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CYTOKINES AND MMP-9 LEVELS IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS PATIENTS WITH PERSISTENT PARVOVIRUS B19, HHV-6 AND HHV-7 INFECTION

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Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes erosive changes and ankylosis of joints and may cause internal injuries. Osteoarthritis (OA) is a degenerative process of the articular cartilage. However, inflammatory mediators may play a pivotal role in the initiation and perpetuation of the OA process. It is necessary to continue to study possible factors that may promote the development of the disease. The goal of this study was to evaluate the frequency and activity stage of parvovirus B19 (B19V) and persistent human herpes virus (HHV)-6 and HHV-7 infection in RA and OA patients, and healthy persons, in relation to cytokine levels and presence or absence of viral infections. RA patients with active B19V infection had the highest levels of tumour necrosis factor alpha (TNF-α), which may contribute to the development of RA. In the case of OA, the TNF-α level was higher in patients with active persistent B19V infection, suggesting that B19V reactivation affects also OA. Interleukin (IL)-6, IL-10 and metalloproteinase (MMP)-9 levels were higher in RA patients with latent HHV-6/-7 infection in comparison with active HHV-6/-7 infection, whereas in OA patients levels of all studied cytokines were very variable, ranging from low to high but without significant differences. This suggests that also latent HHV-6 and -7 viral infections can promote development of RA.

Key words: *arthritis, B19V, herpes viruses, cytokines, MMP-9.*

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by progressing aseptic, symmetric polyarthritis, which causes erosive changes and ankylosis of joints. Prolonged and aggressive course of the disease may also cause internal injuries. RA is the most common inflammatory arthritis in adults. It is known that around 0.5–1% of adults worldwide suffer from this disease. Due to various reasons, RA frequently is diagnosed too late, when irreparable changes in joints and outside joints have already occurred, making the use of effective therapy more difficult. As the disease progresses in a patient, gross joint deformations develop, which significantly restrict work and self-care ability. Over time, inflammation may affect almost any joint. There are drugs that act on different mechanisms in pathogenesis of the disease, providing more effective control of the disease course and preventing its progression. Although RA is not completely curable and its treatment is life-long, it is possible to achieve disease remission. Every patient may have different progress of the disease — from mild, slowly progressing arthritis with periodic remissions to aggressive, quickly progressing and very active arthritis with early development of deformations.

Osteoarthritis (OA) is a degenerative arthritis characterised by severe cartilage degeneration and secondary inflammation. The development of OA can be fostered by various factors. A distinction is made between primary OA, the development of which is primarily influenced by hereditary factors, and secondary OA, which develops as a result of other diseases or conditions. The development of secondary OA is facilitated by: various metabolic diseases; traumatic joint damage; anatomical reasons; any inflammatory arthritis and septic arthritis. A persistent infection, such as latent herpesvirus or chronic bacterial infection, can contribute to the development of OA inflammation (Firestein, 2009a).

RA is an autoimmune disease, which is based on immune system disorders. RA develops in genetically predisposed individuals affected by a range of different external and internal factors. Awareness of these factors can facilitate the treatment of RA. Smoking, large consumption of caffeine, cold injury and psycho-emotional or physical stress, as well as different infectious agents, including both bacteria and viruses, are external factors that can promote the disease. The most well-studied viral agents are parvovirus B19 (B19V), rubella virus, human herpes viruses (HHVs) and others. B19V (*Parvoviridae* family, *Parvovirinae* subfamily, *Erythrovirus* genus) is a non-enveloped single-stranded DNA virus found worldwide. Productive B19V infection is restricted to erythroid progenitor cells and its clinical presentation in patients may vary. The most common clinical syndromes of B19V are *erythema infectiosum*, polyarthritis, transient aplastic crisis and pure red cell aplasia, as well as *hydrops fetalis*, while skin lesion, hepatitis, neurological diseases, changes in blood cell composition, as well as rheumatic diseases, including RA, are less common. B19V increases levels of inflammatory cytokines (Feldmann *et al*., 1996; Kerr *et al*., 2001; Isa *et al*., 2006), it activates CD4+ T lymphocytes (Struyk *et al*., 1994, von Poblotzki *et al*., 1996; Isa *et al*., 2006), and participates in the formation of immune complexes (Ahrem *et al*., 1997; Lehmann *et al*., 2002). In addition, B19V stimulates production of cyclooxygenase-2 (COX-2), secretion of matrix metalloproteinase-9 (MMP-9) and prostaglandin E2 in synoviocytes and induces invasiveness of synoviocytes and the degradation of cartilage by activated synoviocytes (Ray *et al*., 2001; Lu *et al*., 2006).

HHV-6 and -7 (*Herpesviridae* family, *Beta-herpesvirinae* subfamily, and *Roseolovirus* genus) are double-stranded DNA viruses. They are frequently found in persons with neuro-inflammatory diseases. The first infection usually occurs in childhood. As the virus does not become fully eliminated, it creates a persistent life-long infection. An injury, physical and emotional stress, hormonal disbalance or immune suppression may contribute to reactivation of the infection. HHV-6A, HHV-6B, and HHV-7 have profound influence on cytokine production by various immune cells. HHV-6 has been demonstrated to upregulate the production of interleukin (IL) -1β and tumour necrosis factor alpha (TNF-α) by PBMCs (Flamand *et al*., 1991; Gosselin *et al*., 1992).

The correlation between B19V, HHV-6 and -7 and the development of RA has been studied for a long time, however, the published data are ambiguous and often contradictory.

The goal of the study was to assess the role of viral infection in RA and OA comparing the frequency of the B19V infection activity stage, as well as HHV-6 and HHV-7 reactivation in patients and healthy persons.

MATERIALS AND METHODS

Patients and controls. The prospective study included patients in the Rīga East Clinical University Hospital, and the Hospital of Traumatology and Orthopaedics in the period from 2010 to 2016. Inclusion criteria were primary or remotely diagnosed RA or OA. All RA patients met the RA classification criteria set by the American College of Rheumatology in 1987 (Arnett *et al*., 1988) or early RA classification criteria set by the American College of Rheumatology and the European League Against Rheumatism in 2010 (Aletaha *et al*., 2010). The exclusion criteria were other inflammatory diseases. OA patients corresponded to the OA classification criteria set by the American College of Rheumatology (Altman *et al*., 1986; 1990., Altman *et al*., 1991). Potentially healthy individuals without known chronic inflammatory diseases were included in the study as a control group.

One hundred and three Caucasian RA patients were included in the study: 13 men (12.9%) and 90 women (87.1%), with average age 56.3 ± 12.8 (ranging from 19 to 82). The number of men among RA patients was significantly less than in the OA patient group ($p = 0.001$; OR 0.3, 95% CI: 0.1 to 0.6) and in the group of healthy control individuals ($p = 0.0047$; OR 0.3, 95% CI: 0.1 to 0.6). RA patients were significantly older compared to healthy individuals ($p = 0.0047$; OR 4.6, 95% CI: 0.1 to 9.1). The average duration of the disease in this group was 93.7 (0–576) months.

The RA patient group included 37 patients with early RA with the duration of symptoms 24 months or less (Emery, 1994) and 66 remotely diagnosed patients.

Since inflammation plays a certain role in OA development and course, causing subhondral bone and cartilage damage (Berenbaum, 2013), OA patients also were included in this study as a second group. Seventy-eight OA patients were examined in the study: 26 Caucasian men (33%) and 52 Caucasian women (67%), with average age 64.6 ± 12.0 (ranging from 35 to 86). OA patients were significantly older than RA patients ($p < 0.0001$; OR 8.3, 95% CI: 4.6 to 11.9) and healthy control individuals ($p < 0.0001$; OR 12.9, 95% CI: 8.4 to 17.4). The average duration of the disease in the group was 78.6 (2–300) months.

