Parvovirus B19 DNA detectable in hearts of patients with dilated cardiomyopathy, but absent or inactive in blood

Anne Russcher*, Job Verdonschot, Marijke W.A. Molenaar-de Backer, Stephane R.B. Heymans, Aloys C.M. Kroes and Hans L. Zaaijer

1Department of Medical Microbiology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands; 2Department of Cardiology, Maastricht University Medical Centre, Maastricht, The Netherlands; and 3Department of Blood-borne Infections, Donor Medicine Research, Sanquin Blood Supply Foundation, Amsterdam, The Netherlands

Abstract

Aims Parvovirus B19 (B19V) is often assumed to be a cause of dilated cardiomyopathy (DCM), based on the quantification of B19V DNA in endomyocardial biopsies (EMB). Whether the presence of B19V DNA correlates with active infection is still debated. Application of the enzyme endonuclease to blood samples results in degradation of B19V DNA remnants but leaves viral particles intact, which enables differentiation between active and past infection. In this study, the susceptibility to degradation by endonuclease of B19V DNA in blood was compared between DCM patients and a control group of recent B19V infections.

Methods and results Twenty blood samples from 20 adult patients with DCM, who previously tested positive for B19V DNA in EMB and/or blood, were tested with B19V PCR before and after application of endonuclease to the samples. Six blood samples tested positive for B19V DNA with a mean viral load of $2.3 \times 10^4$ IU/mL. In five samples, B19V DNA became undetectable after endonuclease (100% load reduction); in one sample, DNA load showed a 23% log load reduction (viral load before endonuclease: $9.1 \times 10^4$ IU/mL; after: $6.5 \times 10^3$ IU/mL). Presence of cardiac inflammation did not differ between patients with B19V DNAemia (1/4) and patients without B19V DNAemia (6/14) ($P$ value = 1.0). In all 18 control samples of proven recent B19V infections, DNA remained detectable after application of endonuclease, showing only a mean log load reduction of 2.3% (mean viral load before endonuclease: $8.1 \times 10^{11}$ IU/mL; after: $8.0 \times 10^{11}$ IU/mL). Load reduction differed significantly between the DCM group and the control group; indicating the presence of intact viral particles in the control group with proven active infection and the presence of DNA remnants in the DCM group ($P$ value = 0.000).

Conclusion During recent B19V infection, viral DNA levels in blood were unaffected by endonuclease. In contrast, B19V DNA in blood in patients with DCM became undetectable or strongly reduced after application of endonuclease. Circulating viral DNA in this subset of patients with presumed parvovirus-associated DCM does not consist of intact viral particles. Viral replicative activity cannot be assumed from demonstrating B19V DNA in cardiac tissue or in blood in DCM patients.

Keywords Parvovirus B19; Dilated cardiomyopathy; Viral activity; Endonuclease

Received: 6 July 2020; Revised: 24 March 2021; Accepted: 26 March 2021

*Correspondence to: Anne Russcher; Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands. Tel: +0031 338502099; +0031 6 52420895. Email: a.russcher@lumc.nl

Introduction

Dilated cardiomyopathy (DCM) has a broad spectrum of aetiologies, with damage to cardiomyocytes by viral infection as a prominent cause.¹ For certain viruses, such as Coxsackie B virus, the causal relationship between presence of viral nucleic acid in cardiac tissue and cardiac disease is widely accepted, but in case of parvovirus B19 (B19V), this relationship is still debated. After initial infection by B19V, the presence of B19V DNA can be demonstrated in peripheral blood for months or even years, without apparent disease activity.²,³ In tissue, B19V DNA may remain detectable for life as has...
been demonstrated in various tissues, including heart, brain, bone, liver, kidney, skin, and lymphoid tissue. Without additional clinical or laboratory signs of persistent infection, numerous reports remained cautious about assuming a causal relationship between the presence of B19V DNA in cardiac tissue or in blood, and cardiac disease. Furthermore, several studies did not demonstrate a higher incidence of B19V DNA in endomyocardial biopsies (EMB) of DCM patients, as compared with control subjects at autopsy. On the other hand, evidence for a causal role of B19V in cardiac disorders is reported, particularly when the cardiac viral load is quantified. Therefore, the exact role of B19V in cardiac pathology remains unclear. It is suggested that additional factors need to be considered to determine the pathogenicity of B19V, such as viral activity.

