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**Identification and characterization of acute infection with
Parvovirus B19 genotype 2 in immunocompromised patients
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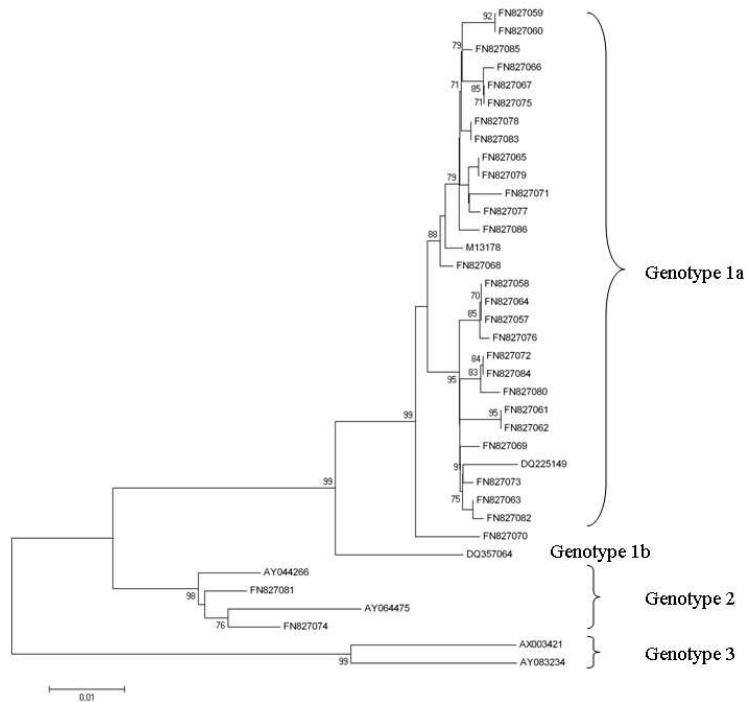
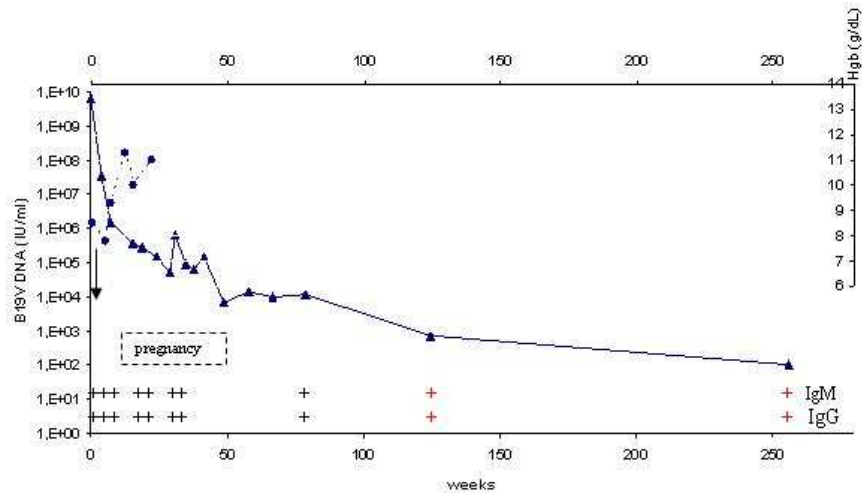


Figure1. Phylogenetic analysis of the B19V strains identified in the Polish patients. The analysis is based upon a 704 bp sequence in the NS1/VP1 region of the B19V genome. Bootstrap values greater than 70 are shown.

A.



B.

