MAJOR ARTICLE

Early Detection of *Toxoplasma* Infection by Molecular Monitoring of *Toxoplasma gondii* in Peripheral Blood Samples after Allogeneic Stem Cell Transplantation

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(See the editorial commentary by Chandrasekar on pages 79–81)

Background. Isolated case reports have shown that recipients of allogeneic hematopoietic stem cell transplants (HSCTs) who develop toxoplasmosis may have circulating *Toxoplasma gondii* DNA in peripheral blood before the onset of clinical symptoms.

Methods. We prospectively studied 106 *T. gondii*–seropositive adult recipients of HSCTs for the incidence of reactivation of toxoplasmosis in the first 6 months after transplantation. Toxoplasmosis infection (TI) was defined by a positive result of polymerase chain reaction (PCR) of peripheral blood specimens, whereas toxoplasmosis disease (TD) was defined as an invasive infection.

Results. The incidence of TI was 16% (95% confidence interval [CI], 8%–21%), whereas the incidence of TD was 6% (95% CI, 1%–10%). In the 16 patients with TI, the incidence of disease was 38%, whereas it was 0% in patients without TI ($P < .0001$). In most patients, the onset of TD or treatment for TI was preceded by an increase in the parasite load in peripheral blood samples, as determined by quantitative PCR.

Conclusions. Toxoplasmosis occurs more commonly after HSCT than has previously been suggested, and routine PCR testing of peripheral blood specimens may be an appropriate tool for guiding preemptive therapy in patients at very high risk of developing invasive disease.

Toxoplasmosis is an opportunistic infection caused by the parasite *Toxoplasma gondii*. Primary infection in immunocompetent hosts leads to the latency of the parasite as cysts in muscle and other organs [1]. Severe infections in immunodeficient patients appear to occur mainly through reactivation of latent cysts into the invasive tachyzoites. Despite being a very common severe infection in *T. gondii*–seropositive patients with advanced AIDS, toxoplasmosis was formerly considered a rare infection in recipients of hematopoietic stem cell transplants (HSCTs) [2–4]. However, recent single-center and multicenter retrospective studies have suggested that invasive disease may be more common than has previously been believed, with incidences among *T. gondii*–seropositive allogeneic transplant recipients of up to 4% and an estimated mortality rate of 60%–90% [5–11]. Most cases, however, are only diagnosed at autopsy, because histological evidence of invasive toxoplasmosis of the CNS or other sites is rarely obtained before death. Recently, several case reports and small case studies have suggested that the finding of *T. gondii* DNA in peripheral blood by PCR might help in the

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early diagnosis of at least some cases of invasive disease [6, 12–14]. Bretagne et al. [12] performed a pilot prospective study evaluating possible asymptomatic reactivation (or infection) among allogeneic HSCT recipients. Three of 24 *T. gondii*–seropositive recipients had positive PCR results, which became negative after trimethoprim-sulfamethoxazole (TMP-SMZ) was given for *Pneumocystis jiroveci* (formerly *carinii*) prophylaxis. Unfortunately, there are no other studies that analyze the incidence of, natural history of and risk factors for *Toxoplasma* infection and disease in *T. gondii*–seropositive patients after allogeneic HSCT.

Herein, we describe the results of a prospective study performed in 5 European transplantation centers with the primary aims of estimating the incidence of *Toxoplasma* infection and *Toxoplasma* disease, as well as determining the risk factors for both infection and disease in *T. gondii*–seropositive adults undergoing allogeneic HSCT.

PATIENTS AND METHODS

Patients. This prospective study was performed by the Infectious Disease Working Party of the European Group for Blood and Marrow Transplantation. Five European Group for Blood and Marrow Transplantation centers with experience in the diagnosis of toxoplasmosis by PCR methods participated in the study. Eligible patients were adults (age, >15 years) who received an allogeneic HSCT and were seropositive (i.e., IgG positive) for *T. gondii.* Patient enrollment occurred during the period of 1 November 2001 through 31 December 2002. All local ethics committees approved the study, and informed consent was obtained from subjects, as required by each institution's policy.

PCR analyses for* T. gondii *and quality controls. Each participating center used its own in-house PCR technique for obtaining results as soon as possible, which were based on previously published techniques [12–19]. To assure reproducibility and to rule out any false-positive results, 2 quality controls were performed before and at the end of the study [19]. Additionally, aliquots of frozen blood samples for each patient who had positive results of a PCR with a local technique were retested centrally by a reference technique. The reference PCR consisted in a real-time PCR test, performed as reported elsewhere, that has a sensitivity of 30 trophozoites/mL [13]. Moreover, this method gave quantitative data on the parasitic burden, which were analyzed in accordance with clinical outcome and therapies given.

