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Dose-by-Dose Virological and Hematological Responses to Intravenous Immunoglobulin in an Immunocompromised Patient with Persistent Parvovirus B19 Infection

Julian W. Tang,[1] June S.M. Lau,[2] Stephanie Y.N. Wong,[3] Jo L.K. Cheung,[1] C.H. Chan,[2] K.F. Wong,[3] Ann Wong,[4] and Paul K.S. Chan[1]*

[1]*Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China*
[2]*Department of Medicine, Queen Elizabeth Hospital, Kowloon, Hong Kong SAR, China*
[3]*Department of Pathology, Queen Elizabeth Hospital, Kowloon, Hong Kong SAR, China*
[4]*Public Health Laboratory Services Branch, Center for Health Protection, Department of Health, Kowloon, Hong Kong SAR, China*

A 42-year-old male with stage IV mantle cell lymphoma received chemotherapy and autologous peripheral blood stem cell transplantation. He developed pancytopaenia, and bone marrow examination indicated a parvovirus B19 (PVB 19)-induced red cell aplasia, confirmed by virological tests. Multiple doses of intravenous immunoglobulin (IVIG) were given over the following months, with blood samples being taken after each dose for quantitative PVB 19 DNA and hematological testing to assess the response. Each dose of IVIG produced a 1–3 $\log_{10}$ drop in PVB 19 DNA levels. Eventually, after the fifth dose of IVIG, the PVB 19 DNA was reduced to $<$10 copies/ml serum, with a gradual improvement in his hematological parameters. This report demonstrates how close monitoring of the virological and hematological response to IVIG therapy for persistent PVB 19 infection in an immunocompromised patient can optimize the usage of this relatively expensive, and sometimes scarce intervention. ***J. Med. Virol. 79:1401–1405, 2007.***

KEY WORDS: pure red cell aplasia; pancytopaenia; response; monitoring

INTRODUCTION

Parvovirus B19 (PVB 19) is a small (22–24 nm), non-enveloped, single-stranded DNA virus and a member of the *Erythrovirus* genus, family *Parvoviridae*. In immunocompetent children it causes 'slapped cheek' or 'fifth disease' also known as 'erythema infectiosum'. In immunocompetent adults it can cause an acute symmetric polyarthropathy mimicking rheumatoid arthritis. In patients with chronic haemolysis or erythropoietic stress it can cause a transient but often severe red cell aplasia [Wong et al., 1993; Heegaard and Brown, 2002]. However, in immunocompromised patients such as solid organ transplant recipients [Moudgil et al., 1997; Wicki et al., 1997; Wong et al., 1999; Lui et al., 2001; Choi et al., 2002], human immunodeficiency virus (HIV)-infected [Yap, 1994; Ramratnam et al., 1995; Hung et al., 2001] and hematological malignancies [Azzi et al., 1993; Sharma et al., 2000; Kaptan et al., 2001; Castelli et al., 2002; Song et al., 2002; Isobe et al., 2004; Mouthon et al., 2005; Eid et al., 2006], PVB 19 infection can persist, resulting in pure red cell aplasia, chronic anemia and pancytopaenia [Heegaard and Brown, 2002].

Intravenous immunoglobulin (IVIG) is a plasma protein derivative obtained from blood donors' pooled plasma, containing mainly immunoglobulin G (IgG) with traces of other immunoglobulins [Darabi et al., 2006]. The seroprevalence of PVB 19 IgG in the Western general population is about 50–70%, explaining the relative success of IVIG in treating persistent PVB 19 infections [Heegaard and Brown, 2002; Mouthon et al., 2005], though there have been reported failures [Lui et al., 2001]. Although IVIG treatment is almost routine

*Correspondence to: Prof. Paul K.S. Chan, Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China. E-mail: paulkschan@cuhk.edu.hk

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for persistent PVB 19-infected immunocompromised patients [Mouthon et al., 2005; Eid et al., 2006], it is still relatively expensive, ranging from 50 to 80 US$/g [Darabi et al., 2006]. However, the evidence for its effectiveness for treating other infections, specifically in the bone marrow transplant (BMT) population, is poor, particularly when specific anti-CMV and other specific therapies, such as anti-fungal drugs, are available and more effective [Darabi et al., 2006]. Also, there have been cases of inadvertent PVB 19 transmission via IVIG treatment [Hayakawa et al., 2002]. It could be argued, therefore, that IVIG is an overused, expensive therapy, of doubtful effectiveness in most situations, with bone marrow transplant patients. Even in the treatment of PVB 19-induced pure red cell aplasia, where IVIG is relatively effective, its use can still be optimized.

