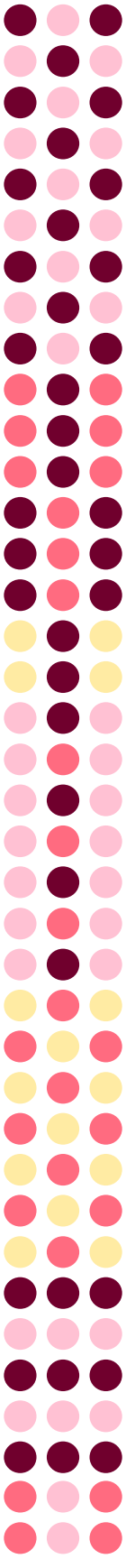
Parvovirus B19: diagnosis, distribution and disease associations

Russcher, A.



5

Detection of parvovirus B19 DNA in blood: viruses or DNA remnants?

Marijke W.A. Molenaar-de Backer[1], Anne Russcher[2], Aloys C.M. Kroes[2], Marco H.G.M. Koppelman[1], Mirthe Lanfermeijer[1], Hans L. Zaaijer[1]

Affiliations
1. Sanquin, Department of Blood-borne Infections, Amsterdam, The Netherlands.
2. Leiden University Medical Center , Department of Medical Microbiology, Leiden, The Netherlands.

J Clin Virol 2016; 84:19-23.

ABSTRACT

Background: Parvovirus B19 (B19V) DNA can be detected in blood over a long period after acute infection. Several reports associate the presence of B19V DNA with disease, irrespective of timing of the initial B19V infection.

Objectives: This study aims to analyze the properties of B19V DNA in blood, in order to differentiate between bare, non-infectious strands of DNA and B19V DNA in viable virions.

Study design*:* Ten blood donors with asymptomatic acute B19V infection were followed and sampled up to 22 months after infection. The samples were treated with and without an endonuclease and tested for B19V DNA, to distinguish between DNA in virions and naked DNA.

Findings*:* In the acute phase of infection, high levels of B19V DNA (> 10^6 IU/mL) were detected, concurrent with B19V IgM antibodies. B19V DNA apparently was encapsidated, as indicated by resistance to endonuclease degradation. Subsequently, B19V DNA was detectable for more than one year in all donors at low levels (< 10^5 IU/mL). Approximately 150 days after infection B19V DNA became degradable by an endonuclease, indicating that this concerned naked DNA. In some donors a second endonuclease-resistant peak occurred.

Discussion*:* Detection of B19V DNA in blood by PCR does not necessarily imply that B19V replication takes place and that infectious B19V virions are present. We propose that remnant B19V DNA strands can be released from tissues without active replication. This finding urges to reconsider an assumed role of B19V infection based on B19V DNA detection in blood, a much debated subject in clinical syndromes such as myocarditis and arthritis.

1. BACKGROUND

Parvovirus B19 (B19V) is a small, nonenveloped, single-stranded DNA virus, replicating primarily in erythroid progenitor cells in the bone marrow. B19V infections are widespread in the human population and transmission predominantly takes place by respiratory droplets. During acute B19V infection a short-lived exceptionally high peak of B19V DNA occurs in peripheral blood (> 10^{12} IU/mL) [1]. During a much longer period following acute infection low levels of B19V DNA (10^{1}-10^{4} IU/mL) may be detected in the blood of immunocompetent individuals, lasting for months or years, long after the anti-B19V IgG response has developed [2-4]. B19V DNA is lifelong detectable in various tissues of immunocompetent adults testing positive for B19V antibodies, such as skin, synovia, tonsils, liver and heart and even in 70-year-old bone remnants [5-7].