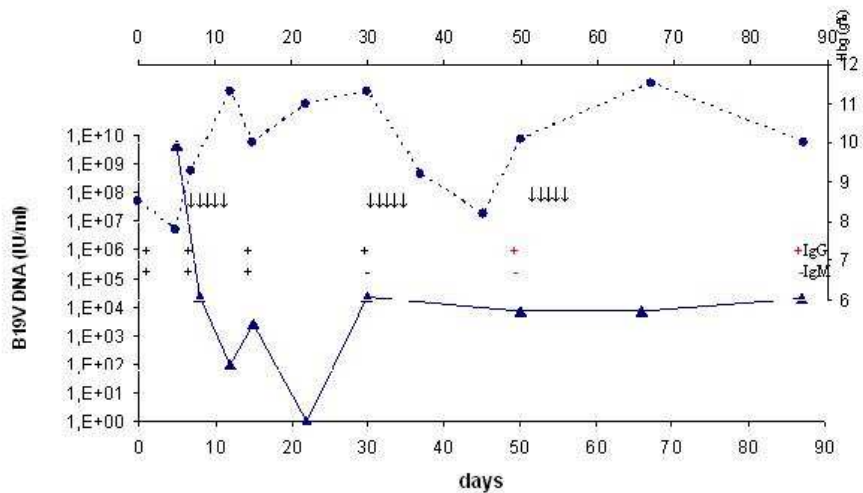


Figure 2. Course of B19V genotype 2 infection in two immunosuppressed women: A) renal transplant patient with pure red cell aplasia and B) leukemia patient with fever, rash, joint aches and anemia at the time of B19V diagnosis. Virological markers are indicated on the figure: viral loads (▲-▲) and antibody status. Levels of hemoglobin are also shown (●-●). In panel A, the transplant recipient, the change in the immunosuppressive regime is shown (↓); the time when the patient was pregnant is indicated. In panel B, the leukemia patient, three rounds of IVIG treatment (Sandoglobulin 24g a day for 5 successive days) are shown (↓↓↓↓).

173x212mm (96 x 96 DPI)

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Abstract

Parvovirus B19 (B19V) is divided into three genotypes. Genotypes 2 and 3 may cause diagnostic difficulties and their epidemiology is not well understood. In the present study the prevalence of B19V genotypes in patients with symptomatic infection in Poland was evaluated and the course of infection in patients infected with non-genotype 1 strains is described.

Real-time PCR, able to detect all three genotypes of B19V was used to screen patient plasma samples. Sixty nine, mainly acute-phase B19V DNA positive cases were identified in patients from hematological and obstetric/gynecological wards between 2004 and 2008. Thirty patients were studied in greater detail and genotyping was performed by analysis of the NS1/VP1u region.

The majority of samples were genotype 1. However two (6.6%) strains were identified as genotype 2, associated with high viraemia and identified in a kidney transplant recipient with anemia and a leukemia patient, following chemotherapy, with pancytopenia. Treatment with intravenous immunoglobulin in the former patient and a change of immunosuppression treatment in latter, resulted in normalization of clinical parameters, and whilst viral loads fell, B19V DNA was still detectable. The kidney transplant recipient subsequently became pregnant with no clinical complications, although persistently infected with B19V genotype 2.

This is the first description of symptomatic cases of genotype 2 B19V infection in Eastern Europe suggesting that acute infection, particularly among immunocompromised patients with these virus strains may be more prevalent than thought.

Abstract: 232 words

Total (without abs): 3 029 words

Background

Human parvovirus B19 (B19V) is distributed worldwide and is associated with a wide spectrum of diseases including: erythema infectiosum (Fifth disease) in children, arthropathy in adults; hydrops fetalis, intrauterine death or other complications during pregnancy; aplastic crisis in patients with increased erythropoiesis, anemia in immunocompromised or immunodeficient patients and occasionally hepatitis, vasculitis, myocarditis and others [Young and Brown 2004; de Jong et al., 2006].

In immunocompetent individuals, viral load rapidly increases during the first week of infection and may exceed 1×10^{12} genome equivalents/ml. The humoral immune response plays a crucial role in viral clearance. In the following 7-14 days, viremia declines, B19-specific immunoglobulin M (IgM) appears and may persist for up to 6 months. Specific IgG is detectable by the third week following infection and remains for years. Viral DNA has been shown to persist in the circulation of immunocompetent individuals for several years accompanying the specific B19V IgG. The virus may also persist in bone marrow, synovial membrane, skin and other tissues [Parsyan and Candotti 2007].