We report an immunocompromised patient with persistent PVB 19 infection-associated pancytopaenia, treated with multiple doses of IVIG, with quantitative PVB 19 DNA testing after each dose. This dose-by-dose monitoring of the virological response to IVIG therapy allowed us to optimize the number of doses required of this relatively expensive intervention.

MATERIALS AND METHODS

Case Report

A 42-year-old male patient with stage IVA mantle cell lymphoma with bone marrow involvement was treated with rituximab-cyclophosphamide, adriamycin, vincristine and prednisolone. Partial remission of disease was achieved with the marrow being clear of lymphoma. He was then given etoposide, cisplatin, methylprednisolone and cytarabine followed by autologous peripheral blood stem cell transplantation, using cyclophosphamide, carmustine and etoposide as the conditioning regimen. However, the disease progressed 3 months after the autologous peripheral blood stem cell transplantation. Three cycles of cyclophosphamide, adriamycin, vincristine and dexamethasone alternating with high dose methotrexate and cytarabine, were then given.

Seventy-eight days after chemotherapy he developed persistent pancytopaenia: hemoglobin 8.5 g/dl, total white cell count 1.1×10^9 L^{-1} with neutrophils 0.5×10^9 L^{-1} and reticulocytes 2.3×10^9 L^{-1}, 1%, and platelets 12×10^9 L^{-1} (see Table I). A bone marrow biopsy taken then showed a mildly hypocellular marrow with increased megakaryocytes, active granulopoiesis, almost absent erythroid precursors except for occasional giant pronormoblasts, compatible with a PVB 19-induced pure red cell aplasia. A CT scan taken then showed no evidence of lymphoma. Intravenous immunoglobulin was started, with a serum sample taken for PVB 19 IgM, IgG and DNA PCR testing after each dose. Packed red cell transfusion was required every 3–8 days before and after the first two courses of IVIG just to maintain a steady hemoglobin level. After the third course of IVIG, the patient became transfusion independent, though IVIG was continued because of the persistent thrombocytopaenia, neutropaenia and positive serum PVB 19 DNA. At the time of writing (8 weeks from last dose of IVIG), the patient no longer required packed cell and platelet transfusions (hemoglobin 10.5 g/dl, total white cell count 3.6×10^9 L^{-1}, with neutrophils 2.0×10^9 L^{-1} and reticulocytes 30×10^9 L^{-1}, 1% and platelets 22×10^9 L^{-1}).

Virological Testing

Parvovirus B19 serology was performed using a commercial immunofluorescent slide kit (Biotrin, Dublin, Ireland), following the manufacturer's instructions. Parvovirus B19 DNA quantitative PCR (qPCR) was performed using an in-house method. Briefly, total DNA was extracted from 200 μl of serum using a commercial kit (QIAamp DNA mini kit, Hilden, Germany). Parvovirus B19 DNA was detected and quantified by real-time PCR using the Power Sybr® Green PCR master mix (Applied Biosystems, Foster City, CA) with the 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The samples were tested with known PVB 19 DNA load standards, with all tests performed in duplicate. The specificity of the amplification was confirmed by checking the dissociation curve against the expected melting temperature of the amplification product.

RESULTS

The virological and hematological results are summarized in Table I. The hemoglobin levels remained low and required supportive packed red cell transfusions between February 27 and May 14, despite the repeated administration of IVIG, whilst the parvovirus DNA levels remained $>10^3$ copies/ml. The parvovirus-specific IgM and IgG levels remained undetectable during this time. However, after the parvovirus DNA levels dropped to about 10^3 copies/mL (around May 18), the hemoglobin levels remained above 9 g/dl, without the need for further supportive packed red cell transfusions. In addition, at this time, the parvovirus-specific IgM became detectable, but the IgG remained undetectable, during this same period.