Since normally no B19V DNA is detectable in blood, considerable attention was given in the last two decades to the frequent detection of parvovirus B19V DNA in blood and tissues. This has triggered interest in the role of B19V in several clinical conditions, particularly in a number of cardiac disorders, but also in chronic arthropathy and liver failure [8,9]. In many reports, a causal relationship between the detection of viral DNA in blood or tissue and the clinical disorder was considered likely [10, 11]. In this way, parvovirus is often classified among the causes of myocarditis and cardiomyopathy [11-13]. On the other hand, based on the absence of further support by serology, clinical signs and epidemiological evidence, several reports remained cautious about the presumed causal relationship [14-17]. In addition, the persistence of viral DNA on the tissue level was considered an alternative source for the release of viral DNA, suggesting that tissue damage could lead to passive release of B19V DNA [6,18,19]. Most likely, in some cases passive release of viral DNA explains its occurrence in blood, while in other cases active viral infection is responsible. Since no further analysis is available which could differentiate between these conditions, divergent views on the relevance of B19V viremia still coexist in literature.

To establish the nature of parvovirus DNA in a patient is a matter of theoretical and practical importance. Passively released viral DNA can be interpreted as causally irrelevant and non-infectious. Viral DNA produced by viral replication indicates active infection and infectivity. This differentiation is relevant for transfusion safety, transplantation purposes and disease management. The availability of a simple test determining the nature of B19V DNA in various conditions would be valuable to interpret the presence of B19V DNA correctly.

2. OBJECTIVES

This study explored potential differences in the properties of parvovirus DNA as detected in different settings: immediately during an established acute infection, and in later stages. The differentiation of B19V DNA as present in infectious viral particles and B19V DNA as bare fragments, released from various cell types, is attempted by determining the effects of a nuclease treatment on samples.

3. STUDY DESIGN

3.1. Blood samples

Ten series of follow-up samples during and after acute B19V infection were obtained as follows. Dutch plasmapheresis donors, who donate frequently, are routinely screened for B19V DNA. Ten plasmapheresis donors, who tested strongly positive for B19V DNA (> 10^6 IU/mL), were selected. Follow-up samples from their routine blood donations were obtained from the blood bank repository. All donors agreed that (a part of) their donation could be used for research. The follow-up samples cover a period of 11-22 months after the index donation. In addition, a sample just before the index donation was obtained from the repository. The ten series of donor samples cover an average period of 18 months, including on average 10 serial samples, with an average inter-donation interval of 62 days. In addition to the 10 donor series, 18 serial samples from a technician in the laboratory who experienced B19V infection were available, covering a period of 25 months.

3.2 Endonuclease treatment and dual target B19V PCR

To investigate whether detected B19V DNA concerns packaged DNA in B19V virions, or whether it is naked, free B19V DNA, a treatment step using Benzonase® (Sigma-Aldrich, The Netherlands) was developed. Benzonase® is an endonuclease which can degrade all forms of DNA and RNA, including 'naked', unpacked B19V DNA, but not the B19V DNA in virions [20,21]. The optimized treatment for EDTA plasma samples was as follows: To 210 µL EDTA-plasma 3.2 µL 1M $MgCl_2$ was added to obtain a final concentration of 15 mM $MgCl_2$. This extra $MgCl_2$ is necessary for the endonuclease to have an optimal activity in EDTA-plasma. Subsequently, the sample was split in 2x 100 µL, and to one of the 100 µL samples 250 Units endonuclease was added. The other 100 µL sample was not treated with endonuclease, to determine the effect of the endonuclease treatment. Both samples were incubated in a shaking incubator at 37°C at 120 rpm for 1 hour. Next the samples

were allowed to cool to room temperature and B19V DNA PCR procedure was performed (see below). In each experiment a negative control and 2 positive controls (naked, extracted B19V DNA and B19V DNA in virions, obtained from an acute B19V infection [load 2 x 10^{13} IU/mL without B19V IgM and IgG]) were tested with and without endonuclease to determine the efficiency of the endonuclease treatment and to control the B19V PCR. B19V DNA was extracted from the samples and controls and the extracts were used for dual target NS1-VP2 B19V PCR, this entire procedure was described previously[22].