Previously, it was demonstrated that application of endonuclease to circulating parvoviral DNA leads to its degradation, except when DNA is packaged and thus protected in viral particles during an active viral infection. This enabled differentiation between active infection, where intact viral particles are present, and past infection, where mere parvoviral DNA remnants are released from persistence in cells. In this study, the susceptibility to degradation of B19V DNA in blood was compared between patients with confirmed DCM and a control group of well-defined recent B19V infections as a measure for viral activity.

Methods

Blood samples of dilated cardiomyopathy patients

Twenty plasma samples were selected from 20 adult patients diagnosed with DCM from a cohort of inpatients and outpatients of the Maastricht University Medical Centre in the period 2006–2016. The cohort was part of a randomized controlled trial investigating intravenous immunoglobulins (IVIG) in the treatment of B19V-positive patients with DCM (NCT: 00659386). Sample selection was based on the highest B19V viral loads in EMB as these patients were expected to have B19V detectable in blood. Eighteen patients previously tested positive for B19V DNA in EMB; two patients did not undergo EMB, but they had previously tested positive for B19V DNA in blood.

Blood samples of patients with recent parvovirus B19 infection

Eighteen plasma samples from 18 B19V PCR-positive patients from different patient groups were selected. If plasma was not available, serum was used as the alternative. This selection consisted of patients from the Leiden University Medical Center with well-characterized, active B19V infection in the period 2011–2017. Patients were categorized by clinical presentation: recent B19V infection in a normal host; severe B19V infection due to underlying haematological pathology; patients presenting with arthropathy; hydropic foetuses and B19V infection in the severely immunocompromised.

Endonuclease (Benzonase®) assay and PCR

The test principle of the endonuclease assay is depicted in Figure 1. Samples were tested as described by Molenaar-de Backer et al. Both plasma and serum are suitable for B19V PCR and the endonuclease assay. In short, each plasma or serum sample was split in two 100 μL aliquots. In case of plasma, MgCl₂ was added to oppose the chelating effects of EDTA on nucleases. To one of the aliquots 250 units of Benzonase® (Sigma-Aldrich, The Netherlands) were added. Subsequently both aliquots were incubated at 37°C in a shaking incubator at 120 rpm for 1 h. After cooling to room temperature, DNA extraction and dual target NS-VP2 PCR were performed as described previously. Viral loads differed between samples of patients with recent B19V infection and patients with DCM. Therefore, the detection of B19V DNA with and without application of endonuclease was also performed on samples after standardizing the B19V DNA load to 10⁴ IU/mL by adding B19V-negative plasma.

Statistical analysis

The data were analysed using IBM SPSS Statistics 26. Categorical data between unrelated groups were compared using Fisher’s exact test. Numerical data between unrelated groups were compared using the Mann-Whitney U test. Results were considered statistically significant at the P = 0.05 level.

Ethics

The investigation conforms with the principles outlined in the Declaration of Helsinki. All cardiomyopathy patients gave written informed consent as part of the Maastricht Cardiomyopathy Registry (for inclusion and exclusion criteria of the registry; refer to Verdonschot et al.). For control patients, samples were collected with patient’s informed consent for the use of the samples for B19V diagnostics.
Results

Dilated cardiomyopathy patients

Six of the 20 DCM patient blood samples tested positive for B19V DNA. Fourteen DCM patients with high cardiac viral loads had no detectable B19V DNA in their blood. Patient characteristics are described in Table 1. Mean viral load was $2.3 \times 10^4$ IU/mL. In five patients B19V DNA became entirely undetectable after application of endonuclease; one remained detectable but showed a viral load reduction from $9.1 \times 10^4$ to $6.5 \times 10^3$ IU/mL, corresponding to a log reduction of 23% (refer to Figure 2). In this patient, B19V DNA had already been detected with a higher load ($5.9 \times 10^5$ IU/mL) in a blood sample taken 6 weeks previously, which was not available for testing with endonuclease. Presence of cardiac inflammation did not differ between patients with B19V DNAemia (1/4) and patients without B19V DNAemia (6/14) ($P$ value $= 1.0$).

