



Disseminated toxoplasmosis following T cell-depleted related and unrelated bone marrow transplantation

TN Small¹, L Leung², J Stiles³, TE Kiehn³, SA Malak⁴, RJ O'Reilly¹ and K Sepkowitz⁴

Departments of ¹Pediatrics (Bone Marrow Transplant Service), ³Clinical Laboratories (Microbiology Service), and ²Medicine (Infectious Disease Service) Memorial Sloan-Kettering Cancer Center, New York City, NY; and ²Cornell University Medical School, New York City, NY, USA

Summary:

More than 95% of reported cases of disseminated toxoplasmosis following BMT have occurred following an unmodified transplant. Most have been fatal, diagnosed at autopsy and without antemortem institution of specific therapy. From 1989 to 1999, we identified 10 cases of disseminated toxoplasmosis, in 463 consecutive recipients of a T cell-depleted (TCD) BMT. Transplants were from an unrelated donor ($n = 5$), an HLA-matched sibling ($n = 4$) or an HLA-mismatched father ($n = 1$). In 40%, both the donor and recipient had positive IgG titers against *T. gondii* pre-transplant; in 30%, only the recipient was sero-positive. Three recipients of an unrelated TCD BMT developed toxoplasmosis despite both donor and host testing negative pretransplant. All 10 patients presented with high grade fever. CNS involvement ultimately occurred in seven patients, with refractory respiratory failure and hypotension developing in nine. Eight of 10 cases were found only at autopsy, involving the lungs ($n = 7$), heart ($n = 5$), GI tract ($n = 5$), brain ($n = 8$), liver and/or spleen ($n = 5$). The only survivor, treated on the day of presentation with fever and headache, was diagnosed by detection of *T. gondii* DNA by polymerase chain reaction (PCR) performed on the blood and spinal fluid. This study demonstrates the similar incidence of toxoplasmosis following TCD BMT and that reported post T cell-replete BMT, and underscores the need for rapid diagnostic tests in an effort to improve outcome. *Bone Marrow Transplantation* (2000) 25, 969–973.

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verent oral exposure to fecally contaminated hands or food, or transplacentally (reviewed in Ref. 7). Toxoplasmosis has been reported following solid organ transplantation^{8,9} and rarely from infused blood products (reviewed in Ref. 10). Whereas acute toxoplasmosis following solid organ transplantation usually represents a primary infection,^{8,9} most cases occurring post hematopoietic stem cell transplantation are due to reactivation of latent infection.^{1,2,4} Disseminated toxoplasmosis following BMT is usually diagnosed at autopsy, often without specific anti-microbial therapy being given prior to death. Of the 55 cases of toxoplasmosis in BMT recipients recently reviewed,¹ 96% of cases developed following T cell non-depleted bone marrow transplantation, generally in association with grade II–IV graft-versus-host disease (GVHD). Fifty-four of the 55 reported cases were fatal.¹

Strategies to reduce the risk of disseminated toxoplasmosis in solid organ transplant recipients and patients with AIDS have focused on identifying high risk groups. Following heart–lung transplantation, for example, highest rates of disseminated toxoplasmosis are seen in seronegative recipients transplanted from a seropositive donor.^{8,9} In patients with AIDS, those with a toxoplasma titer greater than 150 IU/ml, especially when associated with a CD4 cell count <100 cells/ μ l, appear to be at greatest risk.¹¹ These observations have led to the recommendation to use prophylaxis in these settings.^{11,12} In contrast, no such guidelines exist for BMT. In this report, we present 10 patients who developed disseminated toxoplasmosis following TCD bone marrow transplantation. Importantly, the only survivor was treated empirically on the day of presentation with fever and headache. Toxoplasmosis in this patient was diagnosed utilizing PCR analysis of blood and cerebrospinal fluid. Thus, early empiric treatment, pending results of a sensitive test, may help improve survival rates.^{13,14}

Materials and methods

Patient population

From 1 January 1989 to 1 January 1999, 463 patients at Memorial Sloan-Kettering Cancer Center received an allogeneic bone marrow transplant depleted of T cells by soybean agglutination followed by rosetting with sheep red cells as previously described.¹⁵ This results in a 2–3 log depletion of bone marrow T cells.¹⁵ Patients were trans-

