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Original article

A matched case–control study of toxoplasmosis after allogeneic haematopoietic stem cell transplantation: still a devastating complication

A. Conrad [1, 2, 3], M. Le Maréchal [4], D. Dupont [3, 5, 6], S. Ducastelle-Leprêtre [7], M. Balsat [7], H. Labussière-Wallet [7], F. Barraco [7], F.-E. Nicolini [7], X. Thomas [7], L. Gilis [7], C. Chidiac [1, 3], T. Ferry [1, 2, 3], F. Wallet [8], M. Rabodonirina [3, 5, 9], G. Salles [3, 7], M. Michallet [3, 7], F. Ader [1, 2, 3, *] on behalf of the Lyon HEMINF study Group

[1] Département de Maladies Infectieuses et Tropicales, Hospices Civils de Lyon, France
[2] Inserm U1111 Centre International de Recherche en Infectiologie (CIRI), Lyon, France
[3] Université Claude Bernard Lyon I, Lyon, France
[4] Université de Lorraine et Paris Descartes, EA 4360 APEMAC, Nancy, France
[5] Institut de Parasitologie et de Mycologie Médicale, Hospices Civils de Lyon, Lyon, France
[6] WAKING-Physiologie intégrée du système d'éveil, Centre de Recherche en Neurosciences de Lyon Inserm U1028, CNRS UMR5292, Lyon, France
[7] Département d'Hématologie, Hospices Civils de Lyon, Pierre-Bénite, France
[8] Département d'Anesthésie et de Réanimation du Centre Hospitalier Lyon-Sud, Hospices Civils de Lyon, France
[9] Emerging Pathogens Laboratory-Fondation Mérieux, CIRI Inserm U1111, CNRS UMR5308, ENS de Lyon, Lyon, France

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ABSTRACT

Toxoplasmosis (TXP) is a life-threatening complication of allogeneic haematopoietic stem cell transplantation (AHSCT). Little is known about the risk factors and there is no consensus on prophylactic measures. To investigate the risk factors, we conducted a single-centre, retrospective matched case–control study among adults who underwent AHSCT from January 2006 to March 2015 in our hospital. TXP cases were identified from the prospectively maintained hospital's database. The 1:2 control population consisted of the two patients who received an AHSCT immediately before and after each case with similar donor relationship (related, unrelated) but who did not develop TXP. Risk factors were identified by conditional logistic regression. Clinical features and outcome of TXP were examined. Twenty-three (3.9%) cases of TXP (20 diseases, three infections) were identified among 588 AHSCT recipients. Twenty (87%) cases had a positive pre-transplant *Toxoplasma gondii* serology. In comparison with 46 matched control patients, risk factors were the absence of effective anti-*Toxoplasma* prophylaxis (odds ratio (OR) 11.95; 95% CI 3.04–46.88; p <0.001), high-grade (III–IV) acute graft-versus-host-disease (OR 3.1; 95% CI 1.04–9.23; p 0.042) and receipt of the tumour necrosis factor-α blocker etanercept (OR 12.02; 95% CI 1.33–108.6; p 0.027). Mortality attributable to TXP was 43.5% (n = 10). Non-relapse mortality rates during the study period of cases and controls were 69.6% (n = 16) and 17.4% (n = 8), respectively. Lung involvement was the dominant clinical feature (n = 14). Two cases were associated with graft failure, one preceded by haemophagocytic syndrome. Given TXP-related morbidity and attributable mortality, anti-*Toxoplasma* prophylaxis is essential for optimized management of seropositive AHSCT recipients. **A. ConradF. Ader, CMI 2016;22:636**

Introduction

Opportunistic infections are a leading cause of non-relapse mortality after allogeneic haematopoietic stem cell transplantation (AHSCT) [1]. Toxoplasmosis (TXP), caused by *Toxoplasma gondii*, is a severe opportunistic infection with an overall mortality

* Corresponding author. F. Ader, Service de Maladies Infectieuses et Tropicales, Hôpital de la Croix-Rousse, 103 Grande Rue de la Croix-Rousse, 69317 Lyon cedex 04, France.
E-mail address: florence.ader@chu-lyon.fr (F. Ader).

