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RĪGAS STRADIŅA UNIVERSITĀTE

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Significance of human parvovirus B19 infection in the etiopathogenesis of rheumatoid arthritis and possible correlations with the disease activity, stage and clinical course

Abstract of doctoral thesis
Speciality – internal diseases

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SIGNIFICANCE OF HUMAN PARVOVIRUS B19 INFECTION IN THE ETIOPATHOGENESIS OF RHEUMATOID ARTHRITIS AND POSSIBLE CORRELATIONS WITH THE DISEASE ACTIVITY, STAGE AND CLINICAL COURSE

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The doctoral thesis is available at the library of Riga Stradins University and on the website of RSU - www.rsu.lv

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Secretary of the Promotion Council 

Scientific relevance

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by a symmetrical polyarthritis and systemic damage to the internal organs. RA is the most common form of inflammatory arthritis. Statistically, it is diagnosed in approximately 0.5-1% of the adult population in the United States and Europe. According to the calculations performed by the Latvian Association of Rheumatologists, approximately 25.000 individuals or 1% of the population in Latvia are affected by RA. At its early stages RA affects cartilage, small and middle-sized bone joints, spreading onto the larger bone joints, internal organs and systems, such as lungs, vessels and the haemopoietic system. Local inflammatory cells invade the relatively acellular synovium, which leads to its hyperplasia and the formation of pannus and this, respectively, leads to the destruction of cartilage, erosion of bone tissues, decreasing the functional capacity of the affected joints. At the same time, systemic inflammatory process strikes the internal organs, increasing the risk of the development of atherosclerosis and malignant tumors (lymphomas for instance). The inevitable risk of multiple negative factors caused by the prolonged anti-rheumatic drug usage periods, early disability, social disadaptation and lifespan reduction for almost 7 years, places the RA among the most notorious social and economic problems. From a clinical perspective, judging both from the clinical manifestations and the disease progression, RA is a heterogeneous disease. In most cases, the classical clinical manifestations of RA are observed; however, there are cases, where the disease "overlaps" with other rheumatic diseases. Moreover, sometimes RA is observed in the association with autoimmune diseases of a different type. Sjorgen's syndrome and Hashimoto's thyroiditis are the typical examples. According to the reasons yet unknown, the course of RA is very variable, it ranges from mild, non-erosive forms with spontaneous remissions, to severe, rapidly progressing and destructive cases. Judging by the results of genetic risk factor analysis and autoantibody production analysis, it is possible to confirm the existence of the pathogenically distinct subgroups of RA, which require personalized treatment strategies. Processes, which qualify as the pathogenesis of RA include: inflammation, autoimmunity, nociperception, angiogenesis, matrix destruction, tissue remodulation and rejuvenation. Depression, changes in the cognitive functions, increased incidence of cardiovascular disease morbidity and mortality, insulin resistance, osteoporosis, cachexia and the classical extraarticular manifestations of RA are the typical signs of the development of systemic processes in RA cases suggesting multigenic and exogenic interactions, which have an effect on the condition of patients. The treatment of RA is based on the timely diagnosis of the disease and of its co-diseases. An interdisciplinary approach is mandatory. During the last 10 years, so-called biologic drugs, which are used in the different

stages of RA were introduced into the treatment process of the disease. Despite the presence of positive results in some cases, long-term observations weren't performed. Thus, currently there is a lack of information about the possible side-effects of the biologic drugs treatment. Furthermore, a great caution is raised by the number of malignant neoplasm, tuberculosis and other severe infection cases, which seem to develop in a greater scale under the use of biologic drugs. From an immunologic perspective, RA is an autoimmune disorder which is caused by the immunologic tolerance disruptions at a certain point in a patient's life. RA develops in a genetically predisposed individual as a result of autoimmune process activity, involving a chain of exogenous factors. However, the trigger, which initiates the disruptions in immunologic tolerance, is not yet clear. The rates of RA morbidity in different ethnic groups, the diversity of the disease activity paths, the variety of clinical, radiologic and laboratory data prove the existence of multiple, both exogenous and endogenous factors, which affect the initial and further development of RA. The most important and the most often suited fields in connection with RA are: the genetic variations, autoantibodies, cell immunity, hormonal background and the interactions between genetic risk factors and environmental variables. The results of these studies are most often discrepant and point out the necessity of further studies into the subject. Bacteria (mycoplasma, mycobacteria) and viruses (parvovirus, retrovirus, measles, Epstein-Barr virus and other herpes viruses) are the factors debated to be the cause of the immunologic tolerance disorders. This, of course, includes the human parvovirus B19 (B19V). The B19 virus was discovered in 1975 in a donor's blood. It is a single-stranded DNA virus commonly spread all over the world. B19V is pathogenic only for human beings – the viral tropism is aimed at the fissile erythrocyte precursors. The clinical picture of B19V varies according to age and immunologic/ hematologic conditions. The clinical syndromes, which are usually associated with B19V infection, are the following: transient aplastic crisis, *Erythema infectiosum*, polyarthritis, *hydrops fetalis* and pure red cell aplasia; in rare cases: skin diseases, hematologic changes, hepatitis, neurologic and rheumatologic diseases, as well as RA. Despite the number of scientific studies devoted to the research of the interactions between B19V infection and RA published in the recent years and at the end of the 20th century [White, 1985; Cohen, 1986; Klouda, 1986; Naides, 1990; Nikkari, 1994; Kerr, 1995, 1996; Lunardi, 1998; Takahashi, 1998; Altschuler, 1999; Murai, 1999; Ray, 1999; Kerr, 2000; Lehmann, 2002; Mehraein, 2003; Cakan, 2004; Carty, 2004; Chen, 2006; Munakata, 2006; Isa, 2007; Sasaki, 2007; Jorgensen, 2008; Lunardi, 2008; Colmegna, 2009; Tzang, 2009; Smith, 2010; and others], most of the data obtained are ambiguous and often contradictory. Practically, there is no information about the impact of B19V infection activity on the clinical course and development of RA. However, it is essential to know the factors, which caused the disease and are still playing a role in its

development, for a successful treatment. This doctoral thesis summarizes the research conducted between 2001 and 2008 at the Oncovirological laboratory of Kirchenstein's Institute of Microbiology and Virology and the Rheumatology center based in Riga Eastern Clinical University Hospital. In spite of the fact that there are a relatively large number of RA patients in Latvia, such studies weren't conducted until now. Possibly, the analysis, summarization and abstraction of the data obtained during this study, could extend the current knowledge of the etiopathogenesis of RA and factors affecting the development of rheumatoid process, RA form and prognosis. Thereby the scientific relevance of the research devoted to the etiopathogenesis of RA and the factors, which exert an influence upon the rheumatoid process activity, RA form and prognosis are the following: RA is a relatively common autoimmune disorder, RA affects not only the joints of infected individual, but also the internal organs and systems; it increases the morbidity of atherosclerosis, cardiovascular diseases and the risk for the development of malignant neoplasms; RA shortens the lifespan for 7 years; RA is a severe incapacitating disease, which causes considerable social and economic problems; individual affected by RA is in need of a constant, ceaseless treatment, meaning serious financial expenses, including not only the drug therapy, but also the funding of the multi-disciplinary expert team, side effect control of the drug course, preventive procedures and the well-timed treatment; anti-infection agents including B19V infection treatment, although the data may be discrepant in some of the relevant literature sources. Also currently there is a lack of data available in the public sources on the impact of the B19 infection activity on RA, and this defines every serious study devoted to this subject as a valuable investment.

Research hypotheses

1. B19V infection is one of the possible triggers, factors which are the cause or the initiation of RA.
2. B19V infection has an impact on RA clinical form or it somehow effects the development, form and prognosis of RA.

Null hypothesis: B19V infection does not have any influence neither on the etiopathogenesis of RA, nor on the rheumatoid process activity, nor on its stage/clinical course.

Aim of the research

The aim of this research was to confirm or to reject the role of B19V infection in the ethiopathogenesis of RA, while comparing the RA patients with potentially healthy blood donors

and patients with other types of arthritis; as well as to compare the RA clinical course with different B19V infection activity phases; and to confirm or to reject the existence of possible influence of the B19V infection on the activity of the rheumatoid process, RA stages and RA clinical course.

Objectives of the research

1. To study the clinical course of RA and the disease activity process, to determine the stage of the disease. Studies should be performed following appropriate standards.
2. To determine the presence and activity of B19V infection using molecular biological and serological methods for each subject involved in the study:
   a) to determine the presence of B19V sequence in the DNA samples, extracted from PBL;
   b) to determine the presence of B19V sequence in the DNA samples, extracted from serum/plasma;
   c) to determine the presence of B19V sequence in the DNA samples, extracted from cells of synovial fluid;
   d) to determine the presence of the virus-specific IgG and IgM-class antibodies in the blood serum/plasma.
3. To examine the activity of rheumatoid process, RA stages and possible correlations between the clinical course of the disease and the B19V infection activity form.
4. To determine the possible differences in the disease morbidity rates by taking into consideration the patients' sex (male, female), age differences and the presence of B19V infection markers.
5. To study the differences between patients with early RA and patients with the disease period exceeding two years, considering the presence or absence of B19V infection markers.
6. To determine the difference between RA patients and patients with other types of arthritis judging by the presence of B19V infection markers.
7. To confirm or to reject the null-hypothesis during the data processing.
8. To develop a list of recommendations for clinical practice based on the acquired data.

Structure of the research

This doctoral thesis is originally written in Latvian. It consists of the following parts: abbreviations and keywords, introduction, literature review, patients, materials and methods, results and statistical analysis, discussion, conclusions, practical recommendations, references and appendix – 186 pages in total. The work includes 29 figures and 35 tables. The statistical data analysis along with a sample patients' questionnaire are available in Microsoft Office Word format (statistical analysis data are also available in SPSS format on the separate disk attached to this study) in the appendix. The reference list of the literature sources used in this study consists of 176 references.