Disseminated toxoplasmosis is an uncommon, but known complication following bone marrow transplantation (BMT), reported in 0.1 to 6% of allogeneic transplant recipients.^{1–6} Its causative agent, *Toxoplasma gondii*, is an obligate intracellular parasite, usually acquired by ingestion of infected, undercooked meat such as pork or lamb, inad-

planted for a hematologic malignancy ($n = 444$), solid tumor ($n = 1$) or aplastic anemia ($n = 18$) (Table 1). The majority of patients were cytoreduced with 1500 cGy of hyperfractionated total body irradiation in 12 fractions over 4 days, cyclophosphamide (60 mg/kg/day \times 2 days), combined with either thiotepa (5 mg/kg/day for 2 days, $n = 342$ (74%) or etoposide (250 mg/m²/day \times 3 days, $n = 71$ (15%). Forty-four (9.5%) of the 463 patients received hyperfractionated TBI and cyclophosphamide alone ($n = 18$), or combined with carboplatinum ($n = 14$) or diaziquone (AZQ) ($n = 19$), as previously described.¹⁶ Six patients (1.2%) received hyperfractionated TBI, thiotepa and etoposide. Of the 10 patients who developed disseminated toxoplasmosis, six were cytoreduced with hyperfractionated total body irradiation, thiotepa and cyclophosphamide; three with hyperfractionated TBI, etoposide and cyclophosphamide, as described above. The patient who developed disseminated toxoplasmosis following an SBA- E- TCD BMT was cytoreduced with busulfan, thiotepa, and melphalan. Ninety-six percent of the patients, including all 10 who developed toxoplasmosis, received a short course of anti-thymocyte globulin and solumedrol, either before or following the transplant for graft rejection prophylaxis.¹⁶ The only GVHD prophylaxis given to these patients consisted of *ex vivo* TCD. The medical records of these

patients, including all pathology reports were reviewed to determine the presence or absence of documented disseminated toxoplasmosis, defined as the presence of toxoplasma cysts or tachyzoites in more than one organ or in the blood.

IgG antibodies to *T. gondii* were initially measured by an enzyme immunoassay (EIA) method (Wampole Laboratories, Cranbury, NJ, USA or Abbott Laboratories, Abbott Park, IL, USA). An indirect-fluorescent antibody method (IFA) (Wampole Laboratories or GenBio Laboratories, San Diego, CA, USA) was utilized to test all EIA-IgG positive specimens, and used for all IgM antibody determinations. PCR tests were conducted at either the Toxoplasma Serology Laboratory (Palo Alto, CA, USA) or MRL Reference Laboratory (Cypress, CA, USA). PCR DNA analysis on blood and cerebral spinal fluid was performed by MRL Reference Laboratory using primers against the B1 gene of *T. gondii*.

Cell preparations

Heparinized blood was collected from patients within 2 weeks of presentation of high spiking fever. Peripheral blood mononuclear cells (PBMNC) were isolated by Ficoll-Hypaque density gradient separation.

Table 1 Clinical characteristics of patients who did or did not develop toxoplasmosis

	No. (%) of patients		OR (CI 95%)
	With toxoplasmosis (n = 10)	Without toxoplasmosis (n = 453)	
Mean age (years)	35.9 (12.1)	33.0 (14.8)	$P < 0.60$
Median age (years)	36.5	35.6	
Sex			
Female	3 (1.4)	206 (98.6)	1
Male	7 (2.8)	247 (97.2)	1.95 (0.44, 11.79)
Diagnosis			
Acute leukemia	4 (1.9)	205 (98.1)	NA
Chronic leukemia (CML, CLL, CMMOL)	3 (2.1)	142 (97.9)	
Non-Hodgkin's lymphoma	1 (2.9)	34 (97.1)	
Myelodysplastic syndrome or aplastic anemia	1 (1.8)	54 (98.2)	
Multiple myeloma	0 (0.0)	7 (100.0)	
Rhabdomyosarcoma	1 (0.0)	0 (0.0)	
Other	0 (0.0)	11 (100.0)	
Donor toxoplasma IgG status			
Negative	7 (2.4)	290 (98.0)	1
Positive	3 (3.6)	80 (96.4)	1.55 (0.25, 6.99)
Patient toxoplasma IgG status ^b			
Negative	3 (1.0)	307 (99.0)	1
Positive	7 (6.5)	85 (93.5)	8.43 (1.86, 51.21)
Patient and donor toxoplasma IgG status ^c			
Patient negative:donor negative	3 (1.3)	221 (98.7)	1
Patient negative:donor positive	0 (0.0)	44 (100.0)	0 (0, 12.45)
Patient positive:donor negative	4 (7.8)	47 (92.2)	6.27 (1.01, 43.79)
Patient positive:donor positive	3 (9.1)	30 (90.9)	7.37 (0.93, 56.78)
Donor type			
Matched-related	4 (1.3)	316 (98.8)	1
Mismatched-related	1 (7.1)	13 (92.9)	6.08 (0.11, 66.68)
Unrelated	5 (3.9)	124 (96.1)	3.19 (0.67, 16.28)

^a83 patients with unknown donor toxoplasma serology.