Proven recent parvovirus B19 patients

All 18 patients with recent B19V-infection tested positive with B19V PCR with a mean viral load higher than the DCM patients ($8.1 \times 10^{11}$ IU/mL). Table 2 shows background information for all 18 patients. Patients were grouped according to their clinical background (refer to Methods). None of the control patients showed cardiological signs or symptoms. After endonuclease, all remained detectable with a mean viral load of $8.0 \times 10^{11}$ IU/mL, corresponding to only a mean 2.3% log reduction of viral load. Figure 3 shows the results of testing with and without application of endonuclease for individual patients in different patient categories. Load reduction differed significantly between the DCM group and the control group ($P = 0.000$).

Standardized testing at $10^4$ IU/mL

Samples with viral loads exceeding $10^4$ IU/mL were standardized to $10^4$ IU/mL by diluting them with B19V-negative plasma. Twelve samples were available for standardization in the control group. After standardizing, the mean log reduction in viral loads in the control group before and after endonuclease was 2.4%. Samples from cardiac patients were not diluted to $10^4$ IU/mL as all six samples already had viral loads not exceeding $10^4$ IU/mL.

Discussion

Control patients with recent or acute B19V infections showed high B19V DNA levels in blood, which were unaffected by endonuclease. In contrast, B19V DNA levels were low or absent in blood samples of patients with DCM. When B19V DNA was
| Patient | Clinical Information | Laboratory Parameters at Time of EMB | Immunohistochemical Parameters in EMB (cells/mm²) | Cardiac Inflammation | B19V Load in EMB (c/μg DNA) | Time from Diagnosis DCM to EMB (weeks) | B19V Load in Blood (IU/mL) | Time from EMB to Blood Sample (weeks) |
|---------|----------------------|-------------------------------------|--------------------------------------------------|----------------------|----------------------------|------------------------------------------|-----------------------------|-------------------------------------|
| 1       | Heart failure, diagnosed since approx. 9 months; declining VL in blood | NA 517 8.3 | 0 0 6.5 0 | 7.2 2.4 | No | 2333 | 40 | 8.9 x 10³ | -3 |
| 2       | DCM (EF 35%); recently diagnosed | <0.01 70 286 | 20 7.4 11.5 0 | 23.1 4.6 | Yes | 1025 | 3 | 1.3 x 10⁴ | 0 |
| 3       | DCM (EF 32%); after acute perimyocarditis (EF 10%) | 33 63 401 | 2.7 5 0.8 0 | 6.1 4.6 | No | 601 | 1 | 6.5 x 10³ | 0 |
| 4       | DCM (EF 10%), recently diagnosed, improving to 45% with CRTD treatment | NA 302 344 | 1.7 1 1.4 0 | 5.2 2.4 | No | 312 | 3 | 1.6 x 10⁴ | 0 |
| 5       | Heart failure, diagnosed since 6 weeks, declining VL in blood | NA NA 14 | NA NA NA NA NA | NA NA | No EMB | NA NA | NA | 9.1 x 10⁴ | — |
| 6       | Heart failure, diagnosed since 1 week, pre-existent hypertensive heart disease | 0.02 84 3070 | NA NA NA NA NA NA | NA NA | No EMB | NA NA | NA | 2.9 x 10³ | — |
| 7       | DCM (EF 33%); dysynchronisation due to LBBB; improving to 51% with medication | NA NA NA | 11 7.5 3.3 0 | 12.2 5.4 | Yes | 9948 | 3 | Neg | 0 |
| 8       | DCM (EF 32%) | 12 111 49.5 | 3.7 1.6 2.1 0 | 8 2.7 | No | 7250 | 2 | Neg | -7 |
| 9       | DCM (EF 10%); improving to EF 38% during IVIG trial; declining VL in EMB; possibly auto-immune component | 120 94 1032 | 2.4 0.6 8.6 0 | 6.1 4.9 | No | 6889 | 9 | Neg | -1 |
| 10      | DCM (EF 46%); recently diagnosed in work-up atrial fibrillation; improving to EF 54% during IVIG trial | 5 232 2.4 | 3.3 0.4 1.4 0 | 4.5 1.4 | No | 5669 | 2 years | Neg | 0 |
| 11      | DCM (EF 45%); diagnosis after cardiac arrest | 176 107 9.9 | 4.4 2.5 5.5 0 | 29.7 19.8 | Yes | 2806 | 0 | Neg | 0 |
| 12      | DCM (EF 20%); recently diagnosed; improving to 55% during IVIG trial | NA NA 38.7 | 2.5 NA 3.3 0 | 23 3.3 | Yes | 2726 | 9 | Neg | 16 |
| 13      | DCM (EF 40%); improving to 52% during IVIG trial | NA NA 16 | 2.5 10.7 0.5 0 | 6.1 1.5 | No | 2436 | 11 | Neg | -7 |
| 14      | DCM (EF 45%); chronic since 7 years | 0.01 NA 4 | 9.3 NA 7.9 0 | 10 NA | No | 2379 | 7 years | Neg | -8 |
| 15      | DCM (EF 40%); recently diagnosed; possibly past myocarditis | <0.01 NA 65 | 35.4 25.8 20.4 0 | 36 12.7 | Yes | 2220 | 4 | Neg | -20 |
| 16      | DCM (EF 35%); recently diagnosed; improving to 51% during IVIG trial | 0.01 866 278 | NA 0.6 0 0 | 1.2 1.5 | No | 2185 | 12 | Neg | -13 |
| 17      | DCM (EF 20%); recently diagnosed; improving to 35% with medication | <0.01 92 77.3 | 1.8 NA 0.7 0 | 3.3 NA | No | 2163 | 2 | Neg | -5 |
| 18      | DCM (EF 44%) after cardiotoxic chemotherapy; recovery to previous 62% | NA NA 10.1 | 25.4 21.2 21.5 1.4 | 42.3 22.6 | Yes | 1829 | 8 | Neg | 0 |
| 19      | DCM (EF 29%); MRI: midmyocardial fibrosis, possibly past myocarditis | 11 61 46.9 | 6.6 8.6 4.6 0 | 12.2 2.3 | Yes | 875 | 3 | Neg | 0 |
| 20      | DCM (EF 30%); chronic since 2 years; improving to EF 51% with medication | 18 24 17.7 | 3.2 1.6 1.6 0 | 3.7 2.4 | No | 691 | 3 years | Neg | 0 |

