original paper
Haematologica 1997; 82:402-405

PARVOVIRUS B19 INFECTIONS IN PATIENTS WITH CHRONIC ANEMIA

ERIK D. HEEGAARD,* JOHN MYHRE,° ALLAN HORNSLETH,* MARYANNE GUNDESTRUP,# HANNE BOYE*
**Department of Virology, Institute of Medical Microbiology and Immunology, University of Copenhagen; °Department of Hematology, Herlev University Hospital, Copenhagen; #Department of Hematology, Rigshospitalet, Copenhagen, Denmark*

ABSTRACT

Background and Objective. Parvovirus B19 has a marked tropism for erythroid progenitor cells and this may lead to chronic anemia in predisposed individuals. It was the purpose of the present study to investigate prospectively the frequency of parvovirus B19 infections in patients with a diagnosis of chronic anemia.

Methods and Results. Evidence of parvovirus B19 infection was found in 13/43 (30%) patients by demonstrating viral DNA and/or specific IgM antibodies through the use of PCR and ELISA techniques. Parvovirus B19 infection was established in 4 of 7 patients with hemolytic anemia, in 2 of 3 patients with pure red cell aplasia, in 2 of 9 patients with myelodysplastic syndrome, and in 2 of 10 patients with aplastic anemia. In 8 of the 13 positive patients only parvovirus B19 DNA could be detected, while 4 patients tested positive for both parvovirus B19 DNA and specific IgM. In the remaining positive patient only specific IgM could be detected.

Conclusions. Since no predictive paraclinical or clinical features were observed we recommend that all cases of chronic anemia be tested for the presence of parvovirus B19 infection. Due to the discrepancies between DNA and IgM results, the diagnostic procedures should include a search for specific DNA by PCR methods if specific IgM has been found to be negative.

Keywords: parvovirus B19, chronic anemia, polymerase chain reaction, immunoglobulins

Parvovirus B19 has a marked tropism for erythroid progenitor cells and this may lead to transient selective red cell aplasia.[1] Giant pronormoblasts may be detected in the bone marrow (BM) 10 days after inoculation.[1] Subsequent studies of its effects in humans have identified this virus as the causative agent of a broad range of diseases,[1] including: i) diseases found among normal hosts (asymptomatic disease, erythema infectiosum, arthropathy, hydrops fetalis); ii) hematologic diseases (aplastic crisis, chronic anemia, idiopathic thrombocytopenic purpura, transient erythroblastopenia of childhood, Blackfan-Diamond anemia), and iii) a heterogeneous group of diseases in which the etiologic role of parvovirus B19 is less clear and sometimes putative (neurologic disease, rheumatologic disease, vasculitic and myocarditic syndromes).

Conditions shown to predispose to parvovirus B19-induced chronic anemia include Nezelof's syndrome, acute lymphatic leukemia, acute myeloid leukemia, chronic myeloid leukemia, Burkitt's lymphoma, lymphoblastic lymphoma, myelodysplastic syndrome, astrocytoma, Wilms' tumor, HIV infected patients, SCID, BM transplantation, organ transplantation, systemic lupus erythematosus, class-switch defects, patients receiving cancer chemotherapy, and patients with defect immunoglobulin (Ig) specificity and neutralization.[1] In a few cases no immunologic defect can be revealed.[2-4]

Parvovirus B19 viremia may be present for several years in some cases.[5] The accompanying anemia is usually of moderate degree, reflecting the low-grade level of infection in these patients. The serum concentration of parvovirus B19 among chronic anemic patients is usually much lower than that detected during an acute aplastic crisis.[6]

The clinical hallmarks of this condition are fatigue and pallor, while immunologic mediated symptoms, such as rash and arthralgia, are generally not present.[1] The mild, non specific nature of the symptoms explains why the cause of anemia may remain undiscovered for years in some patients.

Chronic anemia is frequently characterized by a selective decrease of red cell precursors in the BM, reticulocytopenia and normocytic anemia. Most cases of chronic anemia are thought to be mediated by immune mechanisms and often these patients respond to immunosuppressive treatment.

Immunodeficient patients may be unable to raise an adequate immunological response against a parvovirus B19 infection, resulting in chronic infec-

Correspondence: Erik D. Heegaard, c/o Allan Hornsleth, Institute of Medical Microbiology and Immunology, University of Copenhagen, Panum Institute, Bldg. 24.2, Blegdamsvej 3C, 2200 Copenhagen N, Denmark. Tel. international +45.35.327680.
Acknowledgments: the authors wish to thank The Danish Medical Research Council for financial support.
Received February 27, 1997; accepted May 31, 1997.

tion with red cell aplasia of the bone marrow.[1,5,7-9] In a recent retrospective study, parvovirus B19-induced chronic red cell aplasia was found in 8 of 57 patients.[8]

In the present study we prospectively investigated the frequency of parvovirus B19 infection in a mixed population of patients suffering from chronic anemia. Our aim was to evaluate different diagnostic tools and investigate possible clinical characteristics of this infection.