B19V has been grouped into three distinct genotypes [Nguyen et al., 1999; Nguyen et al., 2002; Servant et al., 2002,] and further sub-genotypes [Toan et al., 2006; Parsyan et al., 2007; Corcoran et al., 2009]. Genotype 1 is distributed worldwide; however data regarding the prevalence of genotypes 2 and 3 are fragmentary. Based up on a limited number of reports, all three genotypes are believed to have similar biological features and clinical roles, however difficulties have been reported concerning diagnosis of genotypes 2 and 3, including underestimation of the viral load, detection failures as a results of viral polymorphisms at the genomic level

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3 [Blümel et al., 2005; Baylis et al., 2009; Baylis et al., 2004; Baylis et al., 2007;
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5 Hokynar et al., 2004]. In the present paper the results of a study on B19V
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7 polymorphism in patients with symptomatic B19V infection in Poland and the clinical
8
9 course of non-genotype 1 infections are presented.
10

11 **Materials and Methods**

12 *Patients and samples*

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16 30 patients infected with B19V were included in the study. They were selected from a
17
18 group of sixty nine B19V DNA positive patients who were identified by testing of
19
20 clinical samples from patients in hematological, obstetric and gynaecological wards.
21
22 Testing was performed between 2004 and 2008 in the Molecular Biology Laboratory
23
24 at Institute of Haematology and Transfusion Medicine (IHBT). Samples were selected
25
26 for further analysis based upon the availability of sufficient volumes of plasma. The
27
28 results of the additional analyses are reported below. Nineteen of the patients studied
29
30 were immunocompromised. The study was approved by the Institute of Haematology
31
32 and Transfusion Medicine Ethics Committee, Warsaw, Poland.
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36 *B19V antibodies detection and DNA analysis*

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38 Testing for B19V-specific antibodies was performed using the recomWell Parvovirus
39
40 B19 IgG/IgM ELISA (Mikrogen GmbH, Neuried, Germany) according to the
41
42 manufacturer's instructions.
43
44

45 B19V DNA was studied by two alternative quantitative real time PCR methods,
46
47 performed using the ABI Prism 7700 (Applied Biosystems, Singapore) or Corbett
48
49 3000 (Corbett Research, Sydney, Australia) platforms, respectively [Aberham et al.,
50
51 2001; Candotti et al., 2004]. Both methods utilize TaqMan hydrolysis probes
52
53 designed within the VP1 and the NS1 genes, respectively [Aberham et al., 2001;
54
55 Candotti et al., 2004]. In the second method, additional primers and probes were
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3 used for the detection of CCR5 in plasma as an internal control. Amplification was
4
5 carried out using TaqMan Universal PCR Master mix (Roche Branchbur, New Jersey,
6
7 USA) in volume of 25 µl including 10 µl of template. The 100% detection limit is 5
8
9 IU/reaction and 20 IU/reaction for the respective VP1 and NS1 assays. Both methods
10
11 detect genotypes 1-3; however the VP1-specific method, used until the end of 2006,
12
13 has since been shown to under quantify genotype 3 [Baylis et al., 2007].
14
15

16 Amplification was combined with one of two DNA extraction procedures: QIAamp
17
18 DNA Blood Mini Kit (Qiagen, Hilden, Germany) or Nuclisens Extractor (Nuclisens
19
20 automated isolation reagents, BioMerieux, Boxtel, the Netherlands). Both procedures
21
22 were performed according to the manufacturer's instructions.
23
24