Surveillance and anti-*Toxoplasma *therapy. A baseline pretransplantation sample was tested for all 106 patients, and the results were negative in all cases. After transplantation, patients were monitored at least once per week during the first 100 days; they were then monitored once every 2 weeks until day 180 after transplantation (i.e., day +180), the end of study follow-up. The duration of follow-up was chosen because of the observation that *Toxoplasma* disease usually begins early after transplantation, with 95% of cases occurring within the first 6 months after the procedure [6–8]. After engraftment, primary prophylaxis with TMP-SMZ was recommended for all patients when possible, but if intolerance or severe neutropenia occurred, any alternative prophylaxis decisions were made by each center. If a patient was found to have PCR results positive for *T. gondii* (i.e., *Toxoplasma* infection), preemptive therapy with pyrimethamine-sulphadoxine, pyrimethamine-clindamycin, or high-dose TMP-SMZ was given for ⩾7 days or until 2 consecutive negative results were attained. If the patient developed *Toxoplasma* disease, therapy was to be continued for 3 weeks. In both instances, secondary prophylaxis with TMP-SMZ or other active agents was recommended. All other supportive care measures were the standard in each institution.

Definitions. *Toxoplasma* infection and disease were defined using the European Group for Blood and Marrow Transplantation–Infectious Disease Working Party guidelines [6, 20]. In brief, *Toxoplasma* infection was defined as a positive result of a PCR of blood samples for a patient with no evidence of organ involvement, with or without fever. *Toxoplasma* disease was considered to be definite if histological evidence of active toxoplasmosis was found in a clinically and radiologically involved organ, whereas probable disease was defined by a positive result of PCR of a blood sample and/or another sample from the involved organ (usually CSF or bronchoalveolar lavage [BAL]) with clinical signs and symptoms and radiological evidence of active disease. To analyze whether more frequent patient sampling may have increased the incidence of *Toxoplasma* infection, the PCR sampling density was calculated, which was the number of days that peripheral blood samples were tested by PCR divided by the number of weeks that the patient remained in the study (i.e., the duration of follow-up).

Statistical analysis. Patients were followed-up for 6 months (to day +180) after HSCT, and data were censored before this date if the patient died of any cause or if there was relapse of the underlying disease. The incidence of *Toxoplasma* infection and disease were calculated using cumulative incidence estimates, taking into account the competing risks (non–relapse-related mortality and disease relapse), whereas the probability of overall survival was estimated using the Kaplan-Meier product-limit estimate with standard methods [21, 22]. Univariate analyses of risk factors for *Toxoplasma* infection or disease were performed using univariate Cox regression models, and the log rank test was used for overall survival. Multivariate analyses were done with Cox proportional hazards regression, with inclusion of variables with a P value of <.1 in the prior univariate testing. Because variables that predispose patients to the development of *Toxoplasma* infection (e.g., positive PCR results for peripheral blood specimens) may also increase the

Table 1. Patient characteristics and overall outcomes of transplantations.

Characteristic	Value
No. of patients	106
Age, median years (range)	46 (16–65)
Sex	
Male	64 (60)
Female	42 (40)
CMV seropositivity (IgG)[a]	72 (68)
Results of donor serological testing for *Toxoplasma* species (IgG)[a]	
Positive	55 (52)
Negative	47 (44)
Not done	4 (4)
Underlying disease	
Chronic myelogenous leukemia	10 (9)
Acute leukemia/myelodysplasia	56 (53)
Lymphoma	15 (14)
Multiple myeloma/CLL	17 (16)
Other	8 (8)
Disease phase at time of transplantation[b]	
Early	45 (43)
Intermediate	29 (27)
Advanced	22 (20)
Receipt of second HSCT	23 (22)
Donor type	
HLA-identical sibling	65 (61)
HLA-matched, unrelated donor	32 (30)
Mismatched related or unrelated donor	9 (9)
Stem cell source	
Peripheral blood	75 (71)
Bone marrow	28 (26)
Cord blood	3 (3)
Receipt of ATG	43 (41)
T cell depletion	8 (8)
Conditioning regimen	
TBI-based ablative	54 (51)
Chemotherapy-only ablative	8 (8)
Reduced-intensity	44 (41)
Development of acute GVHD	66
All grades	
Percentage of patients	66
Cumulative incidence (95% CI)	60 (45–66)
Grade II-IV	
Percentage of patients	54
Cumulative incidence (95% CI)	51 (41–61)
Grade III-IV	
Percentage of patients	24
Cumulative incidence (95% CI)	21 (13–29)
Developed chronic GVHD	
All cases	
Percentage of patients	24
Cumulative incidence (95% CI)	22 (14–30)
Extensive 6-month cGVHD	
Percentage of patients	4
Cumulative incidence (95% CI)	4 (0–8)
Six-month nonrelapse mortality rate	
Percentage of patients	20
Cumulative incidence (95% CI)	19 (10–26)
Six-month incidence of relapse	
Percentage of patients	21
Cumulative incidence (95% CI)	20 (12–30)
Six-month overall survival rate	
Percentage of patients	74
Cumulative incidence (95% CI)	70 (61–78)

NOTE. Data are no. (%) of patients, unless otherwise indicated. ATG, antithymocyte globulin; CLL, chronic lymphocytic leukemia; CMV, cytomegalovirus; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; TBI, total body irradiation.