^b61 patients with unknown toxoplasma serology.

^c111 patients unknown.

Donor and patient toxoplasma serology were not included in the analysis.

Immunofluorescence analysis

Directly fluoresceinated (FITC) antibodies, including HLE-1 (CD45), (common leukocyte antigen, positive control), MsIgG, Leu-4 (CD3, pan T cell), Leu-M3 (CD14), Leu-M3 (CD13) and phycoerythrin-conjugated (PE) antibodies MsIgG, Leu-2 (CD8), Leu-3 (CD4), Leu-4 were purchased from Becton Dickinson (Mountain View, CA, USA).

Immunofluorescence was performed on peripheral blood lymphocytes or on whole blood as previously described.¹⁶ Immunofluorescence samples were analyzed on a FACScan (Becton Dickinson). The lymphoid populations to be analyzed were gated using log 90 degree and forward angle scatter characteristics. The leukocyte-specific monoclonal antibody HLE-1 (CD45) was used to gate out any residual red cells. Monocyte contamination was ruled out by lack of reactivity (<1%) of the gated lymphoid cells with the monocyte specific marker CD14.

Statistical methods

Associations between quantitative and categorical variables were assessed by *t*-tests. To assess associations between categorical variables (eg development of toxoplasmosis and sex, toxoplasma serology, and donor type) chi-square tests were performed. Data regarding donor and patient Toxoplasma serology were recorded as one variable with four possible categories (patient negative/donor negative, patient negative/donor positive, patient positive/donor negative and patient positive/donor positive).

To adjust for potential confounding effects on the association between variables and the outcome toxoplasmosis, multivariate logistic regression analysis was performed. All variables were entered into models.

Results

The proportion of toxoplasma seropositive patients and donors prior to transplant was ascertained to determine the prevalence of prior exposure in these populations. Titers against *T. gondii* were available for 87 and 82% of patients and donors, respectively. Of the 402 recipients for whom toxoplasma titers were available, 92 (23%) were IgG seropositive. Of 380 donors with available results, 90 (21.5%) had detectable IgG titers against toxoplasma antigen (Table 1). No patient or donor had positive IgM titers against *T. gondii* at the time of transplant.

Review of the 463 patient charts identified 10 patients who developed disseminated toxoplasmosis following an SBA- E- BMT (incidence 2.2%). This included eight cases of disseminated toxoplasmosis found at autopsy, one case of CNS toxoplasmosis found at autopsy which was preceded by pulmonary toxoplasmosis documented by immunohistochemistry 22 days prior to death and one patient with CNS toxoplasmosis diagnosed by positive PCR in the blood and spinal fluid, associated with a clinically compatible MRI of the brain.

The pre-transplant characteristics of the patients who did or did not develop toxoplasmosis following a related or unrelated SBA- E- BMT are shown in Table 1. While

patients with toxoplasmosis were almost twice as likely to be male than female (OR, 1.95; 95% CI, 0.44–11.79), this finding was not significant. The median and mean age of patients who did or did not develop toxoplasmosis were similar (Table 1). The median age of the 10 patients (three female, seven male) was 35.6 years (range 15–57). Four patients received transplants from an HLA-matched sibling, one from an HLA-mismatched father, three from a HLA-A,B,DR serologically matched unrelated donor, and two from a 1 HLA antigen mismatched unrelated donor. There were no significant differences in the median (range) of T cells/kg infused in the bone marrow graft in patients who did (median 0.35×10^5 (0.14 – 2.23×10^5)) or did not (median 0.37×10^5 (0.04 – 5.7×10^5)) develop toxoplasmosis post BMT. In univariate analysis, donor type was not a significant predictor of toxoplasma development. In four of 10 cases, both the donor and recipient had positive IgG titers against *T. gondii* pre-transplant; in three cases, only the recipient was sero-positive. Seven of 92 (7.6%) patients with pre-transplant seropositivity developed disease, compared to three of 310 (1%) known to be toxoplasma seronegative prior to transplant. The lowest incidence of toxoplasmosis occurred in patients with patient negative/donor positive toxoplasma serology, 0.0%, and patients with patient negative/donor negative serology, 1.3%. Incidence was higher among seropositive patients with seronegative donors (7.8%) as well as those with seropositive donors, 9.1%. Three unrelated bone marrow transplant recipients developed toxoplasmosis despite both recipient and donor testing negative pre-transplant. At time of presentation, four of the 10 patients were receiving solumedrol for the treatment of acute grade I–II GVHD. The median (\pm standard error) of CD4⁺ cells in these 10 patients was only 25 ± 27 cells/ μ l.