Forty-three potentially healthy Caucasian control group individuals without known chronic inflammatory diseases were included in the study. This group included 15 men

(34.9%) and 28 women (65.1%), with average age 51.7 ± 11.9 (ranging from 38 to 89).

The Ethics Committee of the Rīga Stradiņš University had approved the study protocol and all individuals signed an informed consent form before their inclusion in the study.

Sample collection and preparation. All clinical laboratory indicators were determined in the respective clinics in local laboratories. EDTA anti-coagulated peripheral blood samples from RA, OA and apparently healthy persons were collected. Plasma was separated from peripheral blood by centrifugation.

DNA was isolated from whole blood and cell-free blood plasma by standard phenol-chloroform extraction. Before DNA extraction plasma samples were treated with DNase I. To assure the quality of the whole blood DNA and to exclude contamination of plasma DNA by cellular DNA debris a beta-globin PCR was carried out.

Determination of persistent B19 infection and its stage of activity. The presence of B19V genomic sequences was determined using nested PCR (nPCR) as described previously (Barah *et al*., 2001). Presence of the B19V genomic sequence in DNA isolated from cell-free plasma was used as a marker of active infection (viremia), while presence of the B19V genomic sequence in DNA isolated from whole blood was used as a marker of latent persistent infection. The viral load was determined using real-time PCR technology with a TaqMan probe for VP-1 gene, according to the manufacturer's protocol (Roboscreen, Germany).

Determination of B19 specific antibodies. B19V specific IgM class and IgG class antibodies were determined using the ELISA *recom*Well kit (Microgen, Germany), based on the manufacturer's recommendations. The plasma presence of B19V antibodies to certain B19V antigens was reconfirmed using the *recom*Line Parvovirus IgM and IgG test (Microgen, Germany) guided by the manufacturer's recommendations.

Determination of persistent HHV-6 and HHV-7 infection and its stage of activity. One microgram of whole blood DNA as well as 10 µl of plasma DNA were subjected to nPCR with HHV-6 and HHV-7 specific primers as described previously (Secchiero *et al*., 1995; Berneman *et al*., 1992, respectively). Detection of viral DNA in whole blood was used as a marker of persistent infection while detection of viral DNA in cell-free blood plasma as a marker of active infection (virus reactivation). Number of persons with latent infection was calculated as the difference between numbers of patients with persistent and active infection.

Determination of the cytokine level. Levels of MMP-9 and cytokines TNF-α, IL-6, and IL-10 were determined using enzyme-linked immunosorbent assay (ELISA) kits from Nordic Biosite, Denmark (TNF-α, IL-6), R&D system, USA (MMP-9), and Affymetrix eBioscience, USA (IL-10). ELISA's were performed and the results analysed according to manufacturer's instructions.

Statistical analysis. The quantification limit (QL) for assay of cytokines assessment was set as 1 pg/ml. Cytokine levels below QL were uniformly set as values around QL/2 (Beal, 2001; Rogers *et al*., 2018), i.e., in the range of 0.3–0.6 pg/ml. Mean levels of all clinical, laboratory parameters and plasma cytokines and MMP-9 were expressed as medians with variability characterised by the interquartile region (IQR). The distributions of cytokine levels were mostly lognormal and, therefore, are summarized also in natural units as geometric means. Statistical differences in the detection frequency of B19V, HHV-6, and HHV-7 genomic sequences in the extracted DNA samples of the individuals with RA and OA as well as the control group persons were assessed with the Fisher's exact test and T-test. Significant differences between the groups was assessed by a quantitative approach applying the nonparametric Mann Whitney U test or Kruskal–Wallis test with uncorrected Dunn's test as a post-hoc procedure, as well as by a qualitative approach using the Chi-square test to compare proportions of measurements (< 1/> 1). Statistical significance was set at $p < 0.05$.

All analyses and graphing were performed using Prism 8.01 for macOS software (Graph Pad, San Diego, CA, USA).

RESULTS

Presence of B19V infection markers according to *recom*Well, *recom*Line and PCR test. RA patients. Tests were performed for 96 RA patients. Based on *recomWell* and PCR results, the RA patients were divided into five groups: (1) 37 RA patients with remote B19V infection (presence of B19V IgG class antibodies only), (2) 15 RA patients with acute B19V infection (presence of B19V IgM class antibodies and/or viremia), (3) 14 RA patients with B19V latent persistent infection (presence of B19 IgG class antibodies and B19V genomic sequences in blood DNA), (4) 19 RA patients with B19V active persistent infection (presence of B19V IgG class antibodies and viremia) and (5) 11 RA without B19V infection (without B19V specific antibodies and virus-specific DNA).

OA patients. Testing was carried out for 78 OA patients. Based on *recomWell* and PCR results, OA patients were divided into five groups: (1) 44 OA patients with remote B19V infection (presence of B19 IgG class antibodies), (2) 9 OA patients with active persistent B19V infection (presence of B19V IgG class antibodies and viremia), (3) 9 OA patients with latent persistent B19V infection (presence of B19 IgG class antibodies and B19V genomic sequences in blood DNA), (4) 5 OA patients with acute B19V infection (presence of B19V IgM class antibodies and/or viremia) and (5) 11 OA patients without B19V infection.

Antibodies to 4–5 different B19V antigens were found in 59.8% of OA patients. IgG class antibodies against B19V NS-1 were found in 22.1% of OA patients. In the groups of OA patients with different activity stages of B19V infec-

tion, a viral genomic sequence was more commonly found in the case of latent persistent B19V infection ($p = 0.045$).

Healthy individuals. Testing was carried out for 19 control group individuals. Based on *recomWell* and PCR results evaluation 12 control group individuals were with remote B19V infection, one — with active persistent B19V infection, one — with latent persistent B19V infection, one — with acute B19V infection and four — without B19V infection.

In plasma of healthy control group individuals, virus-specific antibodies against no more than four different B19V antigens were found.

Cytokine and MMP-9 levels of RA, OA patients and healthy individuals with different activity stages of B19V infection. RA patients. The MMP-9 level in RA patients with active persistent B19V infection was found to be significantly lower than in RA patients with remote B19V infection ($p = 0.006$, Fig. 1). The TNF-α level in the group of RA patients with remote B19V infection was lower than in the group of RA patients with active persistent B19V infection ($p = 0.05$) and with acute B19V infection ($p = 0.05$). However, in the group of RA patients with acute B19V infection, the TNF-α level was lower compared to the patients with latent persistent B19V infection ($p < 0.05$) and with active persistent B19V infection ($p < 0.05$, Fig. 2). The IL-6 level was lower in RA patients with active persistent B19V infection than in patients without B19V infection ($p < 0.01$), with remote B19V infection ($p < 0.01$) and in the RA patients with acute B19V infection ($p < 0.05$, Fig. 3). The IL-10 level was lower in RA patients without B19V infection than in patients with acute B19V infection ($p < 0.01$), and lower in RA patients with active persistent B19 infection than in patients with remote B19V infection ($p < 0.05$, Fig. 4).

OA patients. A lower MMP-9 level was observed in the group of OA patients with active persistent B19V infection compared to the group without B19V infection ($p < 0.01$) and with remote B19V infection ($p < 0.05$, Fig. 1). A higher TNF-α level was found in OA patients with active persistent B19V infection compared to OA patients with latent persistent B19V infection ($p = 0.01$), remote B19V infection ($p = 0.01$) and without B19V infection ($p = 0.01$). Also, in the OA patients with acute B19V infection, the TNF-α level was higher than in patients with remote B19V infection ($p = 0.05$, Fig. 2). The level of IL-6 was lower in OA patients with acute B19V infection than in patients without B19V infection ($p < 0.05$, Fig. 3). The level of IL-10 in the groups of OA patients did not differ significantly depending on the activity stage of B19V infection.