Materials and Methods

All patients in the study met standard criteria for chronic anemia: severe normocytic anemia for more than 1 month, a hemoglobin value of less than 9.7 g/dL, and unexplained marked reticulocytopenia (the majority below 1%). A total of 43 consecutive patients, irrespective of the cause of anemia, referred for the first time to 2 hematological centers were included in the study over a 3-year period. The different categories of patients are listed in Table 1. Twenty of the 43 patients were female. When the study began, the median duration of the anemia (as observed by a private physician and/or local hospital) was 1.5 months (range 1-131), and the median patient age 59 years (range 16-89).

Blood samples obtained from all patients at admission were examined for evidence of parvovirus B19-specific DNA and immunoglobulins by menas of a nested polymerase chain reaction (PCR) and immunofluorescence antibody technique.[6] When available, BM slides were examined by a pathologist. Furthermore, the reticulocyte-, neutrocyte-, and platelet counts were analyzed.

Results

Detection of parvovirus B19 DNA and specific immunoglobulins

Evidence of parvovirus B19 infection was found in 13 of the 43 patients (30%) by demonstrating viral DNA and/or specific IgM antibodies (Table 1). In 8 of the 13 positive patients only parvovirus B19 DNA could be detected, while 4 patients tested positive for both parvovirus B19 DNA and specific IgM. In the remaining positive patient only specific IgM could be detected.

Of the 13 parvovirus B19-positive patients, 11 tested positive for specific IgG (85%), while 18 of the remaining 30 parvovirus B19-negative patients were IgG positive (60%).

Laboratory findings and clinical characteristics

A comparison of the reticulocyte-, neutrophil-, and platelet counts between positive and negative patients revealed no significant differences (Table 2). Giant pronormoblasts, investigated in 5 cases with available BM slides, were noted in only one patient; a parvovirus B19 positive patient suffering from hemolytic anemia (Table 3). Overall, the patients received blood and platelet transfusions corresponding to 1429 donor portions. The resulting estimated incidence of blood-borne infection was 0.43.[10] None of the patients received immunoglobulin treatment before or after testing for parvovirus B19 infection. Hemoglobin values were ultimately normalized and remained stable in 6 parvovirus B19-positive patients (46%) and 16 parvovirus B19-negative patients (53%) after median observation times of 3.5 (range 1-6) and 1 (range 1-6) month, respectively.

Five of 8 parvovirus B19-positive patients (63%) showed normal hemoglobin values, at least transiently, within 2-3 months after receiving immunosuppressive treatment (prednisone and/or cyclosporine). Three of these 5 patients suffered from hemolytic anemia, one had pure red cell aplasia and one was diagnosed with myelodysplastic syndrome. Seven of the 13 parvovirus B19-negative patients (54%) receiving immunosuppressive treatment also achieved normal hemoglobin values within a similar time frame.

Uncharacteristic arthralgia and erythema was observed in 1 patient; a woman positive for parvovirus B19 infection. When the study was closed,

Table 1. Parvovirus B19 DNA and specific antibodies (IgM and IgG) detected in serum samples from 43 patients with chronic anemia.

Category of pts.	*No. investigated*	*B19 DNA*	*IgM*	*IgG Parvovirus B19 DNA/IgM⁻*	*IgG Parvovirus B19) DNA/IgM⁺)*
Parvovirus B19	1	1	1*	1	0
Hemolytic anemia	7	4	1*	2	1
MDS	9	2	0	2	6
Pure red cell aplasia	3	2	1*	2	1
Aplastic anemia	10	2	1*	2	4
Malignant lymphoma	1	0	1°	1	0
AML	3	0	0	0	2
Others[#]	9	1	0	1[@]	4
Total	43	12	5	11	18

*Legend: MDS: Myelodysplastic syndrome, AML: Acute myeloid leukemia. *Parvovirus B19 DNA positive; °Parvovirus B19 DNA negative; [#]sideropenic anemia (n=2), toxic hypoplasia (n=2) (carbamazepin and enalapril), chronic lymphocytic leukemia (n=1), idiopathic thrombocytopenic purpura (n=1), alcoholism (n=1), systemic lupus erythematosus (n=1), and rheumatic arthritis (n=1). [@]The patient suffered from toxic hypoplasia due to treatment with carbamazepin.*

Table 2. Reticulocyte-, neutrophil-, and platelet counts in 43 patients with chronic anemia at the time of parvovirus B19 testing.

