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Molecular Diagnosis of Toxoplasmosis in Immunocompromised Patients: a 3-Year Multicenter Retrospective Study

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Toxoplasmosis is a life-threatening infection in immunocompromised patients (ICPs). The definitive diagnosis relies on parasite DNA detection, but little is known about the incidence and burden of disease in HIV-negative patients. A 3-year retrospective study was conducted in 15 reference laboratories from the network of the French National Reference Center for Toxoplasmosis, in order to record the frequency of *Toxoplasma gondii* DNA detection in ICPs and to review the molecular methods used for diagnosis and the prevention measures implemented in transplant patients. During the study period, of 31,640 PCRs performed on samples from ICPs, 610 were positive (323 patients). Blood ($n = 337$ samples), cerebrospinal fluid ($n = 101$ samples), and aqueous humor ($n = 100$ samples) were more frequently positive. Chemoprophylaxis schemes in transplant patients differed between centers. PCR follow-up of allogeneic hematopoietic stem cell transplant (allo-HSCT) patients was implemented in 8/15 centers. Data from 180 patients (13 centers) were further analyzed regarding clinical setting and outcome. Only 68/180 (38%) patients were HIV$^+$; the remaining 62% consisted of 72 HSCT, 14 solid organ transplant, and 26 miscellaneous immunodeficiency patients. Cerebral toxoplasmosis and disseminated toxoplasmosis were most frequently observed in HIV and transplant patients, respectively. Of 72 allo-HSCT patients with a positive PCR result, 23 were asymptomatic; all were diagnosed in centers performing systematic blood PCR follow-up, and they received specific treatment. Overall survival of allo-HSCT patients at 2 months was better in centers with PCR follow-up than in other centers ($P < 0.01$). This study provides updated data on the frequency of toxoplasmosis in HIV-negative ICPs and suggests that regular PCR follow-up of allo-HSCT patients could guide preemptive treatment and improve outcome.

Toxoplasmosis is a widespread parasitic infection that is frequently asymptomatic in immunocompetent patients. However, this obligate intracellular protozoan parasite can evade the immune system (1, 2) and persist for the life of its host in cyst form, predominantly in the brain, retina, and muscles. Reactivation of latent cysts may occur when the immune system fails to maintain cytokine pressure, which mainly relies on gamma interferon (IFN-γ) (3). Cyst reactivation can lead to ocular toxoplasmosis, cerebral toxoplasmosis (CT), or disseminated toxoplasmosis, which involves most frequently the lungs but potentially all organs. Failure of an efficient Th1 immune response mainly results from acquired immunosuppression, through HIV infection or immunosuppressive therapy. Both primary acquired and reactivated infections are life-threatening in immunocompromised patients (ICPs). Definitive diagnosis can be obtained by the detection of parasites in blood, cerebrospinal fluid (CSF), bronchoalveolar lavage (BAL) fluid, or virtually any tissue by using PCR, which is the most sensitive method (4).

Prevention of CT in patients with HIV is an object of consensus, and guidelines recommend co-trimoxazole (sulfamethoxazole-trimethoprim) chemoprophylaxis in *Toxoplasma*-seropositive patients when CD4$^+$ cell counts fall below 200 cells/μl (4), a prophylactic regimen which also protects patients from *Pneumocystis jirovecii* pneumonia. Nevertheless, toxoplasmosis remains

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the most prevalent cause of neurological opportunistic infection in Europe despite the use of highly active antiretroviral therapy (HAART) (5). Due to the mandatory reporting of AIDS cases in many countries, the evaluation of the toxoplasmosis burden is relatively straightforward. In France, the most recent data from the Institut de Veille Sanitaire (http://www.invs.sante.fr/en) reported that CT was associated with the inaugural AIDS stage in 12% of patients, and the annual number of cases was estimated to be about 160 in 2010 (6).