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rate ranging from 22% to 80% after AHSCT [2–5]. In this setting, TXP is defined as *Toxoplasma* infection when the patient is asymptomatic despite a positive blood PCR or as *Toxoplasma* disease in the presence of clinical symptoms with radiological or pathological evidence of organ involvement by *T. gondii* [2]. Incidence of TXP correlates with seroprevalence through reactivation of latent cysts. A prospective study among *T. gondii*-seropositive AHSCT recipients found a 6-month incidence of *Toxoplasma* infection or disease of 16% and 8%, respectively [6]. Besides recipient seropositivity, the major identified risk factor for TXP is cord blood unit transplantation [6,7]. Graft-versus-host-disease (GvHD) and the absence of chemoprophylaxis have been inconstantly associated with TXP [6,8].

In AHSCT recipients, anti-*Toxoplasma* prophylaxis by trimethoprim-sulfamethoxazole is the favoured option, by analogy with the human immunodeficiency virus-infected population [7,9,10]. However, in the absence of controlled studies, uncertainties remain regarding dosing and duration, as well as concerns about myelotoxicity [7,10,11].

We focused on TXP after AHSCT that met the criteria of infection or disease. Using a matched case–control design, we aimed to identify risk factors associated with TXP after AHSCT in a high seroprevalence setting to characterize subsets of patients to be imperatively targeted by prophylactic measures. Secondary objectives were to study the impact of TXP on the outcome of AHSCT recipients and to provide updated reports of TXP clinical presentations.

Patients and Methods

We conducted a retrospective, observational, single-centre matched case–control study among adult AHSCT recipients transplanted at the haematology department of our institution between January 2006 and March 2015. AHSCT recipients diagnosed with TXP were identified by cross-referencing the prospectively maintained databases of the haematology department and the parasitology laboratory and by additional chart reviews. Patients eligible for inclusion were those who matched the criteria previously defined as *Toxoplasma* infection (at least one blood sample positively tested by PCR for *T. gondii*) or *Toxoplasma* disease depending on whether there was evidence of organ involvement or not [2]. *Toxoplasma* disease was further classified into definite (histological or cytological demonstration of tachyzoites in tissue biopsy or bronchoalveolar lavage), probable (detection of *T. gondii* DNA by PCR in a blood sample and/or another sample from the involved organ such as cerebrospinal fluid, bronchoalveolar lavage or bone marrow) or possible disease (suggestive imaging of central nervous system TXP, response to empirical anti-*Toxoplasma* therapy and no other microbiological documentation). Disseminated TXP involved two or more organs. Each case was matched with two controls with similar donor relationship (related or unrelated) and temporal proximity of AHSCT (immediately before and after each identified case). The study was approved by the local ethics committee (Comité d'Ethique, Hospices Civils de Lyon). Because of the retrospective observational nature of the study and the lack of any modification in the patients' management, the need for informed consent was waived.

Pre-transplant *T. gondii* serology was mandatory for both donors (D+ or D−) and recipients (R+ or R−). The *T. gondii* serostatus of cord blood units was considered negative, regardless of maternal serology. From 2008 to 2015, several assays were used for specific IgG and IgM antibody detection such as the Enzygnost® Toxoplasmosis IgG/IgM assay (Siemens, Munich, Germany; cut-off value 4 IU/mL for IgG and determined for each plate for IgM), the AxSYM Toxo IgG/IgM assay (Abbott, Abbott Park, IL, USA; equivocal zone 2–2.9 IU/mL and 0.5–0.6 IU/mL, respectively), and the ARCHITECT Toxo IgG/IgM assay (Abbott; equivocal zone 1.6–2.9 IU/mL and 0.50–0.59 IU/mL, respectively). All equivocal IgG and IgM results were tested *a posteriori* with the Vidas Toxo IgM assay (Biomérieux, Marcy l'Etoile, France; equivocal zone 4–8 IU/mL) and the Platelia Toxo IgM assay (BioRad, Marnes-la-Coquette, France; equivocal zone 0.8–1.0 IU/mL), respectively.

