Human Parvovirus B19 Infection in Infancy Associated with Acute and Chronic Lymphocytic Myocarditis and High Cytokine Levels: Report of 3 Cases and Review

Giovanni Nigro,[1] Vittoria Bastianon,[1] Vincenzo Colloridi,[1] Flavia Ventriglia,[1] Pietro Gallo,[2] Giulia D'Amati,[2] William C. Koch,[3] and Stuart P. Adler[3]

[1]Pediatric Institute and [2]Department of Experimental Medicine and Pathology, "La Sapienza" University, Rome, Italy; and [3]Department of Pediatrics, Medical College of Virginia, Virginia Commonwealth University, Richmond, USA

Human parvovirus B19 infection is occasionally associated with acute lymphocytic myocarditis (ALM). Three infants with B19 virus–associated ALM were followed up clinically, histologically, and immunovirologically. Each infant had B19 virus DNA in the blood or B19 virus–specific IgM antibodies. Two infants with postnatal infection recovered after immunosuppressive therapy. The third infant with possible prenatal infection developed chronic persistent myocarditis associated with persistent B19 virus DNA in the blood. All 3 infants had increased levels of interferon-γ, tumor necrosis factor–α, and interleukins -6 and -8. Four newborns with congenital B19 virus infection and 4 infants and children who had postnatally acquired B19 virus infection without myocarditis all had normal levels of these cytokines. These observations suggest that B19 virus infection in infancy causes ALM in some infants and children.

Acute lymphocytic myocarditis (ALM) is characterized by an inflammatory infiltrate of the myocardium and myocellular necrosis or degeneration that differs from that typical of ischemic damage [1]. According to Dallas criteria for histological classification, ALM may account for 9% of all types of myocarditis [2]. The disease ranges from subclinical to extremely severe, and the outcomes vary from self-limited to chronic with sudden death occurring in 16%–21% of children [3, 4]. Moreover, at a rate ranging from 3% to 60%, the development of unexplained dilated cardiomyopathy in adults is considered to be a consequence of ALM acquired in infancy [1, 2].

Numerous infectious agents can cause ALM, but viruses and subsequent immune responses are the most frequently implicated pathogens [2, 5]. Among several viruses, human parvovirus B19 has been considered as a possible cause of myocarditis [6–16]. We observed ALM without pericardial effusion in 3 infants with B19 virus infection: 2 had acute infection and recovered, whereas 1 developed chronic persistent myocarditis (CPM) associated with persistent B19 virus infection.

Patients and Methods

Patient 1. A 7-month-old girl was hospitalized in Italy in August 1995 because of acute heart failure that occurred 10 days after

a 3-day febrile illness (temperature, 39°C) followed by a slight truncal rash considered as exanthema subitum (sixth disease). At the time of admission, the baby had pallor, perspiration, a heart rate of 160, and a respiratory rate of 64. Normochromic anemia with leukocytosis and thrombocytosis was noted. An electrocardiogram showed left ventricular hypertrophy. Two-dimensional color Doppler echocardiography demonstrated reduction of the shortening fraction and the presence of a thrombus in the left ventricular apex (size, 42 mm^3) without involvement of the coronary arteries. Treatment with digoxin, furosemide, and heparin was started.

Endomyocardial biopsy (EMB) showed diffuse ALM with mild interstitial fibrosis. Serum samples obtained at admission and 1 month later both contained B19 virus DNA and IgG antibodies specific to B19 virus but not IgM antibodies. The serum IgG concentration was 1070 mg/dL. Intravenous immunoglobulin (IVIG) infusions were given at a dose of 400 mg/kg for 5 days. The patient's condition clinically improved, B19 virus DNA was absent from the blood, and treatment was tapered.

Five months after admission, EMB showed ongoing myocarditis, and B19 virus DNA was again detected in the serum. According to international protocols [2], immunosuppressive therapy with azathioprine (2 mg/kg) and prednisone (2 mg/kg) was then started. Both myocarditis and B19 virus DNA in the serum persisted until the child was aged ≥3.8 years. The child's CPM [4] was confirmed by serial EMB performed every 6 months. Two-dimensional color Doppler echocardiography showed an improved shortening fraction but persistence of the thrombus in the left ventricular apex.