B19V, parvovirus B19; CRTD, cardiac resynchronization therapy defibrillator; DCM, dilated cardiomyopathy; EF, ejection fraction; EMB, endomyocardial biopsy; MRI, magnetic resonance imaging; NA, not available; NT-proBNP, N-terminal pro B-type natriuretic peptide; VL, viral load.

Cardiac inflammation is diagnosed according to the criteria of the European Society for Cardiology.
present, it became undetectable after application of endonuclease in the majority of patients. The degradability of circulating B19V DNA in DCM patients indicates that replicating viral particles are absent in blood, suggesting that the detected B19V DNA consists of DNA remnants. In concordance with this finding is the relatively low viral load in blood of these DCM patients. Similar loads were demonstrated in patients after acute infection who were no longer able to transmit infection.\textsuperscript{21} Standardizing testing conditions by diluting samples with very high viral loads to viral loads of 10\textsuperscript{4} IU/mL gave identical results in the patient group with recent infection, that is, application of endonuclease did not result in a significant reduction of viral load. Therefore, the effect of endonuclease does not depend on viral load in this range. Dilution of samples to viral loads lower than 10\textsuperscript{4} IU/mL was not performed; further dilution could result in inaccurate measurements as 10\textsuperscript{5} IU/mL is a relatively low viral load.

B19V DNA resulting from viral replication can be demonstrated in blood in nearly all patients with proven acute infection.\textsuperscript{22} In patients with proven chronic symptomatic B19V infection, DNAemia is also invariably present in blood.\textsuperscript{23–25} The absence of DNAemia is therefore suggestive of the absence of viral replication. In the cohort of DCM patients, the positivity rate in blood was only 33\% (6/18), so most patients with B19V detectable in cardiac tissue did not demonstrate DNAemia. One cardiac patient with a positive B19V PCR in blood showed only partial degradation of B19V. Given that this patient had B19V detectable in an earlier sample and that the level of B19V DNA in blood was decreasing, this is suggestive of a recent infection. A positive IgM would confirm a recent B19V infection, but unfortunately, no additional serology could be performed in this case, due to insufficient material.

