocol. IFA results were assessed by two researchers independently. Slides were observed using a fluorescence microscope (Olympus DP22, Japan) at × 200 magnification.

Statistical analysis

The chi-square test with the Yates correction was used to compare the results obtained for the blood donors and the patients with musculoskeletal complaints. The difference was considered statistically significant at $p < 0.01$.

Results

Clinical characteristics of subjects

The control group included 65 blood donors while the study group included 40 patients of the Rheumatology Clinic with musculoskeletal complaints. The groups are described more fully in Table 1.

The donor group tended to be younger than the patient group. This could be because younger people typically decide to donate blood (mean 34.28 ± 8.3 SD), while older individuals are more likely to suffer from the various signs and symptoms related to problems with the skeletal system, joints, and muscles (mean 52 ± 13.18 SD). In addition, the donor group had a smaller proportion of women (43.1% female blood donors): often women are not able to donate blood due to poor test results, such as low hemoglobin concentration, and women are more likely to seek help at the onset of symptoms (75% female patients vs 25% men).

The questionnaires were completed by 105 blood donors and patients. Of these, 27 (25.7%) reported owning cats, 47 (44.8%) dogs, and 50 (47.6%) having previous contact with dogs or cats. Over 50% of patients suffered from at least one chronic sign: joint or bone or muscle pain. More patients with musculoskeletal complaints were bitten by ticks compared with blood donors (52.5% vs 27.7%). Apart from differences observed resulting from the specificity of tested groups, e.g., symptoms concerning musculoskeletal system are typically observed more frequently in patients, only the reported frequency of *erythema migrans* was significantly higher in patients than in the donors (p value = 0.009).

Culture

Reference strains were successfully grown on recommended media, and growth was observed after 4–14 days as minute colonies. When 500-μL blood samples (from patients or blood donors) were cultivated in duplicate, no typical growth was observed. Each plate with suspected growth (irregular surface) was carefully observed and investigated more thoroughly with PCR (data not included). Contamination was observed in only two of 105 blood samples (1.9%; confirmed by Gram staining)

Immunofluorescence results

In total, 27 of the 105 tested subjects (23%) were seropositive for *B. henselae* IgG (1:320, according to the manufacturer's procedure) and three for *B. quintana* IgG (2.85%); IgMs against *B. henselae* (titer 1:100) were found in three

Table 1 The results of the questionnaires administered to the donors and patients. Age is presented as mean ± SD; *n* number; very often. *Significantly different to healthy subjects

	Healthy subjects	Patients with musculoskeletal complaints
Total number	65	40
Females/males	28 (43.1%)/37 (56.9%)	30 (75%)/10 (25%)
Age	34.28 ± 8.3	52 ± 13.18
Migratory pain	Very often 0	Very often 22 (55.0%)
	Sporadically 0	Sporadically 5 (12.5%)
Muscle pain	Very often 0	Very often 23 (57.5%)
	Sporadically 2 (3.07%)	Sporadically 3 (7.5%)
Bone pain	Very often 0	Very often 21 (52.5%)
	Sporadically 3 (4.61%)	Sporadically 1 (2.5%)
Joint pain	Very often 0	Very often 30 (75.0%)
	Sporadically 6 (9.23%)	Sporadically 2 (5.0%)
Feeling of exhaustion	Very often 0	Very often 20 (50.0%)
	Sporadically 2 (3.07%)	Sporadically 1 (2.5%)
Experience of raising cats	16 (24.6%)	11 (27.5%)
Experience of raising dogs	26 (40.0%)	21 (52.5%)
Previous frequent contact with cats, dogs	29 (44.6%)	21 (52.5%)
Bitten by a cat/dog	12 (18.46%)	7 (17.5%)
History of a tick bite	18 (27.7%)	21 (52.5%)
History of having *erythema migrans*	1 (1.54%)	7 (17.5%)*
Unexplained febrile diseases	0	7 (17.5%)
Unexplained lymphadenopathy	0	3 (7.5%)

individuals (2.85%; in sera of two blood donors and one patient); IgMs against *B. quintana* were found in the serum of one blood donor (1.54%). No statistically significant difference in the prevalence of *B. henselae* was found between blood donors and patients with musculoskeletal complaints (23% vs 30%). *B. henselae* IgG seropositivity was more common in female patients ($n = 10$) than in male patients ($n = 2$), while among the blood donors, eight women and seven men were IgG seropositive. Surprisingly, *B. quintana* seropositivity (at concentrations indicated by the manufacturer, 1:100 for IgM, 1:320 for IgG) was found only in blood donors: three IgG-positive participants were identified, one of whom was also IgM positive.