3.3. IgM and IgG antibodies to B19V

All serial samples were tested for B19V IgM and IgG antibodies using the Biotrin Parvovirus B19 IgM and IgG Enzyme Immunoassays (DiaSorin, Ireland), following the manufacturer's instructions, with the adaptation that IgM values >1.5 were considered positive and IgM values between 0.9 and 1.5 were considered equivocal.

3.4. Estimation of viremic and IgM positive period

An estimate of the viremic and IgM positive period was made as described previously[23]. Briefly, the median proven duration of viremia (endonuclease resistance) was calculated from all blood donors with more than one B19 endonuclease resistant donation. To this calculated interval 0.5 times the median interval between the last negative and first positive donation and 0.5 times the median duration between last positive and first negative donation were added. In addition this was also performed for proven IgM-positive period with donors which had more than 1 positive IgM time point.

RESULTS

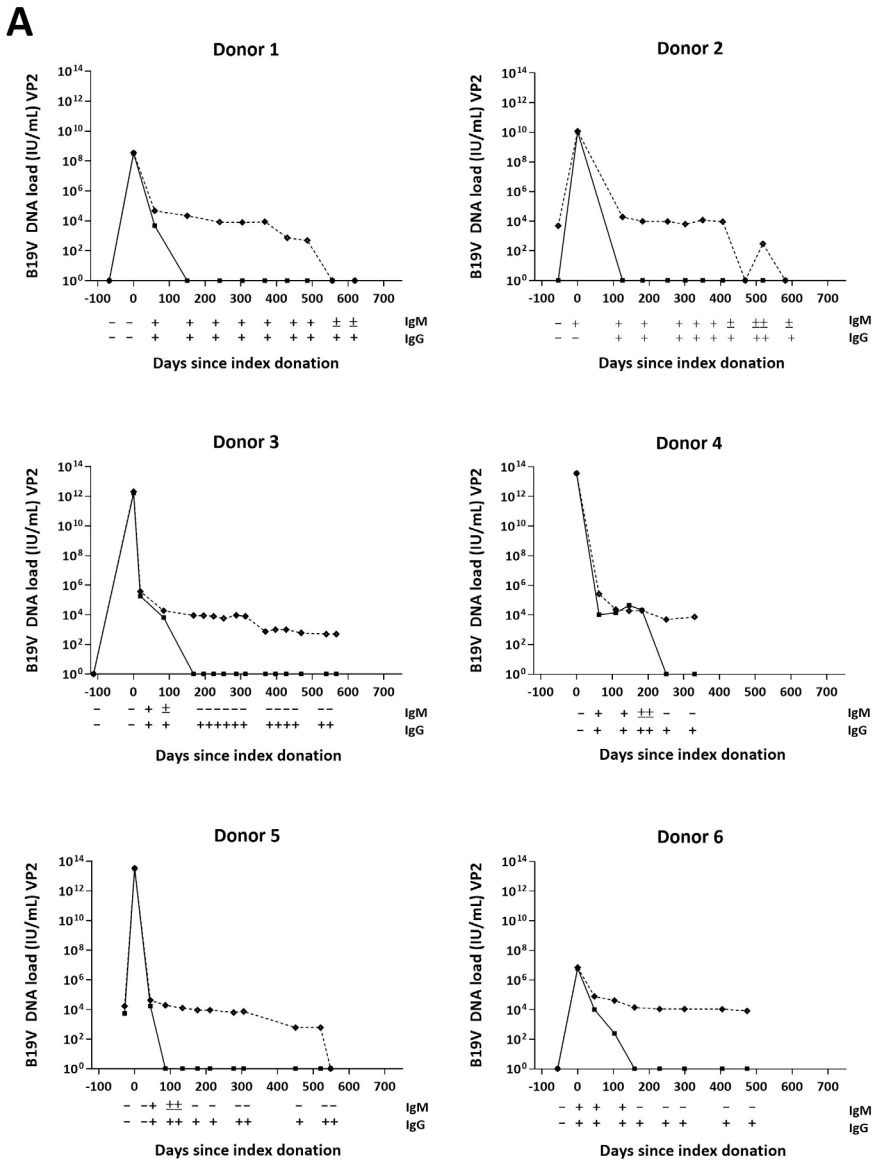
4.1. Effect of endonuclease treatment

There was no reduction observed by endonuclease treatment in any of the 11 high level peaks of acute B19V infections. However, there was a strong reduction, of minimal 4 log10, of B19V DNA levels during later phases of infection and in naked DNA controls.

4.2. Course of B19V DNA levels

The serial samples obtained from 10 B19V infected plasma donors and the B19V infected technician showed the following pattern (see Fig. 1):

1) In the 10 donors the donation before the 'acute' index donation tested negative for B19V DNA and for B19V IgM and IgG antibodies.
2) The index donations showed high B19V DNA levels (>1x10^6 IU/mL) and often these were still negative for B19V IgM (7/10) and B19V IgG (9/10). In the index donations the B19V DNA level was not sensitive to endonuclease treatment, which is compatible with an encapsidated state of B19V DNA in viral particles.