25 *Sequencing and phylogenetic analysis*

26

27 For genotype analysis, a ~1 kb region corresponding to NS1/VP1u was amplified by
28
29 nested PCR [Servant et al., 2002] and both strands were directly sequenced (ABI
30
31 PRISM 3.1 Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Applied
32
33 Biosystems, Warrington, UK). Sequences were aligned using CLUSTAL W [Chenna
34
35 et al., 2003] and a topology tree was constructed using MEGA 4.0 software [Tamura
36
37 et al., 2007], by using the Jukes-Cantor parameter estimation to generate the
38
39 nucleotide distance matrix. To evaluate the phylogenetic relevance, bootstrapping
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41 analysis with 1,000 replicates was performed by neighbour-joining and the Jukes-
42
43 Cantor parameter. Erythrovirus sequences from the GenBank were used as
44
45 reference sequences: genotype 1, the strain Au (M13178) [Shade et al., 1986] and
46
47 two strains from Vietnam (DQ225149 - subtype 1a and DQ357064 - subtype 1b,
48
49 respectively) [Toan et al., 2006]; genotype 2, the strain A6 (AY044266) [Nguyen et
50
51 al., 2002] and the strain LaLi (AY064475) [Hokynar et al., 2002]; and the genotype 3a
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3 virus termed V9 (AX003421) [Nguyen et al., 1999] and the genotype 3b virus D91.1
4
5 (AY083234) [Servant et al., 2002].
6

7 To avoid contamination with DNA amplicons, precautions, which have been
8
9 described previously, were used during all steps connected with DNA detection or
10
11 analysis by PCR [Kwok et al., 1989].
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14 **Results**