The PCR analyses for *T. gondii* were performed on whole blood samples or other biological fluids by an in-house, real-time method targeting the repetitive 529-base-pair DNA fragment of *T. gondii* (GenBank AF 487550), as published elsewhere [12]. DNA was extracted from a 200-μL sample of whole blood or centrifuged pellets using the Qiagen QIAmp DNA Mini Kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. Real-time PCR was performed on a LightCycler 2.0 (Roche Diagnostics, Meylan, France) using FRET probes for detection. Results were classified as positive or negative, with a sensitivity level of 0.1 parasite/mL. Genotyping was not performed. With the exception of R+ cord blood unit recipients (screened weekly for 3 months), systematic PCR testing of AHSCT recipients was performed on demand.

With regard to their potential efficacy against *T. gondii*, we classified the prophylactic regimens as effective (trimethoprim-sulfamethoxazole 160/800 mg thrice weekly), possibly effective (atovaquone 1500 mg daily or pyrimethamine-sulfadoxine 25/500 mg twice-weekly), or ineffective (monthly aerosolized pentamidine or no prophylaxis). According to centre's policy, prophylaxis was initiated after neutrophil engraftment and sustained over a year.

The primary objective of the study was to determine the risk factors associated with TXP after AHSCT. Patients' characteristics were collected to perform a bivariate analysis on selected variables: demographics, underlying haematological disease, disease status at AHSCT, characteristics of AHSCT (conditioning regimen, stem cell source), pre-transplant *T. gondii* serostatus, TXP prophylaxis at engraftment, delay in neutrophil engraftment (> 21 days), absolute lymphocyte count at day 30 after AHSCT, grade of acute GvHD (aGvHD), immunosuppressive treatments (calcineurin inhibitors, high-dose corticosteroids (≥1 mg/kg/day and ≥21 days), tumour necrosis factor-α blocker etanercept and anti-CD25 monoclonal antibody inolimomab, both used for steroid-refractory aGvHD).

Non-relapse mortality and attributable mortality after TXP in the setting of AHSCT were studied. Survival curves were drawn using the Kaplan–Meier method and compared using the log-rank test.

Clinically relevant features affecting target organs (lung, central nervous system, heart, bone marrow, eye) and the severity of the disease (intensive care unit admission, multi-organ failure, graft failure) were detailed. Contribution to graft failure was considered significant when recipients failed to engraft during the course of TXP with no other fatal complication or evidence of disease relapse.

Data were expressed as count (percentage), mean ± standard deviation or median (interquartile range). Descriptive data were compared using Mann–Whitney *U* test, chi-squared test or the Fisher exact test, where appropriate. Odds ratios (95% CI) were calculated by bivariate analyses using conditional logistic regression. Analyses were based on two-sided p values, with statistical significance defined by $p < 0.05$ and conducted with R (version 3.2.1) and the 'survival' package.

Results

During the study period, 588 AHSCT were performed among which 334 (56.8%) were *T. gondii* R+. Twenty-three (3.9%) cases of TXP including 20 (3.4%) diseases and 3 (0.5%) infections were matched with 46 controls (Fig. 1). Study population ($n = 69$) differed from the rest of the cohort ($n = 519$) with respect to the