Figure 1 shows the changes in the hematological parameters (hemoglobin, total white cell count and platelets) on the left-hand axis, against the drop in parvovirus DNA levels on the right-hand axis (note the $\log_{10}$ scale on this axis). The hemoglobin levels fluctuated between 6.9 and 10.4 g/dl, and the total white cell count vary between 0.6 and 2.3×10^9 L^{-1} during this period. The relative change in the platelet counts were the most marked. During the earlier period of monitoring when supportive packed red cell transfusions were being given, the platelet counts rapidly oscillated between 14 and 22×10^9 L^{-1}, probably due to residual platelets in the packed red cells being transfused, as the high-level platelet spikes seem to coincide with the times of these transfusions. After these transfusions ceased and the PVB 19 levels fell below 10^3 copies/ml (after May 15, 154 days post-chemotherapy), the platelet

TABLE I. Results of Patient's Hematological Investigations During the Period of Intravenous Immunoglobulin (IVIG) Administration

Days post-chemotherapy (date, 2006)	IVIG dose	Hb (g/dl) [reticulocyte count $\times 10^9$ L^{-1}, %, where available]	Transfusion of packed red cells (dose)	WCC ($\times 10^9$ L^{-1})	Platelets ($\times 10^9$ L^{-1})	Parvovirus B19 DNA levels (DNA copies/ml) (days post-IVIG)	Parvovirus B19 IgM	Parvovirus B19 IgG
77 (February 27)	—	7.0	—	0.6	8	—	—	—
78 (February 28)	—	8.5 [2.3, 1%] (PT)	1 unit	1.1	12	—	—	—
80 (March 2)	0.4 g/kg/day for 5 days	6.9	—	0.7	12	—	—	—
81 (March 3)	—	10.4 (PT)	2 units	1.4	21	—	—	—
91 (March 13)	—	8.0 (NI)	1 unit	0.9	9.8	—	—	—
94 (March 16)	—	8	—	0.8	8	5.99×10^7 (14)	NEG	NEG
106 (March 28)	—	7.6 (NI)	2 units	0.9	8	—	—	—
107 (March 29)	—	8.3	—	2.3	7	—	—	—
108 (March 30)	1 g/kg/day for 2 days	7.2	—	2.3	4	—	—	—
109 (March 31)	—	8.3 (PT)	1 unit	2.0	17	—	—	—
113 (April 4)	—	7.5 (NI)	1 unit	1.7	9	—	—	—
121 (April 12)	—	8.4 (PT)	1 unit	1.9	6	—	—	—
133 (April 24)	—	7.9 (NI)	2 units	1.2	6	4.63×10^6 (25)	NEG	NEG
137 (April 28)	—	9.7	—	1.7	12	—	—	—
148 (May 9)	—	7.5	—	1.3	4	—	—	—
149 (May 10)	—	—	2 units	—	—	—	—	—
152 (May 13)	1 g/kg/day for 2 days	7.8	—	1.7	22	—	—	—
153 (May 14)	—	—	1 unit	—	—	—	—	—
154 (May 15)	—	8.6	—	1.8	14	—	—	—
157 (May 18)	—	9.2	—	1.8	11	2.78×10^3 (5)	POS	NEG
174 (June 3)	1 g/kg/day for 1 day	9.2	—	1.9	16	—	—	—
185 (June 14)	—	—	—	—	—	3.83×10^2 (11)	POS	NEG
186 (June 15)	—	9.5	—	1.4	14	—	—	—
199 (June 28)	1 g/kg/day for 2 days	9.6	—	1.5	17	—	—	—
204 (July 3)	—	9 [13.4, 0.5%]	—	1.6	16	9.38×10^1 (5)	POS	NEG
211 (July 10)	—	9.8	—	2.2	18	—	—	—

Hb, hemoglobin; WCC, total white cell count; PT, post-transfusion values; NI, whether the sample was taken pre- or post-transfusion was not indicated; '—', test or dose not performed or given, respectively.