[a] Determined by the presence of Ig6.

[b] Disease phase at transplantation was categorized as early (acute leukemia or poor-risk myelodysplasia in first complete remission, untreated good-risk myelodysplasia, first chronic-phase chronic myelogenous leukemia, or lymphoid malignancy in first remission), intermediate (acute leukemia or myelodysplasia in second or higher complete remission, accelerated-phase chronic myelogenous leukemia, or lymphoid malignancy in second or higher remission) and advanced (refractory or relapsed acute leukemia or myelodysplasia, blastic-phase chronic myelogenous leukemia, refractory or relapsed lymphoid malignancy, or any relapse after a previous autograft).

risk of disease, the development of infection was not introduced in the multivariate model for disease, despite being highly significant in univariate testing. For categorical variables, the χ^2 statistic or Fisher's exact test was used to establish differences in their distribution, and the Mann-Whitney U test was used to compare continuous variables. Tests of significance were 2-sided, with a significance level of $P \leqslant .05$.

RESULTS

Table 1 shows patient characteristics and the outcomes of transplantations in detail. During the 6-month post-HSCT follow-up period, 16 patients had $\geqslant$1 PCR of a peripheral blood specimen positive for *T. gondii* (7 patients had only 1 positive sample, 2 had 2 positive samples, 4 had 3 positive samples, 1 had 4 positive samples, 1 had 5 positive samples, and 1 had 6 positive samples); the results were confirmed retrospectively using the reference quantitative real-time PCR test. Six patients developed *Toxoplasma* disease (all probable cases); in 4 cases, a *T. gondii*–positive peripheral blood sample preceded the onset of disease, whereas in the other 2 cases, the first *T. gondii*–positive peripheral blood sample was obtained at the onset of disease (detailed below). The median days of diagnosis of *Toxoplasma* infection and disease were +42 (range, day +2 to day +178) and +57 (range, day +44 to day +91), respectively. The

Table 2. Results of univariate and multivariate analysis of *Toxoplasma* infection and disease.

Variable	No. of patients	Toxoplasma infection[a]					Toxoplasma disease[b]				
		No. of patients	Incidence, % (95% CI)	*P*		HR (95% CI)	No. of patients	Incidence, % (95% CI)	*P*		HR (95% CI)
				Univariate analysis	Multivariate analysis				Univariate analysis	Multivariate analysis	
Participating center (location)				.1	.4	…			.4	…	…
Center 2 (Barcelona)	29	8	27 (10–44)				1	4 (0.1–11)			
Center 3 (Tübingen, Germany)	30	3	11 (0.1–28)				2	7 (0.1–17)			
Center 1 (Créteil, France)	16	2	12 (0.1–22)				1	6 (0.1–18)			
Center 5 (Leuven, Belgium)	14	3	23 (0.1–46)				2	15 (1–35)			
Center 4 (Mainz, Germany)	17	0					0				
PCR density[c]				.04	.2	…			.3	…	…
<0.72	54	5	9 (1.5–17)				2	4 (0.1–11)			
⩾0.72	50	11	27 (12–41)				4	9 (0.5–17)			
Disease status				.003	.1	…			.04	.3	…
Early/intermediate	84	9	13 (4–19)				3	4 (0.1–8)			
Advanced	22	7	36 (15–58)				3	15 (0.1–31)			
No. of transplantations				.04	.5	…			NS	…	…
First HSCT	84	10	13 (5–21)				…	…			
Second HSCT	22	6	30 (10–51)				…	…			
Source of stem cells				<.0001	.01	15 (2.1–100)			<.001	.05	4.1 (1.8–72)
Cord blood	3	2	70 (2–98)				2	70 (2–98)			
Bone marrow/peripheral blood	103	14	15 (8–22)				4	4 (0.2–8)			
ATG in conditioning regimen				NS	…	…			.04	.08	…
Yes	43	…	…				5	12 (2–22)			
No	63	…	…				1	2 (0.1–5)			
Conditioning regimen				.07	.1	…			NS	…	…
Reduced-intensity	44	10	24 (11–38)				…	…	NS		
Standard myeloablative	62	6	11 (3–18)				…	…			
Donor *Toxoplasma gondii* serostatus				.1	.5	…			.05	.07	…
IgG positive	55	7	13 (7–18)				1	2 (0.1–6)			
IgG negative	47	9	21 (10–33)				5	11 (2–20)			

CMV serostatus				NS	…	…			.1	.3	…
Donor and recipient negative	12	…	…				2	14 (0.1–33)			
Donor and/or recipient positive	92	…	…				4	5 (0.2–9)			
Adequate prophylaxis with TMP-SMZ [d]				.01	.07	…			<.01	.08	…
Yes	48	2	4 (0.1–12)				0	…			
No	58	14	25 (11–39)				6	22 (11–33)			
Lymphocytopenia on day 30 after transplantation[e]				NS	…	…			.1	.2	…
Yes	52	…	…				5	10 (2–18)			
No	54	…	…				1	2 (0.1–6)			
Developed *Toxoplasma* infection, as determined by positive PCR results[f]				NA	…	…			<.0001	NA[g]	
Yes	16	…	…				6	38 (14–61)			
No	90	…	…				0				
Developed acute GVHD of grade II–IV[f]				NS	…	…			.1	…	…
Yes	54	…	…				5	10 (2–18)			
No	52	…	…				1	2 (0.1–6)			
Developed extensive chronic GVHD[f]				NS	…	…			.09	.2	…
Yes	4	…	…				1	22 (0.1–62)			
No	102	…	…				5	55 (0.8–10)			

NOTE. ATG, antithymocyte globulin; CMV, cytomegalovirus; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplant; NA, not available; NS, not significant; TMP-SMZ, trimethoprim-sulfamethoxazole.

