Biol Blood Marrow Transplant 25 (2019) 2510–2513

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Toxoplasmosis as an Early Complication of Allogeneic Hematopoietic Cell Transplantation

Christine Robin[1,2,*], Mathieu Leclerc[1,2], Cécile Angebault[2,3], Rabah Redjoul[1], Florence Beckerich[1], Ludovic Cabanne[1], Francoise Foulet[3], Cécile Pautas[1], Andréa Toma[1], Sébastien Maury[1,2], Francoise Botterel[2,3,4], Catherine Cordonnier[1,2]

[1] *Assistance Publique-Hôpitaux de Paris, Henri Mondor Hospital, Department of Hematology and Cellular Therapy, Créteil, France*
[2] *University Paris-Est Créteil (UPEC), Créteil, France*
[3] *Parasitology Unit, Microbiology Department, Henri Mondor Hospital, Créteil, France*
[4] *EA Dynamyc UPEC, Ecole Nationale Vétérinaire d'Alfort (ENVA), Faculté de Médecine de Créteil, Créteil, France*

Article history:
Received 16 May 2019
Accepted 26 July 2019

Key Words:
Toxoplasma gondii
Toxoplasmosis
Allogeneic hematopoietic cell transplantation
Trimethoprim-sulfamethoxazole
PCR

A B S T R A C T

Among 419 consecutive allogeneic hematopoietic cell transplant recipients, we observed 17 (4.0%) cases of toxoplasmosis at a median time of day 45 (range, 6 to 322) after transplant. Seven of these 17 cases occurred before day 30 after transplant. Because of the lack of PCR screening and trimethoprim-sulfamethoxazole prophylaxis before engraftment, the diagnosis of toxoplasmosis was late, and 5 of these 7 patients died. Analyzing these cases, early *Toxoplasma* blood PCR screening, starting from transplant, is crucial.

INTRODUCTION

Toxoplasmosis is a rare but well-known complication that can occur after allogeneic hematopoietic cell transplantation (HCT). It almost exclusively occurs through the reactivation of previously seropositive recipients (R^+), especially when the donor is seronegative or the patient has been transplanted with cord blood [1,2]. The incidence varies widely according to several parameters: (a) the seroprevalence of toxoplasmosis in the country (low in North America, over 50% in France) [3], (b) the administration and compliance to trimethoprim-sulfamethoxazole (TMP-SMX) [1], and (c) whether a regular screening with blood real-time PCR quantitative real-time PCR (qPCR) is performed or not. In centers that do not screen their R^+ patients using a PCR analysis after transplant, the incidence rate is reported to be around 0.2% to 2% [4,5], and the diagnosis is often done at autopsy [2]. In centers screening their R^+ patients, the incidence is much higher, between 6% and 16% [1,4], but with more cases of infection than cases of disease. Indeed, a weekly blood qPCR screening of R^+ patients allows for the detection of the circulating DNA of the parasite before the appearance of symptoms. Despite the lack of a randomized study, qPCR *Toxoplasma* screening is recommended by international HCT guidelines for the early diagnosis of *Toxoplasma* reactivation—especially when TMP-SMX is not given—to anticipate *Toxoplasma* [6].

Most cases of *Toxoplasma* are observed between 2 and 6 months after transplant, and even in the larger studies, early cases occurring before day 30 are very rare [1,5,7]. Because of recent, severe, and early cases of toxoplasmosis in our department, we reviewed all our *Toxoplasma* cases over the past 11 years, focusing on cases observed before day 30 after HCT. The aim of the study was to identify the causes leading to this unexpected finding of early toxoplasmosis and reconsider the pertinence of our procedures accordingly.