Abbreviations and keywords

ANA – antinuclear antibodies

Anti-CCP antibodies – antibodies against cyclic citrullinated peptide

B19V – parvovirus B19

B19V markers – IgG and IgM antibodies against the B19V structural protein VP-2, the presence of B19V genome sequences in the DNA samples extracted from peripheral blood leucocytes and the blood plasma

CRP – C-reactive protein

DNA – deoxyribonucleic acid

ESR – erythrocyte sedimentation rate

HLA – human leukocyte antigen

IgA – immunoglobulin A

IgG – immunoglobulin G

IgM – immunoglobulin M

IL – interleukin

Joints – affected joints

Lym – lymphocytes

MS – morning stiffness

Non-RA – patients with other types of arthritis, not included in the RA patients group.

nPCR – nested polymerase chain reaction

OA – osteoarthritis

PBL – peripheral blood leukocytes

PCR – polymerase chain reaction

RA – rheumatoid arthritis

RF – rheumatoid factor

TNF – tumor necrosis factor

Tr – platelets

Virus-negative patients – patients without B19V infection markers

Virus-positive patients – patients with B19V infection markers

Patients, materials and methods

One hundred and twenty three patients and 94 practically healthy blood donors were enrolled in this study. The research was approved by the local Ethics committee (RSU ethics committee). In addition, each patient signed a participation agreement. Each patient was given a unique study number. Only the patients' initials instead of their full names were used in order to maintain privacy. All patients were allocated either in the Rheumatology Center of the Pauls Stradins Clinical University Hospital, or in the Rheumatology Center of Riga Eastern University Hospital. 100 out of 123 patients had a diagnosis which corresponded to the RA diagnostics criteria. These patients were: 73 Caucasian female patients aged between 21 and 84 years (mean age value 55.01±1.55 years) and 27 Caucasian male patients aged between 29 and 77 years (mean age value 51.85±2.50 years). 23 patients were diagnosed with other, non-RA diseases. This included: 15 patients with osteoarthritis, 4 patients with seronegative spondyloarthropathy and 4 with acute undifferentiated arthritis (all the diagnoses mentioned were consistent with the appropriate criteria). The control-group consisted of 94 practically healthy blood donors: 46 Caucasian females and 48 Caucasian males aged between 19 and 58 (mean age for this group 35±15 years). A separate group of patients was allocated from the total number in order to exclude the immune-suppressive therapy that could be the co-factor of the virus reactivation. This group was formed from 31 (out of 100) RA patients who received neither glucocorticoids, nor any other RA base treatment drugs. Two RA patients were examined twice with the interval of 7 and 24 months in order to determine the infection dynamics and its association with the clinical course, with the subsequent regulation of the drug-therapy. These two patients received their numbers - Nr. 17 (Nr. 63) and Nr. 120 (Nr. 144) – during the examinations, and thus appeared in the statistical data analysis as Nr. 17 and Nr.120. The group of patients with early RA, the group of male RA patients and the group of female RA patients were formed and evaluated. Two groups of patients – patients with (virus-positive RA patients) and patients without (virus-negative RA patients) the B19V infection markers were allocated and compared. Respectively, according to the infection markers, four groups were formed: the 1st group –

created as an "underwent infection" group (although it was not possible to exclude that in some cases the infection was latent – when the target cell was not in PBL, for example), which consisted of 43 patients, who were IgG positive, with no signs of IgM and B19V DNA (hereinafter – IgG positive RA patients); the 2nd group – consisted of 23 patients with an active B19V infection, who were IgM positive with or without IgG-class antibodies and/or B19V DNA in plasma and/or in PBL (hereinafter IgM positive RA patients); the 3rd group – the group of 21 patients with a latent or persistent B19V infection, with the presence of B19V DNA in PBL with or without IgG-class antibodies (hereinafter DNA positive RA patients); the 4th group was formed of 13 patients who had no virus markers (hereinafter virus-negative patients).

The clinical course of RA was evaluated according to the well-established disease identification standards, which include: subjective – the degree of joint pain, morning stiffness, weakness period length and functional restrictions; physical medical examination – the number of joints affected by the disease, mechanical alterations in the affected joints (such as movement restrictions, deformations, crepitation); extra-articular manifestations; laboratory data – erythrocyte sedimentation rate, C-reactive protein level, rheumatoid factor, complete blood count diagram, levels of electrolytes and creatinine, liver enzymes and albumin, urine analysis, synovial fluid analysis, roentgenographic examination of the joints; the definition of the functional state and living standards; a general examination in order to determine the disease activity level. An inquiry form was filled in for each patient, which included the following data: the patient's number, his or her initials, age, sex, the date of examination, drug therapy at the time of examination, the duration of the disease, morning stiffness, the number of affected joints, Ritchie articular index, joint damage symmetry, rheumatoid nodules, RF, CRP, ESR, platelets and lymphocyte counts, hemoglobin levels, ANA, other hematologic alterations, a joint fluid analysis, the virus determination results, roentgenologic stage, extra-articular manifestations.

The determination of B19V antibodies and B19V DNA was performed in the Oncovirological Laboratory of A. Kirchenstein's Institute of Microbiology and Virology (RSU). The presence of IgG and IgM-class plasma antibodies against the main structural protein of B19V – VP2 was established using the anti-VP2 enzymatic immunoassay (Biotrin, Dublin, Ireland), according to the manufacturers' protocol. Nested PCR (nPCR) with the DNA (extracted from the peripheral blood leucocytes, plasma and synovial fluid cells) as the template (both blood and synovial fluid samples of 41 patients were tested, 37 of them were RA patients and, respectively, 4 were non-RA patients) was used to determine the presence of B19V DNA sequence. Cells were incubated with proteinase K and DNA was extracted using a standard phenol-chloroform method. The quality of the extracted DNA was tested using a β-globin PCR

and the concentration was measured by spectrophotometry. The phenol-chloroform method was the key method of extracting DNA from plasma and glycogen was used as a carrier/thickener. The β-globin PCR was performed to exclude undesirable cell DNA fragments from the extracted samples. B19V DNA was determined using nPCR. Oligonucleotides 5'-CTT TAG GTA TAG CCA ACTGG-3' with the nucleotide position 2905-2924 and 5'-ACA CTG AGT TTA CTA GTG GG-3' with 4016-3997 were used as the primers in the first amplification producing the 1112 bp amplimer. On the second amplification cycle the primers were: 5'-CAA AAG CAT GTG GAG TGA GG-3'with the nucleotide position 3187-3206 and 5'-CCT TAT AAT GGT CTG GG-3' with 3290-3271, producing the 104 bp end product. The amplification products were analyzed electrophoreticaly in the 2% agarose gel with ethidium bromide, and the results were visualized using the UV light emission. The B19V-positive serum with the virus load 10^{11} virions/ml was used as a positive control.

Using the state-certified standard-methods, appropriate equipment and reagents, a complete blood analysis, biochemical blood tests, RF, CRP, ESR (Westergen method), ANA, urine analyses and synovial fluid analyses were performed in the E. Gulbja laboratory of Riga Eastern Clinical University Hospital and in the laboratory of Pauls Stradins Clinical University Hospital.

Using state-certified standard-methods and appropriate equipment, radiological examinations were performed in the Roentgenological Departments of Riga Eastern Clinical University Hospital and Pauls Stradins Clinical University Hospital.

A statistical analysis was performed using statistical software SPSS version 13.0 (product of SPSS Inc., Statistical Package for the Social Sciences, USA). The statistical methods used in the study were the conventional descriptive and conclusive statistical methods that are described in the mathematical, biological, medical and general statistics literature. According to the hypotheses, aims of the research and data type, the following methods were used: Chi-square test, Fisher's exact test, Kolmogorov-Smirnov test, Student's t-test, Mann-Whitney test, single factor dispersion analysis ANOVA or Analysis Of Variance, Pirson's correlation coefficient, Spearman's rank correlation coefficient and Factor analysis. The differences in the hypotheses verification parameters were estimated at 95% probability level, which equals to the significance level of p=0.050. In cases where p values equaled to less than 0.050, the null-hypothesis was rejected. If p was greater than 0.050, but at the same time less than 0.100, the value was considered to be a statistical difference tendency.

Results and statistical analysis

Human parvovirus B19 infection marker study results in healthy donors, patients with rheumatoid arthritis and patients with other types of arthritis

The results of the blood tests performed on healthy donors with the purpose of determining the specific immune response and the presence of B19V DNA were: neither specific antibodies against the B19V structural protein VP-2, nor B19V DNA were discovered in 17 (18.1%) of samples, while 77 (81.9%) were positive; 73 of 94 (77.7%) practically healthy blood donors had IgG-class antibodies against the B19V structural protein VP-2; 15 (16%) practically healthy donors had IgM-class antibodies against the B19V protein VP-2, 4 of which had no IgG-class antibodies; B19V genome sequence was discovered in 6 of 94 (6.4%) practically healthy donors' DNA, where 2 – extracted from plasma and 4 – from PBL.

The results of the blood tests performed on the samples of RA patients with the purpose of determining the specific immune response and the presence of B19V DNA were: 13 (13%) patients had no B19V-specific antibodies against the B19V structural protein VP-2 and no B19V DNA present, but 87 (87%) patients were positive for either B19V DNA or the infection-specific antibodies; IgG-class antibodies against the B19V protein VP-2 were detected in 79 out of 100 (79%) of RA patients; IgM-class antibodies against the B19V protein VP-2 were detected in 23 out of 100 (23%) of RA patients, 3 of which had no IgG-class antibodies; 32 out of 100 (32%) had B19V genome sequences in DNA, specifically: in 14 case B19V genome sequence was extracted from plasma, 17 – from PBL and 1 – both from plasma and PBL.

The results of the blood tests performed on the samples of non-RA patients in order to determine the specific immune response and the presence of B19V DNA were: neither the infection-specific antibodies, nor B19V DNA were discovered in 5 cases (21.7%), while in 18 (78.3%) results were positive; 16 out of 23 (69,6%) non-RA patients had IgG-class antibodies against the B19V protein VP-2; IgM-class antibodies against the VP-2 were discovered in 3 out of 23 (13%) non-RA patients, one of whom had no IgG-class antibodies; the genome sequence of B19V was discovered in 4 out of 24 (17.4%) cases, where: 3 – DNA extracted from plasma and 1 – DNA extracted from PBL.

And, finally, the following results were received after studying the samples of OA patients (a nosologically homogeneous group included 15 patients, which was allocated from non-RA patient group and was considered statistically sufficient): 5 patients (33%) had no B19V DNA and no virus-specific antibodies, while 10 (67%) of OA patients were positive; 9 (60%)

had IgG-class antibodies against VP-2; IgM-class antibodies were observed in 3 out of 15 (20%) OA patients' samples, one of whom had no IgG-class antibodies and 3 (20%) had B19V genome sequence in DNA, where: 2 – in DNA extracted from plasma and 1 – DNA extracted from PBL.

Provided data suggested that both, RA patients and practically healthy donors, had almost equal IgG-class antibody prevalence rates – 79% and 77.7%, respectively. However, the prevalence of the virus-specific IgM-class antibodies and B19V genome sequence in DNA, extracted from the plasma and PBL, in RA patients was considerably higher than in practically healthy donors – 23% and 16%, 32% and 6.4%, respectively. RA patients more often had the virus-specific IgG-class antibodies than patients with other types of arthritis. The prevalence rate was 79% and 69.6%, respectively. The percentage of samples with IgM-class antibodies (23% and 13%) as well as with B19V genome sequence (32% and 17.4%) discovered in the plasma and PBL DNA of patients with other types of arthritis was also confirmed to be less than in RA patients. Although RA and OA patients had almost equal percentage of cases where IgM-class antibodies were discovered (23% and 20%), the prevalence of IgG-class antibodies (79% and 60%) and B19V genome sequence presence in PBL and plasma DNA samples (32 and 20%, respectively) was observed significantly less often in samples of OA patients, than in RA patients.

The Chi-Square test revealed statistically significant differences between the following groups: practically healthy donors / RA patients; practically healthy donors / RA patients / non-RA patients; and practically healthy donors/RA patients/OA patients in terms of B19V DNA prevalence rate. The occurrence of the B19V genome sequence was statistically significantly more frequent in the DNA samples of RA patients, which were extracted from PBL and the blood plasma, than in the samples of healthy donors (according to Fisher's exact test $p<0.0001$; odds ratio equaled 1.93; 95% confidence interval between 1.54 and 2.42). A significant difference in B19V genome sequence presence was observed between the DNA samples extracted from PBL and plasma samples of RA patients, healthy donors and non-RA patients, (according to the Chi-Square test $p<0.0001$). Further calculations were considered to be ineffective due to the insufficient number of non-RA patients for statistical processing. Although the further analysis revealed a statistically significant difference in the presence rates of B19V genome sequences in the DNA samples extracted from the PBL and the blood plasma samples of the following groups: RA patients, healthy donors and OA patients, (according to the Chi-Square test $p<0.0001$), subsequent calculations were also considered to be ineffective due to the lack of patients for the statistical analysis in the OA patient group. B19V IgG-class antibodies with a statistically significantly greater frequency were discovered in the blood samples of RA patients than of OA patients and according to the Fisher's exact test, p equaled 0.115, which was not

statistically reliable, possibly, due to the shortage of patients in the OA group, which was essential for the statistical processing (odds ratio equaled 1.15 and 95% confidence interval was between 0.93 and 1.43).