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16 The clinical and virological characterization of the patients are presented in the Table
17
18 1. The patients were immunocompromised (except for P1-3, P9, P11, P16, P17, P19,
19
20 P21, P24 and P25), due to underlying illness, immunosuppressive treatment or
21
22 chemotherapy. The samples studied originated from symptomatic individuals mostly
23
24 during the acute phase of infection. Out of 28 patients, where B19V serology testing
25
26 was available, sixteen samples were positive for both anti-B19V IgM and IgG, one for
27
28 IgM alone, and in the case of 8 immunocompromised patients, anti-B19V was not
29
30 detectable (Table 1). In 3 further individuals specific IgG was found. B19V DNA loads
31
32 ranged from 10^2 to 10^{11} IU/ml. For 17 of the patients, B19V infection markers were
33
34 monitored for between 2 and 255 weeks. For all patients, where data for longer than
35
36 1 month were available, B19V DNA levels declined, however complete
37
38 disappearance of the viral nucleic acid from plasma was not observed. Maximum
39
40 period of observed infection in immunocompetent and immunocompromised patients,
41
42 was 124 and 255 weeks respectively. In one case, where virus was monitored for
43
44 114 weeks, patient (P7) was treated with immunosuppressed drugs and despite high
45
46 doses of intravenous immunoglobulin (IVIG), high levels of B19V viremia and severe
47
48 anemia were observed.
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53
54 The majority of B19V strains sequenced were genotype 1. However, of 30 strains
55
56 sequenced, two (6.7%) genotype 2 viruses were identified and were amongst the 19
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3 immunocompromised patients (10.5%). Variability of a ~700 bp region of the genome
4 (spanning the NS1/VP1u junction) ranged from 0 to 2% for genotype 1 and was 2%
5
6 for genotype 2. Comparing genotype 1 and genotype 2, diversity ranged between 7%
7
8 and 8%. Strains P29 and P30 showed 98% nucleotide identity with genotype 2
9
10 reference strains (A6 and LaLi) and phylogenetic analysis confirmed the clustering of
11
12 P29 and P30 with the genotype 2 reference strains (Figure 1). Twenty-seven of the
13
14 strains grouped with the genotype 1a reference sequence, however one strain (P28)
15
16 was located between the type 1a and 1b sub-groups. The GenBank accession
17
18 numbers of the nucleotide sequences determined in the study are FN827057 to
19
20 FN827097.
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25 For some of the genotype 1 strains, high levels of identity were observed. Sample
26
27 pairs P1 and P2 and P16, P17 were taken from pregnant women with hydrops fetalis
28
29 and their fetuses by cordocentesis. In each case, the respective pairs from mother
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31 and fetus were identical to each other over the region sequenced. In addition, isolate
32
33 P18 was identical to P16 and P17. Strain P18 originated from a patient hospitalized
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35 in a children's ward, in the same city and during same infection season as P16 and
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37 P17. Samples P20 and P21 were identified in a bone marrow donor and recipient,
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39 and were again identical to one another. Identical sequences or with single
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41 nucleotide changes (99.86-100% identity) were isolated from three patients (P6, P7
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43 and P8) in the same children's ward who, prior to the onset symptoms of B19V
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45 infection, had been admitted to hospital at the same time due to hematological
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47 disease.
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51 In Figure 2, the course of the two genotype 2 infections in patients P29 and P30 is
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53 presented. Patient P29 (Figure 2A), a 29 year-old woman, underwent kidney
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55 transplantation in November 2003 and received the graft from 46 year old woman.
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3 Four months later she developed pure red cell aplasia (peripheral blood cell count
4 (RBC) $2.67 \times 10^6/\mu\text{L}$) had with very low levels of hemoglobin (Hb) and reticulocyte
5 count (7.6g/dL and 0.5%, respectively). The patient had a high viral load ($\sim 10^{10}$ IU
6 B19V DNA/ml) and was positive for anti-B19V IgM and IgG. Bone marrow
7 examination revealed depletion of most erythroid elements. Based on the result of
8 B19V testing, a change of immunosuppression treatment from tacrolimus to
9 cyclosporin was introduced and viremia fell (10^7 IU B19V DNA/ml) and hematological
10 parameters normalized (Hb >10.2 g/dL and RBCs $>3.6 \times 10^6/\mu\text{L}$). During the next few
11 months, B19V DNA levels remained around 10^5 IU/ml and infection was
12 asymptomatic. After 10 weeks of B19V infection the woman became pregnant;
13 ultrasound examination of the fetus failed to reveal any signs of B19V infection. The
14 baby was subsequently delivered at full term and in good health. No episodes of
15 symptomatic reactivation were later observed in the mother. In this case B19V DNA
16 was detected for 255 weeks (5 years). Both anti-B19V IgM and IgG were detected in
17 plasma of the patient throughout the five year period. The prolonged infection and
18 atypical seroconversion was probably the result of the immunosuppressive therapy.
19 In Figure 2B, the second case of genotype 2 infection in an 18 year old woman is
20 presented. In January 2008, the patient was diagnosed with T-lymphoblastic
21 leukemia. She was treated according to PALG-4 2002 protocol and later reinduction
22 protocol FLAM, morphology normalized in March 2008. On the 10th May she was
23 hospitalized due to high fever reaching 40°C, rash, aching joints and joint edema.
24 Pancytopenia was observed by blood examination: white blood cells (WBCs) 0.8
25 $\times 10^3/\mu\text{L}$, RBCs $2.77 \times 10^6/\mu\text{L}$, Hb 9.1 g/dl, and platelets (Plt) $48 \times 10^3/\mu\text{L}$. Examination
26 of the bone marrow revealed reticulopenia (reticulocytes 0.08%). Viral loads were 4.6
27 $\times 10^9$ IU B19V DNA/ml and both anti-B19V IgM and IgG were detected. The patient
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3 was treated with intravenous immunoglobulin (IVIg (Sandoglobulin) 24 g /day for 5
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5 days). and the viral load fell (10^2 IU/ml plasma) and fever, rash and joint pain
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7 improved and gradual normalization of peripheral blood parameters was observed
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9 (7th day: RBCs $2.35 \times 10^6/\mu\text{L}$, WBCs $1.94 \times 10^3/\mu\text{L}$, Plt: $190 \times 10^3/\mu\text{L}$; 12th day: RBCs
10
11 $3.70 \times 10^6/\mu\text{L}$, WBC $3.65 \times 10^3/\mu\text{L}$, Plt $251 \times 10^3/\mu\text{L}$, reticulocytes 4.21%). Both anti-B19V
12
13 IgM and IgG were detectable by the 15th day of observation. By the 30th day, until the
14
15 end of the period of serology monitoring, only anti-B19V IgG was observed. Due to
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17 planned chemotherapy and hematopoietic stem cell transplantation, IVIG was
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19 administered twice more to consolidate treatment. No symptoms were observed,
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21 however DNA B19V remained detectable for a total of 85 days until the day of the
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23 patient's death caused by a leukemia relapse. Testing of the last available sample
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25 revealed 2×10^4 IU B19V DNA/ml.
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29 Discussion