Patient 2. A 12-month-old girl was admitted to an Italian hospital in January 1996 because of acute heart failure and hepatomegaly 15 days after the onset of fever (temperature, 38.5°C) that had lasted 7 days. Her heart rate was 147, and her respiratory rate was 51. She also had normochromic anemia, thrombocytosis, and high levels of aspartate aminotransferase and alanine aminotransferase (table 1). The lactate dehydrogenase level (3510 U/

Table 1. Virological and laboratory findings for 3 children with human parvovirus B19–associated clinical myocarditis.

Patient, age in mo	Hemoglobin level, g/dL	Blood cell count, ×10⁶/L			AST level, U/L	ALT level, U/L	B19 virus–specific		
		Leukocytes	Lymphocytes	Platelets			DNA	IgM	IgG[a]
1									
7	9.3	15.2	7.5	852	31	14	+	−	2.0
8	ND	ND	ND	ND	ND	ND	+	−	2.0
9	10.3	24.7	9.1	992	45	19	−	−	2.0
21	12.7	18.6	6.8	436	39	21	+	−	1.9
34	10.6	22.4	5.5	519	34	16	+	−	1.5
2									
12	9.2	14.2	4.5	820	5810	4640	−	+	1.5
13	10.1	7.3	4.7	395	71	35	−	+	2.2
22	13.2	6.5	3.6	369	38	14	−	−	1.9
40	12.4	7.9	2.6	349	35	28	−	−	1.3
3									
17	11.1	18.7	6.9	286	46	17	+	−	0.4
20	12.5	12.3	6.6	312	27	14	−	−	1.3
32	12.7	10.2	4.9	284	23	18	−	−	0.9

NOTE. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ND, not determined.

[a] Values are expressed in absorbance units. Values >0.2 were considered positive.

L) and creatine phosphokinase level (2320 U/L) were also elevated. Two-dimensional color Doppler echocardiography showed a dilated left ventricle with reduction of the shortening fraction but normal coronary arteries. EMB revealed focal lymphocytic myocarditis. The patient was treated with digoxin, furosemide, and immunosuppressive therapy. Her serum contained B19 virus–specific IgM and IgG antibodies but no B19 virus DNA.

One month after admission, aminotransferase levels were normal, the liver was markedly smaller, the concentration of B19 virus–specific IgG antibodies had increased, and there were no B19 virus–specific IgM antibodies. Six months after admission, repeated EMB showed significant improvement in the patient's condition, and full recovery from myocarditis was confirmed by 4 further biopsies.

Patient 3. An 18-month-old girl was admitted to an Italian hospital in December 1996 because of acute heart failure after a 20-day history of low-grade fever (temperature, 37.2°C–37.8°C), fatigue, sleepiness, and perioral cyanosis. Physical examination showed an inflamed throat, hepatomegaly (3 cm below the right costal margin), dyspnea (53 breaths/min), tachycardia (160 beats/min), and edema of the feet. Her temperature was 37.5°C. A chest radiogram showed bilateral lower lobe infiltrates and slight pericardial effusion (size, 3 mm). Laboratory findings were normal except for lymphocytic leukocytosis. Two-dimensional color Doppler echocardiography demonstrated left ventricular enlargement with severe reduction of the shortening fraction and severe mitral valve regurgitation (grade 4). EMB revealed mild focal lymphocytic myocarditis.

B19 virus DNA was present in the first serum sample but not in subsequent samples, and there were low but increasing levels of B19 virus–specific IgG antibodies. B19 virus–specific IgM antibodies were absent. The patient was treated with digoxin, furosemide, prednisone, and azathioprine. Full recovery was demonstrated by serial EMB.