In contrast, the incidence of toxoplasmosis in solid organ transplant (SOT) patients or hematopoietic stem cell transplant (HSCT) patients is far less documented. It is assumed to parallel the seroprevalence in the general population, but it is not subjected to any reporting system, at least in France. Although occasional case reports have been published in the last 10 years (7–11), the incidence of toxoplasmosis in transplant patients is largely unknown. The risk for transplant patients differs according to the type of graft, and prevention measures may differ accordingly (reviewed in reference 12). Reactivation of a chronic infection may occur in a recipient irrespective of the type of graft and is closely related to the duration and degree of immunosuppression, with allogeneic HSCT (allo-HSCT) patients carrying the highest risk. In SOT patients, severe or disseminated toxoplasmosis can result from either reactivation of latent infection in the recipient or from organ-transmitted infection from a seropositive donor to a seronegative recipient (13), a situation for which heart transplant patients are at high risk (12). Prevention measures rely on serologic screening of donors and/or recipients and on chemoprophylaxis (14). In France, determination of the serologic status is mandatory for the donor and highly recommended for the recipient. However, there is no consensus about chemoprophylaxis, except in cases of mismatches in heart transplant patients. Additionally, some authors have advocated laboratory screening of allo-HSCT patients by using PCR on whole blood on a regular basis, with the aim of detecting early circulating parasites and starting preemptive therapy, but the benefit of this practice has not been evaluated (15–17).

In the present multicenter study involving 15 reference laboratories from academic hospitals, we investigated the molecular methods used for the diagnosis of toxoplasmosis in ICPs and the frequency of *Toxoplasma gondii* DNA detection in these patients. The outcome of the disease was examined in 180 patients, allowing us to draw information on the clinical picture and prevention practices.

MATERIALS AND METHODS

Data collection. A 3-year retrospective survey (2009 to 2011) was conducted through the French Network of the Centre National de Référence de la Toxoplasmose (http://cnrtoxoplasmose.chu-reims.fr/). Fifteen academic hospital centers (see the author affiliations) participated in the study and responded to an extensive questionnaire, including the following items: molecular method used for the diagnosis of toxoplasmosis, particular process according to the sample type, total number of samples tested for *Toxoplasma* in ICPs, number of positive samples and sample types over the study period, number of transplantations, type of transplant (heart, kidney, liver, HSCT), type of HSCT (allogeneic or autologous), duration and type of prophylaxis, implementation of iterative PCR follow-up after transplantation or allograft, and, if any follow-up was performed, its frequency and duration. More detailed data were obtained from 13 centers (180 patients) and included immune background, type and number of positive samples, clinical setting, prophylaxis, and clinical outcome at 2 months. Data relative to diagnosis were extracted from laboratory databases or information systems; data relative to patient management were obtained through local physicians and/or information systems.

Data collection relative to routine diagnosis was performed in agreement with the local ethical committee of each center. The coordinator analyzed only aggregated data; thus, no specific permission was required, in accordance with French rules.

Classification of cases. Patients were classified "CT" when the PCR was positive in the CSF or a blood sample with a compatible computerized tomography scan and/or neurological signs. Patients for whom parasites were detected in BAL fluid, bone marrow, or any biopsy specimen from a deep-seated organ (not considered a classical sanctuary for *Toxoplasma* cysts) were considered to have "disseminated toxoplasmosis," regardless of the result of a PCR on whole blood. Patients with a positive PCR in the blood only and who showed no specific imaging signs or symptoms except for fever were classified to have "isolated fever." Patients with a positive PCR in aqueous humor or blood and exhibiting eye lesions were classified as "ocular toxoplasmosis" cases, provided that only ocular signs were present. Patients with a positive PCR in blood but no symptoms/signs at the time of sampling were classified "asymptomatic." Survival was monitored for the 2 months following the first positive PCR result.

Statistical analysis. Qualitative variables were expressed using numbers and percentages, and quantitative variables were expressed as means and standard errors of the means (SEM). Statistical analysis was performed using GraphPad Prism software. A chi-square test or Fisher exact test was used to compare qualitative variables between groups. Quantitative variables were compared using the Mann-Whitney test. A P value of <0.05 was considered significant.