Forty one patients were chosen for both blood and synovial fluid sample tests. 37 out of these patients were RA patients and 4 belonged to the group of non-RA patients. 5 of the samples taken from RA patients were positive for B19V infection markers. In 2 cases the genome sequence of B19V was observed in the DNA extracted from the synovial fluid cells and in 3 cases the presence of B19V genome sequence was stated in both – DNA from the synovial fluid cells and PBL. All the DNA samples of non-RA patients extracted from the synovial fluid cells were negative for the presence of B19V genome sequence.

Drug therapy and its effect on analysis results

A separate group of RA patients was allocated from the total number of RA patients and was called "group B". The group included 31 individuals, who received neither glucocorticoids, nor the RA base therapy (patients, who were recently diagnosed with RA, patients with drug intolerance, and due to the other factors). This was performed to exclude the possibility that the imunne-suppressive therapy is a reactivation co-factor of the virus. The remaining RA patients (hereinafter – group A, which consisted of 69 patients) were receiving the following drugs at the time of the examination: 26 were receiving methotrexate and prednisolone, 6 – sulfasalazine and prednisolone, 14 – only prednisolone, 16 – only methotrexate, 2 – only plaquenil, 2 – methotrexate, sulfasalazine and prednisolone, 2 - solely sulfasalazine and 1 – azathioprine and prednisolone. Soon after the forming, the two groups mentioned (group A and group B) were evaluated. The following data was acquired: the percentage of virus-negative patients in the group A equaled to 14.5%, whereas in group B – 9.7%; thus, virus-positive patients formed 85.5% of the group A and 90.3% of the group B; 78.3% of patients in the group A and 80.6% in the group B had IgG-class antibodies; respectively, 21.7% of patients in the group A and 25.8% in the group B were IgM-class antibody positive; DNA positive patients formed 25.7% of the group A and 41.9% of the group B. 22 patients of the group A were tested for B19V genome sequence both in the blood and synovial fluid samples, 2 (9.1%) of whom were positive for the presence of B19V genome sequence in their synovial fluid cell samples, but the rest (20 or 90.9%) were negative. 15 patients of the group B were tested similarly (the blood and synovial fluid samples were evaluated for the presence of B19V genome sequence) and the following results were obtained: 3 (or 20%) patients had B19V genome sequence in the DNA samples of the synovial fluid cells, but the rest 12 (or 80%) were tested negative. The Chi-Square test results

revealed that (judging by all the values of these groups) groups A and B were not statistically significantly different. And thus, it was possible to conclude, that the drug therapy received by the patients who participated in the study could not be considered as the reactivation co-factor of B19V. So, the division of patients according to the drug-therapy was not essential in further studies.

Monitoring patients in the dynamics

Since only two patients were examined twice, their disease progression was analyzed with no pretensions to make the further conclusions based on these examinations. Two were not considered to be a sufficient evidence for reasoning.

The first patient was a 56 y/o (at the time of the examination) female. She was assigned a Nr. 17 (Nr. 63). Nr. 17 was receiving the methotrexate therapy. Her disease lasted for 12 years. Two examinations, in which she participated, took place with an interval of 7 months. The results of the first examination revealed the presence of both IgG and IgM-class antibodies, but no presence of B19V DNA sequence. At the time of the second examination (seven months from the first one), the B19V-specific genome sequence was found in her PBL, but no IgM-class antibodies were detected in plasma. According to the clinical and laboratory data, her morning stiffness period increased from 15 minutes to 3 hours, the number of affected joints increased from 13 to 28, RF level increased from 166 to 269.2, ESR adjusted upwards from 21 to 37 mm/h, but CRP level was slightly decreased – from 49.5 to 38.2 mg/l. The phase of the viral infection changed from the recently underwent to the persistent infection characterized by the presence of B19V genome sequence in the PNL DNA with IgG, but with no IgM-class antibodies. This transition also coincided with a boost in RA activity.

The second patient, the male patient Nr.120 (Nr.144) was examined twice with an interval of 24 months. At the time of the first investigation Nr.120 was 40, but at the time of the second he was 42 y/o. He was diagnosed with early RA. The patient received neither prednisolone, nor the RA base drug-therapy. At the time of the second examination, he was using sulfasalazine on a regular basis. Initially, only IgG-class antibodies in the patient's plasma samples and B19V genome sequence in the DNA samples extracted from his PBL were found. However, during the second examination, IgM-class antibodies and B19V genome sequence was detected in the DNA extracted from his plasma (viremia). This was a clear sign of the latent/persistent infection's reactivation. Thus, it was possible to observe clinical changes accordingly to the changes in the B19V infection form – the latent form transformed into active

one. Although the viremia and IgM-class antibodies were detected only at the time of the second examination, doubtfully it was the result due to the sulfasalazine therapy. According to the examination data, the patient's morning stiffness period lengthened from 15 minutes to 3 hours, the number of affected joints grew up from 10 to 26, RF level increased from 103.6 to 162.26 U/ml, CRP and ESR levels decreased, Hb level increased from 10.2 to 11.5 g/l and the roentgenologic stage went down from the first to the second.

Sample analysis results for patients with early RA

A group of patients with early RA was allocated from the main group of RA patients. It included 23 patients. Their disease period was between 12 weeks and 2 years. The percentage of patients with virus infection markers was noticeably higher in this group (refer to Table 1). However, there was no statistically significant difference observed in these results, perhaps due to insufficient sampling. A statistically significant connection was observed between the presence of IgM and DNA markers of the infection in patients whose RA duration exceeds two years ($p=0.006$, Fisher's exact test). Such connections were not observed in early RA patients. Thus, the viral genome sequence was observed statistically significantly more frequently in the DNA of PBL and cell-free blood samples of IgM-positive patients with the disease duration exceeding two years than in IgM negative patients with the same disease period.

Table 1

B19V infection marker studies in patients with early RA and in patients with the disease period exceeding two years

Analysis results	Early RA, disease length between 12 weeks and 2 years 23 (100%)	RA patients with the disease length exceeding 2 years 77 (100%)
Negative	2 (8.7%)	11 (14.3%)
Positive	21 (91.3%)	66 (85.7%)
IgG positive	18 (78.3%)	61 (79.2%)
IgM positive	6 (26.1%)	17 (22.1%)
DNA positive	9 (39.1%)	23 (29.9%)
Plasma DNA	4	10
PBL DNA	5	12
Plasma and PBL DNA	0	1
DNA samples of synovial fluid cells	9 (100%)	28 (100%)
Presence of B19V genome sequence	2 (22.2%)	3 (10.7%)

Human parvovirus B19 infection marker study results in male and female RA patients

Seventy three (73) RA patients out of 100 were female patients with a mean age of 55.01±1.55 and 27 were male patients with a mean age of 51.85±2.50 years. The following results were acquired when the samples of the female RA patients were tested: 9 (12.3%) samples were negative for both B19V DNA and the virus-specific antibodies against VP-2, but 64 (87.7%) samples were positive; 58 female patients (79.5%) had IgG-class antibodies against VP-2; 16 (21.9%) had IgM-class antibodies against the virus-specific protein VP-2 and 23 (31.5%) samples were positive for the presence of the B19V genome sequence including 11, which were extracted from plasma and 12 from PBL. The results of the sample tests of male RA patients were: 4 (14.8%) male patients were tested negative for both - B19V DNA and the virus-

specific antibody presence, but 23 (85.2%) samples were positive; 21 (77.8%) male patients had IgG-class antibodies against B19V structural protein VP-2; 7 (25.9%) male patients had IgM-class antibodies; 9 (33.3%) were tested positive for B19V genome sequence in the DNA samples extracted from: 3 – from plasma; 5 from PBL and 1 – both from plasma and PBL. Both blood and synovial fluid samples of 23 female RA patients were analyzed. The analysis returned the following results: 3 samples (13%) were positive for the B19V genome sequence in the DNA extracted from the synovial fluid cells and 20 (87%) were negative. The same number of tests were performed on 14 RA male patients and the results were: 2 male RA patients (14.3%) had B19V DNA in their synovial fluid samples and 12 (85.7%) were tested negative. There were no statistically significant differences observed in terms of the frequency of the B19V marker presence depending on the RA patients' sex. Further studies led to the formation of four separate RA patient groups depending on the infection markers. The average age of female/male patients was calculated per each group. It was the following: the 1st group – 54.97±2.15/57.22±4.28; the 2nd group– 54.94±3.34/48.14±4.84; the 3rd group – 54±4.52/46.14±5.35; the 4th group – 56.89±3.68/56.25±4,23 (refer to Figure 1). There were no significant differences between the mean age of the male and female patients with no B19V infection markers: 56.89±3.68 and 56.25±4.23. A small difference was observed in the mean age of female/male patients who underwent B19V infection or had a latent form of the infection: 54.97±2.15/57.22±4.28. A significant difference was observed in the 2nd and 3rd groups: the mean age of female RA patients with the active infection was 54.94±3.34, whereas the mean age of the male RA patients with the same activity of the infection was 48.14±4.84; the average age values in a group of RA patients with latent/persistent B19V infection were: 54±4.52 for female against 46.14±5.35 for male RA patients. Female RA patients were almost equally aged in all of the four groups, but male RA patients had a moderate difference in the 1st and 4th, 2nd and 3rd groups and the significant one between the 1st and 2nd, 1st and 3rd, 2nd and 4th, 3rd and 4th groups. According to the calculated age values for each group, the differences among female/male patients were observed between the 1st and 2nd group, p= 0.994/0.170; 1st and 3rd group p=0.821/0.097; 1st and 4th group, p=0.705/0.900; 2nd and 3rd group p= 0.850/0.771; 2nd and 4th group, p=0.729/0.320 and between 3rd and 4th group p=0.618/0.218. The average age values of RA male and female patients are available on Figure 3.

A one-factor dispersion analysis suggested that the male patients had statistical differences in terms of age between the 1st and 3rd groups, p=0.097 and in the 1st and 2nd groups, p=0.170. These differences could suggest that male RA patients with the active and latent/persistent form of B19V infection (the male patients with IgM-class antibodies or with the

genome sequence of B19V in the DNA extracted from the cell-free blood plasma and PBL) have a greater morbidity of RA.