Comparison patients. Serum samples from a group of 8 infants and children from Richmond, Virginia, who had B19 virus infection without clinical myocarditis were included for cytokine analysis. Umbilical cord serum samples from 3 asymptomatic infants born to mothers with primary B19 virus infections during pregnancy contained IgM antibodies to B19 virus, and PCR analysis of 1 cord serum sample demonstrated B19 virus DNA. An additional infant with presumed intrauterine B19 virus infection presented at 1 month of age with anemia and reticulocytopenia, and his serum contained B19 virus DNA and IgM antibody to B19 virus. Four infants and children with IgM antibodies to B19 in their sera who had recently acquired symptomatic B19 virus infection were also included in the group of comparison patients.

B19 virus infection. B19 virus–specific IgM and IgG antibodies were detected by a commercial EIA (Biotrin, Dublin). B19 virus DNA was detected in serum specimens by nested PCR analysis with use of primer sequences deduced from the published nucleotide sequence of B19 virus (accession no. M13178; GenBank, Bethesda, MD) as reported elsewhere [17]. First round primers included V1: 5′-ATCCTCTCTGTTTGACTTAGT (nucleotides 2611–2631) and YIR: 5′-GCTTGTGTAAGTCTTCACTAG (nucleotides 2795–2774), which span a 184-bp segment. Second round (nested) primers were V2: 5′-TCTGTTTGACTTAGTTGC (nucleotides 2617–2634) and Y2R: 5′-GTAAGTCTTCACTAGATA (nucleotides 2789–2772), which span a 172-bp segment. Standard reaction mixtures were subjected to initial amplification with external primers (35 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C) followed by reamplification with inner primers (25 cycles). Positive controls included B19 virus complementary DNA and sera known to be positive after the initial or second amplification. Negative controls included reagents without DNA and B19 virus–negative serum. Samples from patients and control patients were examined both separately and together. The limit of detection of the nested PCR assay was 100 copies per sample, as determined by use of a plasmid standard (pY110) that contains the B19 virus genome.

Nested PCR analysis was also used for detection of B19 virus DNA in EMB specimens [18]. An optical microscope was used to analyze hematoxylin-eosin–stained slides for cytopathic effects of B19 virus. B19 virus antigen–specific immunofluorescence was performed with use of fluorescein-conjugated monoclonal antibodies (Dako, Glostrup, Denmark).

Other infections. Paired serum samples from all 3 patients were

negative for CF antibodies to Coxsackie B1 to B6 viruses, echo-viruses, and *Coxiella burnetii.* In addition, these samples were neg-ative for IgM and IgG antibodies specific to cytomegalovirus and Epstein-Barr virus by commercial ELISA (Radim, Pomezia, Italy, and Gull Laboratories, Salt Lake City, UT, respectively). For pa-tient 2, who had acute hepatitis concomitantly with heart failure, examinations for hepatitis viruses were also performed and were negative. They included an ELISA for detection of markers for hepatitis B virus and antibodies to hepatitis A virus (Abbott, North Chicago, IL), and a third-generation ELISA for detection of an-tibodies to hepatitis C virus (Ortho, Raritan, NJ). Assays for IgG and IgM antibodies to human herpesvirus 6 were performed as described elsewhere and were negative [19]. Because most infants are infected with human herpesvirus 7 by ≤2 years of age, and because this virus has not been associated with a specific disease, assays for detection of human herpesvirus 7 infection were not performed.

For the comparison group of control subjects, assays for IgM antibodies to B19 virus and PCR analysis for B19 virus DNA were performed as described elsewhere [20–22].

Immunologic methods. For all patients (including those from Italy and Richmond, VA), TNF-α, IL-6, and IL-8 levels were de-termined by an ELISA (R&D Systems, Minneapolis, MN), whereas IFN-γ levels were measured by an ELISA kit (Endogen, Boston, MA). All tests were done in accordance with the manufacturers' instructions. Serum samples from healthy children were used as negative controls, and values for these specimens were below the normal limits given by manufacturers.