[a] Details of variables with a univariate *P* value of >.2 are not shown and include sex, age, donor type (HLA-identical sibling vs. alternative donor types), presence of ATG in the conditioning regimen, CMV serostatus, CMV infection, acute and chronic GVHD, receipt of steroid therapy, and lymphocytopenia.

[b] Details of variables with a univariate *P* value of >.2 are not shown and include sex, age, transplant number, donor type (HLA-identical sibling vs. alternative donor types), type of conditioning regimen (reduced-intensity regimen vs. conventional myeloablative), CMV infection, and steroid therapy.

[c] No. of days that peripheral blood samples were tested by PCR/no. of weeks that the patient remained in the study.

[d] Adequate anti-*Toxoplasma* prophylaxis was defined as confirmed good compliance with TMP-SMZ therapy (800-mg tablets given 4–6 times per week) for >80% of the study period (i.e., >144 days).

[e] Lymphocytopenia was defined as a lymphocyte count of $<0.5 \times 10^9$ cells/L.

[f] These variables were analyzed as time-dependent covariates.

[g] See the Statistical analysis subsection of Patients and Methods.

Table 3. Characteristics of patients with *Toxoplasma* infection and disease.

Center/patient number	Age in years/sex	Underlying disease	Donor type (stem cell source)	Donor *Toxoplasma* IgG results	GVHD (grade)	No. of positive PCR results for PBs/ no. tested	Day after HSCT of first-last positive PB PCR result (day of prior negative result)
2/29	30/M	ALL	UD (CB)	Negative	Acute (2)	1/22	+59 (+49)
3/46	54/F	MM	UD (PBSCs)	Positive	Acute (2)	1/11	+91 (+86)
3/47	48/M	AA	HLA-identical sibling (PBSCs)	Negative	Acute (3), chronic	4/14	+41 (+34)
1/30	21/M	ALL	UD (CB)	Negative	Acute (2)	5/11	+38 to +49 (+31)
5/76	27/F	AML	HLA-identical sibling (PBSCs)	Negative	Acute (2)	6/28	+47 to +75 (+40)
5/77	55/M	NHL	HLA-identical sibling (PBSCs)	Negative	Acute (1)	3/21	+40 to +47 (+33)
2/12	48/F	AML	HLA-identical sibling (PBSCs)	Positive	Acute (1)	3/19	+34 to +94
2/16	62/M	AML	HLA-identical sibling (PBSCs)	Positive	Not present	1/19	+44
2/17	32/M	HD	HLA-identical sibling (PBSCs)	Negative	Acute (1)	1/19	+74
2/18	40/F	HD	HLA-identical sibling (PBSCs)	Positive	Not present	1/19	+39
2/25	55/M	CLL	HLA-identical sibling (PBSCs)	Positive	Acute (2), chronic	2/24	+178 to +185
2/27	29/M	MDS	HLA-identical sibling (PBSCs)	Negative	Not present	2/21	+83 to +93
2/28	51/F	MM	HLA-identical sibling (PBSCs)	Positive	Acute (1)	1/19	+15
1/50	21/M	AML	UD (PBSCs)	Positive	Acute (3)	1/13	+2
3/32	50/F	CLL	HLA-identical sibling (PBSCs)	Positive	Acute (3)	1/20	+15
5/78	27/M	ALL	UD (PBSCs)	Positive	Acute (3)	3/21	+59 to +66

NOTE. AA, aplastic anemia; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; ARDS, adult respiratory distress syndrome; BAL, bronchoalveolar lavage; CB, cord blood; CLL, chronic lymphocytic leukemia; CMV, cytomegalovirus; GVHD, acute graft-versus-host disease; HD, Hodgkin disease; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; PB, peripheral blood; PBSCs, peripheral blood stem cells; P/C, pyrimethamine/clindamycin; P/S, pyrimethamine-sulphadiazine; P/S/C, pyrimethamine-sulphadiazine plus clindamycin; TMP-SMZ, trimethoprim-sulfamethoxazole; UD, unrelated donor; +, day after transplantation.

[a] Toxoplasma disease diagnosed the first day that PCR results were positive for *T. gondii* in PB and/or in CSF fluid and BAL fluid specimens.

[b] CSF PCR result was negative on day +45.

[c] Administered from day +40 to day +75 and then from day +96 to day +120.