RESULTS

Proficiency of the participating laboratories and validation of the molecular methods used in this study. The reference laboratories in the participating academic hospital centers used a variety of methods for the molecular diagnosis of toxoplasmosis, as reported previously (18) (for details, see Table S1 in the supplemental material). Briefly, DNA extraction was performed using manual commercial methods (Qiagen or Roche Diagnostics), according to the manufacturer specifications, in 11/15 centers; two different manual in-house methods were used in 1/15 centers (19, 20), and an automated method was used in 3/15 centers (Qiagen or Roche Diagnostics). The PCR devices used for amplification and the PCR probes used for detection of amplicons (fluorescent resonance energy transfer or hydrolysis probes) varied between centers, but all centers used the same PCR target, i.e., the repetitive DNA sequence termed rep529 (GenBank accession number AF146527) (see Table S1). Internal controls and negative and positive controls were included in each PCR run. All assays had been previously validated by multicenter evaluations (21, 22) and are routinely evaluated through regular national external quality assessments (18).

The assays were the same as those used for the diagnosis of congenital toxoplasmosis in all centers, except for blood samples, for which a different extraction method was performed in 3 centers. Leukocyte fraction separation from 5 to 10 ml of whole blood was performed before blood DNA extraction in 5 centers (33%), whereas in the 10 remaining centers, DNA was extracted directly from whole blood.

Frequency of *Toxoplasma* DNA detection in immunocompromised patients. Over a 3-year period (2009 to 2011), 610 positive PCR tests were observed from various samples obtained from 323 ICPs in the 15 participating centers. Overall, the mean fre-

TABLE 1 PCR detection of *T. gondii* in 15 centers over the 3-year period (2009 to 2011)

Specimen type (no. of patients with positive PCR result)	No. of positive samples/total samples tested (%)	Mean ± SEM positive PCRs/center
Any specimen (323)	610/31,640 (1.9)	41 ± 33
Blood (152)	337/24,051 (1.4)	22 ± 23
CSF (93)	101/2,293 (4.4)	7 ± 5
BAL fluid (25)	30/3,914 (0.8)	2 ± 1
Aqueous humor (95 patients)	100/836 (12)	8 ± 8
Other specimen (30 patients)	42/546 (NA)[a]	3.5 ± 3
Mean no. of positive PCRs/patient	1.9 (NA)	NA

[a] NA, not applicable.

quency of a positive PCR result was about 3% of all samples analyzed per center (mean, 2.9% ± 1.8%). The most frequently positive sample source was blood (55% of positive samples), followed by CSF (17%), aqueous humor (16%), BAL fluid (5%), and then miscellaneous samples, including various tissue biopsy samples (7%) (Table 1). The frequency of *T. gondii* DNA detection in blood was not significantly different in centers that performed DNA extraction from whole blood versus centers that worked with the leukocyte fraction (data not shown).

Clinical picture and immune background for 180 patients with positive *Toxoplasma* PCR. Clinical data from 13 centers (180 patients) could be analyzed in more detail to determine the clinical significance of *Toxoplasma* DNA detection. Among these 180 patients, 68 (38%) were HIV$^+$ patients, 72 (40%) were HSCT patients, 14 (8%) were SOT patients (4 heart transplants, 7 kidney transplants, and 3 liver transplants), and 26 (14%) had various other causes for immunodeficiency, including 16 (62%) with hematological malignancy (5 with chronic lymphoid leukemia and 7 with lymphoma), 5 (19%) who had a connective tissue disease for which they had been treated with immunosuppressive drugs, 2 who had presented with an acute solid tumor (glioblastoma or breast cancer), 2 who had a congenital immunodeficiency (ataxia telangiectasia or IFN-γ receptor deficiency), and 1 who presented with chronic alcoholism and acute pancreatitis.

All HSCT patients were allograft recipients, except for one who was an auto-HSCT patient. The type of allograft was a matched-related bone marrow donor in 11 cases (15%), a matched-unrelated (national file) donor in 37 cases (51%), cord blood in 13 cases (18%), and undetermined in 7 cases. Overall, the incidence of a positive PCR result was higher in allo-HSCT patients (3.2%) than in other transplant patients (Table 2). PCR-positive (PCR$^+$) samples were associated with clinical symptoms in only 2.2% of allo-HSCT patients (Table 2). On the other hand, PCR results were positive in 23 allo-HSCT patients who were considered asymptomatic (Table 2). Of these, 6 were receiving co-trimoxazole chemoprophylaxis, and a curative therapy was started in view of PCR results in the remaining 17. All asymptomatic PCR$^+$ HSCT patients survived.