The clinical course of the 10 patients was similar (Table 2). Patients typically presented with high grade fever (>39°C) beginning a median of 78.5 (range 38–155) days post transplant. A well appearance despite the high fevers was noted in five patients. In addition to fever, four patients presented with signs of central nervous system dysfunction, two with cough, and one with diarrhea. Seven of the 10 patients ultimately developed signs of central nervous involvement, manifested by headache ($n = 3$), altered mental status ($n = 3$), and/or or seizures ($n = 3$). Retinal lesions were not observed in three of three patients in whom it was evaluated, including the survivor. Nine of 10 patients subsequently developed respiratory failure, requiring mechanical ventilation. Nine also developed profound hypotension necessitating pressor support.

Five of the 10 patients received treatment for toxoplasmosis. Treatment was initiated on the day of presentation in two patients, and late in the course of disease in three (days +6, +7, +20 after high presentation with high fever). Four patients were treated with pyremethamine and clindamycin; one with pyremethamine and sulfadiazine ($n = 1$). Death due to progressive respiratory failure and refractory hypotension occurred in nine of 10 patients a median (range) of 11 (7–17) days after their initial symptoms, including four of the five patients who received specific therapy. At autopsy ($n = 9$), histologic evidence of toxoplasmosis was found in the lungs ($n = 7$), heart ($n = 5$),

Table 2 Clinical characteristics of patients with documented toxoplasmosis

UPN	Age (years)/Sex	Race	Primary diagnosis	BMT date	Donor	Patient/Donor toxoplasma serology	GVHD Yes/No	Tx GVHD	Onset of fever (days)	Death (days)
1032	37/M	Caucasian	CML	30/8/90	MM-Rel	Pos/neg	No	None	36	58
1695	57/M	Caucasian	PNH, AA	24/8/95	UnRel	Neg/neg	Yes, Grade II	Steroids	38	55
2039	15/M	Hispanic	RMS	23/4/98	Sibling	Pos/pos	Yes, Grade II	Steroids	50	
1700	26/M	Hispanic	AML	22/9/95	UnRel	Neg/neg	Yes, Grade II	Steroids	62	78
1492	43/M	Caucasian	CML	13/1/94	UnRel	Neg/neg	Yes, Grade I	Steroids	78	87
2001	27/M	Hispanic	AML	23/12/97	Sibling	Pos/pos	No	None	79	88
2004	46/F	Caucasian	NHL	21/1/98	Sibling	Pos/pos	No	None	80	87
1568	34/M	Hispanic	Acute biphenotypic leukemia	25/8/94	Sibling	Pos/pos	No	None	106	122
873	21/F	Caucasian	ALL	18/1/89	UnRel	Pos/neg	No	None	116	127
925	42/F	Caucasian	CML	8/6/89	UnRel	Pos/neg	No	None	155	163

gastro-intestinal tract ($n = 5$), brain ($n = 8$), liver and/or spleen ($n = 5$). In one patient, CNS involvement could not be determined due to restrictions placed on the extent of autopsy.

The only survivor (UPN 2039) was treated on the day he developed fever to 39°C and headache. His head MRI revealed an extensive area of right frontal edema without discrete intra-axial enhancement, associated leptomeningeal and dural enhancement, and a ring-enhancing lesion within the left occipital lobe. His cerebro-spinal fluid (CSF) showed 7 red blood cells per high power field (HPF) and 30 WBCs /HPF of which 12% were neutrophils and 72% were lymphocytes. His CSF protein was elevated at 90 mg/dl (normal 21–38 mg/dl); glucose was normal at 60 mg/dl. Both his blood and spinal fluid were positive for *T. gondii* DNA by polymerase chain reaction, and negative for bacteria, fungi, acid-fast bacilli, CMV antigen, or viruses by culture. This 56 kg male received three daily doses of 125 mg of pyrimethamine, followed by 5 months of maintenance therapy (25 mg/day) combined with intravenous (900 mg i.v. every 8 h) or oral clindamycin (450 mg p.o. every 6 h). His fever and headache resolved within 4 days of treatment. His head MRI improved within 1 month. However, he died 6 months later due to recurrence of rhabdomyosarcoma.