Results

The virological and laboratory findings for patients 1–3 with B19 virus–associated myocarditis are listed in table 1. Each patient initially had mild anemia. Myocarditis was associated with B19 virus DNA in the blood in patients 1 and 2, whereas it was associated with B19 virus–specific IgM antibodies in patient 3. Resolution of myocarditis was associated with clear-ance B19 virus DNA or IgM antibodies from the blood in patients 2 and 3. In patient 1 who developed CPM, B19 virus DNA persisted in the blood for >3 years, despite the fact that the child had high levels of IgG antibodies to B19 virus that were detected by EIA (table 1). However, IVIG administration at 8 months of age was associated with transient clearance of B19 virus from the blood.

For all 3 patients, nested PCR analysis of EMB specimens was negative for B19 virus. Optical microscopy of hematoxylin-eosin–stained slides was performed to detect cytopathic effects of B19 virus. B19 virus antigen–specific immunofluorescence with use of fluorescein-conjugated monoclonal antibodies did not reveal B19 virus particles or antigens in EMB specimens from each patient.

Because cytokines can inhibit myocardial contractility or im-pair regulation of the phagocytic system resulting in bone marrow suppression and hepatic dysfunction, we measured the levels of 4 cytokines in the 3 patients. In all 3 patients, increased levels

of TNF-α, IFN-γ, IL-6, and IL-8 coincided with acute or chronic myocarditis (table 2). Patients who had either acquired or con-genital B19 virus infection without clinical myocarditis did not have elevated levels of these cytokines (table 2).

Discussion

Human parvovirus B19, a small single-stranded DNA virus, is ubiquitous in humans. B19 virus causes mainly erythema infectiosum, nonimmune fetal hydrops, and anemia. Anemia associated with B19 virus infection is due to a specific viral tropism for progenitor erythroid cells, specifically P antigen (which is found on these cells) [23]. However, clinical and lab-oratory evidence has been accumulated to suggest that B19 virus has a wider tropism for cells other than erythroblasts [24]. Direct infection of myocardial cells after fetal B19 virus infec-tion of extramedullary erythroid progenitor cells has been dem-onstrated by in situ DNA hybridization or electron microscopy [9, 14, 25, 26]. This finding is not surprising because fetal my-ocardial cells contain P antigen [24].

B19 virus infection of the heart is probable because previous case reports have described 8 fetuses, 2 children, and 4 adults with myocarditis associated with concurrent B19 virus infection [7–16, 25, 26]. One case occurred after transplantation [7]. In 5 of these cases, B19 virus DNA was identified in cardiac tissue by PCR analysis [7, 8, 14, 16].

A recent retrospective study of endomyocardial tissue spec-imens from 360 children with suspected myocarditis, 200 chil-dren with suspected posttransplantation cardiac rejection, and 250 control patients identified parvovirus genomes in myocar-dial tissue specimens from 9 additional children (6 transplant

Table 2. Levels of IL-6, IL-8, TNF-α, and IFN-γ in serum samples from 3 children with human parvovirus B19–associated myocarditis and 8 children who had human parvovirus B19 infections without myocarditis.

Patient, age in mo	Level of indicated cytokine, pg/mL			
	IL-6	IL-8	TNF-α	IFN-γ
1, 7	3831	2147	31	16
1, 9	2827	1961	18	1
1, 21	1284	1137	27	14
1, 34	3118	3183	15	9
2, 12	719	597	17	12
2, 14	821	303	15	7
2, 40	3	0	0	0
3, 18	317	614	11	9
3, 20	219	378	4	5
3, 33	1	4	0	1
C1, 0	0	22	0	0
C2, 0	1	82	4	0
C3, 0	16	66	7	0
C4, 1	1	68	3	1
C5, 24	3	57	3	0
C6, 72	2	47	9	0
C7, 84	8	110	6	0
C8, 96	19	<10	7	0

NOTE. C, comparison.

recipients and 3 normal children with myocarditis) [6]. Of these 9 children with B19 virus–associated myocarditis, some had cardiac arrest or dilated cardiomyopathy or recovered, but no detailed clinical or serological data were given. Our study, however, is the first to describe the clinical and immunovirologic follow-up of the progression to CPM in a child with early, persistently active B19 virus infection.

