

The Investigation of Parvovirus B19 Infection in Patients with Haematological Disorders by Using PCR and ELISA Techniques

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Parvovirus B19 has a marked tropism for erythroid progenitor cells. This may lead to chronic anemia in predisposed individuals. The purpose of the study was to investigate the frequency of parvovirus B19 infections in patients with diagnosis of haematological disorders. In order to determine the diagnostic use of different markers of parvovirus B19 infection, serum specimens obtained from 79 patients with haematological disorders were tested for specific antibodies and viral DNA through the use of ELISA and PCR techniques. Evidence of parvovirus B19 infection was found in 23/79 (29.1%) patients by demonstrating viral DNA and/or specific IgM antibody. B19 infection was established in 3 of 11 patients with chronic myeloid leukemia, in 3 of 11 acute myeloid leukemia, in 2 of 11 patients with multiple myeloma, in 3 of 8 patients with Hodgkin's lymphoma, in 5 of 10 patients with non-Hodgkin's lymphoma, in 1 of 6 patients with myelodysplastic syndrome, in 4 of 11 patients with chronic lymphocytic leukemia, and in 2 of 11 patients with acute lymphocytic leukemia. In 4 of 23 positive patients, only parvovirus B19 DNA could be detected, while 7 patients were tested positive for both parvovirus B19 DNA and specific IgM. Nine patients were tested positive for both B19 DNA and specific IgG. In the remaining 3 positive patients only specific IgM could be detected. 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.

Key-Words: PCR, ELISA, haematological disorder.

Human parvovirus B19 (hereafter referred to as B19), which was first recognized in 1975 [1], causes a variety of disease syndromes determined by the age and haematological status of the host. B19, the only Parvovirus known to be pathogenic for humans, is a small DNA virus with a single-stranded linear DNA which encodes one non-structural protein, namely NS-1, and two viral capsid proteins, VP1 (83 kDa) and VP2 (58 kDa) [2]. The virus exhibits a remarkable tropism for erythroid progenitor cells [3] and also is frequently associated with anemia. B19 infection has also been implicated in a wide range of clinical manifestations, the outcome of which depends heavily on the physiological status of the individual and the immune response against the virus [4].

In immunologically healthy hosts, B19 may cause a number of acute, generally self-limited diseases, namely, fifth disease or erythema infectiosum in children, acute polyarthritis in adults and aplastic crisis in children with chronic hemolytic anemia such as sickle cell anemia or hereditary spherocytosis [5,6]. In pregnant women, B19 infection may result in the lysis of fetal nucleated red cells, hydrops fetalis and subsequent spontaneous abortion and fetal death [7,8]. B19 has also been found to be associated with glomerulonephritis, vasculitis, peripheral neuropathies, myocarditis and fulminant hepatic failure [9]. In immunocompromised hosts, B19 infection may persist and

lead to chronic anemia, red cell aplasia, and less frequently, thrombocytopenia, neutropenia, and pancytopenia [10,11]. Chronic anemia is frequently characterized by a selective decrease of red cell precursors in the bone marrow, reticulocytopenia, normocytic anemia. Most cases of chronic anemia are thought to be mediated by immune mechanisms and often these patients respond to immunosuppressive treatment. In the present study, we prospectively investigated the frequency of B19 infection in a selected population of patients suffering from haematological disorders using different laboratory diagnostic tools.

Materials and Methods

Patients

Serum samples were collected from 79 adults (n = 79) with haematological diseases, including acute and chronic leukemia, malignant lymphoma, hemolytic anemia, myelodysplastic syndrome, and multiple myeloma. During the study, the majority of the patients received multiple blood transfusions and immunosuppressive treatment. All patients in the study met standard criteria for chronic anemia; severe normocytic anemia for more than 1 month, hemoglobin value less than 9.7 g/dL. Fifty of the 79 patients were female and the median patient age was 36 years old (range 20-80 years).

All the serum samples had been stored at -20°C until tested for B19 immunoglobulin M (IgM) antibody, IgG antibody and B19 DNA.