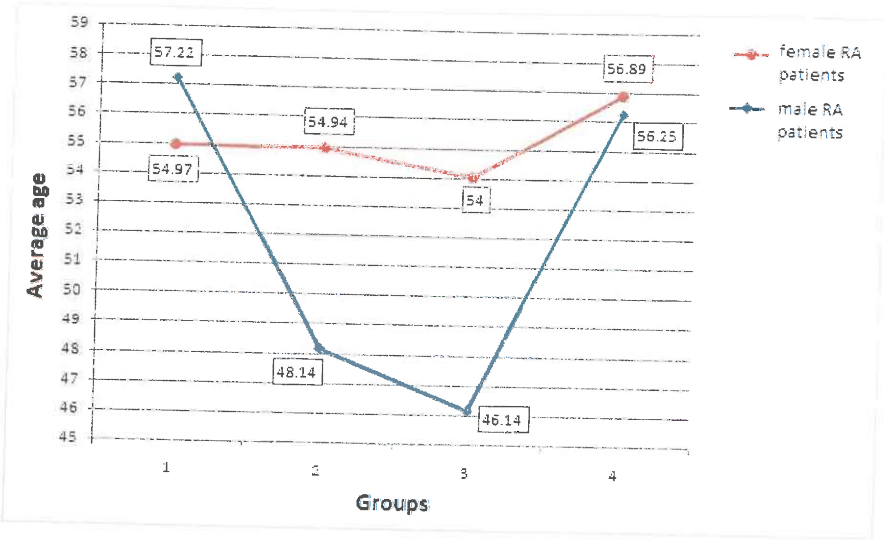


Figure 1. The mean age of the male and female RA patients in the 1st, 2nd, 3rd and the 4th group

Comparison of the clinical and laboratory parameters for rheumatoid arthritis patients

Thirteen RA patients out of 100 had no B19V infection markers, two of whom were patients with early RA. As for the other eleven, their disease duration exceeded 2 years. The clinical values for these virus-negative patients were: the morning stiffness lasted for 1.4±0.4 h; the number of affected joints equaled to 12±1; the average CRP level was 21.3±6.5 mg/l; the mean platelet count was 302±21 x 10^9/l; the average lymphocyte count was 1.7±0.2 x 10^9/l; 3 patients had a seronegative RA and 10 had the seropositive form of the disease; for the seropositive patients, the average RF level equaled 174.3±66.2 U/ml, ESR – 42±5 mm/h and Hb – 12.5±0.3 g/l. All the patients had bone erosion and the II, III or IV roentgenologic stage; however, only one had peripheral nerve disorders.

Eighty seven RA patients were positive for markers of B19V. 21 had early RA, while the rest had the disease period exceeding two years. The clinical values of these virus-positive patients were: the average morning stiffness period lasted for 4.4±0.4 h; the number of affected joints was 14±1; the average CRP level was 35.1±4.1 mg/l; the platelet count – 337±11 x 10^9/l;; lymphocyte count 1.98±0.08 x 10^9/l; a seronegative form of RA was observed in 15 and a seropositive form in 72 patients; the average RF level in seropositive patients was 346.9±61.8

U/ml; ESR – 44±3 mm/h; Hb – 11.5±0.2 g/l. 11 patients had no bone erosion according to the roentgenologic data, others had the II, III or the IV roentgenologic stages. Extra-articular manifestations were observed in 29 patients: 7 had peripheral nerve disorders, 3 – muscle disorders, 4 – Sjoergen's syndrome, 4 – kidney disorders, 2 – lung disorders, 1 – carpal tunnel syndrome, 1 had rheumatoid nodules, 1 – liver disorders and 5 had combined visceral manifestations (Nr.2 – peripheral nerve, muscle disorders and secondary vasculitis; Nr. 58 – peripheral nerve, muscle and kidney disorders; Nr. 65 – peripheral nerve and kidney disorders; Nr. 96 – peripheral nerve, kidney disorders and rheumatoid nodules; Nr. 157 – peripheral nerve and muscle disorders).

Virus-positive and virus-negative patients were compared according to the following parameters: the morning stiffness period, the number of affected joints, CRP level, platelet and lymphocyte count, RF levels in seropositive RA patients, ESR, hemoglobin level (refer to Table 2), extra-articular manifestations, roentgenologic stage (refer to Table 3) and the occurrence of anemia in the RA patients in each group.

Table 2

Virus-positive and virus-negative RA patients

	MS	Joints	CRP	Tr	Lym	RF*	ESR	Hb
Virus-	1.4±0.4	12±1	21.3±6.5	302±21	1.7±0.2	174.3±66.2	42±5	12.5±0.3
Virus-	4.4±0.4	14±1	35.1±4.1	337±11	1.98±0.008	346.9±61.8	44±3	11.5±0.2

*for RF positive patients; MS – morning stiffness period, hours; Joints – the number of affected joints; CRP – C-reactive protein, mg/l; Tr – platelets, x 109/l; ESR – erythrocyte sedimentation rate (Westergren method), mm/h; Hb – hemoglobin, g/l; RF – rheumatoid factor, U/ml

A statistically significant difference was observed in: the morning stiffness period (p=0.005, Mann-Whitney test), which was statistically significantly longer in virus-positive RA patients than in virus-negative RA patients; Hb levels were statistically significantly lower in the virus-positive than in virus-negative RA patients (p=0.047, Student's t-test). A statistical difference tendency was observed in the RF level value, where p equaled to 0.096 according to Mann – Whitney test. The RF level detected in virus-positive RA patients was significantly higher, than in virus-negative RA patients.

There was only one virus-negative and 29 virus-positive RA patients who had extraarticular manifestations, thus a statistically probable difference tendency was observed (p=0.101, Fisher's exact test); odds ratio – 1.17 and 95% probability interval was observed between 1.03 and 1.32. The occurrence of the extraarticular manifestation was significantly more frequent in the virus-positive than in virus-negative RA patients.

A difference in terms of roentgenologic stage was observed in the virus-positive and the virus-negative RA patients (refer to Table 3).

Table 3

Roentgenologic stage distribution of the virus-positive and the virus-negative RA patients

	I stage	II stage	III stage	IV stage
Virusnegative	0%	7.7% (1)	61.5% (8)	30.8% (4)
Viruspositive	12.6% (11)	16.1% (14)	69% (60)	2.3% (2)

A statistically significant roentgenologic stage division was found between the virus-negative and the virus-positive RA patients (p=0.001, Chi-Square test). The prevalence of the III roentgenologic stage was observed in virus-positive RA patients (refer to Figure 2).

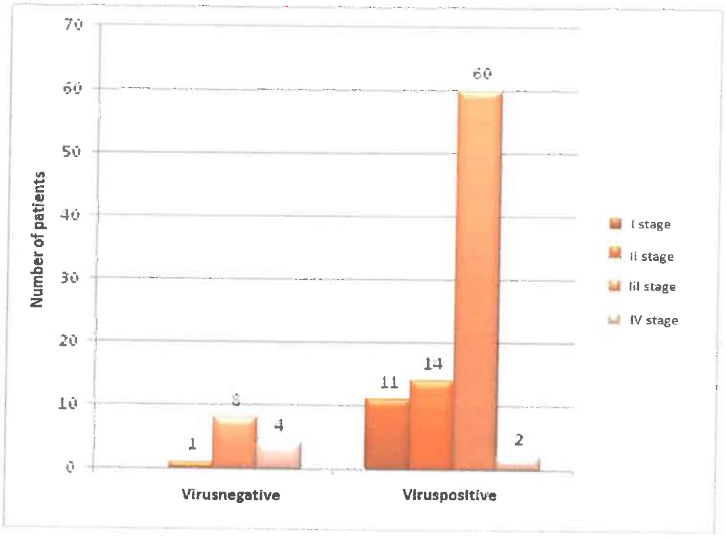


Figure 2. Roentgenologic stage distribution of the virus-positive and the virus-negative RA patients

Although nine or 69.2% of the virus-negative RA patients had no anaemia, it was observed in 4 (30.8%) virus-negative patients. At the same time, anaemia was not present in 36 or 41.4%, while appearing in 51 (58.6%) virus-positive RA patients. Thus, anaemia was observed significantly more frequently in virus-positive RA patients (a statistical difference detected) than in virus-negative RA patients (p equaled to 0.076, according to Fisher's exact test; odds ratio=1.16 and 95% probability interval was between 0.98 and 1.37).

Correlation analysis

The correlation analysis was performed using the following parameters of the virus-positive and virus-negative RA patients: MS, Joints, CRP, Tr, ESR and HB. Statistically significant results are available in Table 4.

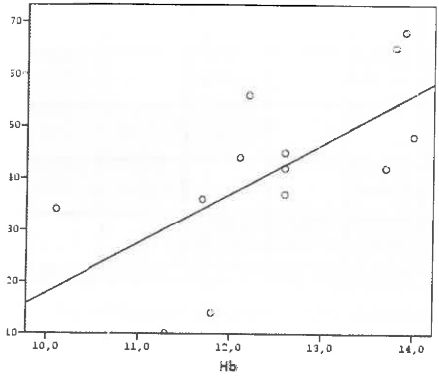
Table 4

	MS		Joints		CRP		Tr		ESR		Hb	
	Virus-	Virus +	Virus-	Virus +	Virus-	Virus +	Virus-	Virus +	Virus-	Virus +	Virus-	Virus +
MS						+0.217* p=0.044				+0.292** p=0.006		
Joint						+0.221* p=0.040						
CRP		+0.217* p=0.044		+0.221* p=0.040				+0.325** p=0.002		+0.476** p<0,0001		-0.223* p=0.038
Tr						+0.325** p=0.002				+0,387** p<0,0001	-.785** p=0.001	-0.352** p=0.001
ESR		+0.292** p=0.006				+0.476** p<0,0001		+0.387** p<0,0001			+0.780** p=0.002	-0.443** p<0,0001
Hb						-0.223* p=0.038	-.785** p=0,001	-0.352** p=0.001	+0.780** p=0.002	-0.443** p<0,0001		

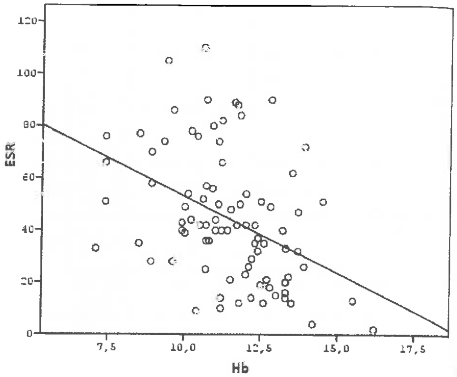
MS – morning stiffness period, hours; Joint – the number of affected joints; CRP – C-reacrive protein, mg/l; Tr – platelets, x 10^9/l; ESR – erythrocyte sedimentation rate (Westergren method), mm/h; Hb – hemoglobin, g/l; RF – rheumatoid factor, U/ml; ** – 1% correlation level; * – 5% correlation level; + positive correlation; – negative correlation; Virus- virusnegative; Virus+ viruspositive

There were some correlations between the activity values of the disease process observed in virus-positive RA patients. These correlations were between: CRP and MS, +2.17*: a greater CRP level corresponded to a longer morning stiffness period, p=0.044; CRP and Joints, +0.221*: the more elevated CRP was, the greater number of joints was affected, p=0.040; CRP and Tr, +0.325**: a higher level of CRP corresponded to a higher platelet count, p=0.002; CRP and ESR, +0.476** – a higher CRP corresponded to a higher ESR, p<0.001; CRP and Hb, -0.223*: a higher level of CRP indicated a lower level of Hb, p=0.038; ESR and MS, +0.292** the more elevated ESR level was, the longer was morning stiffness period, p=0.006; ESR and Tr, +0.387**: the greater ESR led to the higher platelet count, p<0.001; ESR and Hb, -0.443**: a higher ESR meant a lower Hb level, p<0.001; Tr and Hb values, -0.352**: a higher platelet count resulted in a lower Hb level, p=0.001. Respectively, the following correlations of the disease values were observed in virus-negative RA patients: between Tr and Hb, -0.785**: a higher thrombocyte count resulted in a lower Hb level, p=0.001; between ESR and Hb, +0.780** – the higher ESR was, the higher Hb level was, p=0.002.