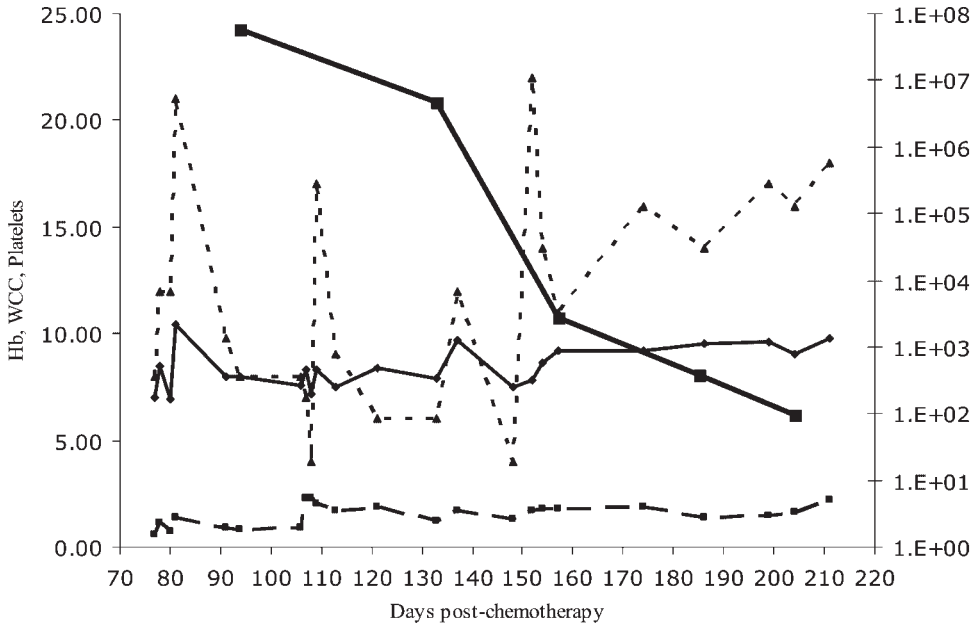

Fig. 1. Left *y*-axis shows the values of hemoglobin (Hb, g/dl, thin solid line), total white cell count (WCC, $\times 10^9 L^{-1}$, long-dashed line) and platelet count ($\times 10^9 L^{-1}$, short-dashed line). Right *y*-axis is a $\log_{10}$ scale and shows a semi-logarithmic plot of the parvovirus B19 levels (DNA copies/ml, thick solid line).

levels climbed steadily, as the PVB 19 DNA levels fell still further.

DISCUSSION

We have demonstrated the effectiveness of serial IVIG dosing with post-dose viral load monitoring on reducing PVB 19 DNA levels in a stage IV mantle cell lymphoma patient with persistent pancytopaenia. Although the absolute number of PVB 19 DNA copies/ml may vary between individual patients, in this patient there seems to be a consistent 1–3 $\log_{10}$ reduction in PVB 19 DNA load after each IVIG dose. The interval between IVIG dosing and serum sampling for PVB 19 DNA qPCR varied between 5 and 25 days, so it is difficult to estimate more precisely the effect of the IVIG at specific PVB 19 loads.

The parvovirus-specific IgG/IgM responses were delayed in this patient, with the IgG never becoming detectable during the period of study and the IgM only appearing in May, even though the PCR results indicate that the patient was infected with parvovirus by March 16 or earlier. Normally, parvovirus IgM and IgG appear after 7 and 14 days after infection, respectively [Heegaard and Brown, 2002]. Mantle cell lymphoma is a B-cell neoplasm, which may partially explain this poor humoral response. However, as the disease was in remission after multiple chemotherapy regimens, the treatment itself may have been the cause of this immune paresis.

The P-antigen receptor for PVB 19 is present on bone marrow erythroid progenitor cells, potentially making PVB 19 infection a severe problem in new bone marrow transplant patients [Keller and Stiehm, 2000; Heegaard and Brown, 2002; Chisaka et al., 2003]. Its clearance requires an effective humoral (B-cell) response with the generation of virus-specific neutralizing (IgG) antibodies. Their absence in this patient correlates with the persisting PVB 19 DNA, indicative of ongoing replication and damage to hematological cell precursors. As symptomatic PVB 19 infection is mainly immune-mediated, such immunocompromised patients may not exhibit the typical fever, rash and arthritis, leaving the detection of anemia as the first indication of an acute PVB 19 infection [Heegaard and Brown, 2002; Chisaka et al., 2003].

The amount of PVB 19-specific neutralizing antibodies in each IVIG batch varies with different blood donor populations, though the PVB 19 IgG seropositivity rate is usually 50–70% of the adult population [Mouthon et al., 2005]. This maybe one factor determining how many IVIG doses are required to clear the PVB 19 infection. Another is the level of the PVB 19 viraemia, which in turn depends on the severity of the patient's immunosuppression. In primary PVB 19 infection, the viral load may be as high as 10^{12} to 10^{14} DNA copies/ml in immunocompetent individuals (e.g. blood donors) [Weimer et al., 2001; Blumel et al., 2002; Gallinella et al., 2002], which may be even higher in the immunocompromised. In such patients, their chemotherapeutic immunosuppression may also be reduced, to allow the host immune system to clear the virus naturally [Lui et al., 2001; Eid et al., 2006].

To conclude, in this case of PVB 19 infection in an immunocompromised patient with a hematological malignancy, monitoring the virological response to each dose of IVIG can optimize the use of this relatively expensive intervention. If there is little or no response

after one to two IVIG doses, it can be withheld earlier rather than later, since it may not be effective for clearing PVB 19 infection in every immunocompromised patient.

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