METHODS

From the laboratory data, we retrospectively collected all cases of *Toxoplasma* infection or disease in allogeneic HCT recipients in our department between January 2007 and June 2018, identifying all patients with a positive qPCR on any body fluid and/or a microscopic examination showing tachyzoites. All patients and donors were assessed for *Toxoplasma* serology before transplant. *Toxoplasma* infection and disease were defined according to the European Society for Blood and Marrow Transplantation's guidelines [8]. Briefly, *Toxoplasma* infection was defined as a positive qPCR with or without fever but with no organ involvement. *Toxoplasma* disease was defined as *Toxoplasma* infection plus clinical, radiologic, or pathologic evidence of organ involvement by *Toxoplasma gondii*. Proven *Toxoplasma* disease was defined as the histologic or cytologic demonstration of tachyzoites in tissue samples obtained either by biopsy or bronchoalveolar lavage (BAL) or at autopsy. Patients who had clinical and radiologic evidence of *Toxoplasma* disease plus

Financial disclosure: See Acknowledgments on page 4.
* Correspondence and reprint requests: Christine Robin, MD, PhD, Hematology Department, Henri Mondor Hospital, 94000–Créteil, France.
E-mail address: christine.robin@aphp.fr (C. Robin).
https://doi.org/10.1016/j.bbmt.2019.07.035
1083-8791/

≥1 positive PCR test from blood, cerebrospinal fluid (CSF), or BAL but who had no histologic confirmation were classified as having probable disease. Disseminated toxoplasmosis was defined as clinical, radiologic, or histologic evidence of disease in >1 organ. The cases were considered early before day 30 after transplant.

All R^+ patients were screened, irrespectively of prophylaxis, by qPCR in blood, targeting a 529-bp repeat-DNA fragment (AF146527) per previously reported methods [9] and with the same qPCR technique over the study period. According to our local procedures from 2000, this screening was performed weekly from engraftment to day 100, then every 1 to 2 weeks from 3 to 6 months, then at each consultation, according to the outpatient's visits, until the patient was withdrawn from any immunosuppressant.

A positive blood qPCR triggered a diagnostic workup, including fundoscopic eye examination, brain computed tomography (CT) scan, magnetic resonance imaging, lung CT scan, and additional investigations according to the clinical presentation. Asymptomatic but ≥1 positive qPCR patients were preemptively treated until there were 2 consecutive negative qPCR tests; the treatments were by daily doses of TMP-SMX if they did not receive TMP-SMX at diagnosis or by pyrimethamine with sulfadiazine, atovaquone, or dapsone if the diagnosis was done under TMP-SMX. Patients with *Toxoplasma* disease were treated with pyrimethamine and sulfadiazine, pyrimethamine and clindamycin, or high-dose TMP-SMX for 3 weeks and then put on secondary prophylaxis. According to French Health Public Law (CSP Art L1121-1.1), the current type of investigation does not require specific informed consent or ethics committee approval.

RESULTS

In total, 419 patients received an allogeneic HCT during the study period. Data were censored starting on October 30, 2018, so that all patients had a minimal follow-up of 4 months after transplant. Among these 419 patients, 17 (4.0%) developed toxoplasmosis, 7 (1.6%) before day 30 (median [range] time, day 22 [6 to 28]) and 10 (2.4%) after day 30 (median [range] time, day 172 [43 to 322]) (Table 1). Briefly, all 17 patients were seropositive (R^+) for *Toxoplasma,* and all donors (D) except 1 were seronegative.

Among the 7 patients with early toxoplasmosis, none had received TMP-SMX prophylaxis just before or since transplant, and only 2 had been screened for qPCR before diagnosis. In these 2 patients, the second qPCR of the screening was positive at day 25 and day 28, respectively. These patients were preemptively treated, and none of them developed *Toxoplasma* disease. In the 5 other early cases, the diagnosis was suspected based on clinical manifestations—mostly fever or pneumonia—when qPCR blood screening was not routine. Three patients were neutropenic, and only 1 had graft-versus-host disease (GVHD). All 7 patients developed febrile neutropenia between transplant and the diagnosis of toxoplasmosis, including 6 cases of fever of unknown origin. Two patients had another documented infection before the diagnosis of toxoplasmosis, which could have delayed the diagnosis: 1 probable pulmonary aspergillosis and 1 *Pseudomonas* bacteremia. Only 1 patient had a coinfection at the time of *Toxoplasma* infection (pulmonary aspergillosis with pleural effusion, which was positive for galactomannan and negative for *Toxoplasma* PCR in the pleural liquid). The delay between first symptoms and toxoplasmosis diagnosis was 0 to 6 days. All 5 (71%) patients with *Toxoplasma* disease (proven, 1; probable, 4) had pulmonary toxoplasmosis diagnosed at a median of 22 days (range, 6 to 28). All 5 patients died 1 to 33 days after the diagnosis from respiratory (n = 2) or multivisceral failure because of disseminated toxoplasmosis (n = 3).