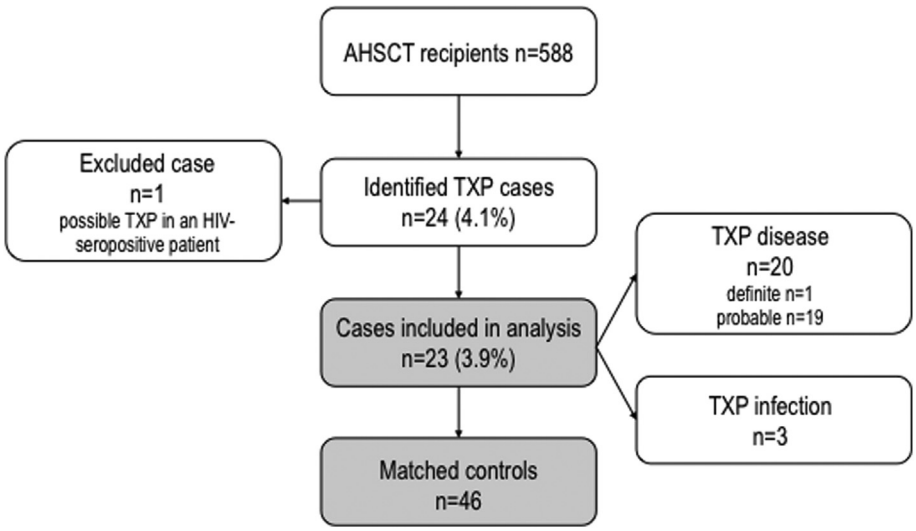

Fig. 1. Flow chart of patients included in the study. Toxoplasmosis (TXP) infection and disease defined according to the European Group for Blood and Marrow Transplantation–Infectious Diseases Working Party [2]. AHSCT, allogeneic haematopoietic stem cell transplantation; HIV, human immunodeficiency virus; TXP, toxoplasmosis.

proportion of cord blood unit recipients (36.2% versus 11.6%, p <0.001, data not shown). Cases ($n = 23$) significantly differed from the control population ($n = 46$) with respect to age, sex, advanced disease status and distribution of unrelated donor types, owing to matching criteria (Table 1).

In bivariate analysis, the lack of effective prophylaxis was strongly associated with TXP occurrence (OR 11.95; 95% CI 3.04–46.88; p <0.001), whereas a trend to higher TXP occurrence was observed with possibly effective prophylaxis (OR 6.28; 95% CI 0.75–52.82; p 0.091) (Table 2). The most frequent R/D pre-transplant serostatus pattern was R+/D− ($n = 15$, 65.2% among cases). After carefully assessing the R− cases ($n = 3$), two had a pre-transplant serology slightly below the positivity threshold in addition to being diagnosed with TXP before engraftment (while in sterile confinement), which invalidates a primary infection mechanism. The underlying haematological condition of the third R− case required numerous treatment lines before AHSCT, hampering antibody detection in the pre-transplantation assessment phase. When examining factors contributing to impaired immune function post-AHSCT, high-grade aGvHD (III–IV) and the use of etanercept were significantly associated with TXP (OR 3.1; 95% CI 1.04–9.23; p 0.042 and OR 12.02; 95% CI 1.33–108.6; p 0.027, respectively) (Table 2).

Non-relapse mortality rates among TXP cases and control patients over the study period were 69.6% ($n = 16$) and 17.4% ($n = 8$), respectively. Attributable mortality of TXP was 43.5% ($n = 10$). Median follow up of the study population was 12 months (interquartile range 4.2–35.4 months). Probability of overall survival of cases was significantly decreased compared with that of controls (log-rank, p <0.001) (Fig. 2). Overall probability of survival for cases at 1, 3, 6 and 12 months was 83%, 55%, 37% and 35%, respectively.

Among TXP cases, 19 (82.6%) recipients were not on prophylaxis at diagnosis whereas 4 (17.4%) were breakthrough TXP while under trimethoprim-sulfamethoxazole ($n = 3$) or pyrimethamine-sulfadoxine ($n = 1$) (see Supplementary material, Table S1). Systematic *T. gondii* blood PCR monitoring revealed TXP in 9 (39.1%) cases. Median time to TXP onset was 62 (interquartile range 34.5–148.5) days. Two patterns were individualized: (i) early-onset TXP occurring before engraftment for 7 (30.4%) recipients of a mismatched unrelated donor; (ii) late-onset TXP (beyond day 100) for 9 (39.1%) recipients, among whom five were experiencing concurrent steroid-refractory high-grade aGvHD.

