

CASE REPORT

Bacteriological and Serological Findings in a Further Case of Transfusion-Mediated *Yersinia enterocolitica* Sepsis

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A 13-year-old patient developed severe shock due to administration of a *Yersinia enterocolitica*-contaminated red blood cell concentrate. *Y. enterocolitica* (serotype O:9, biotype II) was cultivated from the residual blood in the blood bag and from a stool sample of the blood donor. In the donor's plasma immunoglobulin M (IgM), IgA, and IgG antibodies against *Yersinia* outer proteins (YopM, -H, -D, and -E) were found. Since the donor remembered a short-lasting, mild diarrhea 14 days prior to blood donation, a transient attack of *Yersinia* enteritis may be associated with a longer than expected period of asymptomatic bacteremia that causes contamination of donor blood. Serological screening for IgM antibodies against *Yersinia* outer proteins might offer a way to reduce the risk of transfusion-associated *Y. enterocolitica* sepsis.

CASE REPORT

We report the case of a 13-year-old girl suffering from lung metastases of an osteoblastic sarcoma. She required chemotherapy and consecutive substitution of blood products. About 30 min after the beginning of the transfusion of a red blood cell concentrate (RBCC) she developed chills, abdominal pain, nausea, vomiting, pallor, tachypnea, dyspnea, and cough. The transfusion was stopped immediately, and the patient was admitted to the intensive care unit. Blood pressure was 95/60 torr, and heart rate was 170 beats/min. Arterial blood gas analysis showed hypoxemia (partial pressure of O₂, 53 torr). Chest X-ray revealed pulmonary edema. Echocardiographically, the function of both ventricles was markedly impaired (acute deterioration of a preexisting adriamycin-induced cardiomyopathy). There was no evidence of acute intravascular hemolysis (normal haptoglobin and total bilirubin values). Red blood cell compatibility was reexamined immediately after the event and found to be inconspicuous. Half a day after the transfusion, a Gram-stained blood smear was prepared directly from the residual blood in the blood bag. It showed numerous gram-negative rods. Intravenous antibiotics (ceftazidime, gentamicin, and ampicillin) were started, and preexisting oral therapy with cotrimoxazole, polymyxin, and nystatin was continued. The patient's condition improved slowly, and she was discharged from the intensive care unit after 8 days.

Microbiological investigation. Blood from the RBCC was plated on MacConkey, sheep blood (aerobe), and Schaedler

(anaerobe) agar plates. After 1 day of incubation (at 37°C), small colonies that were identified as *Yersinia enterocolitica* by using a numeric profile based on 20 biochemical reactions (API 20E) were found. The fresh frozen plasma from the same donation showed no growth, as it had been stored in a frozen state. Twenty-five days after the blood donation, a stool specimen from the donor was examined by a cold-enrichment technique (10 days in phosphate-buffered saline at 4°C) (28), which also revealed *Y. enterocolitica*. Direct inoculation of the stool sample on cefsulodin-irgasan-novobiocin agar (28°C) (22) had been negative. The isolates from the blood bag and from the donor's stool were characterized as serotype O:9 (by agglutination with anti-*Y. enterocolitica* sera) and as biotype II (by using commercial tests with 20 and 49 biochemical reactions [API 20E and API 50CHE] and by additional testing of the indole reaction with a prolonged incubation of 4 days at 29°C). The antimicrobial susceptibility patterns of the isolates were in concordance with data reported in the literature (18). By genomic macrorestriction analysis using *NotI* digestion of chromosomal DNA and pulsed-field gel electrophoresis, identical restriction fragment patterns were obtained from both isolates (26).

The donor's plasma (from the time of blood donation) contained agglutinating antibodies against *Y. enterocolitica* O-antigen O:9 at a titer of 1:160 as measured by the Widal method (normal level, $\leq 1:80$). In the immunoblot analysis we found antibodies of the immunoglobulin M (IgM) class against YopD and antibodies of the IgA class and of the IgG class against YopM (weak), YopH, YopD, and YopE (13, 15, 17) (Fig. 1).