Viral infections are one of the causes of DCM. In addition to the direct effect of viral replication causing tissue damage, it has been proposed in the case of B19V and DCM that the virus elicits a chronic inflammatory process or that molecular mimicry plays a role.\textsuperscript{16,26,27} IVIG are being studied in the treatment of presumed B19V-induced DCM. Dennert \textit{et al.}\textsuperscript{28} treated DCM patients with relatively high cardiac B19V loads with IVIG. Viral load in EMB decreased, and cardiac functions improved significantly after IVIG treatment. However, the uncontrolled pilot study included only 17 patients, and the absolute improvement in cardiac function was modest. Therefore, a double-blinded randomized trial has been started to investigate any beneficial effects of IVIG in B19V-associated DCM (NCT:00659386). The results of the study are expected to be published in 2021. However, the exact mode of action of the beneficial effects in this application is unknown. If viable viral particles are indeed absent in presumed B19V-induced DCM patients, then a direct antiviral effect of IVIG will not be the primary mechanism. In addition, IVIG also has anti-inflammatory and immunomodulating properties, which could also explain its beneficial effect in DCM patients.\textsuperscript{29} Determining viral activity will be an important factor to consider during the analysis and interpretation of the results of the randomized clinical trial.

Recently, it was observed that immunosuppressive, anti-inflammatory medication (prednisolone and azathioprine) to treat inflammatory cardiomyopathy (DCMi) did not have an adverse outcome in patients with cardiac B19V persistence but was equally beneficial for both DCMi patients with and without B19V DNA detectable in EMB (median viral load 80 c/μg DNA, range 1–5074).\textsuperscript{30} Similar observations were made in a recent Swedish cohort of DCM patients, where the frequent occurrence of B19V DNA in cardiac tissue in both patients with DCM and in healthy donor hearts was confirmed. Prognosis of patients with DCM did not differ between patients with or without B19V DNA in cardiac tissue in this study, without taking viral load into account.\textsuperscript{31} This suggests that the mere presence of B19V DNA does not contribute to the disease process. In reaction, it has been suggested that methods of determining viral activity should be further investigated to understand the pathogenic role of B19V.\textsuperscript{27} The endonuclease test could be a new and easy instrument to contribute to assessment of viral activity. Its application could be useful in the etiological work-up of DCM, but also in cases of acute myocarditis where B19V genomes are detected in EMB and blood and where a strong B19V-specific immune response is elicited.\textsuperscript{32} There are limitations to this study. We have investigated the nature of B19V DNA in peripheral blood but not in cardiac tissue, as the endonuclease test can only be applied to blood samples. To expose intracellular viral particles or DNA in

---

**Figure 2** Parvovirus B19 (B19V) DNA absent or degradable in blood of patients with dilated cardiomyopathy. Blood samples of dilated cardiomyopathy patients with and without endonuclease treatment. Dark bars indicate DNA load without endonuclease treatment; light bars and/or arrows indicate DNA load with endonuclease treatment (DNA load absent after endonuclease in five out of six samples).
tissue to endonuclease, samples would need to be treated with a protease. This would already affect any intact viral particles present, degrading their capsid and therefore interfering with a subsequent endonuclease assay, that is, the application of protease would produce naked DNA. Future research could aim to develop a specific endonuclease assay suitable for cardiac tissue. Another limitation is the difference in disease duration between DCM cases and controls with recent infection. The pathophysiological process in DCM often unfolds silently for a period of time before becoming clinically apparent, as opposed to acute infection with B19V. Although the results from this study do not point to continuing viral replication in the course of DCM, the relation between timing of initial B19V infection and the development of DCM cannot be specified. Therefore, a possible role of B19V in the induction of DCM remains to be elucidated. Also, our study focused on determining viral activity by the method of endonuclease. Additional methods may also be employed to prove the presence or absence of replicating, infectious virus. Viral culture can be considered a gold standard for viral activity, although viral culture in case of B19V is notoriously challenging and insensitive, partly due to its specialized tropism for erythroid progenitor cells. Previous research also proposed the evaluation of mRNA intermediates as a biomarker for B19V activity. The use of mRNA intermediates can still be considered a pioneering technique and sensitivity and specificity are not yet established. It would however be valuable to employ complementary approaches such as mRNA intermediates to gain a complete understanding of the replicative status of B19V.