Our patients developed the sudden onset of ALM with heart failure as the initial clinical feature, but none manifested the typical signs and symptoms associated with erythema infectiosum. We initially considered B19 virus infection in patients 1 and 2 because of mild anemia and thrombocytosis. The absence of the typical clinical manifestations of B19 virus infection in patients with myocarditis may reduce the frequency of diagnosis of B19 virus–associated myocarditis in patients similar to ours.

Although each of our patients had active or recent systemic B19 virus infection, we could not find evidence for direct viral infection of the myocardium even by nested PCR analysis. Other researchers have, however, found B19 virus DNA in myocardial tissue, although no one has convincingly demonstrated viral replication in myocardial tissue [9, 14, 25, 26]. B19 virus is mildly cardiotropic, and ALM probably results from the cellular response to either myocardial tissue or the virus infecting the heart tissue. We found elevated levels of IL-6, IL-8, TFN-α, and IFN-γ in the acute phase of the disease in both children who recovered and persistently high levels of these cytokines in the patient who developed CPM. The levels of these cytokines correlated with the course of ALM and were not associated with B19 virus infection without clinical myocarditis. If elevated cytokine levels occur in cases of myocarditis not associated with B19 virus is unknown.

Two of our patients recovered, and 1 did not. It is possible that recovery was related to the time of exposure of the immune system to B19 virus. Patients 2 and 3 recovered fully with immunosuppressive therapy, and both probably had postnatal B19 virus infection, as suggested by the age at onset and the low but increasing levels of IgG antibodies to B19 virus. Patient 2 also had specific IgM antibodies and acute self-limiting hepatitis. Because acute and occasionally severe hepatitis has been associated with B19 virus infection and because no other agents were identified, it is possible that B19 virus caused this patient's hepatitis, although an immune-mediated mechanism is also possible [27–29].

For patient 1, the persistence of B19 virus infection may have resulted from an infection acquired in utero, which is suggested by the fact that at 7 months of age the infant already had high levels of IgG antibodies to B19 virus without IgM antibodies to B19 virus. We have previously reported virological and serological evidence for frequent intrauterine transmission of B19 virus, which may cause asymptomatic or recurrent postnatal infection [21, 30]. In some infants, prenatal infection may predispose to immune tolerance and chronic postnatal B19 virus infection, even in the presence of high levels of antibodies to the virus. This predisposition certainly occurs with other viral infections acquired in utero, such as rubella, cytomegalovirus infection, and HIV infection, as well as with other types of in utero infections such as toxoplasmosis and syphilis. Chronic persistent B19 virus infections also occur in a variety of immunocompromised patients who cannot make neutralizing antibody and have also been described in immunocompetent adults [23, 31, 32].

Analysis of the first EMB specimen from patient 1 showed signs of severe myocarditis that was concomitant with persistent B19 virus DNA in the blood. She was first treated with IVIG infusions and then with immunosuppressants, because of worsening myocarditis. IVIG therapy was followed by clinical improvement and viral clearing, suggesting both the beneficial effect of this agent and the pathogenic role of B19 virus. In fact, IVIG contain neutralizing antibodies specific to B19 virus and also produce an immunomodulatory effect [33]. At the time of admission, patient 1 had B19 virus DNA in the blood and also had high levels of B19 virus–specific IgG antibodies. Given the persistence of B19 virus DNA in the blood, it is therefore unlikely that her IgG antibodies were able to neutralize the virus [34]. For patient 1, additional IVIG therapy may have been beneficial but was declined by the child's primary physician and family.

The development of CPM in patient 1 may have been related to an ongoing immune-mediated process triggered by B19 virus infection, as suggested by persistently high levels of IL-6, IL-8, and IFN-γ and the presence of abundant cellular infiltrates in the myocardium. Therefore, B19 virus infection, which has been associated with immune-mediated vasculitis, may have played an indirect pathogenic role, and the intracardiac thrombus may have been a consequence of coronary vasculitis [35–37].