The maximal number of correlations was observed in RA patients with the B19V infection markers. These correlations outlined the connections in and between the disease activity parameters. However, the correlations observed in virus-negative RA patients were numerous and didn't indicate any connections in the disease activity values. Almost the same results were acquired, while comparing correlations between Hb and Tr in the virus-positive and virus-negative RA patients. However, comparing the correlation between Hb and ESR in the virus-negative and virus-positive RA patients, the results were opposite. The low levels of Hb in RA patients with B19V markers correlated with higher ESR levels whereas in RA patients without the infection markers, the low levels of Hb correlated with the lower levels of ESR (refer to Figure 3 and Figure 4).



igure 3. Positive correlation between Hb and ESR in virus-negative RA patients



4. attēls. Negative correlation between Hb and ESR in virus-positive RA patients

Test results in patients with osteoarthritis, seronegative spondyloarthropathy and acute undifferentiated arthritis

Eighteen (78.3%) non-RA patients out of 23 were positive for the infection markers of B19V, while negative results were obtained in 5 or 21.7% of non-RA patients. As for the B19V-specific antibodies, 16 (69.6%) non-RA patients were positive for IgG and 3 or 13% were positive for IgM-class antibodies, the genome sequence of B19V was found in the samples of 4 (17.4%) non-RA patients, including 3 samples, which were extracted from plasma and 1 from PBL. The DNA extracted from the synovial fluid samples of four non-RA patients were tested for the presence of B19V DNA. The presence was not confirmed. The following mean values were calculated: Hb level equaled to 12.9±0.3 g/l, platelets count was 277±19 x 10^9/l and the average lymphocyte count was 1.9±0.1 x 10^9/l.

In order to perform further studies, in accordance with nosology, the non-RA group was divided into subgroups. The results of the division mentioned were: 10 OA patients of 15, which is 67%, were positive for the B19V infection markers, while the other 5 were tested negative; nine patients (60%) had IgG and 3 (20%) IgM-class antibodies, but 3 were tested positive for the presence of B19V genome sequence in their DNA samples, 2 of which were extracted from plasma and 1 – from PBL. The DNA extracted from the synovial fluid samples of three patients were tested for the presence of B19V genomic sequence – the presence was not confirmed. Hb level was approximately 12.6±0.3g/l, the platelet count was 246±17 x 10^9/l, while the lymphocyte count equaled to 1.9±0.1 x 10^9/l. All four patients with a seronegative spondyloarthropathy were positive for the B19V infection markers: three were IgG-positive and 1 had B19V genome sequence in plasma without the virus-specific antibodies. Only one patient was subjected to further analysis for the presence of B19V DNA. It returned a negative result. Hemoglobin levels were: Nr. 72–12.6 g/l; Nr. 82 – 11.8 g/l; Nr. 112 – 14.6 g/l; Nr. 152 – 11.6 g/l, platelets count was: Nr. 72 – 437 x 10^9/l; Nr. 82 – 394 x 10^9/l; Nr. 112 – 440 x 10^9/l; Nr. 152 – 257 x 10^9/l and lymphocyte count was: Nr. 72 – 3.2 x 10^9/l; Nr. 82 – 1.11 x 10^9/l; Nr. 112 – 2.35 x 10^9/l; Nr. 152 – 1.6 x 10^9/l. Four patients with an acute undifferentiated arthritis were positive for IgG-class antibodies, suggesting underwent or possibly latent B19V infection. Their hemoglobin levels were: Nr. 33 – 14.0 g/l; Nr. 45 – 13.1 g/l; Nr. 46 – 15.2 g/l; Nr. 54 – 12.2 g/l, the platelet count was: Nr. 33 – 300 x 10^9/l; Nr. 45 –29 x 10^9/l; Nr. 46 – 179 x 10^9/l; Nr. 54 – 289 x 10^9/l and lymphocyte count: Nr. 33 – 1.4 x 10^9/l; Nr. 45 – 1.3 x 10^9/l; Nr. 46 – 2.0 x 10^9/l; Nr. 54 – 2.4 x 10^9/l.

positive RA patients (the 1st group), p=0.042. Also, these numbers were higher in B19V DNA-positive patients (the 3rd group) than in virus-negative RA patients (the 4th group), p= 0.057. Thus, a statistical difference tendency was identified in the differences mentioned.

The one-factor dispersion analysis displayed the statistical differences in platelet count between the 2nd/3rd and the 3rd/4th groups. The mean platelet count was statistically significantly higher in B19V DNA-positive RA patients (the 3rd group) than in IgM positive RA patients (the 2nd group), p= 0.027 and, again, it was statistically probably higher in the 3rd group (DNA-positive RA patients) than in the 4th group, p= 0.044.

The one-factor dispersion analysis displayed the statistical differences in all groups in terms of the morning stiffness duration, p= 0.040. For the 1st and 4th, the 2nd and 4th and the 3rd and 4th groups p equaled to 0.048, 0.008 and 0.013, respectively. The morning stiffness duration was significantly longer in the patients of each virus-positive group, than in virus-negative group.

The same analysis performed on the Hb values, displayed the statistical difference between the 3rd and 4th, the 1st and 4th groups. B19V DNA-positive RA patients (the 3rd group) had the lower levels of Hb than virus-negative RA patients (the 4th group), p= 0.057. Respectively, Hb levels of IgG-positive RA patients (the 1st group) were lower than the levels of virus-negative RA patients, p=0.067. Both cases are statistical difference tendencies.

Finally, the one-factor dispersion analysis was applied on the values of RF levels (only for RF-positive RA patients). The statistical differences were found between the 2nd and 3rd, the 3rd and 4th groups. Thus, the 3rd group or B19V DNA-positive RA patients had the higher values of RF than patients of the 2nd group also known as IgM-positive RA patients, p= 0.059. RF level values were also more elevated in the patients of DNA-positive patient group (the 3rd group) than in virus-negavite RA patients (the 4th group), p= 0.055. A statistical difference tendency was established in both correlation cases.

The number of extra-articular manifestations was calculated for each RA patient group (refer to Figure 5). A Fisher's exact test, which was applied on the extra-articular manifestation data, revealed the presence of the statistical difference between the 2nd and 4th groups, p= 0.011; odds ratio equaled to 1.93 and the 95% probability interval was observed between 1.23 and 3.04. In other words, IgM-positive RA patients (the 2nd group) had the statistically significantly larger number of extra-articular manifestations than virus-negative RA patients (the 4th group).

Anemia in RA patients and in patients with other types of arthritis and 819V infection markers

Thirty six or 41.4% of the virus-positive RA patients had no signs of anemia, while 51 (58.6%) were tested positive. Anemia was also discovered in 5 (27.8%) non-RA patients (Hb Ievel values available for 18 of 23 non-RA patients), although 13 virus-positive non-RA patients did not show si$_{g\ n}$s of anemia. As for virus-positive OA patients, 7 of them were tested negative for anemia, while it was observed in 3 (Hb Ievel data available for 10 of 15 OA patients). Thus, the occurrence of anemia was statistically probably more frequent in virus-positive RA patients, than in the virus-positive non-RA patients (p=0.021, according to Fisher's exact test; odds ratio equaled to 1.24 and 95% probability interval detected between 1.03 and 1.50). A statistical difference tendency, however, was observed in the different correlation: anemia was observed more often in virus-positive RA patients, than in virus-positive OA patients (p=0.103, Fisher's exact test; odds ratio equaled to 1.13 and 95% probability interval detected between 0.97 and 1.31).

Grouping of the RA patients

Four groups of RA patients were compared using the values of the moming stiffness duration, the number of affected joints, CRP Ievel, platelet and lymphocyte counts, RF Ievel (for seropositive RA patients), ESR, Hb Ievel (refer to Table 5), extraarticular manifestations and roentgenologic stage.

Table 5

The average values of clinical parameters in RA patients per each group

	1st group	z^{na} roup	3rd group	4^{in} QIOUD
ESR	43±4	47±6	45±4	42±5
Lvm	1.8±0.1	2±0.2	2.2±0.2	1.7±0.2
CRP	33.7±5.9	31.9±6.3	41.5±9.6	21.3±6.5
Joints	13±1	13±2	17±2	12±1
Tr	335±18	306±15	375±22	302±21
MS	3.8±0.6	5±1	4.8±0.7	1.4±0.4
Hb	11.5±0.3	11.6±0.4	11.3±0.6	12.5±0.3
RF•	355.3±48.8	242.4±64.2	576.2±264. 7	174.3±66.2

*for RF pos1tive patients; MS - the momtng stiffness durat10n, hours; Jomts - number of affected Jomts; CRP - C-reacrive protein, mg/1; Tr - platelets, x 10^9/l; E S R - erythrocyte sedimentation rate (Westergren method), mm/h; Hb - hemoglobin, g/l; RF - rheumatoid factor, U/ml

A one-factor dispersion analysis established the statistical differences in the numbers of affected joints between the 1st and 3rd groups and between the 3rd and 4th groups, which were statistically significantly higher in B19V DNA-positive RA patients (the 3rd group) than in IgG-

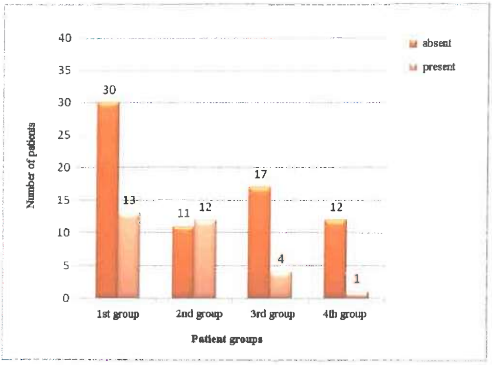


Figure 5. The number of Extra-articular manifestatios for each group of RA patients

The roentgenologic stage distribution was observed in every group of RA patients (refer to Figure 6). A statistical difference tendency was observed between the 3rd and 4th, and also between the 1st and 4th groups, where p values were 0.022 and 0.012, respectively, according to Chi-Square test.

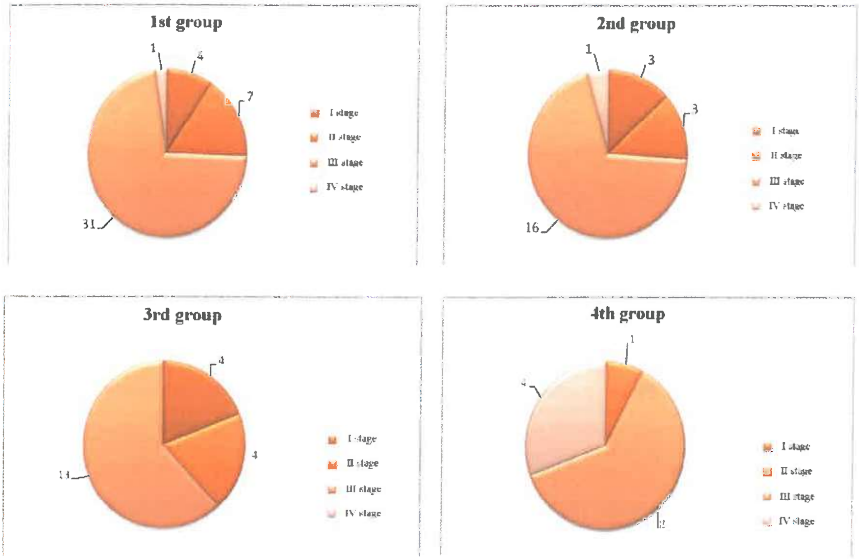


Figure 6. The distribution of roentgenologic stages in groups of RA patients

The correlation data analysis was applied on the clinical parameters of RA patients in each group. The clinical parameters mentioned were the following: MS, Joints, CRP, Tr, ESR and Hb. Results available in table from 6 through 9.