Healthy individuals. No significant difference in levels of MMP-9 and cytokines was found among control group individuals with different activity stages of B19V infection.

No significant difference in MMP-9 and cytokine levels was found between healthy control group individuals with remote B19V infection and RA patients with remote B19V infection.

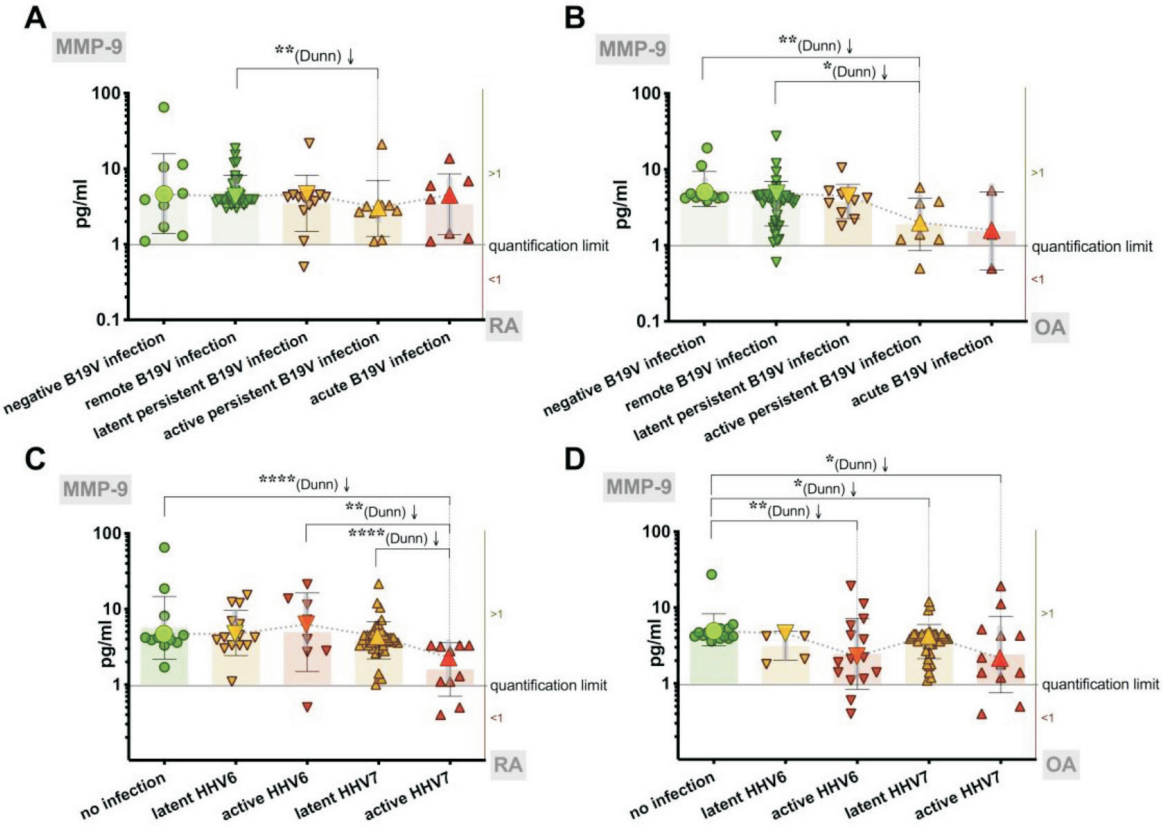

Fig. 1. Level of MMP-9 in RA (A, C) and OA (B, D) patients with various B19V (A, B) and HHV-6 and HHV-7 (C, D) infection activity stages; bars represent geometric mean with geometric SD, gray dotted line connects medians with IQR (bold gray lines), *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

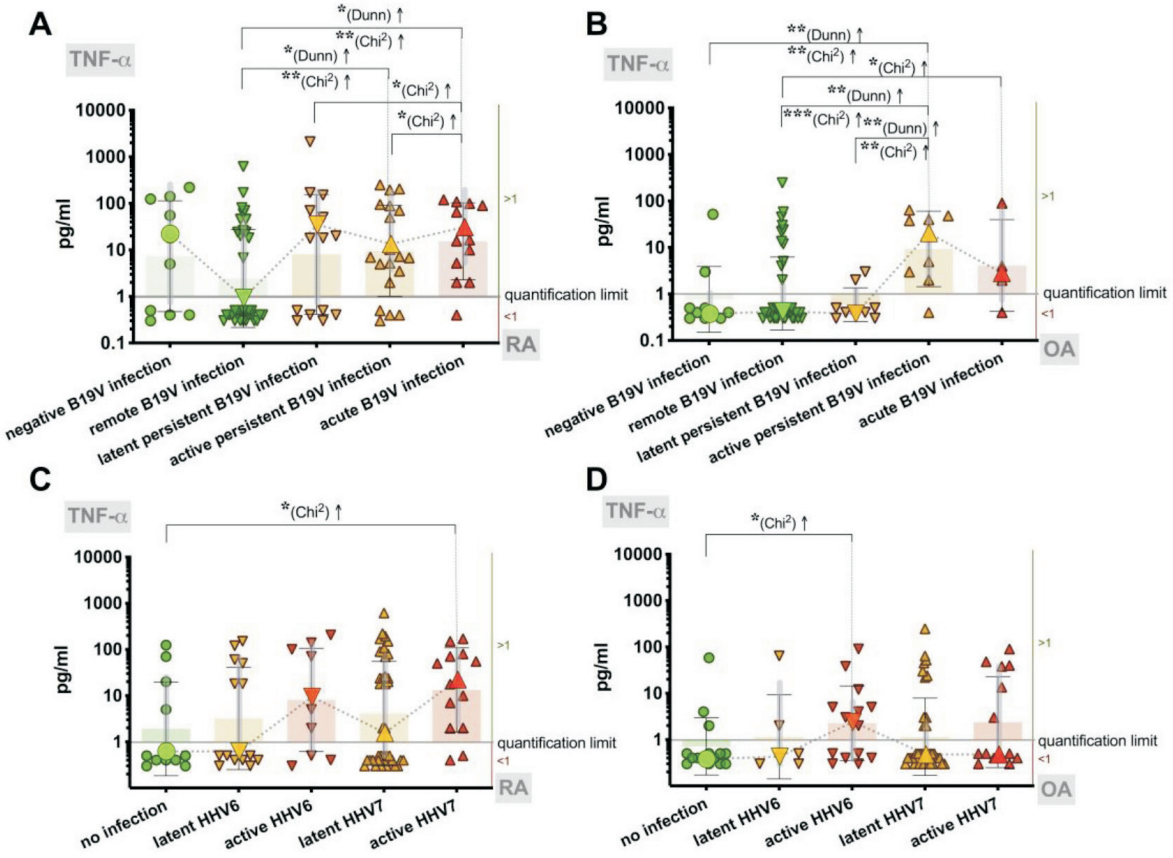

Fig. 2. Level of TNF-α in RA (A, C) and OA (B, D) patients with various B19V (A, B) and HHV-6 and HHV-7 (C, D) infection activity stages; bars represent geometric mean with geometric SD, gray dotted line connects medians with IQR (bold gray lines), $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