Most *B. henselae* IgG-seropositive blood donors were in the 30–39-year-age group and the 40–49-year-age group (5/22, 22.7%; 5/18, 27.8%), while the *B. henselae* IgM-seropositive blood donors were split between the 20–29-year group ($n = 1$) and the 40–49-year group ($n = 1$). Among the patients, most *B. henselae*-seropositive participants were found in the 30–39-year (4/5, 80%) and 40–49-year (5/13, 38.5%) groups (Table 2).

Bartonella seroprevalence and risk factors

The individuals who owned cats, or had been scratched by them, were not more likely to be *B. henselae* seropositive (IgG titer of 1:320). Among all the tested individuals, 27 were *B. henselae* IgG positive and 11 of them (10.5%) owned cats; eight of the seropositive participants owned dogs. None of these results were statistically significant ($p > 0.01$) (Table 3).

Discussion

Bartonella spp. are responsible for a range of diseases in humans, and the host immune response plays a crucial role in stopping the development of infection to more dangerous forms; indeed, *B. henselae* may cause severe disseminated disease and pathologic vasoproliferation in immunocompromised patients [12]. Therefore, the prevalence of infections should be monitored, especially considering the zoonotic potential of *Bartonella* spp.

Unfortunately, as seroprevalence testing in blood donors is not obligatory and diagnostics of infections caused by *Bartonella* spp. are mostly limited to endemic regions, the data concerning the occurrence of anti-*Bartonella* spp. antibodies in healthy populations is scarce [25]. Seroprevalence may differ by region, e.g., 16.1% of blood donors in Sweden were found to be seropositive to all *Bartonella* spp. (immunoreactivity to *B. elizabethae* (14.1%); *B. grahamii* (2.6%); *B. henselae*

Table 2 IgG and IgM immunofluorescence test results with regard to age group

	Age group (years)	Anti-*B. henselae* antibodies				Anti-*B. quintana* antibodies				Total number
		IgG (+)	IgG (−)	IgM (+)	IgM (−)	IgG (+)	IgG (−)	IgM (+)	IgM (−)	
Healthy subjects (*n*)	20–29	3; 13.0%	20; 87%	1; 4.3%	22; 95.6%	2; 8.69%	21; 91.3%	1; 4.3%	22; 95.6%	23
	30–39	5; 22.7%	13; 77.8%	0; 0%	22; 100%	0; 0%	22; 100%	0; 0%	22; 100%	22
	40–49	5; 27.8%	13; 72.2%	1; 5.5%	16; 94.5%	1; 5.5%	16; 94.5%	0; 0%	18; 100%	18
	50–59	2; 100%	0; 0%	0; 0%	2; 100%	0; 0%	2; 100%	0; 0%	2; 100%	2
	60+	0; 0%	0; 0%	0; 0%	0; 0%	0; 0%	0; 0%	0; 0%	0; 0%	0
Total (blood donors)		20; 30.8%	45; 69.2%	2; 3.1%	63; 96.9%	3; 4.6%	62; 95.4%	1; 1.54%	64; 98.5%	65
Rheumatology clinic patients	20–29	0; 0%	2; 100%	1; 50%	1; 50%	0; 0%	2; 100%	0; 0%	2; 100%	2
	30–39	4; 80%	1; 80%	0; 0%	5; 100%	0; 0%	5; 100%	0; 0%	5; 100%	5
	40–49	5; 38.5%	8; 61.5%	0; 0%	13; 100%	0; 0%	13;100%	0; 0%	13;100%	13
	50–59	1; 14.3%	6; 85.7%	0; 0%	7; 100%	0; 0%	7; 100%	0; 0%	7; 100%	7
	60 +	2; 15.4%	11;84.6%	0; 0%	13; 100%	0; 0%	13; 100%	0; 0%	13; 100%	13
Total (patients)		12; 30%	28;70%	1; 2.5%	39;97.5%	0; 0%	40;100%	0; 0%	40;100%	40
Altogether		32 (30.5%)	73 (69.5%)	3 (2.85%)	102 (97.15%)	3 (2.85%)	102 (97.15%)	1 (0.95%)	104 (99.05%)	105 (100%)