Symptomatic toxoplasmosis was acquired through the transplanted organ in 3 of the SOT patients (1 heart transplant, 1 kidney transplant, and 1 liver transplant patient), was due to *Toxoplasma* reactivation in 7, and was due to late primary infection (probable oral infection) in 4 SOT patients. Actually, 7/14 (50%) patients for whom a positive PCR result was observed were seronegative prior to transplantation; these 7 patients included 4 kidney, 2 liver, and 1 heart transplant patient. Parasite DNA was also detected in 3 asymptomatic SOT patients (2 heart transplant and 1 liver transplant) in one center that performed systematic blood PCR follow-up. Of these 3 SOT patients, 1 was treated, 1 was not (both of these patients survived), and 1 was lost to follow-up.

HIV patients mostly presented with cerebral toxoplasmosis (65% of cases) or ocular toxoplasmosis (22%), whereas HSCT and SOT patients were more likely to have disseminated toxoplasmosis (41% and 43%, respectively; $P < 0.01$) (Table 2).

The overall outcomes did not differ statistically between the groups of patients (Table 2). However, after excluding the 23 HSCT patients detected through systematic PCR in the absence of clinical signs, survival proved to be significantly lower in allograft patients than in HIV-infected patients (67% versus 78%; $P < 0.05$) (Table 2). Similarly, the outcome was worse in patients with a miscellaneous immunodeficiency background than for HIV patients after excluding ocular toxoplasmosis from this first category (survival rates of 53% and 78%, respectively; $P < 0.05$).

Heterogeneous prevention practices in transplant patients. Eight out of 13 allograft centers (61%) had implemented a systematic follow-up that included PCR on blood (Table 3). The frequency and duration of blood sampling after allo-HSCT were variable, ranging from twice a week to once a month, for 3 to 6 months or even lifelong in cases of graft-versus-host disease. Iterative follow-up of heart transplant patients via blood PCR has been suggested by some authors in cases with serologic mismatch (positive donor/negative recipient) (12); here, it was done in only 1 out of 10 centers. Chemoprophylaxis regimens also varied among centers. In fact, a prophylaxis regimen specifically targeting toxoplasmosis was rarely applied, except in heart transplant patients and in cases with a serologic mismatch for other SOT patients. Most allo-HSCT patients usually benefited from *Pneumocystis jirovecii* prophylaxis guidelines that included co-trimoxazole; these guidelines were applied in most centers. Overall, kidney transplant, heart transplant, and allo-HSCT patients were given co-trimoxazole in 14/14, 9/10, and 10/13 centers, respectively (Table 3). One additional center declared use of spiramycin in heart transplant patients, and another one used pyrimethamine-sulfadoxine in allo-HSCT patients. The chemoprophylaxis was prescribed irrespective of the recipient's serologic status, but the duration was longer or for life in heart transplant patients with serologic mismatch. Liver transplant patients usually were not given any chemoprophylaxis (7/12 centers) and auto-HSCT patients never were, except in one center. There seemed to be a consensus on the starting date for co-trimoxazole in HSCT patients, at about 20 to 30 days following allograft. Overall, a 6-month duration was the most frequently used scheme, whatever the type of graft (51% of all responses) (Table 3).

Impacts of PCR follow-up on the diagnosis and management of toxoplasmosis in HSCT patients. Data from the 72 PCR$^+$ allo-HSCT patients were analyzed, taking into account the implementation or not of systematic PCR follow-up, with the aim of searching for a benefit from such screening. In centers where a systematic PCR follow-up was implemented, the mean annual number of PCRs performed for HSCT patients was 16-fold higher than in other centers, and the mean number of PCRs per allo-HSCT patient was 10 ± 2.8 (Table 4). The prevalence of patients with *Tox-*

TABLE 2 Characteristics of 180 immunocompromised patients with a positive qPCR test for *T. gondii* (13 centers)