In conclusion, the results confirm the presence of circulating viral DNA in all cases with clinically proven active B19V infection. This viral DNA cannot be degraded enzymatically, probably because it is contained in protective viral

---

Table 2 Characteristics of patients with recent B19V infection

| Patient | Age | Sex | Symptoms | Serology at time of sampling | Duration of clinical symptoms at time of sampling | B19V load in blood (IU/mL) | Sample type |
|---------|-----|-----|----------|-------------------------------|---------------------------------------------|-----------------------------|-------------|
| 1       | 57  | V   | Fever, influenza-like illness | Pos, Neg                       | 11 days                                     | 1.9 × 10^12                 | s           |
| 2       | 36  | V   | Mother of hydropic foetus; sample at time of intra-uterine transfusion | Pos, Pos                       | Unknown                                      | 4.4 × 10^5                   | s           |
| 3       | 63  | V   | Fever and erythema            | Pos, Pos                       | 5 days                                      | 9.5 × 10^6                   | s           |
| 4       | 4   | M   | Severe anaemia; underlying sickle cell disease | Pos, Pos                       | 6 days                                      | 7.5 × 10^6                   | s           |
| 5       | 31  | M   | Severe anaemia; underlying spherocytosis | Pos, Neg                       | 4 days                                      | 1.3 × 10^11                   | p           |
| 6       | 43  | M   | Severe anaemia; underlying aplastic anaemia | Pos, Pos                       | 24 days                                     | 1.1 × 10^5                   | p           |
| 7       | 40  | V   | Transient arthropathy         | Pos, Pos                       | 3 days                                      | 6.5 × 10^5                   | p           |
| 8       | 33  | V   | Transient arthropathy         | Pos, Pos                       | 5 days                                      | 5.7 × 10^5                   | s           |
| 9       | 41  | V   | Transient arthropathy         | Pos, Neg                       | 10 days                                     | 8.9 × 10^5                   | s           |
| 10      | 41  | V   | Transient arthropathy         | Pos, Pos                       | 7 days                                      | 2.3 × 10^7                   | p           |
| 11      | 0   | NA  | Foetal hydrops; sample at time of intra-uterine transfusion | NA, NA                         | Unknown                                     | 2.9 × 10^10                   | s           |
| 12      | 0   | NA  | Foetal hydrops; sample at time of intra-uterine transfusion | NA, NA                         | Unknown                                     | 4.8 × 10^8                   | p           |
| 13      | 0   | NA  | Foetal hydrops; sample at time of intra-uterine transfusion | NA, NA                         | Unknown                                     | 8.2 × 10^10                   | p           |
| 14      | 0   | NA  | Foetal hydrops; sample at time of intra-uterine transfusion | NA, NA                         | Unknown                                     | 5.0 × 10^9                   | p           |
| 15      | 68  | M   | Asymptomatic; underlying multiple myeloma after allogeneic SCT | Pos, Pos                       | Unknown                                     | 4.0 × 10^7                   | s           |
| 16      | 52  | V   | Fever and anaemia; relapse malignancy after allogeneic SCT | Pos, Neg                       | Unknown                                      | 5.1 × 10^10                   | p           |
| 17      | 47  | M   | Recurrent anaemia and B19V viraemia after renal transplantation | Pos, Neg                       | 1 day                                       | 1.2 × 10^13                   | p           |
| 18      | 32  | V   | Fever and lymphadenopathy in patient with renal transplantation | Pos, Neg                       | 6 days                                      | 7.3 × 10^9                   | s           |

B19V, parvovirus B19; NA, not available; SCT, stem cell transplantation.

*S Sample type: p = plasma, s = serum.

*No symptomatology; initial diagnosis by hydropic foetus.

*Onset of symptoms unclear due to multimorbidity; recent infection can be presumed because of positive IgM and increasing titres of IgM in the weeks following sampling.
particles. In patients suspected of chronic B19V-associated DCM, there is a much lower incidence of circulating viral DNA. When viral DNA is present in blood in such cases, it is degradable by endonuclease. These findings show that viral replicative activity cannot be assumed from demonstrating B19V in cardiac tissue or in blood. Multidisciplinary approaches are necessary to further investigate the role of B19V in the pathogenic mechanisms in the development of DCM.

Conflict of interest
None declared.

Funding
The authors received no specific funding for this work.