3) After the index donation the samples showed significant lower levels of B19V DNA (10^3-10^4 IU/mL) during several months. Most donors (7/10) were still B19V DNA positive in the last available sample. However, 150 days following the index donation most samples were 100% sensitive to endonuclease, suggesting that B19V DNA in these samples is no longer encapsidated, but 'naked', non-infectious B19V DNA. The median B19V viremic (endonuclease resistant) period was estimated at 137 days. The median B19V DNAemic period could not be estimated since most donors were still positive for B19V DNA in their last donation.
4) All samples following the index donation tested B19V IgG positive, and IgM antibodies gradually disappeared in most donors. The disappearance of B19V IgM seemed to correlate with the transition from B19V virions to naked B19V DNA. The median time of IgM presence was estimated at 142 days. In two donors no IgM seroconversion was detected. This was probably due to the fact that the time between the index donation and subsequent donation was too long (68 and 122 days) to detect IgM.
5) Strikingly, three donors and the technician showed a second endonuclease-resistant B19V DNA peak, on average 10 months after acute infection (see Fig. 1B). This second peak seemed to reflect a temporary return of B19V virions. The presence of an endonuclease-inhibiting factor was ruled out by adding Parvovirus 4 (PARV4) DNA extract in these samples, which was subsequently degraded by endonuclease. In addition, adding a higher concentration of endonuclease (1250 and 2500 units) did not degrade the B19V DNA in the second peak either.


A
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
Donor 6
B19V DNA load (IU/mL) VP2
Days since index donation
IgM
IgG