Basis of analysis	No. (%) with positive qPCR						
	HIV$^+$ patients ($n = 68$)	HSCT patients ($n = 72$)	SOT patients[a]			Other[b] ($n = 26$)	*P* value[i]
			Heart ($n = 4$)	Kidney ($n = 7$)	Liver ($n = 3$)		
Clinical diagnosis							<0.0001
Cerebral toxoplasmosis	44 (65)	12 (16.5)	0	4 (57)	0	6 (23)	<0.0001
Disseminated toxoplasmosis	8 (12)	30 (41.5)	2 (50)	2 (29)	2 (67)	9 (35)	0.007**
Ocular toxoplasmosis	15 (22)	2 (3)	0	0	0	9 (35)	<0.0001
Isolated fever	1 (1)	5 (7)	0	1 (14)	0	2 (7)	0.409 (NS)
Asymptomatic	0	23 (32)	2 (50)	0	1 (33)	0	<0.0001
No. of cases/no. of grafts (%)[c]	NA[h]	71/2,220 (3.2)[d]; 1/2,940 (0.03)[e]	4/282 (1.4)	7/3,180 (0.2)	3/1,896 (0.16)	NA	NA
No. of symptomatic cases/no. of grafts (%)	NA	48/2,220 (2.2)[d]; 1/2,940 (0.03)[e]	2/282 (0.7)	7/3,180 (0.2)	2/1,896 (0.11)	NA	NA
Chemoprophylaxis [no. (%)]							
Yes	4 (6)	14 (19)	1 (25)	0	0	0	NA
No	17 (25)	37 (51)	3 (75)	6 (86)	2 (67)	13 (50)	NA
Unknown	47 (69)	21 (29)	0	1 (14)	1 (33)	13 (50)	NA
Treatment [no. (%)]							0.0075**
Yes	64 (94)	54 (75)	2 (50)	7 (100)	2 (67)	18 (69)	
No	0	13 (18)	2 (50)	0	1 (33)	2 (8)	
Unknown	4 (6)	5 (7)	0	0	0	6 (23)	
Outcome [no. (%)]							0.626 (NS)
Survival at 2 mo	53 (78)	56 (78)[f]	4 (100)	4 (57)	2 (67)	18 (69)[g]	
Death	11 (16)	16 (22)	0	3 (43)	1 (33)	7 (27)	
Unknown	4 (6)	0	0	0	0	1 (4)	

[a] Recipients were seropositive for *Toxoplasma* prior to transplantation in 7/14 cases.
[b] Consisting of patients with hematological malignancies (16), connective tissue diseases receiving immunosuppressive drugs (5), solid tumors (2), congenital immunodeficiencies (2), or chronic alcoholism (1).
[c] Based on cumulative data from all centers during the study period.
[d] Data are for allo-HSCT patients.
[e] Data are for auto-HSCT patients.
[f] Survival was 67% when asymptomatic patients detected through systematic screening were excluded ($P < 0.05$, compared to HIV$^+$ patients).
[g] Survival was 53% when patients with ocular toxoplasmosis were excluded ($P < 0.05$, compared to HIV$^+$ patients).
[h] NA, not applicable.
[i] Variables were compared using chi-square or Fisher exact tests. **, $P < 0.01$; NS, not significant.

oplasma DNA detection was estimated by dividing the number of PCR$^+$ allo-HSCT patients by the total number of allograft patients in a given center during the study period. The percentage of PCR$^+$ allograft patients was about 3-fold higher in centers performing systematic PCR screening than in nonscreening centers (4.9% ± 1.6% compared to 1.7% ± 0.6%, respectively) (Table 4). All PCR$^+$ asymptomatic patients were detected in centers that performed systematic PCR follow-up. The overall survival of allograft patients was better in centers with regular PCR screening than in other centers (86% versus 50% survival, respectively; $P < 0.01$). When asymptomatic patients, who all survived, were excluded from the analysis, there remained a similar trend, with a better outcome for patients who benefited from PCR screening than the outcome for other patients (76% versus 50% survival) (Table 4).