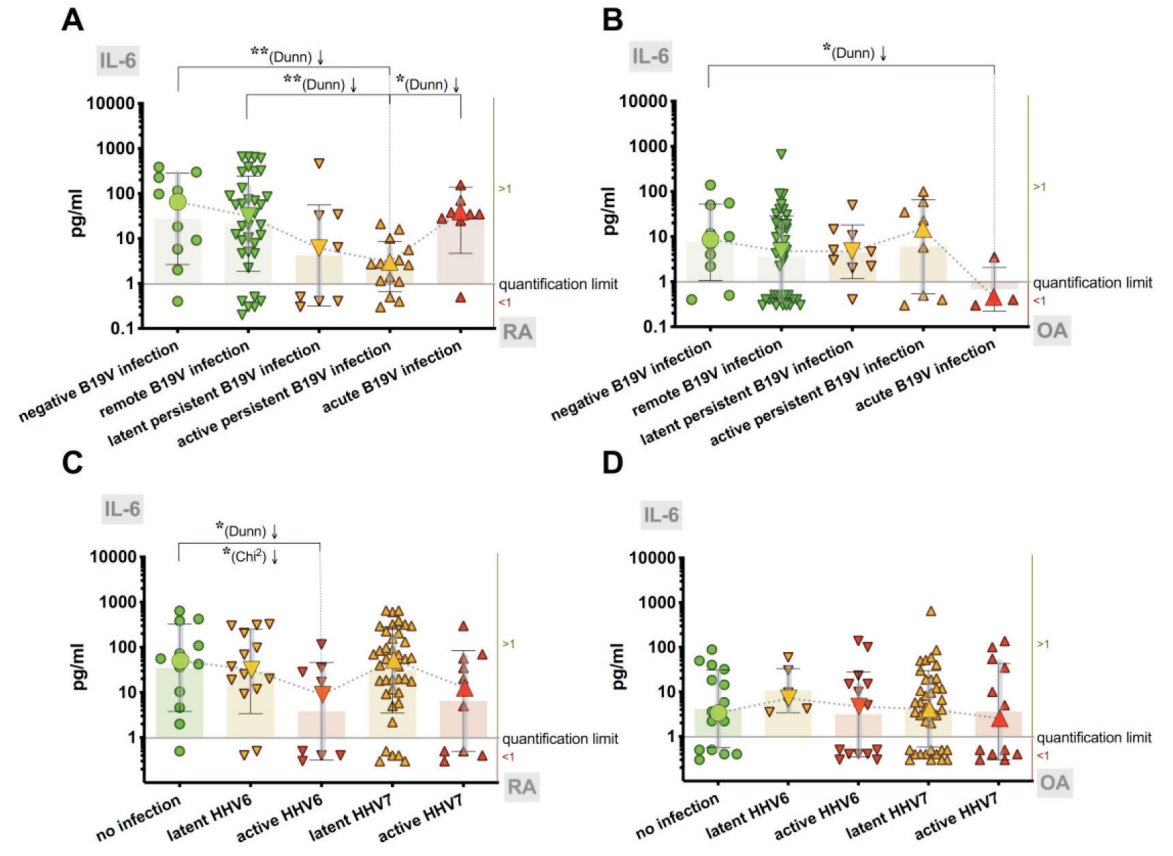

Fig. 3. Level of IL-6 in RA (A, C) and OA (B, D) patients with different B19V (A, B) and HHV-6 and HHV-7 (C, D) infection activity stages; bars represent geometric mean with geometric SD, gray dotted line connects medians with IQR (bold gray lines), $*p < 0.05$, $**p < 0.01$.

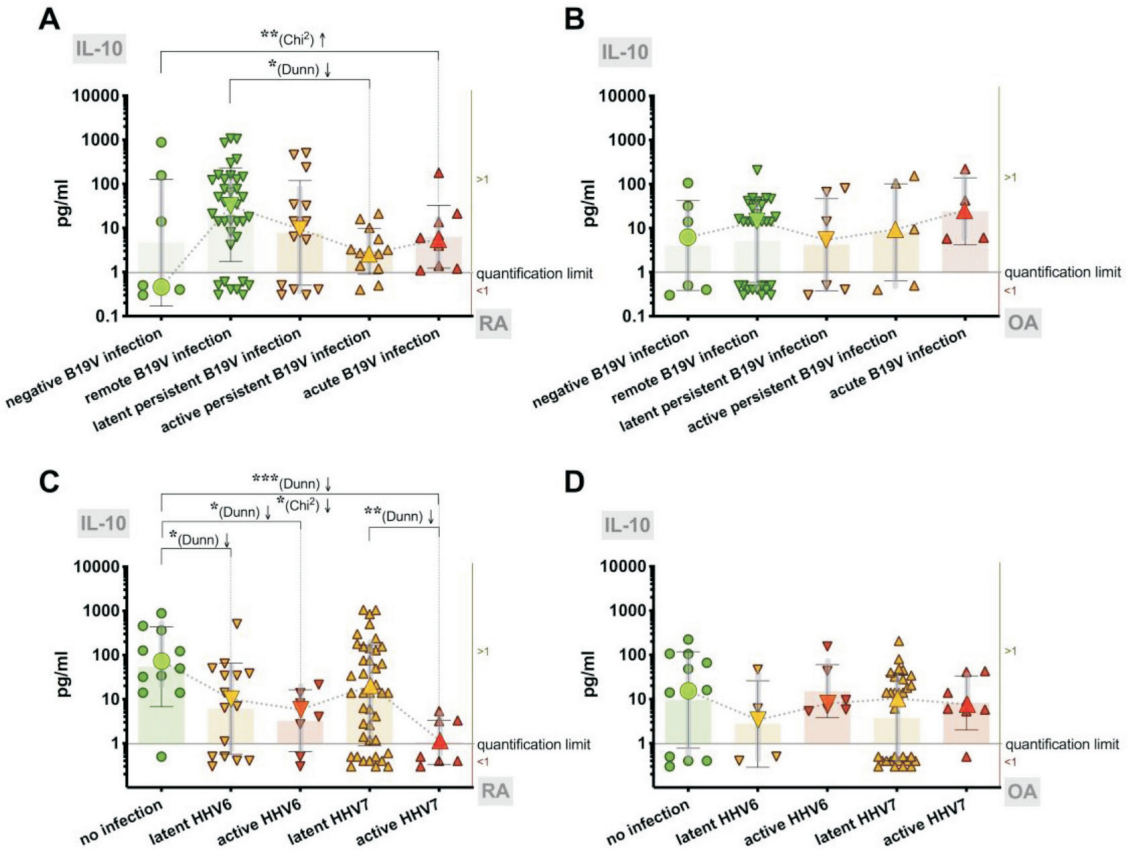

Fig. 4. Level of IL-10 in RA (A, C) and OA (B, D) patients with different B19V (A, B) and HHV-6 and HHV-7 (C, D) infection activity stages; bars represent geometric mean with geometric SD, gray dotted line connects medians with IQR (bold gray lines), $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

No significant differences in MMP-9 and cytokine levels were found between the OA patient group and the healthy control group individuals, in the case when both had remote B19V infection.

HHV-6 and HHV-7 infection markers. RA patients. Persistent HHV-6 and/or persistent HHV-7 infection was found in 67/80 (81.3%) RA patients. Of these cases, 6/67 (9.0%) had single HHV-6 infection, 42/67 (62.7%) — single HHV-7 infection and 19/67 (28.4%) — concurrent HHV-6 and -7 infection. Reactivation of single HHV-6 was found in 10/67 (14.9%) cases with persistent infection, and reactivation of HHV-7 — in 11/61 (18.0%) cases with persistent HHV-7 infection. Two of 19 RA patients with persistent concurrent HHV-6 and HHV-7 infection demonstrated reactivation of both HHV-6 and HHV-7 viruses (10.5%).