The RBCC (buffy coat reduced, suspended in the additive solution SAG-M) causing the adverse transfusion reaction had been stored for 14 days in the refrigerator and had stayed at room temperature for some hours before transfusion. The donor was apparently healthy at the time of donation. After the event he was questioned again. He then remembered a very mild and short-lasting diarrhea without any other symp-

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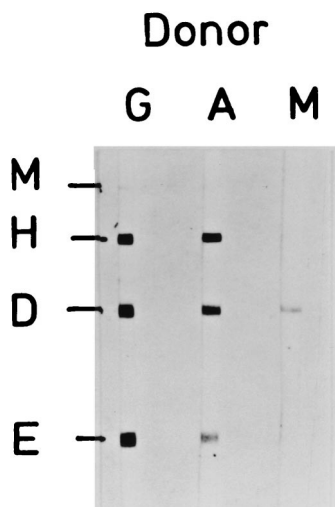


FIG. 1. Immunoblot analysis of donor plasma (dilution, 1:200) for antibodies of the IgG (lane G), IgA (lane A), and IgM (lane M) classes against the Yops YopE, -D, -H, and -M.

toms about 14 days before blood donation. This had not reduced his fitness for work so that he had forgotten it in the meantime.

The first case of transfusion-associated sepsis caused by *Y. enterocolitica* was reported from The Netherlands in 1975 (4). Since that time more than 35 further cases from all over the world have been reported (16). Autologous as well as homologous blood products were found to be contaminated with *Y. enterocolitica* (12; C. Richards, J. Kolins, and C. D. Trindade, Letter, *JAMA* 268:1541–1542, 1992). Most reports concerned red blood cell products (whole blood or RBCC) (16). Between April 1987 and November 1996, 20 cases of transfusion-associated *Y. enterocolitica* sepsis (12 fatal) were reported in the United States (6, 7). The true incidence of this complication of blood transfusion is not known, because the report of nonfatal cases was not obligatory. Although transfusion-associated *Y. enterocolitica* sepsis seems to be a rare event, it has received attention for its high fatality rate among the reported patients.

From the results of our microbiological investigation we conclude that *Yersinia* contamination of the blood donation was the result of a transient bacteremia of the donor, who suffered from mild enteric yersiniosis 2 weeks before blood donation. There are only a few reports on coisolation of *Y. enterocolitica* from donor blood and feces that suggest this way of infection, but our study adds a further case in which identical strains were isolated from the blood bag and the stool sample. Obviously, *Y. enterocolitica* is endowed with properties enabling the pathogen to cause symptomless transient low-level bacteremia during convalescence (25). Recently, the persistence of *Yersinia* antigens in peripheral white blood cells from patients with *Y. enterocolitica* infection for up to 4 years has been demonstrated (10).

Several approaches, such as reducing the storage time (1, 8) or the storage temperature of RBCCs (3), asking donors about gastrointestinal illness in the last weeks before blood donation (11), and inspecting the blood in the bags for color change a short time before the release of the units (19), have been suggested for preventing transfusion-associated *Y. enterocolitica* sepsis but have not yet been generally accepted. Removal of *Y. enterocolitica* from donated blood can be achieved by leukocyte filtration (5, 9, 20, 29). However, large numbers of

bacteria can exceed the capacity of this method to prevent bacterial growth during the storage of RBCCs (5, 29). Testing for antibodies against *Y. enterocolitica* O antigens in donor serum by the bacterial agglutination method (23) or by an enzyme-linked immunosorbent assay (A. J. Morris and D. G. Woodfield, Letter, *Transfusion* 38:1122, 1998) has not been recommended, either because of an unacceptably high rate of positive results for healthy donors (23) or because of an apparent lack of antibody response in some patients (Morris and Woodfield, Letter).