DISCUSSION

This study provides a global insight into the molecular diagnosis of toxoplasmosis in ICPs and shows for the first time that the parasite is detected with a higher frequency in non-HIV ICPs than in HIV-infected ICPs (62% and 38% of cases, respectively). As for *Pneumocystis jirovecii* pneumonia (23), the present data show that toxoplasmosis is of increasing importance in non-HIV ICPs, who represent a growing at-risk population due to the wide use of immunosuppressive therapies and the increasing number of transplant patients. In HIV-infected patients, prevention measures are well codified, and the prevalence of the disease has remained stable since the start of the use of HAART (6). In contrast, the prevention of toxoplasmosis in non-HIV ICPs is not standardized, even though it often benefits from *Pneumocystis* pneumonia prevention in transplant patients, as shown here for heart and kidney transplant patients. However, we found that chemoprophylaxis regimens and durations vary greatly among centers, underlining the need for consensus-based guidelines according to the type of graft. Of great interest was the observation that 26 of 112 non-HIV patients (23%) were neither SOT nor HSCT patients but had miscellaneous immunodeficiency backgrounds, including a high proportion of hematological malignancies. This observation stresses the need for evaluating the risk factors for toxoplasmosis in more detail, which could lead to consideration of chemoprophylaxis in targeted patient populations. Indeed, in our

TABLE 3 Prevention of toxoplasmosis and PCR follow-up practices for transplantation patients (15 centers)

Type of transplantation center, PCR follow-up, or prevention practice	Result for transplantation patient group
Type of transplant [total (mean ± SEM) no. of patients]	
Solid organ transplantation (2009–2011)	
Kidney (14 centers)	3,711 (265 ± 97)
Heart (10 centers)	549 (50 ± 32)
Liver (12 centers)	2,262 (174 ± 86)
Hematopoietic stem cell transplantation (2009–2011)	
Allograft (13 centers)	2,463 (189 ± 79)
Autograft (14 centers)	3,318 (237 ± 101)
PCR follow-up [no. of centers with characteristic/total no. of centers (%)]	
Systematic PCR follow-up	
Allograft patients	8/13 (61)
Heart transplant patients	1/10 (10)
Frequency of blood PCR follow-up in allo-HSCT patients	
2×/wk	1/8 (12.5)
1×/wk	4/8 (50)
2×/mo	2/8 (25)
1×/mo	1/8 (12.5)
Chemoprophylaxis [no. of centers using it/total no. of centers (%)]	
Kidney transplant[a]	14/14 (100)
Heart transplant[b]	10/10 (100)
Liver transplant[c]	5/12 (42)
Allograft transplant[d]	11/13 (85)
Autograft transplant[e]	1/14 (7)

[a] Duration of chemoprophylaxis was variable across centers: 4 to 6 weeks (2 centers), 3 months (4 centers), 6 months (7 centers), unknown (1 center).
[b] Duration of chemoprophylaxis was variable across centers: 4 to 6 weeks (1 center), 3 months (1 center), 6 months (6 centers), 1 year (1 center), lifelong if mismatch (1 center).
[c] Duration of chemoprophylaxis was variable across centers: 6 months (3 centers), variable (1 center), lifelong (1 center).
[d] Duration of chemoprophylaxis was variable across centers: 6 months (4 centers), guided by $CD4^+$ T cell count (4 centers), 1 year (3 centers).
[e] Duration of 3 months.

study, 7/26 patients died, of whom 6 showed disseminated toxoplasmosis after a delayed diagnosis.