B19 infection (DNA or IgM)	*No. of pts.*	*Ret (%) <0.5*	*Ret (%) 0.5-1.0*	*N x10⁹/L <0.5*	*N x10⁹/L 0.5-1.0*	*Plts x 10⁹/L <20*	*Plts x 10⁹/L 20-100*
Positive	13	8 (62%)	2 (15%)	2 (15%)	2 (15%)	5 (38%)	3 (23%)
Negative	30	20 (69%)	2 (7%)	8 (28%)	8 (28%)	8 (28%)	11 (38%)

Ret = reticulocytes; N = neutrophils; Plts = platelets.

Table 3. Gender, age, associated conditions and clinical outcome in 13 parvovirus B19-positive patients.

Pt #	*Gender*	*Age (yrs)*	*Associated condition*	*Treatment and outcome*
1	F	62	Hemolytic anemia	Prednisone Remission
3	F	42	Pure red cell aplasia	Prednisone Periodic remission
4	F	52	Hemolytic anemia	Prednisone Remission
7	M	26	Hemolytic anemia	Blood transfusion Remission
10	F	73	Malignant lymphoma	Prednisone Cytostatics Died
12	M	70	Myelodysplastic syndrome	Prednisone Periodic remission
17	M	76	Myelodysplastic syndrome	Cytostatics Died
21	M	61	Pure red cell aplasia	Blood transfusion No remission
32	M	55	Toxic hypoplasia (carbamazepine)	Blood transfusion Remission
36	F	59	Aplastic anemia	Cyclosporin Prednisone Died
39	F	79	Aplastic anemia	Cyclosporin Prednisone Died
40	M	43	Hemolytic anemia	Prednisone Remission
41	F	41	None	Spontaneous remission

Periodic remission: only brief periods of normal hemoglobin despite continued treatment.

9 of the parvovirus B19-positive (69%) and 20 of the parvovirus B19-negative patients (67%) were alive after a median observation time of 24 (range 2-124) and 23 (range 1-39) months, respectively.

Discussion

As mentioned above and elsewhere[1,5] a growing number of cases involving parvovirus B19 infections among patients suffering from chronic anemia is being reported. A retrospective study by Frickhofen *et al*. found evidence of parvovirus B19 infection in 8/57 (14%) patients with acquired chronic pure red cell aplasia by demonstrating viral DNA in their serum.[8] In our prospective search for parvovirus B19 infections among a consecutive group of chronic anemic patients we found evidence of infection in 13/43 (30%) patients. The number of patients involved in both studies is limited but nevertheless these results suggest that parvovirus B19 infection may be a relatively common finding in patients suffering from chronic anemia, irrespective of the cause.

It is therefore important that cases of chronic anemia in which parvovirus B19 is implicated be diagnosed, since optimal therapy and outcome could differ widely. Specific treatment with Ig may in the future prove to be beneficial as an alternative or adjuvant to the usual long-term immunosuppressive treatment and red cell transfusion with their many potential side effects. Further studies are needed to elucidate this aspect.

The significance of parvovirus B19 infection in these patients remains unknown. The study by Frickhofen *et al.* showed that 7 of 8 parvovirus B19-positive patients presented associated conditions.[8] Similarly, the majority of our patients (12/13) were eventually diagnosed as suffering from a variety of specific diseases with parvovirus B19 thought to represent a coincidental complication. It was hypothesized by the participating clinicians that the parvovirus B19 infection was either due to reactivation of a latent infection at a time of general immunosuppression or it was the result of a prolonged primary infection in an immunocompromised individual. It is possible that the ill effects of parvovirus B19, when it acts as a superimposed infection, help to accentuate the underlying disease, which in turn will prompt the patient to seek medical attention.[5] Consequently, the frequency of parvovirus B19 infections in patients with chronic anemia tested at a much later stage may be lower. In only 1 of our patients was parvovirus B19 thought to be the sole cause of disease: a 41-year-old woman who exhibited an uncomplicated and self-limited episode of anemia with uncharacteristic erythema and accompanying arthralgia lasting for 5 days. As occurred in a few similar cases,[2-4] no immunological deficiencies could be detected in this woman.

It is interesting, however, that 5 of the 8 parvovirus B19-positive patients receiving immunosuppressive therapy actually responded positively to steroid treatment. The reason for this remains obscure. It has been hypothesized that parvovirus B19 could induce autoimmune mechanisms leading to autoimmune pure red cell aplasia, based on the development of anti-DNA and antilymphocyte antibodies during the acute infection.[11,12] The positive effect of cyclosporine observed in a few cases supports this theory.[5,8,13] Accordingly, a positive clinical response to immunosuppressive treatment does not necessarily exclude parvovirus B19 from being involved in the pathogenesis.