Serology

A commercial antibody capture EIA based on the use of parvovirus B19 recombinant capsid proteins was used for the presence of anti-B19 IgM and IgG antibodies in serum (parvovirus B19 IgM and IgG EIA BIOTRIN International, Dublin, Ireland).

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Parvovirus B19 nested PCR

Following DNA extraction from 200 µL serum samples using a highly pure viral nucleic acid isolation kit (Roche Diagnostics, Germany), a fragment of the DNA sequence coding for the major capsid protein (VP2) of parvovirus B19 was amplified by nested PCR as described by Cassinotti et al. [12]. The first amplification consisted of 35 cycles carried out at an annealing temperature of 45°C using the outer primers TJI (direct primer, nucleotides 3775-3792, 5'-TTCTTTTCAGCTTTT TAGG-3') and R4154 (reverse primer, nucleotides 4154-4171, 3'-TTTATACAGTGTCCTTAT-5'). The inner nested primers, 968 (direct primer, nucleotides 3818-3837, 5'-TATAAGTTTCTCCAGTGCC-3') and TJII (reverse primer, nucleotides 3956-3975, 3'-GTACTTCTGGTACGTTAAGT-5') were used for the second amplification, consisting of 35 cycles undertaken at an annealing temperature of 60°C. The 158 base-pair (bp) diagnostic fragment amplified with the inner primers 968 and TJII was subsequently detected by agarose gel electrophoresis. Each sample was tested in duplicate. A 10⁸-fold dilution of a viremic reference serum (N3787) containing approximately 10 to 100 parvovirus B19 genome copies was used as a positive control. Negative controls consisting of phosphate buffered saline (PBS) were extracted concomitantly with the diagnostic specimens during the extraction step for the control of contamination. Additional negative controls were included in each PCR run to test for the absence of carryover contamination.

Results

Evidence of B19 infection was found in 23 (29.1%) of 79 patients by demonstrating viral DNA and/or specific IgM antibody. In 4 of the 23 positive patients only parvovirus B19 DNA could be detected, while 7 patients were tested positive for both parvovirus B19 DNA and specific IgM. Nine patients were tested positive for both B19 DNA and specific IgG. In the remaining 3 positive patients, only specific IgM could be detected (Table 1).

Of the 56 B19 negative patients, 32 were positive for specific IgG. As a result, the exposure ratio of B19 in patients was 69.6% (55/79).

B19 infection was established as follows: in 3 of 11 patients with chronic myeloid leukemia, in 4 of 11 patients with chronic lymphocytic leukemia, in 3 of 11 patients with acute myeloid leukemia, in 2 of 11 patients with acute lymphocytic leukemia, in 2 of 11 patients with multiple myeloma, in 3 of 8 patients with Hodgkin's lymphoma, in 5 of 10 patients with non-Hodgkin's lymphoma, in 1 of 6 patients with myelodysplastic syndrome (Table 1).

In the patient group, 56 (70.9%) received multiple blood transfusions or blood products before collecting serum samples; 23 (29.1%) patients did not receive multiple transfusions. In patients who received blood transfusion and who were not transfused, seropositivity of B19 was 69.6% and 69.5%, respectively (Table 2).

Discussion

As mentioned above and elsewhere, increasing numbers of cases involving B19 infection among patients suffering from haematological disorders have been reported [13,14]. A retrospective study by Frickhofen et al. has found evidence of B19 infection in 8/57 (14%) patients with acquired chronic pure red cell aplasia by demonstrating viral DNA in their sera [15]. In our prospective study of B19 infections among a selected group of chronic anemic immunocompromised patients with severe underlying haematological diseases, evidence of infection was found in 23/79 (29.1%) patients. A prospective study by Heegaard et al. found evidence of B19 infection in 13/43 (30%) patients with chronic anemia [16]. Our results are in agreement with this study, and these results suggest that B19 infection may be a relatively common finding in patients suffering from chronic anemia in predisposed subjects.

Therefore, cases of chronic anemia should be evaluated for B19 positivity so that if parvovirus B19 is implicated, the patients should be diagnosed for optimal therapy, resulting in an outcome that could differ widely. Specific treatment with immunoglobulin might, in the future, prove to be beneficial as an alternative or adjuvant to the usual long-term immunosuppressive treatment and red cell transfusion, which have many potential side effects [14].

