Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent-treated pooled plasma

Ulrike F. Koenigbauer, Ted Eastlund, and John W. Day

BACKGROUND: Lipid-enveloped viruses such as HIV, HBV, and HCV can be inactivated by treatment with solvents and detergents. HAV and human parvovirus B19 lack lipid envelopes and are not inactivated. Solvent/detergent-treated pooled plasma (S/D plasma) contains neutralizing antibodies, but it is not known whether the parvovirus B19 antibody content is sufficient to prevent transmission of the disease. A patient is described who developed a clinical illness due to parvovirus B19 infection after the infusion of S/D plasma.

CASE REPORT: A 36-year-old woman with myasthenia gravis underwent five plasma exchange procedures from January 15 to January 25, 1999, using albumin, except for 5 units of S/D plasma given because of a low fibrinogen level. Four of the 5 units were implicated in a recall after high levels of parvovirus B19 DNA were found in several lots. Two weeks after the infusion, the patient developed fatigue, a rash, and severe polyarthralgias. Parvovirus B19 IgG and IgM antibody titers were consistent with an acute infection.

CONCLUSION: Clinically apparent parvovirus B19 infection can follow the use of S/D plasma that contains high levels of parvovirus B19 DNA.

ABBREVIATIONS: IMIG = IM immune globulin; INR = international normalized ratio; IVIG = IV immune globulin; S:CO = signal-to-cutoff; S/D plasma = solvent/detergent-treated pooled plasma.

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Received for publication January 12, 2000; revision received April 13, 2000, and accepted April 19, 2000.

found by PCR in plasma-derived clotting factor concentrates from various manufacturers and treated with different virus-inactivation methods by many investigators. Clinically evident transfusion-transmitted B19 infection, however, is infrequent, even in susceptible hosts such as HIV-infected hemophilia patients receiving clotting factor concentrates. There have been at least two reports of transfusion-transmitted parvovirus infection from single-donor components, namely RBCs and platelets. We describe a case of symptomatic parvovirus B19 infection after the infusion of solvent/detergent-treated pooled plasma (S/D plasma), which was subsequently implicated in a voluntary recall after high levels of infectious parovirus B19 DNA were detected in the corresponding plasma lots.

CASE REPORT

The patient is a 36-year-old woman with a 10-month history of myasthenia gravis that was poorly responsive to mestinon, prednisone, and azathioprine, as well as to IV immune globulin (IVIG), which had been administered on December 10 and 11, 1998. She also had a history of Hashimoto's thyroiditis and systemic lupus erythematosus with only mild arthralgias in the past that were readily treated with ibuprofen. She did not have any dermatologic manifestations. As treatment for her myasthenia gravis, she underwent five plasma exchange procedures from January 15 to January 25 using 2300 to 2900 mL of 5-percent albumin (Buminate 5%, Baxter Healthcare Corp., Glendale, CA; Albumarc 5%, Baxter; and Albutein 5%, Alpha Therapeutic Corp., Los Angeles, CA) as exchange fluid, which resulted in mild improvement of her proximal muscle weakness. The medications the patient received during the period of the plasma exchange procedures were azathioprine, mestinon, thyroxine, and estrogen. Her fibrinogen level was 98 mg per dL before the fourth procedure, and, even though she had no bleeding, she was given a total of 5 units (1000 mL total volume) of S/D plasma (PLAS+SD, V.L. Technologies, Melville, NY) at the end of her fourth and fifth treatment. Preprocedure international normalized ratio (INR) and partial thromboplastin time were in the normal range, and platelet counts were 159 and 166 × 10^9 per L. Fibrinogen was 145 mg per dL on January 25, 1999. Her Hb level ranged from 10.7 to 11.5 g per dL and her WBC count from 5.1 to 6.7 × 10^9 per L. One unit of S/D plasma infused on January 22, 1999, and all 3 units infused on January 25, 1999, were implicated in a subsequent recall of S/D plasma lots conducted by the American Red Cross after high levels of infectious parovirus B19 DNA had been detected by the manufacturer in several lots. We subsequently interviewed the patient, who reported that, during the second week of February, approximately 2 to 3 weeks after receiving S/D plasma, she had developed an illness, that began with mild rhinorrhea and fatigue. This was followed by severe generalized symmetric polyarthralgias that were poorly relieved by ibuprofen and a lacy erythematous rash on her extremities, which lasted about 7 days. She had no fever, sore throat, or gastrointestinal symptoms. When her symptoms started to resolve, she resumed work and was tested for parvovirus B19 antibodies on February 19, 1999. Her test results were positive for IgM and IgG. The signal-to-cutoff (S:CO) ratio for IgM was 1.68 and that for IgG was 1.49. A S:CO of ≥1.20 is interpreted as positive. The antibody test used was an EIA (Microbiology Reference Laboratory, Cypress, CA) with confirmation testing of IgM antibodies by an indirect immunofluorescence assay (Microbiology Reference Laboratory). On a follow-up test on May 27, 1999, performed in the same laboratory with the same test system, the IgM was no longer positive (IgM, 0.89 S/CO) and IgG rose to 5.42 S/CO, which is consistent with a recent infection. She recalled no history of contact with others infected with or recently exposed to parvovirus B19 infection. Her illness resolved without further treatment. Two weeks after her illness, her blood counts were similar to those performed previously: platelet count 206 × 10^9 per L, WBC count 5.3 × 10^9 per L, and Hb 10.9 g per dL.