Among the 10 cases observed after day 30, there was only 1 *Toxoplasma* disease (10%) in a patient who was being treated for TMP-SMX prophylaxis but had gut GVHD. At day 319, this patient, who refused to come to the hospital during 1 month, developed seizures, revealing brain toxoplasmosis, which was proven by histology and qPCR on a brain biopsy specimen, whereas a previous blood qPCR tested negative 4 weeks before. This patient was successfully treated and survived. The other 9 patients were preemptively treated based on positive qPCR results. Two of them died within 3 months after the diagnosis of *Toxoplasma* infection, 1 from leukemia relapse (n = 1) and the other from unexplained multivisceral failure with pericarditis (n = 1), but both of these patients had a negative blood qPCR at the time of death. A third patient developed *Toxoplasma* infection at day 45 after HCT, concomitantly with uncontrolled acute grade III GVHD and multivisceral failure, and remained positive for qPCR in blood until death despite preemptive treatment and without evidence of *Toxoplasma* disease. The median (range) number of toxoplasma PCRs performed before the diagnosis (first positive PCR) of *Toxoplasma* infection or disease was 0 (0 to 1) in the 7 early cases and 25 (5 to 29) in the 10 cases occurring after day 30.

DISCUSSION

We report that 41% of our toxoplasmosis cases occurred within 30 days after allogeneic transplant when the patients usually did not receive any post-transplant TMP-SMX prophylaxis and were not screened by qPCR, both being started after engraftment according to our procedures. Although the small size of our patient cohort precludes any statistical comparison, the concomitant lack of prophylaxis and of screening probably explains the high proportion of *Toxoplasma* disease and of the early deaths with or from toxoplasmosis in these early cases compared with the later cases. Noteworthy, 6 of these 7 patients developed toxoplasmosis when they had no GVHD and had not received any steroid therapy. On the other hand, the 10 patients with toxoplasmosis after day 30 benefited from a regular screening so that 9 of them could have been treated preemptively before the onset of any symptom. The only patient who developed central nervous system (CNS) toxoplasmosis 10 months after transplant while on treatment of active GVHD had refused consultations during four weeks while he should have benefited from a weekly PCR screening.

In previous series, most toxoplasmosis cases occurred during the second or third months after allogeneic HCT, with a risk persisting across the first year of transplant and sometimes even later [10,11]. Cases occurring during the first 4 weeks of transplant have been rarely reported but seem to have a high mortality rate [1,5,7]. Our series clearly identifies toxoplasmosis as an early complication of allogeneic HCT.

However, these cases should have been detected earlier, before any toxoplasma disease, using a weekly blood qPCR screening from transplant. Therefore, in R^+ patients, we recommend starting this screening immediately after transplant, not from the point of engraftment. Whether the administration of TMP-SMX given before transplant for *Pneumocystis jirovecii* pneumonia prophylaxis in some centers may prevent early toxoplasmosis in HCT recipients has not been addressed in the literature and cannot be determined from our series but seems unlikely as TMP-SMX cannot be efficient on dormant cysts.

The main limit of the current study is that because of the small number of cases, it was not possible to search for the risk factors for developing early—rather than late—toxoplasmosis among our whole cohort of 419 patients. However, our findings are consistent with previous studies identifying cord blood transplant, donor's seronegativity, and lack of TMP-SMX prophylaxis as risk factors for *Toxoplasma* reactivation [1,4].