A substantial portion of the 13 parvovirus B19-positive patients were IgG positive, indicating a possible chronic state of infection. The discrepancies between DNA and IgM findings indicate that searching for specific IgM may be a cheap and easy

diagnostic tool for basic screening, but the sensitivity may be very low in selected groups of patients, e.g. 38% in our study. It is advisable, if IgM turns out to be negative, to continue the search for possible parvovirus B19 infection by employing the more sensitive (92%) PCR method as a second line of diagnostics. Giant pronormoblasts were not helpful in diagnosing parvovirus B19-positive patients, as reported in earlier studies.[8,13] Nor could parvovirus B19-positive patients be identified clinically, as previously noted in other disease settings.[8,13]

In the present study parvovirus B19 infection was prevalent among groups of patients suffering from hemolytic anemia, myelodysplastic syndrome (MDS), aplastic anemia and pure red cell aplasia. We consequently investigated selected disease categories to test the statistical significance of these findings in larger populations. We examined the presence of parvovirus B19 DNA in a series of children (n=19) and adults (n=39) with a diagnosis of MDS.[9] Only 1 patient tested positive for parvovirus B19 DNA. We concluded that parvovirus B19 infection only rarely mimics MDS or is a superimposed infection in MDS. We have recently conducted a search for parvovirus B19 infections among children with pure red cell aplasia (Blackfan-Diamond anemia).[13] We found evidence of parvovirus B19 infection in 3/11 (27%) patients by demonstrating viral DNA in BM. This study represents the most exhaustive of its kind. The 3 parvovirus B19 DNA-positive patients were the only children who experienced a remission of their anemia and who are free of medication.

In summary, we found a high incidence of parvovirus B19 infections in cases diagnosed as chronic anemia. Since no firm clinical or paraclinical features were predictive of a parvovirus B19 infection, we recommend that all cases of chronic anemia be examined for specific antibodies and tested for the presence of parvovirus B19 DNA in serum or bone marrow by means of the PCR technique.

References

1. Heegaard ED, Hornsleth A. Parvovirus: the expanding spectrum of disease. Acta Pædiatr 1995; 84:109-17.
2. Faden H, Gary GW, Anderson LJ. Chronic parvovirus infection in a presumably immunologically healthy woman. Clin Infect Dis 1992; 15: 595-7.
3. Murray JC, Gresik MV, Leger F, McClain KL. B19 parvovirus-induced anemia in a normal child. Am J Pediatr Hematol Oncol 1993; 15: 420-3.
4. Kerr JR, Curran MD, Moore JE, Coyle PV, Ferguson WP. Persistent parvovirus B19 infection. Lancet 1995; 345:1118.
5. Hasle H, Kerndrup G, Jacobsen BB, Heegaard ED, Hornsleth A, Lillevang ST. Chronic parvovirus infection mimicking myelodysplastic syndrome in a child with subclinical immunodeficiency. Am J Pediatr Hematol Oncol 1994; 16:329-33.
6. Hornsleth A, Carlsen KM, Christensen LS, Gundestrup M, Heegaard ED, Myhre J. Estimation of serum concentration of parvovirus B19 DNA by PCR in patients with chronic anaemia. Res Virol 1994; 145: 379-86.
7. Young NS. B19 Parvovirus. In: Young NS, ed. Viruses and Bone Marrow: Basic Research and Clinical Practice. New York: Marcel Dekker, 1993. p. 75-117.
8. Frickhofen N, Chen ZJ, Young NS, Cohen BJ, Heimpel H, Abkowitz JL. Parvovirus B19 as a cause of acquired chronic pure red cell aplasia. Br J Haematol 1994; 87:18-24.
9. Hasle H, Heegaard ED, Kerndrup G, Jensen IM, Peterslund NA, Hornsleth A. Parvovirus B19 infection infrequently involved in children and adults with myelodysplastic syndrome. Leuk Res 1996; 20:81-3.
10. McOmish F, Yap PL, Jordan AHH, Cohen BJ, Simmonds P. Detection of parvovirus B19 in donated blood: a model system for screening by polymerase chain reaction. J Clin Microbiol 1993; 31:323-8.
11. Sasaki T, Takahashi Y, Yoshinaga K, Sugamura K. Shiraishi H. An association between human parvovirus B-19 infection and autoantibody production. J Rheumatol 1989; 16:708-9.
12. Solonínka CA, Anderson MJ, Laskin CA. Anti-DNA and antilymphocyte antibodies during acute infection with human parvovirus B19. J Rheumatol 1989; 16:777-81.
13. Heegaard ED, Hasle H, Clausen N, Hornsleth A, Kerndrup GB. Parvovirus B19 infection and Diamond-Blackfan anaemia. Acta Pædiatr 1996; 85:299-302.