Values are presented as follows: number; %

Table 3 *Bartonella henselae* seroprevalence and risk factors

Type of risk factor	Seropositive subjects *B. henselae*		
	Risk factor		*p* value
	(+)	(−)	
Dog ownership	8	19	0.2723
Cat ownership	11	16	0.0692
Had been bitten by an animal (dog, cat)	4	14	0.6805
Dog and cat ownership	5	22	0.4327
Had contact with cats or dogs	16	11	0.8406
Had been bitten by a tick	14	13	0.1978
Had *erythema migrans*	2	30	0.9671

(1.2%); *B. henselae* (Marseille) (1.8%); *B. quintana* (0.2%); *B. vinsonii* subsp. *vinsonii* (0.0%)) [26]; 8.7% of healthy adults were seropositive to *B. henselae* in Spain [27], 19.6% in Zhejiang Province in China [28], and 15.0% in Korea [29].

No recent studies have examined the seroprevalence of *Bartonella* spp. in blood donors in Poland. The present study uses a commercially available test (Euroimmun) which detects the most common *Bartonella* species: *B. henselae* and *B. quintana*. Our results indicate that the tested individuals have frequent contact with *Bartonella* spp.: antibodies against *B. henselae* were found in 23.07% ($n = 15$ of 65) of blood donors. No significant difference in seroprevalence was observed between genders, as noted previously [30].

Such high result indicates that *Bartonella* spp. may be a hazard factor for blood recipients. Bearing in mind the ability of these species to cause asymptomatic infection in healthy individuals [27] and serious infections in immunocompromised patients, blood recipients may face a risk of receiving blood or blood products contaminated with these species. *B. henselae* transmission by transfusion has been confirmed in a mouse model, even when the donor animals have undetectable bloodstream infection [31]. Therefore, more detailed investigation is needed to confirm our present findings, as antibody detection may not offer sufficient predictive value in confirming or excluding *Bartonella* species bacteremia [32]. The presence of bacterial DNA in donor blood samples can be confirmed more accurately by molecular methods, such as PCR and sequencing. Studies based on a combination of culture methods and PCR assays may allow the true risk of *Bartonella* spp. transmission through blood samples to be confirmed in this population. Previous studies have reported *Bartonella* seropositivity in 16.1% of asymptomatic blood donors in Sweden [26] and 13.6% of donor blood samples to be positive for *B. henselae* by PCR in Chile [33]. The high number of seropositive individuals identified in the present

study, by comparison, a Turkish study only found 3.3% prevalence [30], may be a consequence of high ownership of pets, such as cats and dogs, in Poland.

Some studies have examined various groups of patients displaying anti-*Bartonella* antibodies. Among patients with acute undifferentiated febrile diseases in Korea, 23.7% (n = 37) had raised IgG levels against *B. henselae* (≥ 1:160) [34]; 32.4% of them (12/37) had contact with animals and had developed arthralgia (29.5%), headache (25%), or lymphadenopathy (15.9%) [34]. Of the rheumatology clinic patients suffering from unexplained musculoskeletal symptoms examined in the present study, 12 were *B. henselae* IgG seropositive (30%), and ten of these seropositive patients had animals (a cat (5), a dog (4), cows (1)), and all of them had developed arthralgia. Higher seroprevalence was also observed in a study of pediatric patients in Argentina who were suspected to have CSD; it was found that 29 children (31.5%) were IgG (+)/IgM (+) and nine (9.8%) were IgG (+)/IgM (−), which highlights the significant role played by infections caused by *Bartonella* spp. [35]. Elsewhere, IgG antibodies were found in 33.3% of patients with inflammatory conditions of the eye [36], and in 21% of 38 patients awaiting heart transplant, among whom IgM immunoglobulins were also detected in 8% [37]. Anti-*Bartonella* spp. IgG was also identified in 37.5% of immunocompromised individuals with hematologic cancer (n = 51) [38]. All these results suggest that *B. henselae*, and other species from this genus, are important pathogens in immunocompromised patients; in addition, our findings indicate that these species may also play a significant role in patients with musculoskeletal complaints.