Table 6

Results of correlation analysis for the 1st group

	MS	Joints	CRP	Tr	ESR	Hb
MS						
Joints						
CRP				+0.482** p=0.001	+0.485** p=0.001	
Tr			+0.482** p=0.001		+0.435** p=0.004	-0.352* p=0.020
ESR			+0.485** p=0.001	+0.435** p=0.004		-0.403** p=0.007
Hb				-0.352* p=0.020	-0.403** p=0.007	

** – 1% correlation level
* – 5% correlation level

For the first group of RA patients, positive correlations were detected between the values of Tr and CRP, +0.482** (p=0.001), ESR and CRP, +0.485** (p=0.001) and ESR and Tr, +0.435** (p=0.004), while negative between Hb and TR values, 0.352* (p=0.020) and Hb and ESR values, -0.403** (p=0.007).

Table 7

Results of correlation analysis for the 2nd group

	MS	Joints	CRP	Tr	ESR	Hb
MS			+0.524* p=0.010		+0.605** p=0.002	
Joints			+0.469* p=0.024			
CRP	+0.524* p=0.010	+0.469* p=0.024			+0.430* p=0.040	
Tr						
ESR	+0.605** p=0.002		+0.430* p=0.040			-0.555** p=0.006
Hb					-0.555** p=0.006	

** – 1% correlation level
* – 5% correlation level

The following positive correlations were observed in the 2nd group of RA patients: between ESR and CRP, +0.430* (p=0.040), ESR and MS, +0.605** (p=0.002); CRP and MS, +0.524*

(p=0.010); CRP and Joints, +0.469* (p=0.024), while negative correlation was observed between Hb and ESR, -0.555** (p=0.006).

Table 8

Results of correlation analysis the 3rd group

	MS	Joints	CRP	Tr	ESR	Hb
RS						
Loc						
CRO					+0.541* p=0.011	
Tr					+0.461* p=0.035	-0.494* p=0.023
EGĀ			+0.541* p=0.011	+0.461* p=0.035		-0.479* p=0.028
Hb				-0.494* p=0.023	-0.479* p=0.028	

** – 1% correlation level
* – 5% correlation level

The following positive correlations were detected in the 3rd group of patients: positive - between ESR and CRP, +0.541* (p=0.035) and negative – between Hb and Tr, -0.494* (p=0.023) and between Hb and ESR, -0.479* (p=0.028).

Table 9

Results of correlation analysis for the 4th group

	MS	Joints	CRP	Tr	ESR	Hb
RS						
Loc						
CRO						
Tr						-0.785** p=0.001
EGĀ						+0.780** p=0.002
Hb				-0.785** p=0.001	+0.780** p=0.002	

** – 1% correlation level
* – 5% correlation level

As for the 4th group, a positive correlation was observed between the values of ESR and Hb, +0.780** (p=0.002) and a negative correlation – between Tr and Hb, -0.785** (p=0.001).

Thereby, a positive correlation between the values of Tr and CRP was only observed among the patients of the 1st group; a positive correlation between ESR and CRP in patients of the 1st, 2nd and the 3rd groups, a positive correlation between ESR and platelet count was found in

the 1st and 3rd groups. In addition, negative correlations between Hb and ESR were observed in the patients of the 1st, 2nd and the 3rd groups, positive – between CRP and MS, ESR and CRP and Joints only among the patients of the 2nd group and, finally, a positive correlation between Hb and ESR was estimated only in patients of the 4th group.

The maximal number of correlations was observed in the patients of the 1st (mostly 1% correlation level) and 2nd (mostly 5% correlation level) groups, where these correlations reflected the connections in the disease activity parameters. Although the number of correlations observed in the patients of the 3rd group was lower, these correlations also reflected the connections between the disease activity parameters. The 4th group of patients had the minimal number of correlations, in other words, it was less than in any other group and these correlations, contrary to the correlations in other groups, did not reflect the connections between the disease activity parameters.

Factor analysis

During the data processing, an exploratory factor analysis was applied using the values of the clinical parameters determined for each patient. These values were: ESR, CRP, the duration of morning stiffness, the number of affected joints, Hb level and platelet/lymphocyte counts. The validity of the use of the exploratory factor analysis was justified by KMO index also known as the Kaiser-Meyer-Olkin index of Sampling Adequacy, which in this case was 0.661 (must be greater than 0.5). After the analysis was completed, the following parameters were considered to be the values of the disease activity: ESR, CRP, the duration of morning stiffness, the number of affected joints and Hb level. The values mentioned were used to form the so-called "F1 factor" (refer to Figure 7) which could relatively be the disease activity general-factor. Another factor – F2 was formed during the factor analysis besides F1. F2 included platelet and lymphocyte counts. On contrary to F1, F2 was not found statistically probable and thus was omitted in the further studies.

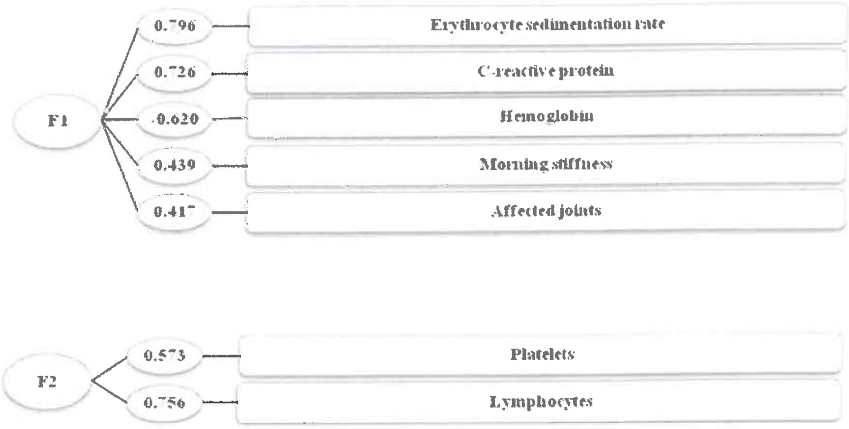


Figure 7. Factors F1 and F2, the disease activity parameters and factor loadings

Three sublevels of the disease activity general-factor F1 were distinguished: low, average and high (refer to Table 10) and low, average and high rheumatoid process activity, respectively. According to all parameters, each group was unique and statistically probably different, $p<0.0001$.

Table 10

Sublevels of the disease activity general-factor F1

	Low	Average	High
MS	2.3±0.4	3.1±0.5	6.6±0.8
Joints	12±1	13±1	17±1
CRP	14.8±2.4	20.8±3	64.7±8
ESR	24±3	42±3	65±4
Hb	12.8±0.2	11.6±0.3	10,3 ± 0,2

MS – morning stiffness period, hours; Joints – the number of affected joints; CRP – C-reactive protein, mg/l; ESR – erythrocyte sedimentation rate (Westergren method), mm/h; Hb – hemoglobin, g/l

Every patient with an elevated level of the disease activity general-factor was positive for B19V infection markers. This was based on the result of the comparison of the virus-negative RA patients and patients with B19V infection markers and according to Chi-Square test was statistically significant ($p=0.024$).

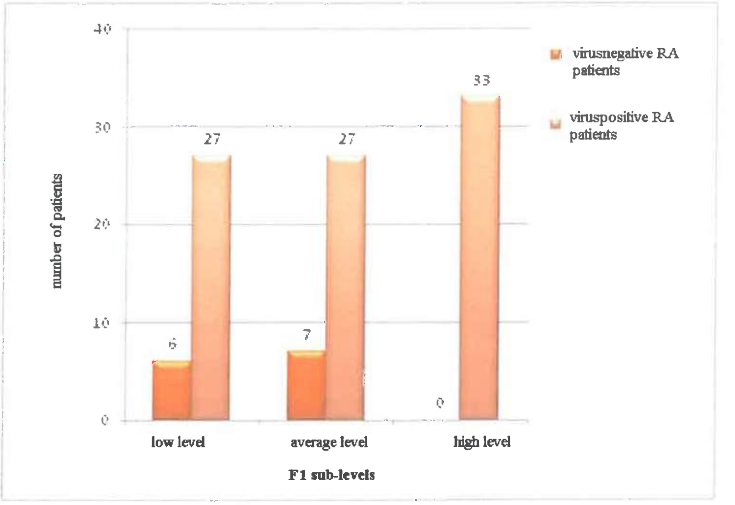


Figure 8. F1 sublevels and RA patients with or without B19V infection markers

While examining possible connections between the sublevels of F1 and RA patient groups, two statistically significant differences were observed: between the 3rd and 4th groups, where p equaled to 0.008 according to Chi-Square test (refer to Figure 9) and between the 1st and 4th groups, where p=0.032, similarly, according to Chi-Square test (refer to Figure 10).

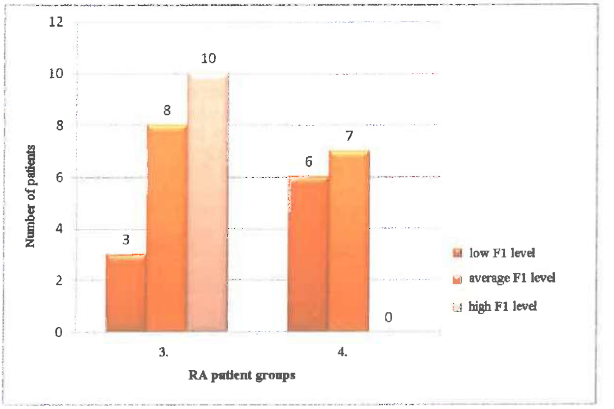


Figure 9. F1 sublevel distribution in the 3rd and the 4th group of RA patients

The 3rd group had a statistically significantly greater number of patients with high levels of the disease activity general-factor F1 than the 4th group, which was also known as the group of virus-negative RA patients.

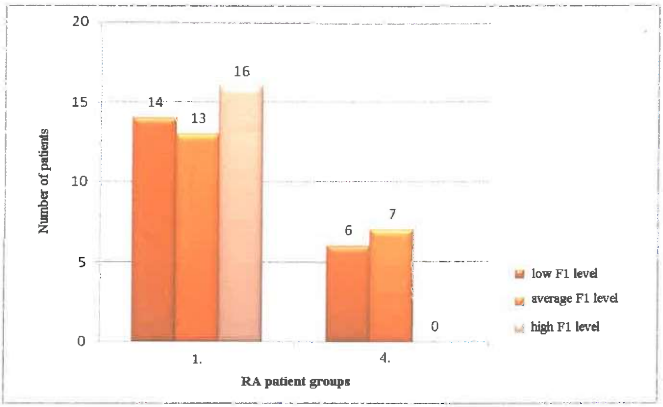


Figure 10. F1 sublevel distribution in the 1st and the 4th group of RA patients

The number of patients with high levels of the disease activity general-factor F1 in the 1st group was statistically significantly greater than in the group of virus-negative RA patients (the 4th group).