References

1. Verdonschot JAJ, Hazebroek MR, Ware JS, Prasad SK, Heymans SRB. Role of targeted therapy in dilated cardiomyopathy: the challenging road toward a personalized approach. J Am Heart Assoc 2019; 8: e012514.
2. Kerr JR, Curran MD, Moore JE, Coyle PV, Ferguson WP. Persistent parvovirus B19 infection. Lancet (London, England) 1995; 345: 1118.
3. Lindblom A, Isa A, Norbeck O, Wolf S, Johansson B, Brolden K, Tolfvenstam T. Slow clearance of human parvovirus B19 viremia following acute infection. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2005; 41: 1201–1203.
4. Soderlund-Venermo M, Hoknar K, Nieminen J, Rautakorpi H, Hedman K. Persistence of human parvovirus B19 in human tissues. Pathol Biol 2002; 50: 307–316.
5. Adamsson-Small LA, Ignatovich IV, Laemmerhirt MG, Hobbs JA. Persistent parvovirus B19 infection in non-erythroid tissues: a possible role in the inflammatory and disease process. Virus Res 2014; 190: 8–16.
6. Kuethe F, Lindner J, Matschke K, Wenzel JJ, Norja P, Ploetz E, Schaall S, Kamvissi V, Bornstein SR, Schwanebeck U, Modrow S. Prevalence of parvovirus B19 and human bocavirus DNA in the heart of patients with no evidence of dilated cardiomyopathy or myocarditis. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2009; 49: 1660–1666.
7. Lotze U, Egerer R, Gluck B, Zell R, Sigusch H, Erhardt C, Heim A, Kandolf R, Bock T, Wutzler P, Figulla H-R. 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: 1449–1457.

8. Stewart GC, Lopez-Molina J, Gottumukkal RA, Rosner GF, Anello MS, Hecht JL, Winters GL, Padera RF, Baughman KL, Lipes MA. Myocardial parvovirus B19 persistence: lack of association with clinicopathologic phenotype in adults with heart failure. Circ Heart Fail 2011; 4: 71–78.

9. Koepsell SA, Anderson DR, Radio SJ. Parvovirus B19 is a bystander in adult myocarditis. Cardiovasc Pathol 2012; 21: 476–481.

10. Schenk T, Enders M, Polnik S, Hahn R, Huzly D. High prevalence of human parvovirus B19 DNA in myocardial autopsy samples from subjects without myocarditis or dilatative cardiomyopathy. J Clin Microbiol 2009; 47: 106–110.

11. Nielsen TS, Hansen J, Nielsen LP, Baandrup UT, Banner J. The presence of enterovirus, adenovirus, and parvovirus B19 in myocardial tissue samples from autopsies: an evaluation of their frequencies in deceased individuals with myocarditis and in non-inflamed control hearts. Forensic Sci Med Pathol 2014; 10: 344–350.

12. Pankuweit S. Prevalence of the parvovirus B19 genome in endomyocardial biopsy specimens. Hum Pathol 2003; 34: 497–503.

13. Kuhl U, Pauschinger M, Seeberg B, Lassner D, Noutsias M, Poller W, Schultheiss H-P. Viral persistence in the myocardium is associated with progressive cardiac dysfunction. Circulation 2005; 112: 1965–1970.

14. Bock CT, Klingel K, Kandolf R, Human parvovirus B19-associated myocarditis. N Engl J Med 2010; 362: 1248–1249.

15. Dernert R, van Paassen P, Wolfs P, Bruggeman C, Velthuis S, Felix S, van Suylen RJ, Crijns HJ, Cohen Tervaert JW, Heymans S. Differences in virus prevalence and load in the hearts of patients with idiopathic dilated cardiomyopathy with and without immune-mediated inflammatory diseases. Clinical and vaccine immunology: CVI 2012; 19: 1182–1187.

16. Verdonschot J, Hazebroek M, Merken J, Debing Y, Dernert R, Brunner-La Rocca HP, Heymans S. Relevance of cardiac parvovirus B19 in myocarditis and dilated cardiomyopathy: review of the literature. Eur J Heart Fail 2016; 18: 1430–1441.

17. Molenaar-de Backer MW, Russcher A, Kroes AC, Koppelman MH, Lanfermeijer M, Zaanier HL. Detection of parvovirus B19 DNA in blood: viruses or DNA remnants? Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology. 2016; 84: 19–23.

18. Reber U, Moser O, Dilloo D, Eis-Hubinger AM. On the utility of the benzonase treatment for correct laboratory diagnosis of parvovirus B19 infection. Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology 2017; 95: 10–11.