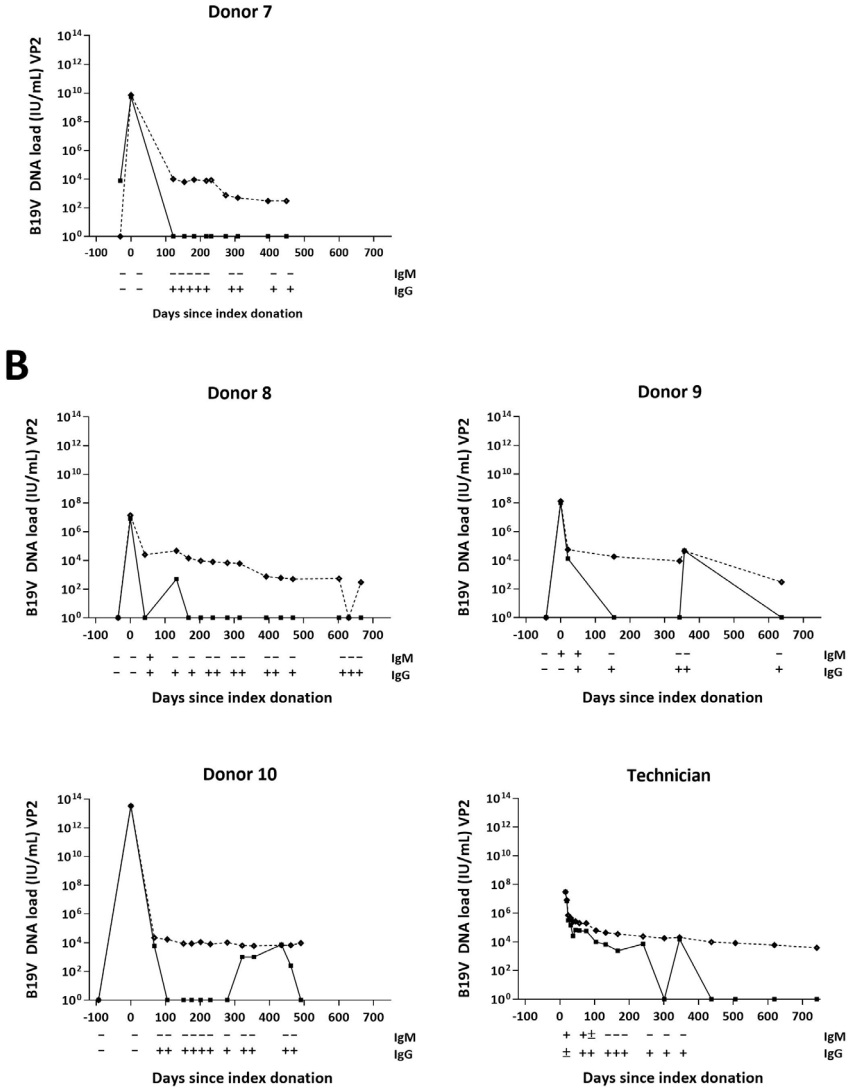


***Figure 1**: Follow-up of B19V DNA levels and B19V serology after acute B19V infection in 11 persons. The B19V DNA level was determined without (diamonds) and with (squares) endonuclease treatment. Panel A shows the predominant pattern: initially B19V DNA is endonuclease-resistant, indicative of encapsidated viral DNA. Subsequently a longer episode of endonuclease-degradable B19V DNA follows, indicating the presence of bare B19V DNA strands. Panel B shows 4 persons with a second endonuclease-resistant peak, suggestive of a second episode of virus replication.*

DISCUSSION

This study provides evidence that the presence of B19V DNA in blood not necessarily correlates with active B19V replication. The serial samples from acutely B19V-infected, asymptomatic blood donors demonstrated that B19V DNA can be detected for a long period after acute B19V infection in accordance with previous studies [2,3,24]. Endonuclease treatment demonstrated that only in the first months after acute B19V infection presence of B19V DNA is indicative of the presence of B19V virions. Five months after the acute peak almost all B19V DNA could be degraded by endonuclease, indicating that predominantly naked B19V DNA was present. The transition from virions to naked DNA correlated with the disappearance of B19V IgM in most cases. Naked B19V DNA was detected when B19V DNA levels decreased below 10^5 IU/mL. This finding is in agreement with the observation that after acute infection, blood donors with B19V levels below 10^4 IU/mL do not transmit B19V infection to recipients of blood and blood products [25,26]. Until now it was assumed that this lack of infectivity is due to concurrent neutralising B19V antibodies, or an inadequate amount of infused virions [24]. We hypothesize that such low 'viremic' blood donors simply do not carry B19V virions anymore.

Interestingly in 4 out of 11 persons a second temporary endonuclease-resistant B19V DNA peak occurred between 6 and 12 months after primary infection. Spiking of one of these samples with naked PARV4 DNA showed that this was not due to endonuclease-inhibiting factors, since naked PARV4 DNA was degraded. The possibility of a second phase of replication is confirmed by Lindblom et al, who reported that 1 of 5 patients with B19V infection showed increasing B19V DNA levels 50 weeks after infection [27]. Why this second peak arises and whether it has clinical consequences remains to be elucidated.