Discussion

RA is a chronic systemic disease with a progressive development characterized by joint disorders, which manifest as a chronic, aseptic synovitis and destructive, erosive joint damages. Progressive joint damages, the disease process affecting the internal organs, and the persistent disease activity – all of these factors substantially decrease the quality of life, produce a prolonged invalidity, and decrease a life span. RA is the most common inflammatory arthritis. The incidence of the disease is increasing with age. The facts mentioned suggest RA as one of the greatest problems both medicine-wise and economy-wise, also suggesting the significance, relevance and the necessity of studies devoted to the etiopathogenesis and the influencing factors of the disease. Moreover, despite the large number of studies, the etiopathogenesis of the disease is not completely studied. Viral infections (including B19V infection) are considered to be one of the initializing factors in the development of the disease. B19V involvement in the etiopathogenesis of RA is confirmed by the following data: clinically, chronic arthritis observed along with concommitant B19V infection doesn't differ from the classical form of RA [Colmegna, 2009; Kerr, 2000; Khouqeer, 2009]; the development of arthritis is more frequent in the HLA-DR*4-positive patients, who underwent B19V infection [Klouda, 1986]; RF and anti-CCP antibodies were detected in B19V-positive patients [Barzilai, 2007; Cohen, 1986; Kerr, 1996, 2000]; the occurrence rates of the IgM-class virus-specific antibodies and the B19V DNA

presence in the blood samples of RA patients are elevated, comparing to the control-group [Murai, 1999]; the increased invasivity of synovial fibroblasts after the incubation in serum-containing B19V [Ray, 1999]; the cross-reactivity of IgG-class antibodies and the II-type human collagen, cytokeratin and cardiolipin [Lunardi, 1998, 2008; Sekine, 1999]; the epidemiologic data, which suggest that RA and B19V infection are the New World-specific diseases [Altschuler, 1999; Carty, 2004; Rothschild, 1988, 1992]; the increase in IL-6, IL-8 un TNFα production in RA patients is connected to B19V NS-1 [Sasaki, 2007; Tzang, 2010]; the decrease in joint process activity and in the numbers of B19V-containing cells in the bone marrow of RA patients, who underwent intervenous immunoglobulin treatment [Maeda, 2001, Muscat, 1995] and many other studies. However, the data available is not completely definite. This can be partially explained by the multiplicity of RA clinical forms. It is possible that the age of the patients whose samples were involved in these studies was varying, thus meaning that the initialization moment of RA was also different, or perhaps, that the organism's reaction is different in the patients of the diverse ethnic groups. The other examples that should be mentioned are: the reservoir of B19V is not yet found, B19V and its protein activity are not completely studied; no studies completely cover the ways in which and whether other infections have an impact on RA. There is a probability that the genotype of B19V is significant, and from a clinical point of view, the presence of B19V depends upon the type of sample material. The analysis of possible correlations between B19V infection and the clinical course of RA or its development practically is a blank spot in the available literature. Besides, the numbers of studies devoted to the subject is low both in Latvia and in the rest of the world. Thus, the results of this study bear both theoretical and practical novelty. This study is a summary of the research performed by the Laboratory of Oncovirology of RSU A. Kirhenshtein's Institute of Microbiology and Virology, the Rheumatology Center of Clinical University Hospital of Pauls Stradins and the Rheumatology Center of Riga Eastern Clinical University Hospital during the period of eight years from 2001 to 2008. It involved 123 patients and 94 practically healthy blood donors. The following groups of RA patients were formed and studied: the 1st group – created as an "underwent infection" group (although it was not possible to exclude, that in some cases, the infection was latent – when the target cell was not in PBL, for example), which consisted of 43 patients, who were IgG-class anti-body positive, with no signs of IgM-class antibodies or B19V DNA; the 2nd group - consisted of 23 patients with active B19V infection, who were tested positive for IgM-class antibodies with or without IgG-class antibodies and/or B19V DNA, where B19V DNA was in plasma and/or in PBL; the 3rd group – a group of 21 patients with latent or persistent B19V infection, with the presence of B19V DNA in PBL with or without IgG-class antibodies; the 4th group was formed of 13 patients who had no virus

markers. The analysis of B19V antibodies and the detection of B19V DNA were performed in the Laboratory of Oncovirology of A. Kirhenshtein's Institute of Microbiology and Virusology, RSU.

The study results and the analysis of sources allow us to conclude that B19V infection is an important factor influencing the ethiopathogenesis, activity, stages and clinical course of RA.

The assessment of the significance of B19V infection in the ethiopathogenesis of RA is based on the following data:

1) The presence of B19V genome sequence in the DNA samples extracted from the blood plasma and PBL of RA patients is observed statistically significantly more frequently than in the samples of healthy donors, $p<0.0001$. B19V DNA occur almost 1.93 times more frequently in RA patients than in healthy donors (odds ratio 1.93; 95% probability interval between 1.54 and 2.42).
2) The occurrence rate of the virus-specific IgG-class antibodies in RA patients is higher, than in OA patients. Despite the fact, that p is equal to 0.115, which is not statistically probable and perhaps due to the lack of OA patients, the detection frequency is almost 1.15 times higher in RA patients than in OA patients. Odds ratio – 1.15 and 95% probability interval between 0.93 and 1.43.
3) B19V DNA is detected only in synovial fluid DNA samples of RA patients.

Although similar suggestions have already been published [Cakan, 2004; Chen, 2006; Colmegna, 2009; Kerr, 1995, 2000; Sasaki, 2007; Takahashi, 1998], there is no expressed difference described between RA patients and the control-group, judging by the B19V genome sequence presence in the samples extracted from PBL and the blood plasma. The detection of B19V DNA in synovial fluid samples, listed in this study, is purely descriptive, due to the lack of the material diversity for statistical analysis.

4) B19V-specific IgM-class antibodies are found statistically more frequently in RA patients than in practically healthy donors (23% and 16%, respectively). Yet, according to the number of authors, the IgM-class antibody detection frequency varies between 2 and 6% [Cohen, 1986; Nikkari, 1994; Harrison, 1998]. At the same time, authors like Murai and his colleagues report 18% [Murai, 1999], but other, like Tzang and his co-authors for example, observe a high occurrence of B19V specific IgM-class antibodies in RA patients [Tzang, 2009].

5) There is a significant connection between the presence of B19V IgM-class antibodies and B19V genome sequences in PBL and the blood plasma DNA samples in patients with the disease duration exceeding two years, p=0.006. There is a statistically greater number of patients with IgM-class antibodies who are positive for B19V DNA in their PBL and the blood plasma, than it is amongst the IgM-negative patients. The connection mentioned is not observed in patients with early RA. It is certainly essential to mention the single patient with early RA, who was examined twice. At the time of the first examination, he had B19V-specific IgG-class antibodies in plasma and the presence of B19V genome sequence in PBL DNA. IgM-class antibodies appeared in this patient at the time of the second examination, along with the occurrence of B19V genome sequences, which are observed in his plasma DNA samples, allowing us to consider that the virus reactivation is affecting the future form of rheumatoid process. These facts, however, require more detailed attention and further research.
6) The intentional distribution of RA patients according to their sex, utilized in this study, clearly reflects the epidemiologic RA morbidity data available (the morbidity rate is 3 times higher in female than in male patients). Thus, seventy three out of 100 chosen RA patients are female and the other 27 - male. It seems that there are no statistically probable differences in B19V infection markers among both male and female RA patients, according to the morbidity data available. The average age of female RA patients equals to 55.01±1.55, which also correlates with RA morbidity rates in women at the age of menopause. The average age of male patients enrolled in this research is 51.85±2.50. It is also accurate regarding the RA morbidity rates in younger (<45) males. According to the distribution of female and male patients in the groups, neither male nor female patients' age values diverge from the epidemiologic data available. The acquired data suggest that the maximum probability to develop the disease is observed in younger males with an active or latent/persistent form of B19V infection (p is equal to 0.170 and 0.097, respectively). However, such associations are observed neither in male RA patients who underwent the infection with no B19V markers, nor in female patients without the markers of B19V infection, allowing us to suggest the virus (B19V) as the causative agent of RA in younger males. This suggestion is supported by the statistical analysis data designating it as a statistical difference tendency, which requires a further research. Unfortunately, a genetic predisposition or smoking habits haven't been taken into consideration in this research. These factors undoubtedly require a further research, including risk factors, clinical course of the disease and auto-antibody levels.

7) Virus-positive RA patients are different from virus-positive non-RA patients in terms of the anaemia occurrence rates, which are statistically significantly higher in virus-positive RA patients (p=0.021). Thus, anaemia is detected 1.24 times more frequently in virus-positive RA patients. Odds ratio equals to 1.24 and 95% probability interval is observed between 1.03 and 1.50. A difference tendency is also present in the anaemia morbidity rates between virus-positive RA patients and virus-positive OA patients, where p=0.103. It is 1.13 times more probable to observe anaemia in a RA patient than in an OA patient. Odds ratio equals to 1.13 and 95% probability interval is between 0.98 and 1.30. None of these differences is described in the available literature sources.

The obtained results describe the connection between B19V infection and the ethiopathogenesis of RA, which allows to consider the B19V as a RA trigger-factor. The direct evidences are available in the 1st, 2nd and the 3rd, but the indirect – in the 4th, 5th and the 6th paragraphs.

Despite that in daily clinical practice the viral infections are not paid much attention (including B19V infection), in other words, neither laboratory diagnostics, nor any special attention is required in RA cases, the obtained data suggest B19V infection as the factor, potentially responsible for the appearance of immunologic disorders with the possible further development of RA. A special attention is drawn to patients with polyarthritis, which is developing after the B19 virus infection. It can possibly be either the beginning of RA or it can transform into RA in the future. During the time of the research, such development was observed in a patient with the classic form of RA, which advanced due to the acute B19V infection. However, the diagnostics of the rheumatic diseases, including polyarthritis, is not an easy task, especially with the lack of classical criteria. The presence of B19V genome sequence observed in patients with undifferentiated polyarthritis allows suggesting the probability of RA diagnosis.

The evaluation of the mutual correlations between the infection (B19V) and the disease (RA) activity, its stage and clinical course is based on the following data: RA patients with the B19V infection markers have a higher rheumatoid process activity rate, their prognoses are worse and the RA form is more severe than in patients with no virus markers. The highest degree of the rheumatoid process activity is supported by the following facts:

1) the duration of morning stiffness is longer in each group of RA patients with B19V infection markers than in the group of virus-negative RA patients, p=0.005 and is statistically significant;

2) the level of Hb are lower in RA patients with B19V infection markers than in virus - negative RA patients, p=0.047 and is statistically significant;
3) there are higher numbers of affected joints observed in RA patients with latent/persistent B19V infection than in RA patients with no markers of the infection, p=0.057 and is a statistical significance tendency.
4) the mean platelet count is higher in RA patients with the latent/persistent form of B19V infection than in patients with no markers of the infection, p=0.044, and is statistically significant;
5) anemia is observed more frequently in RA patients with the infection markers of B19V than in virus-negative RA patients, p=0.076, that is a statistical significance tendency. The incidence of anemia in RA patients with B19V infection markers is 1.16 times higher, than in virus-negative patients with RA; odds ratio equals to 1.16 and 95% confidence interval is between 0.98 and 1.37;
6) statistically significantly greater number of extra-articular manifestations is observed in RA patients with the active form of the B19V infection than in virus-negative RA patients, p=0.011. The occurrence rate of extra-articular manifestations in patients with B19V infection is 1.93 times higher than in virus-negative RA patients; odds ratio equals to 1.93; 95% confidence interval is between 1.23 and 3.04;
7) the maximum number of correlations between the parameters is observed in the RA patients who either underwent or are with an active B19V infection. These correlations display the connections between the disease activity parameters. Even though the number of correlations in patients with latent/persistent B19V infection is insignificantly lower, it also reveals the connections between the disease activity parameters. A significantly lower number of correlations are observed in virus-negative RA patients than in any group of virus-positive RA patients, thus revealing no connections between the disease activity parameters. The opposite results are obtained comparing the correlation between the levels of Hb and ESR in virus-positive and virus-negative RA patients;
8) all patients with high levels of the disease activity general-factor F1 are positive for the presence of the B19V infection markers, p=0.024 which is statistically significant.

A worse prognosis for the RA patients with the B19V infection markers than in virus-negative RA patients is clearly visible comparing the RF levels in both groups of patients, p=0.096, which is a statistical difference tendency. A more severe form of the disease in patients with the B91V infection markers is suggested by the division of roentgenologic stages between virus-positive and virus-negative patients, p=0.001. The third roentgenolgic stage dominates among the RA patients who are found positive for B19V infection markers.