Although B19 virus–associated myocarditis appears to occur infrequently, there is now sufficient evidence, based on our study and previous reports, to consider B19 virus as a cause of lymphocytic myocarditis [6, 7, 9, 14, 16, 25, 26]. In patients with ALM, clinicians should search for evidence of concurrent B19 virus infection. Additional prospective studies starting from infancy are now appropriate to determine the true incidence of cardiac involvement associated with B19 virus infections and the long-term consequences of B19 virus–associated cardiac disease.

References

1. Aretz HT, Billingham ME, Edwards WD. Myocarditis: a histopathologic definition and classification. Am J Cardiovasc Pathol **1987**;1:3–14.

2. Drucker NA, Newburger JW. Viral myocarditis: diagnosis and management. Adv Pediatr 1997;44:141–71.

3. Mason JW. Distinct forms of myocarditis. Circulation 1991;83:1110–1.

4. Lieberman GB, Hutchins GM, Herskowitz A, Rose NR, Baughman KL. Clinicopathologic description of myocarditis. J Am Coll Cardiol 1991;18: 1617–26.

5. Martin AB, Webber S, Fricker FJ, et al. Acute myocarditis: rapid diagnosis by polymerase chain reaction (PCR) in children. Circulation 1994;90: 330–9.

6. Schowengerdt KO, Ni J, Denfield SW, et al. Association of parvovirus B19 genome in children with myocarditis and cardiac allograft rejection: diagnosis using the polymerase chain reaction. Circulation 1997;96:3549–54.

7. Heegaard ED, Eiskjaer H, Baandrup U, Hornsleth A. Parvovirus B19 infection associated with myocarditis following adult cardiac transplantation. Scand J Infect Dis 1998;30:607–10.

8. Enders G, Dötsch J, Bauer J, et al. Life-threatening parvovirus B19–associated myocarditis and cardiac transplantation as possible therapy: 2 case reports. Clin Infect Dis 1998;26:355–8.

9. Respondek M, Bratosiewicz J, Pertynski T, Liberski PP. Parvovirus particles in a fetal heart with myocarditis: ultrastructural and immunohistochemical study. Arch Immunol Ther Exp (Warsz) 1997;45:465–70.

10. Orth T, Herr W, Spahn T, et al. Human parvovirus B19 infection associated with severe acute perimyocarditis in a 34-year-old man. Eur Heart J 1997;18:524–5.

11. Chia JKS, Jackson B. Myopericarditis due to parvovirus B19 in an adult. Clin Infect Dis 1996;23:200–1.

12. Moore L, Chambers HM, Foreman AR, Khong TY. A report of human parvovirus B19 infection in hydrops fetalis: first Australian case confirmed by serology and immunohistology. Med J Aust 1993;159:344–5.

13. Brandenburg H, Los FJ, Cohen-Overbeek TE. A case of early intrauterine parvovirus B19 infection. Prenat Diagn 1996;16:75–7.

14. Porter HJ, Quantrill AM, Fleming KA. B19 parvovirus infection of myocardial cells. Lancet 1988;1:535-6.

15. Malm C, Fridell E, Jansson K. Heart failure after parvovirus B19 infection. Lancet 1993;341:1408–9.

16. Saint-Martin J, Choulot JJ, Bonnaud E, Morinet F. Myocarditis caused by parvovirus. J Pediatr 1990;116:1007–8.

17. Nigro G, Piazze J, Taliani G, Mazzocco M, Cassinotti P, Cosmi EV. Postpartum lupus erythematosus associated with parvovirus B19 infection. J Rheumatol 1997;24:968–70.

18. Griffin LD, Ni J, Kearney D, et al. Analysis of formalin-fixed and frozen myocardial autopsy samples for histology-proven myocarditis using polymerase chain reaction (PCR). Cardiovasc Pathol 1995;4:3–11.