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32 In the present study, where patients with clinical manifestation of B19V were
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34 studied, two genotypes of B19V were found in Poland. Genotype 1 was the
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36 predominant type, with some genotype 2 strains being detected less frequently. To
37
38 the best of our knowledge, this is the first report of genotype 2 cases in Eastern
39
40 Europe. The data presented imply that in Poland genotype 2 could be responsible for
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42 a significant proportion of acute symptomatic infections, especially in
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44 immunocompromised patients. A similar case of genotype 2 infection has been
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46 described in a kidney transplant recipient by Liefeldt et al. [2005]. The patient
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48 developed persistent viremia associated with chronic anemia and pure red cell
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50 aplasia combined with recurrent high viremic periods [Liefeldt et al., 2005]. These
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52 nosocomial cases – the two described in present study and the one described by
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54 Liefeldt et al. [2005] occurred within a fairly close geographical area (i.e. Poland and
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3 South Eastern Germany) and may favor the hypothesis that the circulation of B19V
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5 variants may be epidemiologically restricted to certain geographical areas. In a
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7 previous report, Hübschen et al. [2009] found two single cases of genotype 3b B19V,
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9 but not genotype 2, in rash/fever patients from South-East Europe (Greece and
10
11 Bulgaria) and suggested spread of genotype 3b from Africa to Europe.
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14 In the study presented, the clinical spectrum associated with genotype 2 was
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16 found to be similar to that observed with genotype 1 infection. This was not
17
18 unexpected, since the study aimed to detect variants in groups of patients with
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20 symptoms suggestive of B19V infection. The genotype 2 B19V infection in the kidney
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22 transplant recipient did not manifest clinically during pregnancy. However, it is likely
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24 to be due to the presence of protective antibodies at the start of pregnancy rather
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26 than lack of pathogenic potential in the fetus.
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30 These observations together with problems in genotype 2 detection described
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32 previously [Hokynar et al., 2004; Cohen et al., 2006; Baylis et al., 2009] demonstrate
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34 that proper diagnosis, particularly in the case of immunosuppressed patients is very
35
36 important. In addition, the data highlighted in the present study indicate that lack of
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38 detectable antibodies to B19V in immunocompromised patient is a frequent
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40 phenomenon, emphasizing the importance of B19V DNA analysis in patients [Amiot
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42 et al., 1998; Oeda et al. 1994; Liefeldt et al. 2005].
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46 Previously genotype 2 B19V DNA has been detected in 2.5% of plasma-
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48 derived clotting factors, more frequently in older concentrates than those
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50 manufactured more recently [Schneider et al., 2004]. Genotype 2 B19V has been
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52 identified in blood donors and in plasma for fractionation albeit only rarely [Blümel et
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54 al., 2005; Koppelman, et al., 2007; Baylis 2008]. Genotype 2 strains have been found
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56 occasionally in patients in France [Servant et al., 2002], England [Cohen et al., 2006],
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3 Italy [Corcioli et al., 2008], Germany [Liefeldt et al., 2005; Schneider et al., 2008],
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5 United States [Nguyen et al., 2002; Wong et al., 2003], Brazil [Sanabani et al., 2006],
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7 Vietnam [Toan et al., 2006] and South Africa [Corcoran et al., 2010] and represents a
8
9 minority of strains. Although in a significant number of these reports viremia was the
10
11 result of persistent infections characterized by the presence of a low viral load or
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13 DNA B19V detectable in certain tissues from older subjects [Schneider et al., 2008;
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15 Sanabani et al., 2006; Toan et al., 2006; Corcioli et al., 2008]. Analysis of the
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17 proportion of B19V variants in tissues in relationship to patients' age led to the
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19 proposal that genotype 1 and 2 circulated widely and with equal frequency in
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21 Northern and Central Europe, from the 1930s until the 1950s. However, genotype 2
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23 appears to have disappeared thereafter, as confirmed by patient sera collected
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25 during the 1980s and 1990s [Norja et al., 2006]. Norja et al. [2008] suggested that the
26
27 infrequent number of cases of genotype 2 infections recorded in Europe may have
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29 arisen by the transmission of reactivated virus from persistently infected individuals
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31 rather than by the continued low-level circulation of this genotype in the community.
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33 In the case of the kidney transplant recipient described in present paper, no data are
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35 available regarding blood transfusion, however transmission of persistent genotype 2
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37 virus from the kidney donor (a 46 year old woman) cannot be excluded as it was
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39 described previously by Yango and colleagues [2002]. The leukemia patient infected
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41 with genotype 2, had not been transfused for at least for two months prior to the
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43 onset of infection and this case may suggest current circulation of the variant virus in
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45 the community, and not the effect of reactivation of persistent virus infection from the
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47 blood or the graft from the donor.
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54 In present study we observed persistent viremia in both immunocompetent
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56 and immunocompromised patients, lasting at least ~2.5 and ~5 years, respectively.
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3 Similar observations have been reported previously [Tang et al. 2007; Liefeldt et
4 al.2005; Wąsak-Szulowska et al. 2007; Dobec et al. 2007; Lefrère et al. 2005] and
5 the probable causes of such infections have been reviewed by Parsyan and Candotti
6 [2007]. In case of kidney graft recipient infected with genotype 2 B19V it seems that
7 lack of viral clearance was mainly connected with immunosuppressive therapy
8 impairing the humoral immune response. Consequently, no seroconversion was
9 observed. Similar prolonged infection with B19V genotype 2, lasting ~2.5 years, was
10 observed previously in a renal transplant recipient, with levels 4.2×10^{11} IU/ml of
11 B19V DNA and severe anemia [Liefeldt et al. 2005].