Another possibility of serological testing is the detection of antibodies against *Yersinia* outer proteins (Yops) (13). Yops are clearly associated with virulence properties of pathogenic strains (24). They are borne by the virulence plasmid pYV, which is present in all human pathogenic *Yersinia* species and serotypes (15). In contrast to testing for antibodies to O antigens (2), screening for antibodies to Yops is not restricted to single serotypes (21). Antibodies of the IgM class—especially those directed against YopD—may indicate an acute infection (27). A preliminary study with a small number of healthy blood donors had shown a specificity of 97% for immunoblot analysis for IgM antibodies against Yops (14). Further studies are required to evaluate the suitability of the detection of IgM antibodies against Yops for reducing the risk of transfusion-associated *Y. enterocolitica* sepsis.

REFERENCES

1. Arduino, M. J., L. A. Bland, M. A. Tipple, S. M. Aguero, M. S. Favero, and W. R. Jarvis. 1989. Growth and endotoxin production of *Yersinia enterocolitica* and *Enterobacter agglomerans* in packed erythrocytes. *J. Clin. Microbiol.* 27:1483–1485.
2. Bottone, E. J., and D. J. Sheehan. 1983. *Yersinia enterocolitica*: guidelines for serologic diagnosis of human infections. *Rev. Infect. Dis.* 5:898–906.
3. Bradley, R. M., R. M. Gander, S. K. Patel, and H. S. Kaplan. 1997. Inhibitory effect of 0°C storage on the proliferation of *Yersinia enterocolitica* in donated blood. *Transfusion* 37:691–695.
4. Bruining, M., and C. C. M. De Wilde-Beekhuizen. 1975. A case of contamination of donor blood by *Yersinia enterocolitica* type 9. *Medikon* 4:30–31.
5. Buchholz, D. H., J. P. AuBuchon, E. L. Snyder, R. Kandler, S. Edberg, V. Piscitelli, C. Pickard, and P. Napychank. 1992. Removal of *Yersinia enterocolitica* from AS-1 red cells. *Transfusion* 32:667–672.
6. Centers for Disease Control and Prevention. 1991. Update: *Yersinia enterocolitica* bacteremia and endotoxin shock associated with red blood cell transfusions—United States, 1991. *Morb. Mortal. Wkly. Rep.* 40:176–178.
7. Centers for Disease Control and Prevention. 1997. Red blood cell transfusions contaminated with *Yersinia enterocolitica*—United States, 1991–1996, and initiation of a national study to detect bacteria-associated transfusion reactions. *JAMA* 278:196–197.
8. Franzin, L., and P. Gioannini. 1992. Growth of *Yersinia* species in artificially contaminated blood bags. *Transfusion* 32:673–676.
9. Gong, J., C. F. Högman, A. Hambraeus, C. S. Johansson, and L. Eriksson. 1993. Transfusion-transmitted *Yersinia enterocolitica* infection. *Vox Sang.* 65:42–46.
10. Granfors, K., R. Merilahti-Palo, R. Luukkainen, T. Möttönen, R. Lahesmaa, P. Probst, E. Märker-Hermann, and P. Toivanen. 1998. Persistence of *Yersinia* antigens in peripheral blood cells from patients with *Yersinia enterocolitica* O:3 infection with or without reactive arthritis. *Arthritis Rheum.* 41:855–862.
11. Grossman, B. J., P. Kollins, P. M. Lau, J. L. Perreten, R. J. Bowman, S. Malcolm, and W. M. Palko. 1991. Screening blood donors for gastrointestinal illness: a strategy to eliminate carriers of *Yersinia enterocolitica*. *Transfusion* 31:500–501.
12. Haditsch, M., L. Binder, C. Gabriel, P. Müller-Urli, R. Watschinger, and H. Mittermayer. 1994. *Yersinia enterocolitica* septicemia in autologous blood transfusion. *Transfusion* 34:907–909.
13. Heesemann, J., C. Eggers, J. Schröder, and R. Laufs. 1986. Serological diagnosis of yersiniosis by the immunoblot technique using plasmid-encoded antigens of *Yersinia enterocolitica*, p. 79–88. In C. Simon and P. Wilkinson (ed.), *Diagnosis of infectious diseases—new aspects*. Schattauer, Stuttgart, Germany.
14. Heesemann, J., C. Eggers, and J. Schröder. 1987. Serological diagnosis of yersiniosis by immunoblot technique using virulence-associated antigen of enteropathogenic *Yersiniae*. *Contrib. Microbiol. Immunol.* 9:285–289.
15. Heesemann, J., U. Gross, N. Schmidt, and R. Laufs. 1986. Immunochemical analysis of plasmid-encoded proteins released by enteropathogenic *Yersinia* species grown in calcium-deficient media. *Infect. Immun.* 54:561–567.