19. Nigro G, Luzi G, Krzysztofiak A, D'Orio F, Aiuti F. Detection of IgM antibodies to human herpesvirus 6 in Romanian children with nonprogressive human immunodeficiency virus disease. Pediatr Infect Dis J 1995; 14:891–4.

20. Koch WC, Adler SP. Detection of human parvovirus B19 DNA using the polymerase chain reaction. J Clin Microbiol 1990;28:65–9.

21. Koch WC. A synthetic parvovirus B19 capsid protein can replace viral antigen in antibody-capture enzyme immunoassays. J Virol Methods 1995;55: 67–82.

22. Koch WC, Harger JH, Barnstein B, Adler SP. Serologic and virologic evidence for frequent intrauterine transmission of human parvovirus B19 with a primary maternal infection during pregnancy. Pediatr Infect Dis J 1998; 17:489–94.

23. Brown KE. Human parvovirus B19 infections in infants and children. Adv Pediatr Infect Dis 1997;13:101–26.

24. Heegaard ED, Hornsleth A. Parvovirus: the expanding spectrum of disease. Acta Paediatr 1995;84:109–17.

25. Naides SJ, Weiner CP. Antenatal diagnosis and palliative treatment of nonimmune hydrops fetalis secondary to fetal parvovirus B19 infection. Prenat Diagn 1989;9:105–14.

26. Morey AL, Porter HJ, Keeling JW, Fleming KA. Non-isotopic in situ hybridisation and immunophenotyping of infected cells in investigation of human fetal parvovirus infection. J Clin Pathol 1992;45:673–8.

27. Langnas AN, Markin RS, Cattral MS, Naides SJ. Parvovirus B19 as a possible causative agent of fulminant liver failure and associated aplastic anemia. Hepatology 1995;22:1661–5.

28. Yoto Y, Kudoh T, Hasyama K, Suzuki N, Chiba S. Human parvovirus B19 infection associated with acute hepatitis. Lancet 1996;347:868–9.

29. Sokal EM, Melchior M, Cornu C, et al. Acute parvovirus B19 infection associated with fulminant hepatitis of favourable prognosis in young children. Lancet 1998;352:1739–41.

30. Koch WC, Adler SP, Harger J. Intrauterine parvovirus B19 infection may cause an asymptomatic or recurrent postnatal infection. Pediatr Infect Dis J 1993;12:747–50.

31. Kurtzman G, Frickhofen N, Kimball J, Jenkins D, Nienhuis A, Young N. Pure red-cell aplasia of 10 years' duration due to persistent parvovirus B19 infection and its cure with immunoglobulin therapy. N Engl J Med 1989;321:519–23.

32. Kerr JR, Curran MD, Moore JE, Coyle PV, Ferguson WP. Persistent parvovirus B19 infection. Lancet 1995;345:1118.

33. Nigro G, D'Eufemia P, Zerbini M, Krzysztofiak A, Finocchiaro R, Giardini O. Parvovirus B19 infection in a hypogammaglobulinemic infant with neurologic disorders and anemia: successful immunoglobulin therapy. Pediatr Infect Dis J 1994;13:1019–21.

34. Kurtzman GJ, Cohen BJ, Field AM, Oseas R, Blaese RM, Young NS. Immune response to B19 parvovirus and an antibody defect in persistent viral infection. J Clin Invest 1989;84:1114–23.

35. Nigro G, Zerbini M, Krzysztofiak A, et al. Active or recent parvovirus B19 infection in children with Kawasaki disease. Lancet 1994;343:1260–1.

36. Finkel TH, Török TJ, Ferguson PJ, et al. Chronic parvovirus B19 infection and systemic necrotising vasculitis: opportunistic infection or aetiological agent? Lancet 1994;343:1255–8.

37. Ferguson PJ, Saulsbury FT, Dowell SF, Török TJ, Erdman DD, Anderson LJ. The prevalence of human parvovirus B19 infection in children with Henoch-Schönlein purpura. Arthritis Rheum 1996;39:880–1.