Among RA patients persistent HHV-6 mono-infection at latent stage was found in 4/6 (66.7%) patients. Persistent HHV-7 infection at latent stage was found in 31/42 (73.8%) and concurrent persistent HHV-6 and HHV-7 infection at latent stage in 11/19 (57.9%) RA patients.

OA patients. Persistent HHV-6 and/or persistent HHV-7 infection was found in 63/78 (80.8%) OA patients. Among these 63 OA patients, one (1.6%) had HHV-6 infection only, 41/63 (65.1%) — HHV-7 infection only, and 21/63 (33.3%) patients had concurrent HHV-6 and -7 infection.

Reactivation of HHV-6 was found in 16 (25.4%) and reactivation of HHV-7 in 14 of 63 (22.2%) OA patients with persistent HHV-6 and/or HHV-7 infection. In 7/21 (33.3%) OA patients with persistent concurrent HHV-6 and HHV-7 infection, simultaneous reactivation of both viruses were observed.

There were no OA patients with persistent HHV-6 mono-infection at latent stage, but persistent HHV-7 infection at latent stage was found in 34/41 (82.9%) and concurrent persistent HHV-6 and HHV-7 infection at latent stage in 6/21 (28.6%) cases.

There were no OA patients with persistent HHV-6 mono-infection at latent stage, but HHV-7 persistent infection at latent stage was found in 34/41 (82.9%) and concurrent persistent HHV-6 and HHV-7 infection at latent stage in 6/21 (28.6%) cases.

Healthy individuals. Persistent HHV-6 and/or persistent HHV-7 infection was found in 15/19 (78.9%) healthy control group individuals. Of these cases, single HHV-6 infection was not found in any, single HHV-7 infection in 10/15 (66.7%) and concurrent HHV-6 and/or HHV-7 infection in 5/15 (33.3%) individuals.

Reactivation of HHV-6 was observed in 4/15 (26.7%) and reactivation of HHV-7 infection in 3/15 (20.0%) healthy control group individuals. Simultaneous reactivation of both

viruses was found in 1/5 (20.0%) healthy individuals with persistent concurrent HHV-6 and HHV-7 infection.

There were no control group subjects with persistent HHV-6 mono-infection at latent stage, but persistent HHV-7 infection at latent stage was found in 8/10 (80.0%) healthy control group individuals. Persistent infection of both viruses (HHV-6 and HHV-7) at latent stage was found in 1/5 (20.0%) healthy control group subjects.

Cytokine and MMP-9 levels of RA, OA patients and healthy individuals with different activity phase of HHV-6 and HHV-7 infections. RA patients. The mean level of MMP-9 was considerably lower in RA patients with active HHV-7 infection compared to RA patients with latent HHV-7 infection ($p < 0.0001$), patients with active HHV-6 infection ($p = 0.01$) and RA patients without HHV-6 and HHV-7 infection ($p < 0.0001$, Fig. 1). The level of TNF-α was higher in RA patients with active HHV-7 infection than in RA patients without HHV-6 and HHV-7 infection ($p < 0.05$, Fig. 2). The mean level of IL-6 was considerably lower in RA patients with active HHV-6 infection than in patients without HHV-6 and HHV-7 infection ($p < 0.05$, Fig. 3). The mean level of IL-10 was significantly lower in RA patients with active HHV-7 infection compared to RA patients with latent HHV-7 infection ($p < 0.01$). However, the IL-10 level was higher in RA patients without HHV-6 and HHV-7 infection than in patients with active HHV-7 infection ($p < 0.001$), patients with active HHV-6 infection ($p < 0.05$) and in patients with latent HHV-6 infection ($p < 0.05$, Fig. 4).

OA patients. A higher mean MMP level was detected in OA patients without HHV-6 and HHV7 infections compared to OA patients with active HHV-7 infection ($p < 0.05$), with latent HHV-7 infection ($p < 0.05$) and compared to OA patients with active HHV-6 infection ($p < 0.01$, Fig. 1). A higher TNF-α level was occurred in the OA patient group with active HHV-6 infection than in patients without HHV-6 and HHV-7 infection ($p < 0.05$, Fig. 2). The mean level of IL-6 and IL-10 in OA patients at different activity stages of persistent HHV-6 and HHV-7 infection did not significantly differ.

Healthy individuals. No significant difference in MMP-9 and cytokine mean levels was found among healthy control group individuals with different stages of B19V infection activity.

DISCUSSION

Viruses and viral infections are an important risk factor for the development of autoimmune diseases, especially in genetically predisposed persons. Two-thirds of RA patients have been observed to be positive for HLA-DR * 4 (Colmegna and Alberts-Grill, 2009). The development of autoimmune diseases, including connective tissue diseases, multiple sclerosis and Hashimoto thyroiditis, has been associated with HHV-6 A/B infection (Chapenko *et al*., 2003; Nora-Krukle *et al*., 2011; Caselli *et al*., 2012; Hayem and Hayem, 2012; Broccolo *et al*., 2013, Sultanova *et al*., 2017). It has been shown that several viral infections, including B19V, HHV-6 and HHV-7, may trigger the development and progression of arthritis. B19V-infection-induced arthritis often meets RA criteria (Kerr, 2000; Colmegna and Alberts-Grill, 2009; Khoqueer, 2009) and prolonged B19V viremia may contribute to the development of pronounced and prolonged arthritis (Ogawa *et al*., 2008). B19V-induced arthritis is similar to RA progression with symmetrical small joint damage (Kerr, 2000; Takasawa *et al*., 2004; Franssil and Hedman, 2006).

Several studies are being conducted to reconfirm the response of cells to B19V antigens and potential expression of the B19V genome in patients (Takahashi *et al*., 1998; Murai *et al*., 1999; Stahl *et al*.; 2000; Kozireva *et al*., 2008). Although the B19V-specific immune response of cells is being studied, there have been several studies on the development of response in cases of acute and persistent B19V infection. The role of persistent B19V infection in the development of RA is shown by high incidence of B19V infection among RA patients, lack of anti-B19 IgM class antibodies in most RA patients with viremia and presence of anti-B19 IgG class antibodies in these patients, frequent finding of anti-B19 NS-1 IgG class antibodies in RA patients compared to healthy individuals, and relatively low viral load in RA patients with viremia (von Poblotzki *et al*., 1995; Hemauer *et al*., 2000; Kerr and Cunniffe, 2000; Lee *et al*., 2011).

Some reports suggest that B19V infection also increases the level of IL-6 (Tzang *et al*., 2009; Kerr, 2016;). Naciute and colleagues (2016) showed association of IL-6 with higher disease activity by the Disease Activity Score 28 (DAS28) and C reactive protein (CRP). Our data also suggest that B19V can alter the aggressiveness of RA and promote its progression. The overall aggressiveness of the disease estimated by the level of rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) has been shown to be higher in RA patients with acute B19V infection than in patients without B19V infection and in patients with remote B19V infection.

