Rev. Inst. Med. Trop. Sao Paulo
52(4):225-227, July-August, 2010
doi: 10.1590/S0036-46652010000400012

CASE REPORT

SEROLOGICAL MONITORING OF A *Toxoplasma* INFECTION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

Cláudio L. ROSSI(1), Fernanda S. NASCIMENTO(1), Sílvia de BARROS-MAZON(1), Daniela F. DIAS(2), Antonio C. VIGORITO(2) & Cármino A. de SOUZA(2)

SUMMARY

We report a primary response to *Toxoplasma gondii* following a hematopoietic stem cell transplantation in a patient with multiple myeloma. The primary response to *T. gondii* was supported by IgM, IgG and IgA seroconversion. The patient was promptly treated and there were no complications related to toxoplasmosis in the subsequent months.

KEYWORDS: Hematopoietic stem cell transplantation; Toxoplasmosis; Serological diagnosis.

INTRODUCTION

Toxoplasmosis, a worldwide infection caused by the intracellular parasite *Toxoplasma gondii*, is generally asymptomatic or is associated with mild, non-specific clinical manifestations in immunocompetent subjects. The parasite can, however, cause serious illness in congenitally infected infants[2] and in immunocompromised patients[1,4]. We report a primary response to *T. gondii* following a non-myeloablative allogeneic hematopoietic stem cell transplantation in a patient with multiple myeloma.

CASE REPORT

A 53-year-old Brazilian man was diagnosed in December 1998 as having multiple myeloma (stage IIIA according to Durie and Salmon's classification). The patient achieved partial remission after VAD chemotherapy. In April 2000, he underwent an autologous hematopoietic stem cell transplantation (HSCT) (8.94 x 10^6/kg CD34+ mononuclear cells) under a conditioning regimen of high-dose melphalan (200 mg/m^2). There were no important complications in the post-transplantation period and no transfusion was necessary. Neutrophil recovery was observed on day +10. However, because of disease progression, thalidomide (200-400 mg/day) was started in June 2003. In June 2004, the patient underwent a non-myeloablative allogeneic stem cell transplantation (NST) from an HLA-identical sibling donor and mismatched ABO group (patient O+ and donor B+). Pretransplant serology for toxoplasmosis and cytomegalovirus (CMV) was IgM-negative and IgG-positive. The conditioning regimen included total body irradiation (200 cGy) and fludarabine (90 mg/m^2). Graft-versus-host disease (GVHD) prophylaxis with cyclosporine-A and a short course of mycophenolate were initiated. The patient received 8.32×10^8 mononuclear cells/kg and 3.29×10^6 CD34+ cells/kg. Neutrophil recovery was observed on day +5. Complete chimerism was documented by VNTR on days +28, +56, +122, +211 and +909 after NST. The immunosupressor therapy was continued until day +56, when the GVHD prophylaxis was tapered off.

In July 2005 (390 days post-NST), the patient presented fever, myalgia and headache. Serology for syphilis, hepatitis A, B and C and HIV was negative. The serology for CMV was IgM-negative and IgG-positive, whereas the serology for toxoplasmosis was positive for IgM, IgG and IgA antibodies. The donor's serology for toxoplasmosis was negative for IgM and IgG antibodies. The patient was promptly treated with pyrimethamine, sulfadiazine, and folinic acid, and no complications related to toxoplasmosis have been observed up to now.

The antibody concentrations for several infectious agents, including *T. gondii*, were measured in recipient serum samples at periodic intervals during follow-up (Table 1). *Toxoplasma*-specific IgM and IgG antibodies were determined by indirect immunofluorescence (IIF) and enzyme-linked fluorescent assay (ELFA), using the VIDAS® system (BioMérieux, France). *Toxoplasma*-specific IgG avidity was also determined by ELFA using the VIDAS system. Anti-*T. gondii* IgA was measured by enzyme-linked immunosorbent assay (ELISA) using Platelia™ Toxo IgA kits (Bio-Rad, France). IIF was done as previously described[9], and titers ≥ 32 were considered positive. ELFA and ELISA were performed according to the manufacturers' instructions. For the VIDAS system, IgM indices ≥ 0.65 and antitoxoplasma IgG titers ≥ 8 international units per milliliter (IU/mL) were considered positive. The IgG avidity test allows specimens

(1) Department of Clinical Pathology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, São Paulo, Brazil.
(2) Bone Marrow Transplantation Unit, Hematology and Hemotherapy Center, State University of Campinas (UNICAMP), Campinas, São Paulo, Brazil.
Correspondence to: Cláudio Lúcio Rossi, Department of Clinical Pathology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), P.O. Box 6111, 13083-970 Campinas, São Paulo, Brazil. Phone: +55-19-3521-7064. E-mail: clr@fcm.unicamp.br

Table 1
Serological monitoring of the *Toxoplasma* infection

Days after transplant	Chimerism	IgM-IIF (titer)	IgG-IIF (titer)	IgM-ELFA (index)	IgG-ELFA (IU/mL)	IgG avidity (index)	IgA-ELISA (POS/NEG)
0	autologous hematopoietic stem cell transplantation						
1211		NR	512	NR	216	0.55	NEG
0	non-myeloablative allogeneic stem cell transplantation						
28	complete	ND	ND	ND	ND	ND	ND
41		NR	256	NR	244	0.63	NEG
56	complete	ND	ND	ND	ND	ND	ND
122	complete	ND	ND	ND	ND	ND	ND
156		NR	64	NR	46	0.58	NEG
211	complete	ND	ND	ND	ND	ND	ND
390				fever, myalgia and headache			
398		NR	NR	2.70	30	0.37	POS
531		256	8 192	4.67	5 734	0.09	ND
551		256	16 384	3.93	6 682	0.09	ND
607		256	131 072	2.82	8 487	0.11	POS
622		128	131 072	2.54	7 985	0.11	POS
775		32	16 384	1.01	2 212	0.14	NEG
909	complete	NR	8 192	0.81	1 210	0.18	NEG
945		NR	8 192	0.67	1 078	0.19	ND
972		NR	4 096	NR	1 060	0.19	ND
1036		ND	ND	NR	1 052	0.29	NEG
1044		ND	ND	NR	1 041	0.30	ND
1063		ND	ND	NR	1 000	0.30	ND
2135		ND	ND	NR	451	0.32	ND