DISCUSSION

Treatment of plasma with the solvent tri(n-butyl)phosphate and the detergent Triton-X 100 inactivates lipid-enveloped viruses such as HIV, HBV, and HCV. Viruses such as HAV or parovirus B19 that lack the lipid envelope are not inactivated. Because S/D plasma is prepared from the blood of donors who are representative of the general community, about half of the units of donated plasma contain antibodies to parovirus. Neutralizing antibodies are therefore contained in S/D plasma, which is derived from pooled plasma from up to 2500 volunteer donations. These antibodies could potentially prevent infection in the recipient. However, the amount of antibody needed to prevent transmission by a blood component containing parvo virus B19 has not been established.

IVIG is recommended as therapy in chronic parvovirus B19 infection, as it has effectively eliminated viremia and symptoms. The amount of antibody required to protect against or cure parvovirus B19 infection with IVIG is not known, and one possible parvovirus transmission from IVIG has been reported. Using PCR, B19 DNA has been detected in 3 of 4 lots of IM immune globulin (IMIG) and 3 of 15 lots of IVIG by one group, whereas McOmish et al. did not find B19 DNA in 10 lots of IVIG preparations. Our patient had received two infusions of IVIG to treat myasthenia gravis approximately 60 days before the onset of her symptoms. Because rash and arthralgias occur 2 to 3 weeks after infection by the virus, IVIG is unlikely to be the cause

KOENIGBAUER ET AL.
of her parvovirus infection. Passive acquisition of the antibody from either IVIG or S/D plasma would not explain her seroconversion pattern of a declining IgM parvovirus antibody and a rising IgG level.

Our patient received albumin during the course of her treatment, and this should be considered as a potential source of the infection. Saldanha et al.28 found parvovirus B19 DNA in 1 to 3 of 12 lots of albumin, which contained the lowest levels of B19 DNA of various tested blood components (<10^5-10^7 genome equivalents/mL). Higher levels were found in IVIG, IMIG, and clotting factor concentrates that had levels of 10^4 to 10^7 genome equivalents per mL. In contrast, Lefrère et al.37 had negative results in all 29 albumin batches from two manufacturers. It is interesting that B19 DNA has also been found in 5 of 30 lots of recombinant factor VIII concentrates that were not derived from human plasma.38 This finding was attributed to the albumin used as stabilizer.

The presence of B19 DNA in plasma derivatives, especially if in low concentrations, does not mean that these products can transmit infection, as the viability of the virus may have been destroyed during processing.24,26-29 This may explain the results of PCR testing, which did not correlate with infectivity assays in factor VIII concentrates spiked with canine parvovirus before terminal dry-heat treatment.38 B19 DNA is infrequently found in albumin, and, when it is detected, the B19 DNA content is low. In addition, albumin is heat-treated for 10 hours at 60°C, which results in a reduction of at least 10^4 genome equivalents per mL. Conversely, the parvovirus B19 content of some of the S/D plasma lots used in our case was reported by the manufacturer to be greater than 10^7 genome equivalents per mL, which was the level that was found by the manufacturer to have caused B19 viremia and seroconversion in healthy subjects. Thus, we do not consider albumin to be the source of parvovirus infection in our patient.

Our patient had been diagnosed with systemic lupus erythematosus 2 years previously, on the basis of antinuclear antibodies and mild arthralgias that were readily treated with ibuprofen. Before her recent parvovirus infection, she had never had severe joint pain, lupus skin eruptions, or any other severe manifestation. The rash she developed during her acute illness after S/D plasma treatment was not suggestive of lupus, and her severe symmetric polyarthralgias were consistent with acute parvoviral infection.