The significance of B19 infection in these patients remains unknown. The study by Heegaard et al. [14] showed that 12 of 13 B19 positive patients presented associated conditions (which were mentioned above). Similarly, all of our patients were diagnosed as suffering from a variety of specific diseases and B19 was thought to represent a coincidental complication.

It is known that the B19 infection is either due to reactivation of a latent infection in a general immunosuppression situation or it is the result of a prolonged primary infection in an immunocompromised individual [17].

In this study, the exposure ratio of B19 was 69.6% in patients. This ratio was too high, because all of the patients involved in this study were in a high-risk population (haematological malignancy, immunocompromised status, etc.). It was shown that B19 infection was prevalent among groups of patients with leukemia, malignant lymphoma, aplastic anemia, hemolytic anemia, thalassemia. Among 23 B19 patients, B19 DNA has been detected in 20 patients; and IgM and DNA were positive in 7 patients. Three patients were positive only for specific IgM. Together B19 DNA and specific IgG seropositivity were detected in 9 of 23 patients which were B19 infected. These markers present the reactivation of latent infection or persistence of infection. Only B19 DNA positivity was shown at 4 patients. It was possible that these patients were in early viremic period or they were immunosuppressive patients who have not enough immune response.

Follow-up samples were not available so it was not possible to make any interpretations about these patients. Naturally, the ideal procedure for detecting the viral load is quantitative

Table 1. Results of B19 DNA and specific antibodies (IgM and IgG) detected in serum samples of patients with B19 infection among 79 patients with haematological disorders

Category of patients	Number investigated	B19 DNA +	B19 DNA + IgM+	B19 DNA + IgG+	Only IgM +	No. of B19 infection
CML	11	1	1	1	-	3
AML	11	-	1	1	1	3
MM	11	1	-	1	-	2
HL	8	1	-	1	1	3
NHL	10	1	1	3	-	5
MDS	6	-	1	-	-	1
CLL	11	-	3	1	-	4
ALL	11	-	-	1	1	2
Total	79	4	7	9	3	23

CML=chronic myeloid leukemia; AML=acute myeloid leukemia; MM=multiple myeloma; HL=Hodgkin's lymphoma; NHL=Non-Hodgkin's lymphoma; MDS=myelodysplastic syndrome; CLL=chronic lymphocytic leukemia; ALL=acute lymphocytic leukemia.

Table 2. The transfusion status and seropositivity rate of 79 patients

	Seropositive		Seronegative		Total	
	N	%	N	%	N	%
Transfusion (+)	39	69.6	17	30.4	56	100
Transfusion (-)	16	69.5	7	30.5	23	100
Total	55	69.6	24	30.4	79	100

PCR with intervals. However, our results confirmed that parvovirus B19 infection is relatively in high frequency in patients, most of which were immunosuppressed and had haematological malignancies.

A total of 23 patients were tested positive for B19 infection by using PCR and ELISA techniques. Twenty of 23 patients were positive with the detection of B19 DNA and 10 of 23 patients were positive with specific IgM seropositivity. 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 (43.4% in our study). It is advisable that if IgM turns out to be negative, to continue searching for possible B19 infection by employing more sensitive (86.9% in our study) PCR method as a second line diagnostic assay.

In this study, 70.9% of the patients were multiple transfused subjects. There was no significant difference for B19 positivity between patients who received blood transfusion and who did not ($p>0.05$). Additionally, there are several studies which have reported values of 1/167-1/35,000 of B19 positivity in blood donors [18-20]. Although B19 infection is known to be transmitted via transfusion, it appears to be unrelated with transfusion; so studies including larger populations are needed to be performed as our study population was relatively small.

In summary, we have found a high incidence of B19 infection in cases diagnosed with severe haematological diseases associated with coincidental chronic anemia. Since no accurate clinical or paraclinical features were predictive of

a B19 infection, we recommend that all cases of haematological disorders should be examined for specific antibodies and tested for the presence of B19 DNA in serum by means of PCR technique.

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