The endonuclease treatment has some limitations. The interpretation of serial samples is straightforward, but the interpretation of a single sample might be more challenging, if only partial reduction by endonuclease occurs. In addition, multiple freeze-thawing cycles of samples could damage the B19V capsid and cause overestimation of naked B19V DNA.

After the DNA peak, donors show a short rapid decline in B19V DNA to 10^4–10^5 IU/mL. This rapid decline coincides with B19V IgG production and might reflect efficient neutralization of the virus by IgG. This is in accordance with the results of Lindblom et al. which show a rapid decline in B19V DNA, when B19V IgG antibodies

appear. In addition, this coincides with resolution of acute clinical symptoms [27]. After this sharp decline, B19V DNA levels remain stable at 10^4IU/mL and only slowly decrease over years. The B19V DNA becomes endonuclease sensitive after the sharp decline and this seems to correlate to the disappearance of IgM. In addition the clearance rates of virions (endonuclease resistance) and IgM were quite comparable, 137 versus 142 days respectively.

The finding of bare B19V DNA in plasma months or years after acute B19V infection thus seems unrelated to active viral replication and probably has a different origin. During acute infection B19V infects erythrocyte progenitors via the globoside receptor and via co-receptors α5β1 integrins and Ku80 [28,29]. After cell entry, B19V can replicate only when the cell is in late S-phase. Recently, von Kietzell et al reported that B19V can also enter cells via enhanced antibody-mediated endocytosis involving heat-sensitive complement factor C1q [30]. However, antibody-mediated endocytosis does not seem to initiate a productive infection cycle [30]. It is tempting to speculate that antibody-mediated endocytosis leads to accumulation of B19V DNA in several types of cells, which are non-permissive for B19V infection. B19V may not replicate due to the presence of antibodies, or because these cells fail to provide the correct proteins for B19V replication, e.g. are not in S-phase. In addition, the extreme high levels of B19V viremia during acute infection may cause B19V to enter cell types and biocompartments via otherwise inefficient, non-specific receptors. Once in a non-permissive cell, B19V DNA will persist episomally, since cells have no mechanism to degrade DNA. This is confirmed by the fact that many tissues which are non-permissive for B19V have been reported to contain B19V DNA [5,7]. When these cells are damaged by any disease process or when renewed (normal cell turnover), naked B19V DNA will be released into the circulation. For long-lived cells this may occur years or even decades after initial B19V infection. Many acute B19V infections appear to be followed by long-term, slowly declining levels of B19V DNA. As different cell types have different renewal rates, this might cause a stochastic release of B19V DNA, explaining the slowly declining 'viremia' after acute infection. In addition to apoptosis and necrosis, the release of viral DNA via exocytosis might contribute to the presence of naked B19V DNA in plasma [31].

Small amounts of B19V DNA can be detected because PCR assays are very sensitive. However, they do not discriminate between naked and virion-associated DNA. The method presented in this study could prove useful to determine whether

B19V DNA is infectious and linked to replication or not. This question is particularly urgent in myocarditis or cardiomyopathies, often found to be associated with detectable levels of B19V DNA. If indeed B19V DNA in myocarditis patients is not encapsidated, this would suggest that B19V DNA is released from damaged cardiomyocytes years after infection, and that B19V is not the cause of the myocarditis but only a marker. During the entire human life it has been estimated that only 39% of cardiomyocytes is renewed [32]. Thus, they may store B19V DNA passively for decades. Several authors have considered this passive role of released B19V DNA as a mere marker of tissue damage the most likely explanation [14-18]. When B19V indeed is not replicating, antiviral treatment with IVIG, a proposed treatment in B19V myocarditis [33], will be ineffective.