Plasma was infused to our patient at the end of her fourth plasma exchange, because of a fibrinogen level below 100 mg per dL found before the plasma exchange. The patient was not bleeding and the hypofibrinogenemia was due to the use of albumin as a replacement fluid. S/D plasma was also given at the end of her fifth exchange, despite the absence of bleeding and a fibrinogen level above 100 mg per dL. This was inappropriate, and we lowered our threshold to 50 mg per dL in nonbleeding patients.

Viremia in asymptomatic blood donors is of concern, especially in connection with S/D plasma derived from large pools. Titers of 5 x 10^10 genome equivalents per mL have been found in some donors.27 The prevalence of viremia in healthy blood donors has been studied by several groups with variable results that depend on the sensitivity of the assay; the rates are generally higher during epidemics.25 In a study of 20,000 donors, the prevalence of B19 DNA detected by PCR was 1 per 3,300,27 and, during an epidemic in Japan, it was as high as 1 per 167.40 A more recent study in which US voluntary blood donors were screened for B19 DNA indicated a prevalence of 0.1 percent (11/9,568).41 Therefore, most plasma pools from which S/D plasma is manufactured will contain parvovirus B19 DNA. Subsequent to the recall and in conjunction with the FDA, the S/D plasma manufacturer has instituted steps whereby all lots are now screened by PCR for parvovirus B19 DNA. Lots containing viral loads that have been associated with seroconversion in healthy volunteers are no longer distributed. S/D plasma released for transfusion may contain parvovirus B19 DNA and specific antibodies, but the B19 DNA antibody titers, if present, are at low levels that did not lead to seroconversion in experimental subjects. The S/D plasma is therefore considered noninfectious.

We conclude that our patient’s clinical illness, which was consistent with parvovirus infection and accompanied by seroconversion, was acquired via infusion of S/D plasma containing high levels of parvovirus B19.

REFERENCES
血液製剤の安全性の向上について

日本赤十字社では血液製剤の安全対策として、①献血受付時の問診、②血清学的検査（HBS抗原、HBS抗体、HBC抗体、HCV抗体、HIV-1、2抗体、HTLV-1抗体、梅毒トレポネーマ抗体、ヒトパルボウイルスB19）、③核酸増幅検査（NAT: HBV、HCV、HIV）、④新鮮冷結血漿及び血漿分画製剤の原料血漿の6ヶ月間の貯留保管等を実施しています。2008年8月、血清学的検査を凝集法から化学発光酵素免疫法（CLEIA法）へ変更し、また、NATについても新NATシステムへ切り替え、当該システムによる検査を開始しました。

●血清学的検査（CLEIA法）

■測定原理

図像化抗体とALP標識抗体で検体中の抗原を検査抗原査体複合体を形成させる。ALP標識抗体と基質との反応で発光させ、発光量をカウントする。

■検査機器システム

Fe

核酸増幅検査

■検出感度

ウイルスの種類 | 平均検出感度
---|---
HBV | 3.2 IU/mL
HCV | 12.4 IU/mL
HIV-1 Group M | 41.8 IU/mL
HIV-1 Group O | 93.7 copies/mL
HIV-2 | 2.0 copies/mL

新NATシステムは、核酸抽出から増幅、検出まで1台で行う全自動タイプで、従来システムより感度が向上しました。また、HIV-1グループO及びHIV-2の検出も可能となりました。

これらの変更により、血液製剤の安全対策をこれまで以上に充実します。
Mirasol Clinical Experience: Results from the MIRACLE Trial

Raymond P. Goodrich, Ph.D.
Chief Science Officer
Navigant Biotechnologies, LLC
Study Goals and Design

- Multicenter, Prospective, Randomized, Open Label, Blinded Endpoint
- Each investigational site:
  - Blood Establishment – Technical processing of platelet product, randomization of subjects
  - Clinical Site – Selection, transfusion and patient follow-up

- Subjects randomized to receive Mirasol vs. Reference platelets
- Transfusion indications per attending MD
- Transfusion needed
- On study for 28 days, or until no more
- Followed additional 28 days for adverse events

Determine if the Mirasol PRT System for Platelets - Performs safely, and - Maintains adequate platelet performance in a clinical setting.