The study also examines possible risk factors associated with *Bartonella* infection. The best-known reservoirs of *B. henselae* are cats, though other animals may also be a factor. In a study conducted in Korea, cat ownership was significantly associated with *B. henselae* seropositivity; 9.8% of individuals who had cats showed seropositivity, compared with 2.0% of those without contact with cats [29]. In a Spanish study [27], 31.6% of healthy seropositive individuals reported exposure to cats. In the present study, 11 *B. henselae* cat owners were IgG seropositive (11 of 27 seropositive, 40.7%) and 16 were IgG seronegative, which is not a statistically significant result (p = 0.600). Similarly, dog ownership was not significantly associated with *B. henselae* seropositivity, as also noted previously [29].

There are also other risk factors that may correlate with infections caused by *Bartonella* spp., e.g., occupation. According to some research, farm dwellers were 3.6 times more likely to be IgG seropositive than non-farm individuals [38]. The present study included only two farmers, and one of them was seropositive for *B. henselae* IgG; none of the tested individuals worked as a vet or a forestry worker. It has been found that 60.5% of veterinary personnel in Chile were positive for anti-*B. henselae* IgG antibodies, although no significant relationship was found between IgG seroprevalence and cat scratch or bite [39]. In Spain, 56% of veterinary staff displayed high seroreactivity against *B. v. berkhoffii* genotype III and 11.2% against IgG for *B. quintana* [40]. Elsewhere, antibodies against *B. henselae* were found in only 1.7% of 2975 tested forestry workers in France [41]; in contrast, 41.2% of 722 tested forestry workers in the German state of North Rhine-Westphalia displayed anti-*B. henselae* IgG in 2011–2013 [42]. Interestingly, Muller et al. [43] report anti-*B. quintana* IgG in 23% of hunters (vs 22% in blood donors) and anti-*B. quintana* and anti-*B. henselae* in 2% and conclude that exposure to ticks does not substantially increase the risk of *Bartonella* infection [43]. In Poland, anti-*B. henselae* IgG were identified in 27.7% of farmers, 31.5% of forestry workers (vs 8.9% in the control group), and 3% of ticks; the authors propose a weak positive correlation between the infections of ticks and humans living in the same geographic area [44]. In the present study, 28 subjects reported being bitten by a tick: 14 were seropositive for IgG *B. henselae* and 14 seronegative; this result was not statistically significant (p value = 0.1979), perhaps because of the small number of individuals tested.

Conclusion

This is the first study to compare the seroprevalence of *B. henselae* and *B. quintana* between patients with musculoskeletal complaints and healthy blood donors in Poland. It was found that the patients displayed a higher seroprevalence of antibodies against *B. henselae* than healthy individuals, which indicates that *Bartonella* spp. may be an important pathogen responsible for musculoskeletal symptoms. As these bacteria may cause asymptomatic bacteremia in healthy people, there is a possibility that they may negatively influence the functioning of the musculoskeletal system in the presence of underlying disease or any other immunosuppressive conditions. In addition, the tested blood donors also demonstrated high seroprevalence of *B. henselae* and *B. quintana* antibodies, with positive results for IgM. As blood is typically given to patients who may be immunosuppressed, there is clearly a need for further studies to confirm, or exclude, the need of blood testing for the presence of *Bartonella* spp. As this study is limited by the small number of subjects, such further studies are planned to extend the tested groups and include molecular biology methods in the test procedure.

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Compliance with ethical standards

The study was approved by the Ethics Committee of the Medical University of Lodz, Poland (no. RNN227/16KE;KE/1204/18).

Disclosures None.

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