In summary, this study demonstrated that after infection, levels of B19V DNA below 10^5 IU/mL were not based on active B19V replication. Pre-treatment of blood samples with endonuclease enabled discrimination between naked B19V DNA (unrelated to replication) and B19V DNA encapsidated in virions. This method can be useful to settle longstanding controversies on the nature of B19V DNA in various conditions, whether it is the cause of pathology or whether it is just a consequence, an innocent bystander.

Competing interest: The authors do not have a commercial or other association that might pose a conflict of interest.

Funding: This research was funded by Sanquin Blood Supply Foundation, Amsterdam, The Netherlands.

Ethical approval: Not required.

REFERENCES

1. Young NS, Brown KE. Parvovirus B19. N Engl J Med 2004;350(6):586-97.
2. Kerr JR, Curran MD, Moore JE, Coyle PV, Ferguson WP. Persistent parvovirus B19 infection. Lancet. 1995;345(8957):1118.
3. Musiani M, Zerbini M, Gentilomi G, Plazzi M, Gallinella G, Venturoli S. Parvovirus B19 clearance from peripheral blood after acute infection. J Infect Dis. 1995;172(5):1360-3.
4. Lefrere JJ, Servant-Delmas A, Candotti D, Mariotti M, Thomas I, Brossard Y, et al. Persistent B19 infection in immunocompetent individuals: implications for transfusion safety. Blood. 2005;106(8):2890-5.
5. Norja P, Hokynar K, Aaltonen LM, Chen R, Ranki A, Partio Ek, et al. Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. Proc Natl Acad Sci USA. 2006; 103(19):7450-3.
6. Corcioli F, Zakrzewska K, Rinieri A, Fanci R, Innocenti M, Civinini R, et al. Tissue persistence of parvovirus B19 genotypes in asymptomatic persons. J Med Virol. 2008;80(11):2005-11.
7. Toppinen M, Perdomo MF, Palo JU, Simmonds P, Lycett SJ, Soderlund-Venermo M, et al. Bones hold the key to DNA virus history and epidemiology. Sci Rep. 2015;5: 7226.
8. Heegaard ED, Brown KE. Human parvovirus B19. Clin Microbiol Rev. 2002; 15(3): 485-505.
9. Adamson-Small LA, Ignatovich IV, Laemmerhirt MG, Hobbs JA. Persistent parvovirus B19 infection in non-erythroid tissues: possible role in the inflammatory and disease process. Virus Res. 2014;190:8-16.
10. Chia JK, Jackson B. Myopericarditis due to parvovirus B19 in an adult. Clin Infect Dis. 1996; 23(1):200-1.
11. Kuhl U, Pauschinger M, Bock T, Klingel K, Schwimmbeck CP, Seeberg B, et al. Parvovirus B19 infection mimicking acute myocardial infarction. Circulation. 2003;108(8):945-50.
12. Bock CT, Klingel K, Kandolf R. Human parvovirus B19-associated myocarditis. N Engl J Med. 2010;362(13):1248-9.
13. Pankuweit S, Moll R, Baandrup U, Portig I, Hufnagel G, Maisch B. Prevalence of the parvovirus B19 genome in endomyocardial biopsy specimens. Hum Pathol. 2003;34(5):497-503.
14. Kuethe F, Lindner J, Matschke K, Wenzel JJ, Norja P, Ploetze K, et al. Prevalence of parvovirus B19 and human bocavirus DNA in the heart of patients with no evidence of dilated cardiomyopathy or myocarditis. Clin Inf Dis. 2009;49(11):1660-6.
15. Lotze U, Egerer R, Gluck B, Zell R, Sigusch H, Erhardt C, et al. Low level myocardial parvovirus B19 persistence is a frequent finding in patients with heart disease but unrelated to ongoing myocardial injury. J Med Virol. 2010;82(8): 449-57.
16. Stewart GC, Lopez-Molina J, Gottumukkala RV, Rosner GF, Anello MS, Hecht JL, et al.