Toxoplasma gondii was detected in 14 SOT patients after a highly variable period following transplantation; 7 of them were not infected prior to transplantation. In two cases (1 kidney transplant and 1 heart transplant), *Toxoplasma* seroconversion (and parasite DNA detection) occurred several years after transplantation, making organ-transmitted infection unlikely. Toxoplasmosis occurred in 3 kidney transplant patients who benefited from chemoprophylaxis for 3 or 6 months, but the delay from transplantation to parasite DNA detection was not recorded, making it impossible to verify whether toxoplasmosis occurred after stopping chemoprophylaxis. The two remaining cases occurred in liver transplant patients from centers who did not use chemoprophylaxis. Overall, the mortality rate observed here in SOT patients (29%) was 2-fold higher than that observed in a Spanish multicenter study (24), despite a similar incidence of symptomatic toxoplasmosis in these patients (0.14% in the study by Fernandez-Sabé et al., versus 0.2% in our study). We also confirmed that heart transplant patients are more at risk of donor-related toxoplasmosis than other SOT patients, which has been recognized through multiple case reports and cohort studies (reviewed in reference 25). More rarely, *Toxoplasma* seroconversion has been described in SOT patients in the absence of clinical signs (26, 27), even in patients who did not receive prophylaxis (26). Here, we observed a positive blood PCR result in two heart transplant patients who were asymptomatic. Both were *Toxoplasma* seropositive prior to transplantation. In the first patient, PCR was positive 6 weeks after transplantation, while the patient received chemoprophylaxis; thus, co-trimoxazole likely prevented full-blown toxoplasmosis. In the second case, blood PCR was positive twice, 22 years after transplantation; the patient was not under chemoprophylaxis anymore and the episode resolved spontaneously. These data clearly show that low circulating parasite levels can be detected in transplant patients, without any clinical impact, provided that they are given chemoprophylaxis or that they are only mildly immunocompromised.

Not surprisingly, allo-HSCT patients accounted for the major proportion of toxoplasmosis cases in non-HIV patients (64%); most of them, as already described (28), were patients engrafted with a matched-unrelated donor. The frequency of toxoplasmosis in these patients (2.2%) was similar to estimates from areas of high seroprevalence (29), such as France. The overall mortality in allo-HSCT symptomatic patients (67%) (Table 2) was lower here than in the study by Schmidt et al. (30), who included only cases of disseminated toxoplasmosis and found a mortality rate of 95%, and it was higher than in HIV patients, as already observed in the same study (30). Routine PCR testing on blood has been proposed to monitor these patients in the months or even years following allo-HSCT, to allow early treatment and to improve survival (15, 16, 31). We therefore analyzed separately the data from HSCT patients, taking into account whether or not such a policy had been implemented in the participating center. This analysis revealed that survival was indeed better in centers where PCR follow-up was implemented (86% versus 50%; $P < 0.01$).

The potential drawback of such a systematic screening strategy is the possible detection of circulating parasite DNA in asymptomatic patients (17), which raises the question of the necessity to start treatment or not. In the present study, 48% of allo-HSCT PCR^+ patients in centers applying a systematic PCR follow-up policy were asymptomatic. This percentage was lower than that observed in two previous studies, which reported that 10/16 (62%) (16) and 9/13 (69%) (15) patients had a positive PCR result without clinical signs; yet, our result was similar to that reported by Meers et al. (8/18; 44%) (32). Overall, the incidence of PCR^+ asymptomatic allo-HSCT patients detected through systematic PCR follow-up was also lower in our study (23/1,220; 1.9%) than in other studies. Indeed, Martino et al. reported 10 asymptomatic PCR^+ patients out of 106 allo-HSCT patients (16), Fricker-Hidalgo et al. found 9 out of 70 (12.8%) of such allo-HSCT patients (15), and Edvinsson et al. observed 1 out of 12 (8%) of such allo-HSCT patients (17). Divergent attitudes were observed among the French centers regarding the management of these asymptomatic patients: 74% of the patients received a curative therapy, whereas the remaining 26% were left under co-trimoxazole chemoprophylaxis. No patient was left without any specific treatment, making it difficult to have a clear view of the clinical significance of circulating DNA: is it an early sign of toxoplasmosis reactivation or a

TABLE 4 *Toxoplasma* DNA detection in allo-HSCT patients according to PCR follow-up policy[a]