19. Molenaar-de Backer MW, de Waal M, Sjers 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: 440–448.

20. Verdonschot JAJ, Hazebroek MR, Derks KWI, Barandiaran Aizpuruza A, Merken JJ, Wang P, Bierau J, van den Wijngaard A, Challa SM, Abdul Hamid MA, van Bilsen M, van Empel VPM, Knackstedt C, Brunner-la Rocca HP, Brunner HG, Krapels IPC, Heymans SRB. Titin cardiomyopathy leads to altered mitochondrial energetics, increased fibrosis and long-term life-threatening arrhythmias. Eur Heart J 2018; 39: 864–873.

21. Juhl D, Ozdemir M, Dreier J, Gorg S, Hennig H. Look-back study on recipients of parvovirus B19 DNA-positive blood components. Vox Sang 2015; 109: 305–311.

22. Eid AJ, Brown RA, Patel R, Razonable RR. Parvovirus B19 infection after transplantation: a review of 98 cases. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 2006; 43: 40–48.

23. Knoester M, van den Borne PA, Vossen AC, Kroes AC, Claas EC. Human parvovirus B19 genotype 3 associated with chronic anemia after stem cell transplantation, missed by routine PCR testing. Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology. 2012; 54: 356–370.

24. Beckhoff A, Steffen I, Sandoz P, Hirsch HH, Schaub S. Relapsing severe anemia due to primary parvovirus B19 infection after renal transplantation: a case report and review of the literature. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association 2007; 22: 3660–3663.

25. Liefeldt L, Buhl M, Schweickert B, Engelmann E, Sezer O, Laschinski P, Preuschof L, Neumayer H-H. Eradication of parvovirus B19 infection after renal transplantation requires reduction of immunosuppression and high-dose immunoglobulin therapy. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association 2002; 17: 1840–1842.

26. Cooper LT Jr. Myocarditis. N Engl J Med 2009; 360: 1526–1538.

27. Verdonschot JAJ, Cooper LT, Heymans SRB. Parvovirus B19 in dilated cardiomyopathy: there is more than meets the eye. J Card Fail 2019; 25: 64–66.

28. Dernert R, Velthuis S, Schalla S, Eurlings L, van Suylen RJ, van P, Tervaert JWC, Wolfs P, Goossens VAJ, Bruggeman C, Waltenberger J, Crijns HJ, Heymans S. Intraocular immunoglobulin therapy for patients with idiopathic cardiomyopathy and endomyocardial biopsy-proven high PVB19 viral load. Antivir Ther 2010; 15: 193–201.

29. Ballow M. The IgG molecule as a biological immune response modifier: mechanisms of action of intravenous immune globulin in autoimmune and inflammatory disorders. J Allergy Clin Immunol 2011; 127: 315–323 quiz 24–5.

30. Tschope C, Elsanhoury A, Schlieker S, Van Linhout S, Kuhl U. Immunosuppression in inflammatory cardiomyopathy and parvovirus B19 persistence. Eur J Heart Fail 2019; 21: 1468–1469.

31. Hjalmarsson C, Liljeqvist JA, Lindh M, Karason K, Bollano E, Oldfors A, Andersson B. Parvovirus B19 in endomyocardial biopsy of patients with idiopathic dilated cardiomyopathy: foe or bystander? J Card Fail 2019; 25: 60–63.

32. Streitz M, Noutsias M, Volkmer R, Rohde M, Brestrich G, Block A, Klippert K, Kotsch K, Ay B, Hummel M, Kuhl U, Lassner D, Schultheiss HP, Volk HD, Kern F. NS1 specific CD8+ T-cells with effector function and TRBV11 dominance in a patient with parvovirus B19 associated inflammatory cardiomyopathy. PLoS One 2008; 3: e2361.

33. Wolfsberg R, Ruprecht N, Kempf C, Ros C. Impaired genome encapsidation restricts the in vitro propagation of human parvovirus B19. J Virol Methods 2013; 193: 215–225.

34. Kuhl U, Lassner D, Donar R, Rohde M, Escher F, Seeberg B, Hertel E, Tschope C, Skurk C, Gross UM, Schultheiss HP, Poller W. A distinct subgroup of cardiomyopathy patients characterized by transcriptionally active cardiotropic erythrovirus and altered cardiac gene expression. Basic Res Cardiol 2013; 108: 372.

35. Pietsch H, Escher F, Aleshcheva G, Lassner D, Bock CT, Schultheiss HP. Detection of parvovirus mRNAs as markers for viral activity in endomyocardial biopsy-based diagnosis of patients with unexplained heart failure. Sci Rep 2020; 10: 22354.