After performing the data analysis, it is possible to conclude that the presence of the latent/persistent form of the B19 virus infection is one of the most significant factors that have an effect on the RA form. The RA patients who have latent/persistent B19V infection have a higher level of the rheumatoid process activity, worse prognosis and are more likely to develop a severe form of RA than patients with no B19V markers or patients with other forms of B19V infection. The following facts suggest a higher degree of the rheumatoid process activity:

1) there is a greater number of affected joints in RA patients with the latent/persistent form than of B19V infection than in RA patients with no B19V infection markers, where p=0.057, which is evaluated as a statistical difference tendency, or is statistically probable in case of the underwent B19V infection, where p=0.042;
2) the average number of platelets in RA patients with the latent/persistent B19V infection form is statistically significantly greater than in the RA patients with no infection markers, p=0.044, and in the RA patients with active B19V infection than in the RA patients with no infection markers, p=0.027;
3) the RA patients with the latent/persistent form of the infection suffer longer periods of morning stiffness than patients without infection markers, p=0.013, which is statistical significant;
4) the significantly lower levels of Hb are observed in RA patients with the latent/ persistent B19V infection form, than in virus-negative RA patients, p=0.067, which is a statistical difference tendency;
5) there is a greater number of patients with high levels of the disease activity general-factor F1 among patients with the latent/persistent and underwent (latent/persistent cannot be excluded) form of B19V infection than among virus-negative RA patients, p equals to 0.008 and 0.032 and is statistically probable.

A worse prognosis is more probable in RA patients with the latent/persistent form of B19V infection, judging by the RF levels, which are higher in comparison with RA patients without infection markers or with the active form of the infection, p equals to 0.055 and 0.059, respectively, which is a statistical significance tendency. The more severe form of the disease is determined by the statistically significant difference found in the distribution of roentgenologic stages among RA patients with the latent/persistent B19V infection in comparison with the distribution of roentgenologic stages among virus-negative RA patients, p=0.022; the statistically significant difference found in the distribution of roentgenologic stages among the RA patients who underwent the infection (the latent/persistent infection form is not excluded) and among virus-negative RA patients, p=0.012.

Considering the conclusions above, it is essential to mention a certain RA patient whose disease activity process was under observation for seven months. The disease activity developed rapidly since the first examination and the infection form changed. From the "underwent" infection at the beginning, it transformed into the latent/persistent form at the end of the observation period. Thus, it seems that the development of the infection concurs with the increase in RA activity.

It is also essential to mention that the molecular-biological marker of the latent/persistent infection utilized in this study (the presence of the virus-specific sequence in the DNA samples extracted from the peripheral blood or PBL) doesn't actually allow differentiate the latent from persistent form of infection. Although the serologic marker used to identify the latent/persistent infection (the presence of IgG class antibodies in blood serum/plasma) does not allow differentiate the latent from persistent form of infection. It is necessary to point out that upon B19V infection, IgG-class antibodies are created against the multiple antigens (VP-2p, VP-N, VP-1S, VP-2r, VPC and NS-1) and each of them has its own significance. While the anti-B19 IgG-class antibodies against VP-2g remain in the patient's blood for his or her entire life after the infection, the anti-B19 IgG-class antibodies against VP-1S and VP-2r exist only for a limited period of time, usually for some years or even months. Anti-B19 IgG-class antibodies against NS-1 develop when the organism is not able to destroy the virus. This inability results in the virus persistence or viremia in the various internal organs, which, in its turn, results in the chronic form of the disease [van Poblotzki, 1995]. In the recent years a new Western blot-based serologic test system called *recomLine* Parvovirus has become available. The system is based on the detection of multiple recombinant antigens of B19V - VP-2p, VP-N, VP-1S, VP-2r, VPC, NS-1, which gives a better way to understand the current condition of the infection, whether it is an active, latent or persistent form. This doctoral thesis doesn't include such studies. However, the first data on a relatively moderate number of patients are summarized and published emphasizing the significance of the persistent infection in the increased aggressivity and activity of the disease.

According to the data in the number of publications [Bateman, 1999; Ferri, 1999; Lewkonia, 1995; Sfriso, 2010] the autoimmune process activity is decreasing when the patient becomes infected with B19V. The authors are referring to such diseases as idiopathic juvenile arthritis, systemic sclerosis, juvenile dermatomyositis or juvenile systemic sclerosis, which does not give a possibility for the comprehensive comparison with the data acquired in this study. The B19 virus infection and its association with the activity of the rheumatoid arthritis have been examined by Chun and co-authors [Chun, 2001]. CRP and ESR are the parameters used by these authors to determine the rheumatoid process activity. The authors have also determined the

presence of B19V-specific IgM and IgG-class antibodies. And finally, Chun and co-authors concluded that there is no visible connection between the activity of the rheumatoid arthritis and B19V infection which is in contradiction with our results. Such contradictions probably exist due to the following factors: the number of the clinical parameters of rheumatoid arthritis used in this study is greater, it features the assessment of the presence of B19V genome sequence in the samples of the involved patients, and finally, it is possible that there are the geographic and ethnical differences in the target groups enrolled in the studies. Thus, the infection of B19V can either flares-up the existing rheumatoid process or in the case of unknown clinical course, the infection can aggravate the development of RA.

Considering the infection impact on the clinical course of RA, the self-evident question arises: how should a medical doctor chose the correct treatment in cases of B19V infection appearance in patients with acute undifferentiated arthritis or RA. The IVIG therapy should be used both – in patients with pure red cell aplasia and in the patients who received the immune-suppressive therapy. Although the effectiveness of IVIG therapy in the treatment of various autoimmune diseases including RA is confirmed by a number of studies it is not used during this research and in the authors' clinical practice. This topic is debatable and requires further studies.

Performing a statistical analysis, the mathematical method called exploratory factor analysis is used. The appropriateness of the use of exploratory factor analysis in this study is confirmed by the KMO (Kaiser-Meyer-Olkin) index, which was equal to 0.661 (must be greater than 0.5). However, there are some aspects of the exploratory factor analysis that should be clarified. The first point is DAS (short for Disease Activity Score). DAS is a combined index which is introduced to determine the activity of RA [Prevoo, 1995; van Gestel, 1996]. Nowadays the following modifications of DAS exist: DAS28, DAS28-CRP and other. The most commonly used one is DAS28, which includes the values of the following parameters: the number of tender and swollen joints (between 0 and 28), ESR and Visual Analog Scale (0-100mm), which is filled in by a patient according to his or her health condition. DAS is calculated using a certain standard-formula. Unlike DAS, the values of the disease activity general factor F1, F1 levels and, respectively, a high, average and low activity of the rheumatoid process, are obtained in the study process. Secondly, the mathematical parameter F1 or the disease activity general-factor, which includes the main parameters of the disease taken from the material base of the research, is derived using exclusively mathematical statistics methods (such as factor analysis), thus supporting the correctness of the research. Thirdly, a large number of RA patients with underwent B19V infection have a high rheumatoid process activity (a high level of F1), which can be explained by the possible presence of the latent form of B19V infection in cases when the latency is not substituted by the peripheral blood lymphocyte. This also applies to such analysis

results as the prolonged duration of morning stiffness or the decreased level of Hb in RA patients with the underwent or latent B19V infection in comparison to the same values in virus-negative patients; the existence of the statistically significant difference in the distribution of roentgenolic stages in RA patients with the underwent or latent B19V infection and in virus-negative RA patients; the maximal number of correlations in RA patients with the underwent or latent B19V infection. The only difference between the latent/persistent and underwent or latent B19V infection form is the number of affected joints, which, in case of the latent/persistent infection, is statistically significant greater. And finally, although DAS used to determine the activity of the rheumatoid process in clinical practice and in many scientific studies performed in Latvia and worldwide, it is not utilized in this research. The omission of DAS is due to the fact that this study begun in 2001, but at that time DAS wasn't a very common index to deal with and later its introduction was considered to be inefficient i.e. to avoid the splitting of the research material.

Even though the data obtained during this research is sufficient and mostly direct, it is not possible to make a general conclusion about the connection between B19V infection and RA, because some portions of data are only highlighting the tendencies or are of the descriptive nature. It is essential to perform multiple large-scale studies, each with a specific aim, in order to effectively use the data obtained during this research. The specific aims mentioned may be the following: to perform the necessary observations in order to determine the presence of B19V infection in a larger group of OA patients; to observe the presence of the infection markers in RA patients in the dynamics; to determine the prevalence rate of the infection markers in RA patients with early or very early stages of the disease; to receive the confirmation that male patients with the active or latent/persistent B19V infection form tend to develop RA at the relatively young age; to concretize the number of joint changes in RA patients with the B19V infection markers using the Sharp/van der Heijde method [Landewe, 2005]; to trace the distribution of the infection markers for each of the RA extraarticular manifestations separately; to study the synovial fluid DNA samples as well as the synovium in a sufficient scale for the statistical analysis; to study the latency spots of the infection in cases where the presence of B19V genome sequence is detected neither in the plasma, nor in PBL DNA samples upon high titer of the virus-specific IgG-class antibodies and sometimes with the presence of IgM-class antibodies; to examine the possible connections between RA and other viral infections, herpes for example; to compare the exploratory factor analysis data with the data obtained using the DAS-specific standard disease activity assessment methods.

Conclusions

1. B19V infection is connected to RA. It is a trigger-factor in the ethiopathogenesis of RA.
2. The genome sequence of B19V is more frequently observed in the DNA samples (extracted from the blood plasma and PBL) of RA patients than in the samples of healthy donors.
3. B19V DNA is observed only in DNA extracted from the synovial fluid samples of RA patients.
4. The prevalence rate of the virus-specific IgM-class antibodies is higher in the samples taken from RA patients than from healthy blood donors.
5. The greater probability of RA development exists in the younger RA patients with the active or latent/persistent B19V infection.
6. There is a statistically probable connection between IgM-class antibodies and the presence of the genome sequence of B19V in blood plasma and PBL samples, which is observed in patients with RA whose disease duration exceeds two years.
7. Anemia is observed statistically significantly more frequently in virus-positive RA patients than in virus-positive patients with other types of arthritis.
8. B19V infection has an impact on the activity, stage and clinical course of RA.
9. The activity of the rheumatoid process is higher in RA patients with the viral markers of B19V infection than in patients without the infection markers as well as a worse prognosis and the severe form of the disease.
10. According to the data obtained, the latent/persistent B19V infection has the greatest impact on the rheumatoid process activity, form and prognosis. However, it is essential to perform the detection of anti-B19 IgG-class NS1 antibodies in order to differentiate the latent or persistent form of the infection.

Practical recommendations

Considering the data obtained during this study, the recommendations that may be relevant for the clinical practice are:

1) in order to specify the causative agent of the disease in patients with a viral infection followed by anemia and complaints about pain and/or joint swelling, the virological diagnostics should be prescribed, because sometimes, infections, such as B19V, are the trigger-factors of the autoimmune process;

2) to expect the possibility of either the appearance of arthritis at the background of B19V infection, or the infection triggering RA. All available medical and diagnostics arsenal should be used while treating these patients. They should be under constant supervision until the time of the final diagnosis;
3) the presence of B19V infection markers should be checked in patients with undifferential polyarthritis. The probability to diagnose RA increases, when the genome sequence of B19V is detected in the DNA samples extracted from the blood plasma or PBL;
4) when the presence of B19V infection is confirmed in RA patients, it is essential to follow the rheumatoid process activity and to be ready to stop the flare-ups;
5) if rheumatoid arthritis is flaring up with no apparent cause, the verification of the presence of B19V infection markers may be beneficial.

List of references

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