For a long time, because of the length and deepness of neutropenia after myeloablative conditioning, our efforts during the first 3 to 4 weeks of transplant have been focused on the optimal management of bacterial and fungal infections, which were the main fears of the transplanters. More recently, because of the predominant use of reduced-intensity

Table 1
Clinical Characteristics and Outcome of the Patients Who Developed *Toxoplasma* Infection or Disease after Allogeneic HCT within 30 Days (n = 7) or from 30 Days (n = 10) after Transplant

Characteristic	Toxoplasmosis before Day 30 after HCT	Toxoplasmosis from Day 30 after HCT	Total
No. (%) of patients	7 (41)	10 (59)	17
Age, median (range), yr	53 (19-65)	56 (31-65)	55 (19-65)
Male/female, No.	5/2	8/2	13/4
Underlying disease, No.			
Acute myeloid leukemia	4	5	9
Acute lymphoblastic leukemia	1	2	3
Biphenotypic acute leukemia	0	1	1
Lymphoma	2	1	3
Primary myelofibrosis	0	1	1
Toxoplasma serology before HCT, No. (%)			
Positive	7 (100)	10 (100)	17 (100)
Negative	0	0	0
Toxoplasma serology of the donor, No. (%)			
Positive	1 (14)	0 (0)	1 (6)
Negative	6 (86)	10 (100)	16 (94)
Type of transplant, No. (%)			
HLA-identical	0 (0)	1 (10)	1 (6)
Matched unrelated	4 (57)	5 (50)	9 (53)
Mismatched unrelated	1 (14)	3 (30)	4 (23.5)
Cord blood	2 (29)	1 (10)	3 (17.5)
Conditioning regimen, No. (%)			
Myeloablative	1 (14)	3 (30)	4 (23.5)
Reduced intensity	6 (86)	6 (60)	12 (70.5)
Nonmyeloablative	0	1 (10)	1 (6)
Antithymocyte globulin in the conditioning	6 (86)	7 (70)	13 (76)
Type of toxoplasmosis, No. (%)			
Infection	2 (29)	9 (90)	11 (64.5)
Proven disease	1 (14) (lung, gut)	1 (10) (brain)	2 (12)
Probable disease	4 (57) (lung: 4; brain: 1)	0 (0)	4 (23.5)
Median day (range) of toxoplasmosis after HCT	22 (6-28)	172 (43-322)	45 (6-322)
Polymorphonuclear $\leq$500 cells/μL at diagnosis of toxoplasmosis, No. (%)	3 (43)	0 (0)	3 (17.5)
GVHD at time of diagnosis of toxoplasmosis or before (maximal severity), No. (%)			
None	6 (86)	3 (30)	9 (53)
Acute grades I-II	0	1 (10)	1 (6)
Acute grades III-IV	1 (14)	5 (50)	6 (35)
Chronic	NA	1 (10)	1 (6)
Steroids at time of toxoplasmosis, No. (%)	1 (14)	8 (80)	9 (53)
TMP-SMX prophylaxis within the 3 weeks before toxoplasmosis, No. (%)	0 (0)	2 (20)	2 (12)
Atovaquone prophylaxis within the 3 weeks before toxoplasmosis, No. (%)	0 (0)	3 (30)	3 (17.5)
Outcome within 3 months, No. (%)			
Survival	2 (29)	7 (70)	9 (53)
Death	5 (71)	3 (30)	8 (47)
Death with or from toxoplasmosis	5 (71)	1 (10)	6 (35)
Time between toxoplasmosis diagnosis and death, d	1, 4, 7, 18, and 33	4, 20, and 43	

NA indicates not applicable.

conditioning, other infections, initially considered complications of the "second" postengraftment phase of transplant, have emerged as major, early problems. Indeed, we recently reported the early onset of pneumocystosis in 3 of 18 cases because of the lack of TMP-SMX before engraftment [12]. Similarly, *Toxoplasma* infection may develop early after transplant and should be detected using a weekly qPCR screening from transplant to avoid the development of early *Toxoplasma*

disease. From 3 months after transplant, we recommend to go on PCR screening, every 1 to 2 weeks, as long as the patient receives immunosuppressants.