B19V promotes the production of various cytokines (Kerr *et al*., 2004) and inflammatory cytokines and particularly TNF-α plays an important role in the development and progression of RA. In patients with acute and active persistent B19V infection the level of TNF-α is significantly higher than in patients with remote B19V infection. In patients with latent persistent B19V infection and active persistent B19V infection, the level of TNF-α is higher than in RA patients with acute B19V infection. Both Kerr (2001) and Barash *et al* (2003) found that B19V infection at its acute stage and subsequently may increase the level of TNF-α, thus promoting the development of RA after acute B19V infection or reactivation of persistent B19V infection. The detected MMP-9 level is significantly lower in RA patients with active persistent B19V infection than in patients with remote B19V infection, although Tzang *et al*. (2009) re-

vealed an increase of MMP-9 activity under the influence of B19-VP1u. Some reports suggest that B19V infection also increases the level of IL-6 (Tzang *et al*., 2009; Kerr, 2016; Naciute *et al*., 2016). Isa *et al.* (2007) observed that early after acute B19V infection, the level of IL-12 increases, then IL-2 increases, while IL-10 is low. In our study, the mean level of IL-6 level was lower in RA patients with active persistent B19V infection than in RA patients without B19V infection, with remote B19V infection and in patients with acute B19V infection. The different results may be explained by the treatment received in distinct patient groups. The IL-12 level does not significantly differ in patients with different activity stages of B19V infection.

B19V is able to influence the synthesis of cytokines: it has been shown that the non-structural NS-1 protein of B19V activates the expression of pro-inflammatory cytokines (IL-6 and TNF-α) in cells (Moffatt *et al*., 1996; Fu *et al*., 2002; Hsu *et al*., 2006) and the recombinant B19V VP-1u protein increases the expression of IL-6 and IL-1β mRNA in macrophages (Tzang *et al*., 2009). It is possible that the properties of this B19V in genetically predisposed individuals in the case of persistent B19V infection affect the development of RA.

In OA patients, a considerably higher mean level of MMP-9 was found in the groups without B19V infection and with remote B19V infection. A higher TNF-α level occurred in OA patients with active persistent B19V infection compared to OA patients with latent persistent B19V infection, remote B19V infection and without B19V infection, and a higher level also occurred in OA patients with acute B19V infection compared to OA patients with remote B19V infection. The level of IL-6 was lower in OA patients with acute B19V infection than in patients without B19V infection. Aslan *et al*. (2008) observed a higher IL-6 level in OA patients, depending on the degree of OA damage in the joint. Our results would likely have differed if we evaluated OA patients according to OA structural damages. The IL-10 level did not significantly differ in groups of OA patients depending on the activity stage of B19V infection. The data showed that active persistent and acute B19V infection are associated with higher TNF-α level and B19V levels, but not with production of cytokines in the OA patients. No significant difference in MMP-9 and cytokine levels was found among healthy control group individuals with different stages of B19V infection determined according to the *recomWell* test.

B19V may alter plasma cytokine levels in RA patients and healthy subjects (Naciute *et al*., 2016; Naciute *et al*., 2017). In our study no significant differences in MMP-9, IL-6, IL-10, and TNF-α level were found between healthy control group individuals with remote B19V infection and RA patients with remote B19V infection. Furthermore, a higher IL-10 level occurred in the group of RA patients with remote B19V infection than in the group of healthy individuals with remote B19V infection. This indicated that factors other than B19V infection can affect the IL-10 level.

No significant differences in cytokine levels were found between the OA patient group and healthy control group individuals with remote B19V infection, in both studied groups.

In RA patients, the presence of HHV-6 genome and increased viral loads can be statistically significantly higher than in healthy controls (Alvarez-Lafuente *et al*., 2005). Broccoli *et al.* (2013) also found that HHV-6 genomic sequences from cell-free serum (viremic) and anti-HHV-6 IgG antibodies were significantly more common in connective tissue diseases, including RA, compared to healthy control subjects. We did no observe significant differences in reactivation of persistent HHV-6 and HHV-7 infection, as well as reactivation of HHV-6 and HHV-7 in groups of RA and OA patients, and healthy individuals. In the chronic inflammation process, lymphocytes containing HHV-6 and HHV-7 genome sequences are found in the joint, which suggest their potential role in the development of the disease.

The mean level of IL-6 was significantly lower in RA patients with active HHV-6 infection than in patients without HHV-6 infection. The mean level of TNF-α was higher in RA patients group with active HHV-7 infection than in patients without HHV-6 and HHV-7 infection, but active and latent HHV-6 infection and active HHV-7 infection down regulate IL-10. The mean level of MMP-9 was considerably lower in RA patients with active HHV-7 infection compared to RA patients with latent HHV-7 infection, with active HHV-6 infection and without HHV-6 and HHV-7 infection. The data indicate that also latent infection, not only active infection, can affect the course of RA. It was shown previously that HHV-6 increased the production of inflammatory cytokines like IFN-α, TNF-α, IL-1β, IL-8 and IL-15 and down regulated IL-2 and IL-10 (Lusso, 2006). This is in line with our research data.

Among the cytokines and MMP-9 in OA patients at different activity stages of persistent HHV-6 and HHV-7 infection, only the TNF-α level was higher in patients with active HHV-6 infection than in patients without HHV-6 and HHV-7 infection, while the MMP-9 level is higher in patients without HHV-6 and HHV-7 infection than in patients with active HHV-6 or HHV-7 and latent HHV-7 infection. The data indicate a negligible role of these viruses in the course of OA.

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REFERENCES

Ahrem, M. J., Smith, M. D. (1997). Rheumatoid arthritis. *Med. J. Austral.,* **166**, 156–61.

Aletaha, D., Neogi, T., Silman, A., Funovits, J., Felson, D., et al. (2010). Rheumatoid arthritis classification criteria: An American College of

Rheumatology/European League against Rheumatism Collaborative Initiative. *Arthritis Rheum*., **62**, 2569–2581.

Altman, R., Asch, E., Bloch, D., Bole, G., Borenstein, D., Brandt, K., et al. (1986). The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the knee. *Arthritis Rheum*., **29**, 1039–1049.

Altman, R., Alarcón, G., Appelrouth, D., Bloch, D., Borenstein, D., Brandt, K., et al. (1990). The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hand. *Arthritis Rheum*., **33**, 1601–1610.

Altman, R., Alarcón, G., Appelrouth, D., Bloch, D., Borenstein, D., Brandt, K., et al. (1991). The American College of Rheumatology criteria for the classification and reporting of osteoarthritis of the hip. *Arthritis Rheum*., **34**, 505–514.

Alvarez-Lafuente, R., Fernández-Gutiérrez, B., de Miguel, S., Jover, J. A., Rollin, R., Loza, E., Clemente, D., Lamas, J. R. (2005). Potential relationship between herpes viruses and rheumatoid arthritis: Analysis with quantitative real time polymerase chain reaction. *Ann. Rheum. Dis*., **64** (9), 1357–1359.

Arnett, F., Edworthy, S., Bloch, D., et al. (1988). The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum*., **31**, 315–324.

Aslan, B., Serin, M. S., Aslan, G., Kalaci, A., Yanat, A. N., Tezcan, S., Emekdas, G. (1988). Detection of parvovirus B19 in synovial fluids of patients with osteoarthritis. *Diagn. Microbiol. Infect. Dis*., **60** (4), 381–385.

Barah, F., Vallely, P., Chiswick, M., Cleator, M., Kerr, J. (2001). Association of human parvovirus B19 infection with acute meningoencephalitis. *Lancet*, **358**, 729–730.

Barash, J., Dushnitzki, D., Barak, Y., Miron, S., Hahn, T. (2003). Tumor necrosis factor (TNF) alpha and its soluble receptor (sTNFR) p75 during acute human parvovirus B19 infection in children. *Immunol. Lett*., **88**, 109–112.

Beal, S. (2001). Ways to fit a PK model with some data below the quantification limit. *J. Pharmacokin. Pharmacodyn.*, **28** (5), 481–504.

Berenbaum, F. (2013). Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthritis Cartilage*, **21** (1), 16–21.