[d] Adequate anti-*Toxoplasma* prophylaxis was defined as confirmed good compliance with TMP-SMZ therapy (800-mg tablets given 4–6 times per week) for >80% of the study period (i.e., >144 days); intermittent prophylaxis was defined as receipt of TMP-SMZ on and off during the study period but not reaching 80% of the study period (144 days) in total; possible prophylaxis refers to therapy with a drug possibly but not definitely proven to be effective for prophylaxis of toxoplasmosis (e.g., clindamycin and atovaquone).

[e] Also present was a nonspecific brain lesion that was determined by MRI as nonsuggestive of toxoplasmosis.

incidence of and risk factors for *Toxoplasma* infection and disease are shown in table 2.

Incidence and risk factors for* Toxoplasma *infection. The overall 6-month incidence of *Toxoplasma* infection was 16% (95% CI, 8%–21%) (figure 1). Higher PCR density, non-early disease status, second HSCT, cord blood transplantation, and not having received appropriate prophylaxis with TMP-SMZ (tables 2 and 3) were found to have increased the incidence of infection in univariate analysis. In multivariate analysis, only cord blood transplantation retained statistical significance, whereas not having received appropriate prophylaxis with TMP-SMZ showed a nonsignificant trend.

Incidence and risk factors for* Toxoplasma *disease. The overall 6-month incidence of *Toxoplasma* disease was 6% (95%

Prior anti-*Toxoplasma* prophylaxis	Therapy for patients with positive PB PCR results (day of commencement)	Signs of *Toxoplasma* disease (non-PB PCR samples with positive results)	Day of onset of *Toxoplasma* disease	Therapy for *Toxoplasma* disease	Outcome and comments
Intermittent (TMP-SMZ)	P/S (+59)	Probable encephalitis (CSF)	+59[a]	P/S	Cured; alive and well at day +180; received secondary prophylaxis with TMP-SMZ
Possible (clindamycin)	TMP-SMZ (+91)	Probablepneumonitis, hyperacute onset (BAL)	+91[a]	TMP-SMZ	Died on day +93 of ARDS due to toxoplasmosis
Possible (atovaquone)	P/S/C (+55)	Probable encephalitis (CSF)	+55	P/S/C	Toxoplasmosis improved; patient later had progression of disease while receiving therapy and died of toxoplasmosis on day +315
Possible (atovaquone)	TMP-SMZ (+49)	Probable acute dissemuinated disease, hyperacute onset (CSF)	+45	TMP-SMZ	Died on day +51 of disseminated toxoplasmosis and CMV disease
None	P/C (+63)	Probable encephalitis (CSF)	+63	P/C	Cured of toxoplasmosis; died of AML on day +175; had received secondary prophylaxis with TMP-SMZ
None	P/C (+44)	Probable encephalitis (CSF)[b]	+44	P/C	Cured; alive and well on day +180; received secondary prophylaxis with TMP-SMZ
None	TMP-SMZ[c]	None/fever of unkown origin	…	…	Alive and well on day +180; discontinued TMP-SMZ therapy on day +75
Adequate (TMP-SMZ)[d]	P/S (+47)	None/fever of unkown origin	…	…	Alive and well on day +180; continued receiving TMP-SMZ
Adequate (TMP-SMZ)[d]	P/S (+77)	None	…	…	Alive and well on day +180; continued receiving TMP-SMZ
None	P/S (+42)	None	…	…	Alive and well on day +180; continued receiving TMP-SMZ
Intermittent (TMP-SMZ)	P/S (+187)	None/fever of unkown origin	…	…	Alive and well on day +180; continued receiving TMP-SMZ
Intermittent (TMP-SMZ)	P/S (+94)	None/fever of unkown origin	…	…	Alive and well on day +180; continued receiving TMP-SMZ
None	P/C (+18)	None/fever of unkown origin	…	…	Alive and well on day +180; continued receiving TMP-SMZ
None	Clindamycin (+2)	None	…	…	Died on day +71 of ARDS; no autopsy was performed
None	TMP-SMZ (+21)	None/fever of unkown origin[e]	…	…	Alive and well on day +180; continued receiving TMP-SMZ
None	P/C (+67)	None/fever of unkown origin	…	…	Alive and well on day +180; continued receiving TMP-SMZ

CI, 1%–10%). Nonearly disease status, cord blood transplantation, antithymocyte globulin (ATG) in the conditioning regimen, receipt of a transplant from a *T. gondii*–seronegative donor, not having received appropriate prophylaxis with TMP-SMZ, and *Toxoplasma* infection (i.e., positive results of PCR of peripheral blood samples) increased the incidence of disease in univariate analysis. Again, in multivariate analysis, only cord blood transplantation retained statistical significance, whereas the use of ATG, not having received appropriate prophylaxis with TMP-SMZ, and receipt of a transplant from a *T. gondii*–seronegative donor showed a nonsignificant trend. As discussed in the "Statistical analysis" section of Methods, *Toxoplasma* infection was not included in the multivariate model, but this variable was highly significantly associated with the development of disease in univariate analysis (figure 2).