CONCLUSION

Because there is no reliable *Toxoplasma* prophylaxis except for TMP-SMX, which should not be given before engraftment because of its hematologic toxicity, we strongly recommend starting a weekly qPCR screening with the transplant and eventually from conditioning in R^+ patients to catch early reactivation. The timing between the sample and result is also crucial to optimally start a preemptive treatment and avoid the development of *Toxoplasma* disease.

ACKNOWLEDGMENTS

The authors are grateful to the technical staff of the Laboratory of Mycology and to the physicians and nurses of the Department of Hematology. This work has been partly reported as a poster at the 44th Annual Meeting of the European Society for Blood and Marrow Transplantation; March 18 to 21, 2018; Lisbon, Portugal.

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: C.R. and C.C. conceived and designed the study. R.R., M.L., F.B., C.P., A.T., and S.M. generated and provided the clinical data. C.A., F.F., and F.B. provided the laboratory data. C. R., L.C., and C.C. assembled the data and ran the analysis. C.R., S.M., F.B., and C.C. analyzed and interpreted the data and drafted the manuscript. All authors approved the final version.

REFERENCES

1. Martino R, Bretagne S, Einsele H, et al. Early detection of *Toxoplasma* infection by molecular monitoring of *Toxoplasma gondii* in peripheral blood samples after allogeneic stem cell transplantation. *Clin Infect Dis*. 2005;40 (1):67–78.
2. Mele A, Paterson PJ, Prentice HG, Leoni P, Kibbler CC. Toxoplasmosis in bone marrow transplantation: a report of two cases and systematic review of the literature. *Bone Marrow Transplant*. 2002;29(8):691–698.
3. Guigue N, Leon L, Hamane S, et al. Continuous decline of *Toxoplasma gondii* seroprevalence in hospital: a 1997-2014 longitudinal study in Paris, France. *Front Microbiol*. 2018;9:2369.
4. Meers S, Lagrou K, Theunissen K, et al. Myeloablative conditioning predisposes patients for *Toxoplasma gondii* reactivation after allogeneic stem cell transplantation. *Clin Infect Dis*. 2010;50(8):1127–1134.
5. Mulanovich VE, Ahmed SI, Ozturk T, Khokhar FA, Kontoyiannis DP, de Lima M. Toxoplasmosis in allo-SCT patients: risk factors and outcomes at a transplantation center with a low incidence. *Bone Marrow Transplant*. 2011;46(2):273–277.
6. Gea-Banacloche J, Masur H, Arns da Cunha C, et al. Regionally limited or rare infections: prevention after hematopoietic cell transplantation. *Bone Marrow Transplant*. 2009;44(8):489–494.
7. Prestes DP, Mendes C, Batista MV, et al. A case-series of Toxoplasmosis in hematopoietic stem cell transplantation: still a concern for endemic countries. *Bone Marrow Transplant*. 2018;53(10):1336–1339.
8. Martino R, Maertens J, Bretagne S, et al. Toxoplasmosis after hematopoietic stem cell transplantation. *Clin Infect Dis*. 2000;31(5):1188–1195.
9. Costa JM, Bretagne S. Variation of B1 gene and AF146527 repeat element copy numbers according to *Toxoplasma gondii* strains assessed using real-time quantitative PCR. *J Clin Microbiol*. 2012;50(4):1452–1454.
10. Bretagne S, Costa JM, Kuentz M, et al. Late toxoplasmosis evidenced by PCR in a marrow transplant recipient. *Bone Marrow Transplant*. 1995;15 (5):809–811.
11. Gajurel K, Dhakal R, Montoya JG. *Toxoplasma* prophylaxis in haematopoietic cell transplant recipients: a review of the literature and recommendations. *Curr Opin Infect Dis*. 2015;28(4):283–292.
12. Redjoul R, Robin C, Foulet F, et al. *Pneumocystis jirovecii* pneumonia prophylaxis in allogeneic hematopoietic cell transplant recipients: can we always follow the guidelines? *Bone Marrow Transplant*. 2019;54:1082–1088.