16. **Högman, C. F., and L. Engstrand.** 1996. Factors affecting growth of *Yersinia enterocolitica* in cellular blood products. *Transfus. Med. Rev.* **10**:259–275.
17. **Hoogkamp-Korstanje, J. A. A., J. de Koning, and J. Heesemann.** 1988. Persistence of *Yersinia enterocolitica* in man. *Infection* **16**:81–85.
18. **Kanazawa, Y., K. Ikemura, and T. Kuramata.** 1987. Drug susceptibility of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. *Contrib. Microbiol. Immunol.* **9**:127–135.
19. **Kim, D. M., M. E. Brecher, L. A. Bland, T. J. Estes, R. A. Carmen, and E. J. Nelson.** 1992. Visual identification of bacterially contaminated red cells. *Transfusion* **32**:221–225.
20. **Kim, D. M., M. E. Brecher, L. A. Bland, T. J. Estes, S. K. McAllister, S. M. Agüero, R. A. Carmen, and E. J. Nelson.** 1992. Prestorage removal of *Yersinia enterocolitica* from red cells with white cell-reduction filters. *Transfusion* **32**:658–662.
21. **Mäki-Ikola, O., J. Heesemann, R. Lahesmaa, A. Toivanen, and K. Granfors.** 1991. Combined use of released proteins and lipopolysaccharide in enzyme-linked immunosorbent assay for serologic screening of *Yersinia* infections. *J. Infect. Dis.* **163**:409–412.
22. **Nash, P., and M. M. Krenz.** 1991. Culture media, p. 1237. In A. Balows, W. J. Hausler, K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
23. **Prentice, M.** 1992. Transfusing *Yersinia enterocolitica*. *Br. Med. J.* **305**:663–664.
24. **Ruckdeschel, K., A. Roggenkamp, S. Schubert, and J. Heesemann.** 1996. Differential contribution of *Yersinia enterocolitica* virulence factors to evasion of microbicidal action of neutrophils. *Infect. Immun.* **64**:724–733.
25. **Ruckdeschel, K., S. Harb, A. Roggenkamp, M. Hornef, R. Zumbihl, S. Köhler, J. Heesemann, and B. Rouot.** 1998. *Yersinia enterocolitica* impairs activation of transcription factor NF- κ B: involvement in the induction of programmed cell death and in the suppression of the macrophage tumor necrosis factor α production. *J. Exp. Med.* **187**:1069–1079.
26. **Saken, E., A. Roggenkamp, S. Aleksic, and J. Heesemann.** 1994. Characterisation of pathogenic *Yersinia enterocolitica* serogroups by pulsed-field gel electrophoresis of genomic *NotI* restriction fragments. *J. Med. Microbiol.* **41**:329–338.
27. **Stahlberg, T. H., J. Heesemann, K. Granfors, and A. Toivanen.** 1989. Immunoblot analysis of IgM, IgG and IgA responses to plasmid encoded released proteins of *Yersinia enterocolitica* in patients with and without *Yersinia* triggered reactive arthritis. *Ann. Rheum. Dis.* **48**:577–581.
28. **Van Noyen, R., R. Selderslaghs, G. Wauters, and J. Vandepitte.** 1987. Optimal recovery of *Yersinia enterocolitica* O:3 and O:9 from stools of patients with intestinal disorders. *Contrib. Microbiol. Immunol.* **9**:272–278.
29. **Wenz, B., E. R. Burns, and L. F. Freundlich.** 1992. Prevention of growth of *Yersinia enterocolitica* in blood by polyester fiber filtration. *Transfusion* **32**:663–666.