Characteristic	Result for centers with:		*P* value[c]
	PCR follow-up (6 centers)	No PCR follow-up (5 centers)	
Annual no. of allografts	410	330	
Annual no. of PCRs (mean ± SEM)	796 ± 624	48 ± 26	0.0095**
No. of PCRs/no. of allo-HSCT patients (mean ± SEM)	10 ± 2.8	0.6 ± 0.2	0.0043**
No. of patients with PCR^+ test	56	16	NA
No. of PCR^+ patients/no. engrafted (%, mean ± SEM)	4.9 ± 1.6	1.7 ± 0.6	0.177 (NS)
Asymptomatic patients with PCR^+ test			
Total no. of patients	23	0	0.0015**
No. detected/no. engrafted (%, mean ± SEM)	2.1 ± 0.8	NA	
Type of positive sample	Blood	NA	
No. of PCR^+ results/patient (mean ± SEM)	1.7 ± 0.6	NA	
No. of patients under chemoprophylaxis	8	NA	
No. (%) treated	17[b] (74)	NA	
No. (%) that survived	23 (100)	NA	
Symptomatic PCR^+ patients			
Total no. PCR+ patients	33	16	
No. detected/no. engrafted (%, mean ± SEM)	2.8 ± 0.9	1.7 ± 0.6	0.329 (NS)
No. (%) with:			
Disseminated toxoplasmosis	21 (64)	9 (56)	0.756 (NS)
Cerebral toxoplasmosis	8 (24)	4 (25)	1 (NS)
Isolated fever	3 (9)	2 (13)	0.672 (NS)
Ocular toxoplasmosis	1 (3)	1 (6)	1 (NS)
No. (%) that survived	25 (76)	8 (50)	0.106 (NS)
Overall survival (%) of the 72 patients	48 (86)	8 (50)	0.005**

[a] In total, this analysis included data for 72 patients from 11 centers whose policies did or did not include PCR follow-up. NA, not applicable.
[b] The six untreated patients received chemoprophylaxis.
[c] **, $P < 0.01$; NS, not significant.

negligible event? In fact, for most patients, circulating *Toxoplasma* DNA was detected during the first 6 months following engraftment, and thus may correspond to the early detection of *Toxoplasma* reactivation, which usually occurs during this time frame (16, 32). Thus, it can be hypothesized that reactivation does not evolve toward full-blown disease either because the patients receive chemoprophylaxis or because preemptive treatment is started early. The significance of circulating *Toxoplasma* DNA was more debatable for 3 patients who tested positive 18 months (1 patient) and 8 years (2 patients) after allograft. A technical false-positive result can be ruled out in all instances, since these positive PCR tests were observed in centers who routinely use PCR decontamination measures as well as negative controls. To circumvent the question of the significance of positive PCR results in asymptomatic patients, we excluded them for the survival analysis. Interestingly, there remained a trend toward higher survival in symptomatic patients from centers with PCR follow-up compared to centers with no PCR follow-up (76% versus 50%). This suggests again that symptomatic patients could have been detected at an early stage through regular PCR screening, thereby allowing preemptive therapy.

There are obviously limitations in this study; the main one relies stems from the fact that this was a retrospective study. Accurate data about allo-HSCT patient immune backgrounds were not recorded, and various confounding factors might have interfered with the survival comparison. The date of transplantation was not always recorded, and thus the delay of onset of toxoplasmosis could not always be determined. This is an important issue, which would be interesting to address in order to evaluate whether the duration of chemoprophylaxis should be extended. Moreover, chemoprophylaxis data were inconstantly collected through medical charts, making difficult any inference about the compliance to, or efficacy of, chemoprophylaxis and its effects on PCR positivity. Finally, some biases related to biological diagnosis should also be considered: (i) the sensitivity of PCR on CSF was previously estimated to be only about 50% or lower (33–35), and therefore the number of cases with mild cerebral toxoplasmosis may have been underestimated, although the previously published data were obtained with less sensitive PCR methods than now used; (ii) cases of ocular toxoplasmosis may also have been underestimated, since aqueous humor is not always collected for analysis when ocular lesions are typical and the sensitivity of laboratory diagnosis does not exceed 80% (36, 37).

In conclusion, this study points to the need for standardization of prevention policies in transplant patients and for identification of new groups of at-risk patients who may benefit from chemoprophylaxis, such as patients with hematological malignancies, as recently underlined (38). Allo-HSCT clearly appears to be the main risk factor, and the better outcome of these patients in centers who have implemented a regular biological follow-up using blood PCR introduces an interesting perspective. This should be confirmed in a large prospective multicenter study that takes into account the severity of the immune background of the patients and their comorbidities.

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