To control for potential confounding, variables were entered into a multivariate logistic-regression model (Table 3). The model identified an independent association

between the development of toxoplasmosis and the following factors: having an unrelated donor (odds ratio (OR), 5.48; 95% CI, 1.61–55), having patient positive and donor negative IgG toxoplasma serology (OR, 7.80 95% CI, 1.45–41.83), and having patient positive and donor positive IgG toxoplasma serology (OR, 9.41 95% CI, 1.61–55.00).

Discussion

This series demonstrates that disseminated toxoplasmosis is not restricted to unmodified transplant recipients but can occur following TCD marrow transplantation, even in the absence of GVHD. As in patients with AIDS,¹¹ reactivation of toxoplasmosis post BMT was seen only in individuals who had extremely low CD4 counts (<200 cells/ μ l).

The 7.6% incidence of disseminated toxoplasmosis in this *T. gondii* seropositive group of TCD BMT recipients is remarkably similar to the 7.4% reported by Derouin *et al*² and higher than the 2% estimated by Slavin *et al*⁴ following unmodified BMT, despite differences in the prevalence of seropositivity in the three patient populations. Sixty-seven percent of patients in the French study² had detectable IgG toxoplasma titers pre-transplant, compared to 23% in this series and 15% in the Seattle series.⁴ It should be noted that disseminated toxoplasmosis occurred in three patients in whom both donor and host were seronegative prior to transplant, suggesting either blood product transmission,¹⁰

Table 3 Logistic regression model ($n = 349$)

Variable	B	s.e.	Odds ratio (CI 95%)
Age (1 year)	0.01	0.0229	1.01 (0.97, 1.06)
Male sex	0.94	0.7563	2.56 (0.58, 11.25)
Donor type			
Matched-related	0.00		1
Mismatched-related	1.90	1.2727	6.67 (0.55, 80.84)
Unrelated	1.70	0.7631	5.48 (1.23, 24.46)
Toxoplasmosis IgG status			
Patient negative:donor negative	0.00		1
Patient negative:donor positive	-5.53	24.0057	0.00 (0, ∞)
Patient positive:donor negative	2.05	0.8570	7.80 (1.45, 41.83)
Patient positive:donor positive	2.24	0.9009	9.41 (1.61, 55.00)

false negative antibody titers, or primary infection in the host following transplant due to exposure to toxoplasma infected food, water, or animals (reviewed in Ref. 7).

The incidence of toxoplasmosis is probably underestimated following hematologic stem cell transplantation in view of the difficulty in making the diagnosis in patients unable to reliably mount an IgM response or increase in IgG titers in response to an acute infection, coupled with the morbidity and mortality associated with obtaining biopsies in patients who are acutely ill and often thrombocytopenic. In addition, concerns about administering bone marrow suppressive drugs, such as pyremethamine and sulfadiazine, limits the use of empiric therapy, which in turn has likely increased the mortality associated with this opportunistic infection.

Several studies, primarily in solid organ transplant recipients, have shown the value of PCR analysis of spinal fluid in the diagnosis of isolated cerebral toxoplasmosis.^{13,14,17} In a limited number of BMT patients, successful treatment of isolated pulmonary or cerebral toxoplasmosis based on PCR analysis of bronchoalveolar lavage or CSF, respectively, has also been reported.^{13,14,17} However, in severely immunocompromised patients, particularly those with widely disseminated disease, waiting to start therapy until a positive PCR result is available, is unlikely to alter the course of this rapidly progressive infection. The fatal outcome of the majority of patients with disseminated toxoplasmosis following BMT warrants the empiric use of anti-toxoplasma drugs pending PCR results in patients with a compatible presentation, particularly in those with CD4 counts less than 200 cells/ μ l. Depending on the response to treatment and PCR results, therapy can be adjusted accordingly. Future studies testing this strategy as well as pre-emptive treatment based on positive surveillance *T. gondii* DNA PCR performed on the blood of patients at high risk are clearly needed.

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