Broccolo, F., Drago, F., Cassina, G., Fava, A., Fusetti, L., Matteoli, B., Ceccherini-Nelli, L., Sabbadini, M. G., Lusso, P., Parodi, A., Malna, M. S. (2013). Selective reactivation of human herpesvirus 6 in patients with autoimmune connective tissue diseases. *J. Med. Virol*., **85**, 1925–1934.

Broccolo, F., Fusetti, L., Ceccherini-Nelli, L. (2013). Possible role of human herpesvirus 6 as a trigger of autoimmune disease. *Sci. World J.,* **24**, Article ID 867389.

Caselli, E., Zatelli, M. C., Rizzo, R., Benedetti, S., Martorelli, D., Trasforini, G., Cassai, E., degli Uberti, E. C., Di Luca, D., Dolcetti, R. (2012). Virologic and immunologic evidence supporting an association between HHV-6 and Hashimoto's thyroiditis. *PLoS. Path.*, **8** (10), e1002951.

Chapenko, S., Millers, A., Nora, Z., Logina, I., Kukaine, R., Murovska, M. (2003). Correlation between HHV-6 reactivation and multiple sclerosis disease activity. *J. Med. Virol*., **69** (1), 111–117.

Colmegna, I., Alberts-Grill, N. (2009). Parvovirus B19: Its role in chronic arthritis. *Rheum. Dis. Clin. North. Amer*., **35** (1), 95–110.

Emery, P. (1994). The Roche Rheumatology Prize Lecture. The optimal management of early rheumatoid disease: The key to preventing disability. *Brit. J. Rheumatol*., **33** (8), 765–768.

Feldmann, M., Brennan, F. M., Maini, R. N. (1996). Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol*., **14**, 397–440.

Firestein, G. S. (2009). Rheumatoid arthritis. In: Harris, E. D., Budd, R. C., Genovese, M. C., Firestein, G. S., Sargent, J. S., Sledge, C. B. (eds.). *Kelley's Textbook of Rheumatology*. 8th ed. Saunders Elsevier, Philadelphia, Pa, chap. 9.

Flamand, L., Gosselin, J., D'Addario, M., Hiscott, J., Ablashi, D. V., Gallo, D. C., Menezes, J. (1995). Human herpesvirus 6 induces interleukin-1beta and tumor necrosis factor alpha, but not interleukin-6, in peripheral blood mononuclear cell cultures. *J. Virol*., 65, 5105–5110.

Flamand, L., Komaroff, A. L., Arbuckle, J. H., Medveczky, P, G, Ablashi, D, V. (2010). Review, part 1: Human herpesvirus-6-basic biology, diagnostic testing, and antiviral efficacy. *J. Med. Virol.,* **82** (9), 1560–1568.

Franssila, R., Hedman, K. (2006). Infection and musculoskeletal conditions: Viral causes of arthritis. *Best Pract. Res. Clin. Rheumatol*., **20** (6), 1139–1157.

Fu, Y., Ishii, K. K., Munakata, Y., Saitoh, T., Kaku, M., Sasaki, T. (2002). Regulation of tumor necrosis factor alpha promoter by human parvovirus B 19 NS1 through activation of AP-1 and AP-2. *J. Virol.,* **76**, 5395–5400.

Gibofsky, A. (2012). Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. *Amer. J. Manag. Care*, **18** (13), S295–S,302.

Gosselin, J., Flamand, L., D'Addario, M., Hiscott, J., Stefanescu, I., Ablashi, D. V., Gallo, R. C., Menezes, J. (1992). Modulatory effects of Epstein–Barr, herpes simplex, and human herpes-6 viral infections and coinfections on cytokine synthesis. A comparative study. *J. Immunol.*, **149**, 181–187.

Hayem, F., Hayem, G. (2012). Still's disease and the mitochondrion: The other face of an old friend? *Med. Hypotheses*, **79** (2), 136–137.

Hemauer, A., Gigler, A., Searle, K., Raab, U., Broliden, K., Wolf, H., Enders, G., Modrow, S. (2000). Seroprevalence of parvovirus B19-infected and uninfected individuals, and in infected pregnant women. *J. Med. Virol*., **60**, 48–55.

Hsu, T. C., Tzang, B. S., Huang, C. N., Lee, G. J., Lin, G. Y., Chen, M. C., Tsay, G. J. (2006). Increased expression and secretion of interleukin-6 in human parvovirus B19 non-structural protein (NS1) transfected COS –7 epithelial cells. *Clin. Exp. Immunol*., **144**, 152–157.

Isa, A., Lundqvist, A., Lindblom, A., Tolfvenstam, T., Broliden, K. (2007). Cytokine responses in acute and persistent human parvovirus B19 infection. *Clin. Exp. Immunol*., **147**, 419–425.

Isa, A., Norbec, O., Hirbod, T., Lundquist, A., Kasprowicz, P., Bowness, P., Broliden, K., Tolfvenstam. T. (2006). Aberrant cellular immune responses in humans infected persistently with parvovirus B19. *J. Med. Virol*., **78**, 129–133.

Kerr, J. R. (2000). Pathogenesis of human parvovirus B19 in rheumatic disease. *Ann. Rheum. Dis*., **59**, 672–683.

Kerr, J. R. (2016). The role of parvovirus B19 in the pathogenesis of autoimmunity and autoimmune disease. *J. Clin. Pathol*., **69** (4), 279–291.

Kerr, J. R., Cunniffe, V. S. (2000). Antibodies to parvovirus B19 non-structural protein are associated with chronic but not acute arthritis following B19 infection. *Rheumatology*, **39**, 903–908.

Kerr, J. R., Cunniffe, V. S., Kelleher, P., Coats, A. J., Mattey, D. L. (2004). Circulating cytokines and chemokines in acute symptomatic parvovirus B19 infection: Negative association between levels of pro-inflammatory cytokines and development of B19-associated arthritis. *J. Med. Virol*., **74** (1), 147–155.

Kerr, J. R., Barah, F., Mattey, D. L., Laing, I., Hopkins, S. J., Hutchinson, I., Tyrell, D. A. (2001). Circulating tumour necrosis factor–α and interferon–γ are detectable during acute and convalescent parvovirus B19 infection and are associated with prolonged and chronic fatigue. *J. Gen. Virol*., **82**, 3011–3019.

Khouqeer, R. A. (2017). Viral Arthritis. Available from: http://emedicine.medscape.com/article/335692-overview (accessed 04.04.2019).

Kozireva, S., Zestkova, J., Mikazane, H., Kadisa, A., Kakurina, N., Lejnieks, A., Danilane, I. N., Murovska, M. F. (2008). Incidence and clinical significance of parvovirus B19 infection in patients with rheumatoid arthritis. *J. Rheumatol*., **35**, 1265–1270.

Kumar, A., Perdomo, M. F., Kantele, A., Hedman, L., Hedman, K., Franssila, R. (2015). Granzyme B mediated function of parvovirus B19-specific CD4+ T cells. *Clin. Transl. Immunol.,* **4** (7), e39.

Lee, T. H., Kleinman, S. H., Wen, L., Montalvo, L., Todd, D. S., Wright, D. J., Tobler, Busch, M. P. (2011). Distribution of parvovirus B19 DNA in blood compartments and persistence of virus in blood donors. *Trasfusion*, **51**, 1896–1908.

Lehmann, H. W., Kuhner, L., Beckenlehner, K., Muller-Godeffroy, E., Heide, K. G., Kuster, R., Modrow, S. (2002). Chronic human parvovirus B19 infection in rheumatic disease of children and adolescence. *J. Clin. Virol*., **25**, 135–143.

Lu, J., Zhi, N., Wong, S., Brown, K. E. (2006). Activation of synoviocytes by the secreted phospholipase A2 motif in the VP1-unique region of parvovirus B19 minor capsid protein. *J. Infect. Dis*., **193**, 582–590.