Characteristics and outcome of* Toxoplasma *infection and disease. Table 3 shows in detail the characteristics of and outcomes for the 16 patients who developed *Toxoplasma* infection and disease. Only 2 patients were able to adhere to primary prophylaxis with TMP-SMZ, whereas the other 14 patients were not able to receive an appropriate prophylaxis (11 patients) or received drugs that have not been proven to prevent toxoplasmosis (atovaquone or clindamycin; 3 patients). However, all 16 patients with positive PCR results received anti-*Toxoplasma* therapy after the positive results of the PCR were known to the clinicians.

Among the 6 patients with *Toxoplasma* disease, 4 had apparently localized encephalitis, 1 had pulmonary disease with rapid dissemination, and 1 had acute disseminated disease. Two patients with disease had the first peripheral blood sample yield

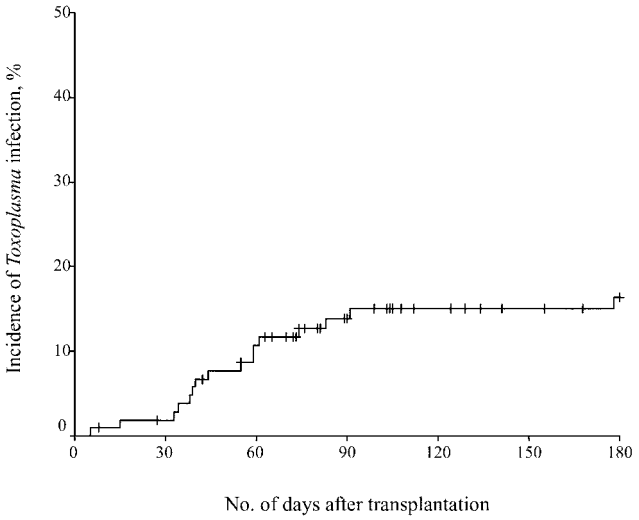

Figure 1. Incidence of *Toxoplasma* infection. The 6-month incidence of infection in all 106 patients was 16% (95% CI, 8%–21%).

positive PCR results on the same day that symptoms of disease began (patients 2/29 and 3/46 [denoted as health care center/patient number]), whereas in the other 4 patients, a positive PCR result preceded disease onset by 14, 7, 16, and 4 days (cases 3/47, 1/30, 5/76, and 5/77, respectively). Patients with disease were treated as shown in table 3. Three patients had complete resolution of toxoplasmosis, whereas 2 died after starting therapy (2 and 6 days later). Patient 2/47 showed an apparent complete response to therapy, but encephalitis progressed, and the patient died 8 months later while receiving secondary prophylaxis. Thus, 3 patients were considered to have died of toxoplasmosis disease, although a postmortem examination was not granted in any case.

Anti-*Toxoplasma* therapy was started a median of 4.5 days (range, 0–11 days) and 5 days (range, 0–16 days) after the first positive result of PCR of a peripheral blood sample in patients who developed only infection and disease, respectively ($P = .2$). However, if we exclude the 2 patients whose peripheral blood PCR results were positive the same day that disease started (2/29 and 3/46), the median times from the first positive PCR result to the commencement of therapy were 4.5 days (range, 0–11 days) and 12.5 days (range, 4–16 days), respectively ($P = .04$). Other possible differences between patients with infection and disease were as follows: receipt of a transplant from a *T. gondii*–seropositive donor (7 patients [70%] vs. 1 patient [17%]; $P = .1$), use of ATG in the conditioning regimen (2 [20%] vs. 5 [83%]; $P = .04$), receipt of a cord blood transplant (0 [0%] vs. 2 [33%]; $P = .1$), graft-versus-host disease of grade II–IV (3 [30%] vs. 5 [83%]; $P = .1$), lymphocytopenia on day +30 (3 [30%] vs. 5 [83%]; $P = .1$), and number of PCR-positive peripheral blood samples (median, 1 [range, 1–3] vs. 3.5 [range, 1–6]; $P = .08$). The small sample sizes precluded multivariate analysis. The estimated quantitative parasite load in peripheral blood samples for patients with disease and for those with infection alone did not differ (data not shown).

Of the 10 patients with *Toxoplasma* infection, 7 had fever of unknown origin, which resolved after receiving anti-*Toxoplasma* therapy. This observation suggests that some patients may have had a subclinical form of disease, but this cannot be determined with certainty, because fever of unknown origin is very common after receipt of an allogeneic HSCT.

Impact of* Toxoplasma *infection and disease on overall survival. The 6-month overall survival rate for the 10 patients who developed *Toxoplasma* infection (50%; range, 10%–90%) or the 6 who developed disease (68%; range, 58%–77%) was not significantly different than that for the other 90 patients (100%) ($P = .3$). In multivariate analysis, the variables that decreased the 6-month overall survival rate were cord blood transplantation ($P = .01$), increasing age ($P = .05$), and development of cytomegalovirus (CMV) disease ($P = .04$).