Among patients with TXP disease ($n = 20$), lung involvement was the dominant feature ($n = 14$, 70%), ranging from mild

Table 1
Patient demographic and haematological characteristics

Characteristic	Cases ($n = 23$)	Controls ($n = 46$)	p-value
Age (years), mean (SD)	51.3 (13.5)	43.1 (14.3)	0.016
Male sex	10 (43.5)	33 (71.7)	0.035
Underlying disease			
Acute leukaemia	12 (52.2)	27 (58.7)	0.606
Myelodysplastic syndrome	1 (4.3)	7 (15.2)	0.253
Myeloproliferative disorder	4 (17.4)	3 (6.5)	0.211
Lymphoproliferative disorder	2 (8.7)	3 (6.5)	1
Multiple myeloma	4 (17.4)	5 (10.9)	0.468
Aplastic anaemia	0 (0)	1 (2.2)	1
Disease status at AHSCT[a]			
Early	9 (39.1)	25 (54.3)	0.233
Intermediate	4 (17.4)	12 (26.1)	0.550
Advanced	10 (43.5)	9 (19.6)	0.036
Prior HSCT	6 (26.1)	7 (15.2)	0.276
Donor type			
Identical sibling	4 (17.4)	8 (17.4)	1
Matched unrelated	3 (13.0)	19 (41.3)	0.027
Mismatched unrelated	16 (69.6)	19 (41.3)	0.029
Stem cell source			
Bone marrow	8 (34.8)	16 (34.8)	1
Peripheral blood	5 (21.7)	15 (32.6)	0.348
Cord blood unit	10 (43.5)	15 (32.6)	0.376
Conditioning regimen			
Myeloablative conditioning	11 (47.8)	28 (60.9)	0.303
Anti-thymocyte globulin	16 (69.6)	36 (78.3)	0.429
Total body irradiation	14 (60.9)	26 (56.5)	0.730

Data are *n* (%) of patients, unless otherwise indicated. Variables were compared using Mann–Whitney *U*-test, χ^2 test or the Fisher exact test, where appropriate.
Abbreviations: AHSCT, allogeneic haematopoietic stem cell transplantation; HSCT, haematopoietic stem cell transplantation; SD, standard deviation.

[a] Disease status at AHSCT classified in early (any underlying disease in first complete remission, untreated myelodysplastic syndrome of good prognosis, myeloproliferative disorder in chronic phase), intermediate (any underlying disease in at least second complete remission, multiple myeloma not in complete remission, myeloproliferative disorder in accelerated phase) or advanced (refractory or relapsing underlying disease, myeloproliferative disorder in blastic phase, any relapsing malignancy after previous HSCT, second AHSCT after previous primary graft failure).

Table 2
Bivariate analysis of risk factors

Variable	Cases ($n = 23$)	Controls ($n = 46$)	OR	95% CI	p-value
Toxoplasma serostatus[a]					
R+	20 (87)	30 (65.2)	3.5	0.91–13.47	0.069
D+	5 (21.7)	12 (26.1)	0.79	0.24–2.57	0.695
Combined serostatus					
R−/D−	3 (13)	13 (28.3)		(reference)	
R−/D+	0 (0)	3 (6.5)	0	0–∞	1
R+/D−	15 (65.2)	21 (45.7)	3.05	0.74–12.48	0.122
R+/D+	5 (21.7)	9 (19.6)	2.38	0.46–12.43	0.304
Anti-*Toxoplasma* prophylaxis at engraftment[b]					
effective	3 (13)	29 (63)		(reference)	
possibly effective	2 (8.7)	3 (6.5)	6.28	0.75–52.82	0.091
none or ineffective	18 (78.3)	14 (30.4)	11.95	3.04–46.88	<0.001
Delayed engraftment[c]	16 (69.6)	23 (50)	2.26	0.79–6.47	0.129
Lymphocytopenia[d]	11 (47.8)	10 (21.7)	3.57	1.2–10	0.022
aGvHD[e]					
all grades	15 (65.2)	34 (73.9)	0.67	0.23–1.95	0.458
grade III-IV	10 (43.5)	9 (19.6)	3.1	1.04–9.23	0.042
Immunosuppressants[f]					
calcineurin inhibitor	21 (91.3)	46 (100)	0	0–∞	1
High-dose steroids[g]	13 (56.5)	23 (50)	1.3	0.48–3.52	0.612
etanercept	5 (21.7)	1 (2.2)	12.02	1.33–108.6	0.027
inolimomab	3 (13)	3 (6.5)	2.12	0.40–11.31	0.377