Lusso, P. (2006). HHV-6 and the immune system: Mechanisms of immunomodulation and viral escape. *J. Clin. Virol.*, **37**, S4–S10.

Moffatt, S., Tanaka, N., Tada, K., Nose, M., Nakamura, M., Muraoka, O., et al. (1996). A cytotoxic on-structural protein, NS1, of human parvovirus B19 induces activation of interleukin–6 gene expression. *J. Virol*., **70**, 8485–8491.

Murai, C., Munakata, Y., Takahashi, Y., Ishii, T., Shibata, S., Murryoi, T., Funato, T., Nakamura, M., Sugamure, K., Sasaki, T. (1999). Rheumatoid arthritis after parvovirus B 19 infection. *Ann. Rheum. Dis*., **58**, 130–132.

Naciute, M., Mieliauskaite, D., Rugiene, R., Maciunaite, G., Mauricas, M., Murovska, M., Girkontaite, I. (2017). Parvovirus B19 infection modulates the levels of cytokines in the plasma of rheumatoid arthritis patients. *Cytokine*, **96**, 41–48.

Naciute, M., Mieliauskaite, D., Rugiene, R., Nikitenkiene, R., Jancoriene, L., Mauricas, M., Nora-Krukle, Z., Murovska, M., Girkontaite, I. (2016). Frequency and significance of parvovirus B19 infection in patients with rheumatoid arthritis. *J. Gen. Virol*., **97** (12), 3302–3312.

Nora-Krukle, Z., Chapenko, S., Logina, I., Millers, A., Platkajis, A., Murovska, M. (2011). Human herpesvirus 6 and 7 reactivation and disease activity in multiple sclerosis. *Medicina* (Kaunas), **47** (10), 527–531.

Ogawa, E., Otaguro, S., Murata, M., Kainuma, M., Sawayama, Y., Furusyo, N., Hayashi, J. (2008). Intravenous immunoglobulin therapy for severe arthritis associated with human parvovirus B19 infection. *J. Infect. Chemother*., **14** (5), 377–382.

Von Poblotzki, A., Gerdes, C., Reischl, U., Wolf, H., Modrow, S. (1996). Lymphoproliferative responses after infection with human parvovirus B19. *J Virol*., **70**, 7327–7330.

Von Poblotzki, A., Gigler, A., Lang, B., Wolf, H., Modrow, S. (1995). Antibodies to parvovirus B19 NS1 protein in infected individuals. *Gen. Virol*., **76**, 19–27.

Ray, N. B., Nieva, D. R., Seftor, E. A., Khalkhali-Ellis, Z., Naides, S. J. (2001). Induction of an invasive phenotype by human parvovirus B19 in normal human synosial fibroblasts. *Arthr. Rheum*., **7**, 1582–1586.

Rogers, C., Scott, L., Reeves, B., Downes, S., Lotery, A., Dick, A., Chakravarthy U. (2018). Serum vascular endothelial growth factor levels in the IVAN trial; Relationships with drug, dosing, and systemic serious adverse events. *Ophthalmol, Retina*, **2** (2), 118–127.

Stahl, H. D., Pfeiffer, R., von Salis-Soglio, G., Emmrich, F. (2000). Parvovirus B19 associated mono-and oligoarticular arthritis may evolve into a chronic inflammatory arthropathy fulfilling criteria for rheumatoid arthritis or spondylarthropathy. *Clin. Rheumatol*., **19**, 510–511.

Struyk, L., Hawes, G. E., Dolhain, R. J., van Scherpenzeel, A., Godthelp, B., Breedveld, F. C. (1994). Evidence for selective *in vivo* expansion of synovial tissue-infiltrating $CD4^+$ $CD45RO^+$ T lymphocytes on the basis of CDR3 diversity. *Int. Immunol*., **6**, 897–907.

Sultanova, A., Cistjakovs, M., Gravelsina, S., Chapenko, S., Roga, S., Cunskis, E., Nora-Krukle, Z., Groma, V., Ventina, I., Murovska, M. (2017). Association of active human herpesvirus-6 (HHV-6) infection with autoimmune thyroid gland diseases. *Clin. Microbiol. Infect.*, **23** (1), 50e1–50e5.

Takahashi, Y., Murai, C., Shibata, S, Munakata, Y., Ishii, T., Ishii, K., Saitoh, T., Sawai, T., Sugamura, K., Sasaki, T. (1998). Human parvovirus B19 as a causative agent for rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA*, **95**, 8227–8232.

Takasawa, N., Munakata, Y., Ishii, K. K., Takahashi, Y., Takahashi, M., Fu, Y., Ishii, T., Fujii, H., Saito, T., Takano, H., Noda, T., Suzuki, M., Nose, M., Zolla-Patzner, S., Sasaki, T. (2004). Human parvovirus B19 transgenic mice become susceptible to polyarthritis. *J. Immunol*., **173** (7), 4675–4683.

Tzang, B., Chiu, C., Tsai, C., Lee, Y., Lu, I., Shi, J., Hsu, T. (2009). Effects of human parvovirus B19 VP1 unique region protein on macrophage responses. *J. Biomed. Sci*., **16**, 13.

Yoshikawa, T., Fujita, A., Yagami, A., Suzuki, K., Matsunaga, K., Ihira, M., Asano, Y. (2006). Human herpesvirus 6 reactivation and inflammatory cytokine production in patients with drug-induced hypersensitivity syndrome. *J. Clin. Virol.,* **37**, S92–S96.

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CITOKĪNU UN MMP-9 LĪMENIS REIMATOĪDĀ ARTRĪTA UN OSTEOARTRĪTA PACIENTIEM AR PERSISTENTU PARVOVĪRUSA B19, HHV-6 UN HHV-7 INFEKCIJU

Reimatoīdais artrīts (RA) ir hroniska autoimūna slimība, kas izraisa erozīvas izmaiņas un ankilozi locītavās un var izraisīt arī iekšējo orgānu bojājumus. Osteoartrīts (OA) ir locītavu skrimšļa deģeneratīvs process, taču iekaisuma mediatoriem var būt būtiska loma OA procesa uzsākšanā un uzturēšanā. Neraugoties uz daudziem ilgtermiņa pētījumiem, precīzs šo slimības izraisītājs nav zināms, tāpēc ir jāturpina pētīt iespējamie faktori, kas var veicināt šo slimību attīstību. Šī pētījuma mērķis bija novērtēt parvovīrusa B19 (B19V) un, persistentas cilvēka herpes vīrusa (HHV)-6 un HHV-7 infekcijas sastopamību un aktivitātes stadiju RA un OA pacientiem, kā arī veseliem cilvēkiem, un analizēt citokīnu un metalloproteināzes (MMP)-9 līmeņa izmaiņas atkarībā no vīrusa klātbūtnes vai neesamības. RA pacientiem ar aktīvu B19V infekciju ir visaugstākais tumora nekrozes faktora alfa (TNF-α) līmenis, kas var veicināt RA attīstību. OA gadījumā TNF-α līmenis ir augstāks pacientiem ar aktīvu persistentu B19V infekciju, un tas liecina, ka B19V reaktivācija ietekmē arī OA attīstību un klīnisko gaitu. IL-6, IL-10 un MMP-9 līmenis augstāks ir RA pacientiem ar latentu HHV-6/-7 infekciju, salīdzinot ar aktīvu HHV-6/-7 infekciju, bet OA pacientiem visu noteikto citokīnu līmenis būtiski neatšķiras. Tas liecina, ka arī latenta HHV-6 un -7 vīrusu infekcija var veicināt RA attīstību**.**