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23 In the present study transmission of certain genotype 1 stains could be
24 identified, not only from mother to fetus, but the same strain was detected in a bone
25 marrow donor and recipient (clinical details were described previously by Wąsak-
26 Szulowska et al., [2008]), in patients from a children's hematological ward and even
27 in patients residing in the same region during the same infection season. These
28 results are in agreement with previous reports where data were presented,
29 concerning strains obtained from acute-phase serum samples of patients with
30 erythema infectiosum and transient aplastic crisis, the B19V viruses circulating during
31 the particular outbreak showed high levels of identity up to 100% [Erdman et al.,
32 1996; Hemauer et al., 1996]. Since seasonal outbreaks of B19V occur which cause
33 epidemics every few years, there is the potential for rapid changes in the
34 epidemiology of the virus. It is possible that similar to the situation for genotype 1
35 B19V, epidemic spread of genotype 2 could occur in the future in Eastern Europe or
36 in other areas, resulting in reemergence of this B19V variant.

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54 In conclusion, in the present study two cases of symptomatic genotype 2
55 infection in immunocompromised patients by prospective testing. Such cases
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3 indicate that variants should be considered when designing and validating B19V
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5 assays and in the development of reference preparations. These tools should help in
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7 selecting diagnostics assays that will be reliable to detect all known genotypes both
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9 blood donor screening as well as B19V diagnostic testing in patients and in a better
10
11 understanding of the epidemiology of B19V both past and present.
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14 15 16 **Acknowledgments**

17
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19
20 helpful discussions.
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23 24 25 **Conflict of interest**

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27 The authors declare no conflict of interest.
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Table 1. Clinical and virological characterization of B19V DNA positive samples.