Data are *n* (%) of patients, unless otherwise indicated. Odds ratios were determined by conditional logistic regression.
Abbreviations: aGvHD, acute graft-versus-host-disease; AHSCT, allogeneic haematopoietic stem cell transplantation; calcineurin inhibitor; D, donor; R, recipient; SD, standard deviation.

[a] Pre-transplant *Toxoplasma gondii* serostatus determined by the presence or not of anti-*Toxoplasma* IgG.

[b] Efficacy prophylaxis against *Toxoplasma gondii* classified as effective (trimethoprim-sulfamethoxazole 160/800 mg thrice-weekly), possibly effective (atovaquone 1500 mg daily or pyrimethamine-sulfadoxine 25/500 mg twice-weekly) or ineffective (pentamidine or no prophylaxis at all).

[c] Delayed engraftment defined as neutrophil recovery (≥0.5 G/L) occurring later than day +21 post-transplant.

[d] Total lymphocyte count ≤0.2 G/L on day +30 post-transplant.

[e] Highest grade of acute graft-versus-host-disease within 3 months preceding toxoplasmosis diagnosis for cases, within the first semester post-transplant for controls.

[f] Immunosuppressive medication in progress or administered within three months preceding toxoplasmosis diagnosis for cases, within the first semester post-transplant for controls.

[g] High-dose steroid therapy, i.e. ≥1 mg/kg/day and ≥21 days.

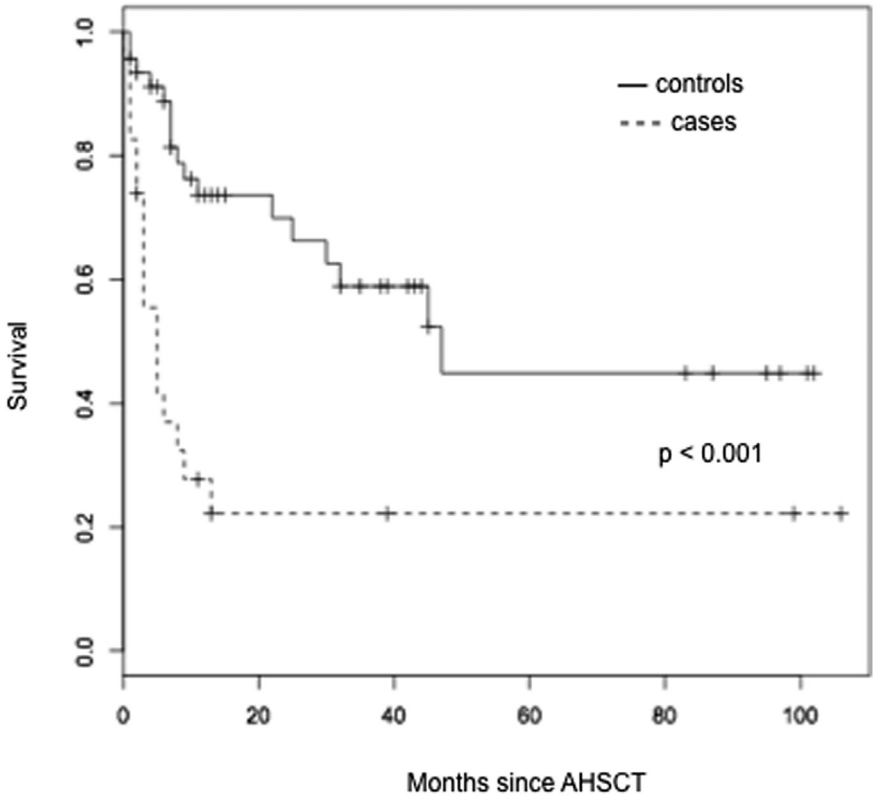

Fig. 2. Overall survival probability of toxoplasmosis cases and control patients. Overall survival probabilities determined by the Kaplan–Meier method and compared by the log-rank test. AHSCT, allogeneic haematopoietic stem cell transplantation.