Quantification of parasite load. In 5 of the 6 patients with *Toxoplasma* disease, quantitative parasitic loads obtained by real-time PCR were analyzed to identify a possible quantitative relationship between increasing parasite load and the onset of disease. Details of the quantitative PCR are shown in table 4. Patient 3/46 was not evaluable, because aliquots from the samples positive in the local laboratory were not available for retesting. In the other 4 patients, there seems to have been a correlation between increasing parasite load and progression to disease and response to therapy. CSF samples tested positive

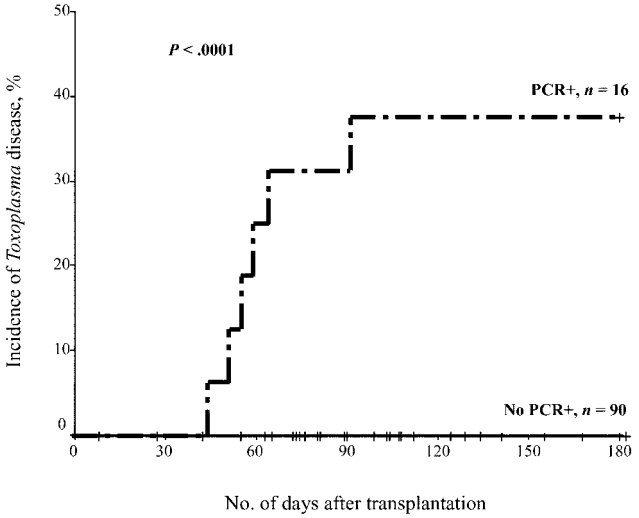

Figure 2. Incidence of *Toxoplasma* disease. The 6-month incidence of disease in all 106 patients was 6% (95% CI, 1%–10%; curve not shown). Among the 16 patients who had ⩾1 peripheral blood sample yield PCR results positive for *T. gondii* (PCR+), the incidence of disease was 38% (95% CI, 14%–61%), while it was 0% in patients with no positive PCR results (overlaps with the *x* axis).

Table 4. Quantitative PCR results for 5 of the 6 patients who developed *Toxoplasma* disease.

Center/patient number, sample	Parasite load in trophozoites/mL, by day after transplantation																					
	31	34	38	41	44	45	47	48	49	50	51	54	55	57	59	61	63	64	66	69	70	75	80
2/29																							
PB	…	…	…	0	…	…	…	…	0	…	…	…	…	…	3220[a,b]	…	0	…	…	…	0	…	…
CSF	…	…	…	…	…	…	…	…	…	…	…	…	…	…	7360[a,b]	…	…	…	…	…	0	…	…
3/47																							
PB	…	0	…	49	…	…	…	…	…	309	…	…	…	40	…	…	…	33	…	…	0	…	…
CSF	…	…	…	…	…	…	…	…	…	…	…	…	73[a,b]	…	…	…	…	…	…	…	…	…	…
1/30[c]																							
PB	0	…	182	984	…	6657[b]	…	1835	426,630[a]	…	…	…	…	…	…	…	…	…	…	…	…	…	…
CSF	…	…	…	…	…	…	…	74	2288	…	…	…	…	…	…	…	…	…	…	…	…	…	…
5/76																							
PB	…	…	…	0	…	…	286	…	…	…	…	428	…	…	…	4059	…	…	1781	1047	…	42	0
CSF	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	11,745[a,b]	…	…	…	…	…	…
5/77																							
PB	…	0	…	46	84[a,b]	…	5	…	…	…	0	…	…	…	0	…	…	…	0	…	…	…	…
CSF	…	…	…	…	…	0[d]	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…

NOTE. Patient 3/46 is not included because aliquots from the positive samples obtained on day 91 were not available for retesting by quantitative PCR. PB, peripheral blood.

[a] Day anti-*Toxoplasma* therapy was started.
[b] Day of onset of *Toxoplasma* disease.
[c] Patient died of taxoplasmosis on day 51.
[d] The CSF sample was obtained after the patient had started therapy.

in 3 of 4 patients with encephalitis, with no apparent correlation between the parasite load in the peripheral blood and that in the CSF. After therapy was started, all patients (except patient 1/30) experienced a decrease in parasite loads or had persistently negative results of real-time PCR during follow-up.

The median parasitic load in the 6 patients who did not develop disease and who had only 1 positive peripheral blood sample was 194 trophozoites/mL (range, 40–360 trophozoites/mL). In the 4 patients with >1 positive PCR result who did not develop disease, there was no systematic increase in the parasitic burden before starting therapy, but again the PCR results were persistently negative thereafter. In these 4 patients, the median parasite loads in the first and last positive samples were 180 trophozoites/mL (range, 46–1520 trophozoites/mL) and 135 trophozoites/mL (range, 68–1780 trophozoites/mL), respectively.