Myocardial parvovirus B19 persistence: lack of association with clinicopathologic phenotype in adults with heart failure. Circ Heart Fail. 2011;4(1):71-8.
17. Koepsell SA, Anderson DR, Radio SJ. Parvovirus B19 is a bystander in adult myocarditis. Cardiovasc Pathol. 2012;21(6):476-81.
18. Schenk T, Enders M, Pollak S, Hahn R, Huzly D. High prevalence of human parvovirus B19 DNA in myocardial autopsy samples from subjects without myocarditis or dilative cardiomyopathy. J Clin Microbiol. 2009;47(1):106-10.
19. Clewley JP. Polymerase chain reaction assay of parvovirus B19 DNA in clinical specimens. J Clin Microbiol. 1989;27(12):2647-51.
20. de Backer MW, Garner KM, Luijendijk MC, Adan RA. Recombinant adeno-associated viral vectors. Methods Mol Biol. 2011;789:357–376.
21. Pyeon D, Lambert PF, Ahlquist P. Production of infectious human papillomavirus independently of viral replication and epithelial cell differentiation. Proc Natl Acad Sci USA. 2005:102(26):9311–9316
22. Molenaar-de Backer MW, de WM, Sjerps MC, Koppelman MH. Validation of new real-time polymerase chain reaction assays for detection of hepatitis A virus RNA and parvovirus B19 DNA. Transfusion. 2016:56(2):440-448.
23. Hogema B, Molier M, Sjerps M, de Waal M, van Swieten P, van de Laar T et al. Incidence and duration of hepatitis Evirus infection in Dutch blood donors, Transfusion. 2016;56(3):722–728.
24. Juhl D, Gorg S, Hennig H. Persistence of Parvovirus B19 (B19V) DNA and humoral immune response in B19V-infected blood donors. Vox Sang. 2014;107(3):226-32.
25. Juhl D, Ozdemir M, Dreier J, Gorg S, Hennig H. Look-back study on recipients of Parvovirus B19 (B19V) DNA-positive blood components. Vox Sang. 2015;109(4):305-11.
26. Hourfar MK, Mayr-Wohlfart U, Themann A, Sireis W, Seifreid E, Schrezenmeier H, et al. Recipients potentially infected with parvovirus B19 by red blood cell products. Transfusion. 2011;51(1):129-36.
27. Lindblom A, Isa A, Norbeck O, Wolf S, Johansson B, Broliden K, et al. Slow clearance of human parvovirus B19 viremia following acute infection. Clin Inf Dis. 2005;41(8):1201-3.
28. Weigel-Kelley KA, Yoder MC, Srivastava A. Alpha5beta1 integrin as a cellular coreceptor for human parvovirus B19: requirement of functional activation of beta1 integrin for viral entry. Blood. 2003;102(12):3927-33.
29. Munakata Y, Saito-Ito T, Kumura-Ishii K, Huang J, Kodera T, Ishii T, et al. Ku80 autoantigen as a cellular coreceptor for human parvovirus B19 infection. Blood. 2005;106(10):3449-56.
30. von Kietzell K, Pozzuto T, Heilbronn R, Grossl T, Fechner H, Weger S. Antibody-mediated enhancement of parvovirus B19 uptake into endothelial cells mediated by a receptor for complement factor C1q. J Virol. 2014;88(14):8102-15.
31. van der Vaart M, Pretorius PJ. Circulating DNA. Its origin and fluctuation. Ann N Y Acad Sci 2008;1137:18-26.

32. Bergmann O, Zdunek S, Felker A, Salehpour M, Alkass K, Bernard S, et al. Dynamics of Cell Generation and Turnover in the Human Heart. Cell. 2015;161(7):1566-75.
33. Dennert R, Velthuis S, Schalla S, Eurlings L, van Suylen RJ, van Paassen P, et al. Intravenous immunoglobulin therapy for patients with idiopathic cardiomyopathy and endomyocardial biopsy-proven high PVB19 viral load. Antivir Ther. 2010;15(2):193-201.