Case	Accession number	Clinical manifestations	Anti-B19V		B19V DNA level (IU/ml)	Year/region
			IgM	IgG		
P1	FN827059	Hydrops fetalis, 21 st week of pregnancy	+	+	8.6x10 ⁶	2007, Mazowieckie
P2	FN827060	Fetus of P1	-	-	3.9x10 ⁸	2007, Mazowieckie
P3	FN827078	Pregnant woman with hydrops fetalis	+	+	6.4x10 ⁶	2005, Mazowieckie
P4	FN827085	Aplasia	no data	no data	1x10 ⁵	2006, Mazowieckie
P5	FN827083	Pancytopenia in patient with acute lymphoblastic leukemia	-	-	1.3x10 ⁷	2005, Mazowieckie
P6	FN827066	Rash	-	-	1x10 ³	2007, Mazowieckie
P7	FN827067	Splenomegaly and reticulocyte count drop in patient with autoimmune hemolytic anemia	+	-	9.6x10 ⁹	2007, Mazowieckie
P8	FN827075	Rash, fever, enlarged spleen and liver, neutropenia	-	-	2x10 ⁶	2007, Mazowieckie
P9	FN827086	Aplastic crisis	+	+	1.8x10 ⁸	2006, Mazowieckie
P10	FN827077	Red cell aplasia	+	+	1.1x10 ⁷	2005, Lubelskie
P11	FN827071	Abortion	+	+	4.7x10 ²	2007, Mazowieckie
P12	FN827065	Fever, rash in patient with acute lymphoblastic	-	+	6.9x10 ¹⁰	2007, Mazowieckie

		leukemia				
P13	FN827079	Anemia, splenomegaly	+	+	1.8×10^4	2007, Mazowieckie
P14	FN827068	Severe anemia after allogeneic hematopoietic stem cell transplantation	-	-	10^{11}	2007, Pomorskie
P15	FN827082	Before allogeneic hematopoietic stem cell transplantation	-	-	2.8×10^6	2008, Mazowieckie
P16	FN827057	Hydrops fetalis	-	+	3.1×10^6	2007, Mazowieckie
P17	FN827058	Fetus of P16	-	+	4×10^8	2007, Mazowieckie
P18	FN827064	Anemia	+	+	1.9×10^3	2007, Mazowieckie
P19	FN827076	Pregnant woman with hydrops fetalis, 22 nd week of pregnancy	+	+	2.6×10^4	2007, Mazowieckie
P20	FN827061	Anemia after allogeneic hematopoietic stem cell transplantation	-	-	2.9×10^8	2004, Mazowieckie
P21	FN827062	Donor for P20	+	+	2×10^4	2004, Mazowieckie
P22	FN827072	Aplasia in patient with lymphoblastic leukemia	+	+	5.7×10^7	2006, Śląskie
P23	FN827084	no data	-	-	8.8×10^2	2006, Śląskie
P24	FN827080	Abortion in 13 th week of pregnancy	+	+	5.1×10^4	2006, Mazowieckie
P25	FN827063	Rash, reticulocyte count drop in patient with Evans syndrome	+	+	2.7×10^6	2008, Mazowieckie
P26	FN827069	Aplasia	+	+	1.3×10^4	2005, Mazowieckie

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P27	FN827073	Aplasia	+	+	9.5x10 ³	2005, Śląskie
P28	FN827070	Aplasia in patient with autoimmune hemolytic anemia	no data	no data	1.4x10 ⁶	2006, Śląskie
P29	FN827081	Anemia after kidney transplantation	+	+	6.5x10 ⁹	2004, Wielkopolskie
P30	FN827074	Anemia in T-lymphoblastic leukemia patient	+	+	4.6x10 ⁹	2008, Mazowieckie

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3 Figure1. Phylogenetic analysis of the B19V strains identified in the
4 Polish patients. The analysis is based upon a 704 bp sequence in the
5 NS1/VP1 region of the B19V genome. Bootstrap values greater than 70
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10 are shown.
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For Peer Review

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3 Figure 2. Course of B19V genotype 2 infection in two immunosuppressed
4 women: A) renal transplant patient with pure red cell aplasia and B)
5 leukemia patient with fever, rash, joint aches and anemia at the time of
6 B19V diagnosis. Virological markers are indicated on the figure: viral
7 loads (▲-▲) and antibody status. Levels of hemoglobin are also shown
8 (●--●). In panel A, the transplant recipient, the change in the
9 immunosuppressive regime is shown (↓); the time when the patient was
10 pregnant is indicated. In panel B, the leukemia patient, three rounds of
11 IVIG treatment (Sandoglobulin 24g a day for 5 successive days) are
12 shown (↓↓↓↓↓).

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