DISCUSSION

Diagnosis of devastating opportunistic infections in an early, nondisseminated stage in HSCT recipients has been a priority for clinical microbiologists and clinicians. The best example of an infection for which this goal has been achieved is CMV infection, for which preemptive treatment at an early stage of infection has drastically reduced the incidence of and mortality associated with disease [23]. More recently, EBV-related lymphoproliferative disease [24], adenovirus disease [25], and invasive aspergillosis [26, 27] have been actively investigated for a possible blood-based test that could be efficiently used for preemptive therapy, but there are few data on toxoplasmosis. PCR techniques have been studied for use in the noninvasive diagnosis of cerebral toxoplasmosis in patients with AIDS, with a reported sensitivity of 50%–65% and specificity of 95%–100% [16, 26, 28, 29].

PCR techniques for detection of the presence of *T. gondii* DNA in peripheral blood samples and other samples are not standardized, and there is no consensus on the optimal protocol to be used in clinical laboratories [17, 26, 28, 30]. With the difficulty of interpreting any PCR test with a nonstandardized technique in mind, we decided to homogenize our data with a quantitative real-time PCR technique, which excludes most of the false-positive PCR results associated with contamination with previously amplified products. In the quality controls, we observed that the PCR assays performed locally did not yield false-positive results. However, we cannot exclude false-negative results, and our results may underestimate the *Toxoplasma* infection rate, although the threshold of positivity observed in all centers in the 2 quality controls performed was 30 trophozoites per mL of blood.

Results from the current study indicate that the incidence of *Toxoplasma* infection in *T. gondii*–seropositive allograft recipients is 16% at 6 months after transplantation (95% CI, 8%–21%) (figure 1). Univariate testing indicated that risk factors for *Toxoplasma* infection in our study were mainly advanced disease status at the time of transplantation, insufficient prophylaxis with TMP-SMZ, and receipt of a cord blood transplant from an unrelated donor (see table 2). Of note, only data from 3 cord blood transplantations were included, so this observation should be interpreted with caution.

In our study, the incidence of *Toxoplasma* disease was 6% (range, 1%–10%), which is much higher than in prior retrospective studies. In univariate testing, prior or concomitant *Toxoplasma* infection was the most important risk factor for disease (table 2 and figure 2). Thus, our study indicates that monitoring for *T. gondii* DNA in peripheral blood specimens helps in identifying patients at high risk of developing an invasive disease. In 4 patients, the peripheral blood *T. gondii* DNA level increased before the onset of disease and the start of anti-*Toxoplasma* therapy, indicating that a quantitative PCR test is probably of greater use for making decisions and managing therapy. However, because of the small numbers of events (infection and disease), the kinetics of the PCR load cannot be studied in more detail. It should be emphasized that cases of probable or documented *Toxoplasma* disease with negative results of peripheral blood PCR have been described elsewhere [11, 14, 26]; thus, a negative result of peripheral blood PCR should not rule out the presence of disease in a seropositive HSCT recipient with a compatible clinical presentation. An interesting finding was that risk factors for *Toxoplasma* disease suggested that the absence of effective anti-*Toxoplasma* cellular immunity may predispose patients to develop severe infection (table 2). The trends observed for a higher risk of disease among patients with a seronegative donor, those who received ATG in the conditioning regimen, those with CMV seropositivity, those with lymphocytopenia early after HSCT, and those who underwent cord blood transplantation all point in this direction, because all of these characteristics are known to predispose to or are surrogate markers for a delay in the development of pathogen-specific cellular immune responses [31–36].

Of the 16 patients we describe, 10 did not have progression to disease when preemptive therapy was started, and of the 6 who developed disease, 3 eventually died of toxoplasmosis. Thus, this study suggests that PCR-based preemptive therapy may avoid death due to this infection in ~80% of patients who develop infection. However, the natural history of untreated *Toxoplasma* infection—and, thus, the risk of progression to disease—in these patients is not known with certainty, and the study was not designed to test whether PCR-based preemptive therapy can reduce the occurrence of *Toxoplasma* disease and improve the overall survival rate for patients with infection. A large number of seropositive recipients with *Toxoplasma* infection would have to be studied in a randomized fashion, similar to the preemptive PCR or antigenemia-based studies that set

the basis for the current recommended strategies for CMV infection [23].

We found that good compliance with TMP-SMZ prophylaxis had a possible protective effect on the development of *Toxoplasma* infection and disease (tables 2 and 3). However, compliance cannot be definitely proven, because patients did not maintain a diary documenting the exact doses of TMP-SMZ taken and their tolerance to the drug, and these data were obtained by reviewing the hospital or outpatient records and are thus only approximate data. Several drugs have been shown to be effective in preventing toxoplasmosis in other patient groups, especially patients with AIDS, but there have been fewer data for these drugs than for TMP-SMZ [37].

In conclusion, our study shows that a PCR-based method for monitoring recipients of an HSCT at risk of developing toxoplasmosis identifies a relatively high incidence of infection, and many patients who develop disease have had prior infections. Patients at high risk of developing disease are especially suitable for further research into the kinetics of *T. gondii* DNA load in peripheral blood samples, the post-HSCT kinetics of reconstitution of specific T cell immunity [38], and the impact of early preemptive therapy in abrogating the progression to disease.

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