Significant antibody results: IgM-IIF ≥ 32; IgM-ELFA ≥ 0.65; IgG-ELFA ≥ 8 IU/mL; IgA-ELISA = POS (POS = positive; NEG = negative); an avidity index ≥ 0.3 is a strong indication of a primary infection dating back more than four months; an index < 0.3 does not allow a recent infection to be differentiated from an old infection. NR = non-reactive; ND = not determined.

to be classified as low (index < 0.2), borderline (0.2 < index < 0.3) or high (≥ 0.3) avidity. An avidity index ≥ 0.3 is a strong indication of a primary infection dating back more than four months. An index < 0.3 does not allow a recent infection to be differentiated from an old infection. In the ELISA for IgA, serum samples with a sample ratio (sample optical density/optical density for cut-off control serum) ≥ 1 were considered positive.

DISCUSSION

Toxoplasmosis is a rare but serious complication in allogeneic HSCT recipients[3,5,7,8,10]. Most cases of *Toxoplasma* infections occur in seropositive HSCT recipients, suggesting that toxoplasmosis usually results from the reactivation of latent tissue parasites[5,8]. In the present case, the seropositive recipient received hematopoietic stem cells from a seronegative donor. The primary response to *T. gondii* was supported by IgM and IgG seroconversion as shown by IIF, and IgA seroconversion as shown by ELISA. The IgG avidity test also detected low-avidity antibodies in the patient after infection with *T. gondii*; the low-avidity indices persisted for more than 14 months after the primary infection.

Most cases of toxoplasmosis after HSCT are only diagnosed at autopsy because histological evidence of organ involvement is rarely obtained before death[6]. The early detection of *T. gondii* DNA in body fluids by PCR methods has been considered an important tool for monitoring *Toxoplasma* infection following HSCT[5,6]. A recent study has shown the importance of monitoring toxoplasmosis after HSCT with biological tests that combine PCR and serological techniques[3]. This case stresses the importance of detecting anti-*T. gondii* antibodies in donors and recipients before transplantation, and of serologically monitoring the recipient during long-term follow-up.

RESUMO

Monitoramento sorológico de uma infecção toxoplásmica após transplante de células progenitoras hematopoiéticas

Esse relato de caso descreve uma resposta primária ao *Toxoplasma gondii* após transplante de células progenitoras hematopoiéticas em paciente com mieloma múltiplo. A resposta primária para o *T. gondii* foi evidenciada pela soroconversão observada na resposta de anticorpos IgM, IgG e IgA. O paciente foi prontamente tratado e complicações relacionadas à toxoplasmose não foram observadas nos meses subseqüentes.

REFERENCES

1. Derouin F, Pelloux H. Prevention of toxoplasmosis in transplant patients. Clin Microbiol Infect. 2008;14:1089-101.

2. Desmonts G, Couvreur J. Congenital toxoplasmosis. A prospective study of 378 pregnancies. N Engl J Med. 1974;290:1110-6.

3. Fricker-Hidalgo H, Bulabois CE, Brenier-Pinchart MP, Hamidfar R, Garban F, Brion JP, *et al*. Diagnosis of toxoplasmosis after allogeneic stem cell transplantation: results of DNA detection and serological techniques. Clin Infect Dis. 2009;48:e9-e15.

4. Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. Clin Infect Dis. 1992;15:211-22.

5. Martino R, Maertens J, Bretagne S, Rovira M, Deconinck E, Ullmann AJ, *et al*. Toxoplasmosis after hematopoietic stem cell transplantation. Clin Infect Dis. 2000;31:1188-95.

6. Martino R, Bretagne S, Einsele H, Maertens J, Ullmann AJ, Parody R, *et al*. Early detection of *Toxoplasma* infection by molecular monitoring of *Toxoplasma gondii* in peripheral blood samples after allogeneic stem cell transplantation. Clin Infect Dis. 2005;40:67-78.

7. Mele A, Paterson PJ, Prentice HG, Leoni P, Kibbler CC. Toxoplasmosis in bone marrow transplantation: a report of two cases and systematic review of the literature. Bone Marrow Transplant. 2002;29:691-8.

8. Roemer E, Blau IW, Basara N, Kiehl MG, Bischoff M, Günzelmann S, *et al*. Toxoplasmosis, a severe complication in allogeneic hematopoietic stem cell transplantation: successful treatment strategies during a 5-year single-center experience. Clin Infect Dis. 2001;32:e1-8.

9. Takahashi EEH, Rossi CL. IgM and IgA antibody responses in 12 cases of human acquired toxoplasmosis. Rev Inst Med Trop Sao Paulo. 1997;39:327-31.

10. Zver S, Černelč P, Mlakar U, Pretnar J. Cerebral toxoplasmosis - a late complication of allogeneic haematopoietic stem cell transplantation. Bone Marrow Transplant. 1999;24:1363-5.

Received: 6 May 2010
Accepted: 24 June 2010