- Multicenter, Prospective, Randomized, Open Label, Blinded Endpoint
- Blood Establishment – Technical processing of platelet product, randomization of subjects
- Clinical Site – Selection, transfusion and patient follow-up

- Each investigational site:
Endpoints

- 1-hour and 24-hour CCI for first 8 transfusions
- Days between platelet transfusions
- Number of platelet transfusions per subject
- Number of platelets per day of support
- Length of time of transfusion support
- Number of platelets per 1-hour and 24-hour CCI

Infections

- Serious adverse events & bleeding (WHO scale)
- Neoantigen analysis in refractory subjects
- Neoantigen analysis in refractory to platelets
- Number of red cell transfusions
- Number of subjects refractory to platelets

For subjects with > 8 transfusions: Longitudinal regression
Platelet Products

- Mirasol treatment done at each site
- Trima collections – 5 sites
- Buffy coat platelets – 1 site
- Gamma irradiated at 2 of the 6 sites
- Mirasol and Reference platelets stored in 100% plasma

- Initial volume = 170 – 360 mL
- Initial Concentration = 1180 – 2100 X 10^3 / μL plasma
- No additive solutions
- Final dose = 3.0 – 5.1 x 10^11 platelets per product

- Stored for up to 5 days

- Mirasol treatment done at each site

- Trima collections – 5 sites
- Buffy coat platelets – 1 site
- Gamma irradiated at 2 of the 6 sites
6 sites in France

Grenoble
Lyon
Bordeaux
Nantes
Besançon
Strasbourg
Overview of Safety Approach

- Protocol required 3 levels of independent external review:
  - Medical Monitor
  - Data Safety Monitoring Board
  - Data Monitoring Committee
- All reviews done independently to assure no introduction of bias between groups
- Reviews completed at defined intervals
- Protocol required 3 levels of independent external review
In total 8 Patients not transfused and 15 others excluded from analysis.

118 Thrombocytopenic Patients Enrolled

80 Patients - Apheresis Platelets

41 Mirasol

7 Mirasol

8 Reference

39 Reference

15 Patients - Buffy Coat Platelets

453

453
<table>
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<tr>
<th>Age of Platelets in Days (Range)</th>
<th>Number of Non-Gamma Irradiated products transfused</th>
<th>Number of Gamma Irradiated products transfused</th>
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<tr>
<td>0.832 (NS) (1-5 Days)</td>
<td>65 (26%)</td>
<td>262 (89%)</td>
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<tr>
<td>2.7 (1-5 Days)</td>
<td>182 (74%)</td>
<td>31 (11%)</td>
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<td>P-value Reference (N=48)</td>
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<td>Mirasol (N=47)</td>
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Platelet Dose in Products

Average Number of Platelets Transfused (x10^11) By Treatment Group with 95% Confidence Limits

p = 0.612
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<td>0.51 (NS)</td>
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<td>0.50 (NS)</td>
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<td>0.58 (NS)</td>
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<tr>
<td>Mean number of platelet units transfused over the 28 day treatment period</td>
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<tr>
<td>Mean number of platelet units transfused per day of platelet support</td>
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<tr>
<td>Mean number of platelet units transfused divided by body surface area</td>
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<tr>
<td>Mean cumulative platelet dose transfused per patient in total (x 10^11)</td>
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By Treatment Group with 95% Confidence Limits

Total Cumulative Number of Platelets Transfused (x 10^11)

Cumulative Platelet Dose Transfused

p=0.511
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<tr>
<td>P-value</td>
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<tr>
<td>Mean number of transfusions per patient</td>
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<tr>
<td>Transfusions 1-8</td>
<td>1.2</td>
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<tr>
<td>Transfusions &gt;8</td>
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<td>Cumulative number of days from transfusion 1 to 8</td>
<td>16.2</td>
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<td>Transfusions &gt;8</td>
<td>5.3</td>
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<td>Mean number of transfusions per day of platelet transfusion support</td>
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<td>Transfusions 1-8</td>
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<td>Number of “off-protocol” platelet transfusions</td>
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<tr>
<td>Mean number of transfusions per day of platelet transfusion support</td>
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Mean number of transfusions per day of platelet transfusion support:

- Transfusions 1-8
- Transfusions >8

Number of “off-protocol” platelet transfusions:

- Transfusions 1-8
- Transfusions >8
Frequency Distribution of Platelet Transfusions

Number of Transfusions

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p = 0.335

% of Patients
% of subjects with initial positive HLA test result

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P-value Reference (N=48) Mirasol (N=47)