interstitial pneumonitis to acute respiratory distress syndrome. Central nervous system involvement ($n = 10$, 50%) was the second clinically relevant manifestation through encephalitis ($n = 6$, 30%) or brain abscesses ($n = 4$, 20%). Two (10%) cases had a unique presentation of TXP-related haemophagocytic syndrome. Neither myocarditis nor retinochoroiditis were recorded. Seven (35%) cases had disseminated disease. Nine (45%) disease cases required admission to the intensive care unit because of respiratory and/or neurological failure(s) leading to death in 8 (88.9%) cases (see Supplementary material, Table S1). Two cases experienced primary graft failure, one being associated with haemophagocytic syndrome.

Discussion

In the present study, although rare, TXP was a devastating complication with an attributable mortality of 43.5% (88.9% when ICU was required), as well as a preventable one. Pre-transplant, *T. gondii* recipient seropositivity (R+) not receiving prophylaxis is the central risk factor for reactivation of TXP after AHSCT. In a review ($n = 356$) of TXP after AHSCT, 259 (73%) were R+ and at least 67% of these patients were not on TXP prophylaxis [7]. Consistently, in our series (n = 23), 87% were R+, of whom 85% were not on prophylaxis at TXP diagnosis, i.e. received no prophylaxis (especially while in sterile confinement) or ineffective pentamidine because of concerns about adverse effects of trimethoprim-sulfamethoxazole or atovaquone. Consequently, a *Toxoplasma*-risk management strategy is necessary for every R+ by prescribing effective prophylactic drug regimen and/or by adopting a *T. gondii* PCR-driven pre-emptive approach when available.

In the setting of AHSCT, trimethoprim-sulfamethoxazole prophylactic efficacy is supported by data showing a lower incidence of TXP reactivation when used at least three times a week [6,7,13]. However, limitations are myelosuppression and breakthrough infections. In our series, 30.4% of the patients experienced pre-engraftment TXP. According to Gajurel *et al*., among R+ developing TXP, 19 (11%) diseases and 13 (32%) infections occurred before day 30 [7]. Hence, prophylaxis should ideally be initiated upon transplantation on condition that it should not compromise engraftment. Long-term exposure to trimethoprim-sulfamethoxazole has been inconsistently associated with haematotoxicity [14,15]. High-dose trimethoprim-sulfamethoxazole (320/1600 mg/day) was shown to delay neutrophil recovery after HSCT [16] and a rather small ($n = 3$) descriptive study reported that trimethoprim-sulfamethoxazole 160/800 mg/day impaired *in vitro* colony formation of peripheral blood stem cells [17]. However, retrospective studies (160/800 mg or 320/1600 mg thrice weekly) did not evidence significant delay in engraftment compared with no prophylaxis or atovaquone (1500 mg/day) [18,19].

In our series, 17.4% were breakthrough TXP, in accordance with 12% of breakthrough TXP reported by Gajurel *et al*. [7]. Breakthrough TXP might reflect suboptimal dosing of prophylaxis related to inadequate oral absorption, mainly caused by gastrointestinal aGvHD, or drug interruptions because of haematotoxicity and gastrointestinal intolerance [7,19,20].

Pre-emptive strategy based on the detection of asymptomatic *Toxoplasma* infection by serial blood *T. gondii* PCR is the path to prevent disease occurrence and to reduce TXP attributable mortality [4–6,13,21]. Our results have led us to consider *T. gondii* blood PCR monitoring useful in R+ irrespective of stem cell source: (i) before engraftment in the absence of prophylaxis; (ii) on prophylaxis, with impaired absorption or with particular risk factors such as high-grade aGvHD; (iii) following drug interruption due to intolerance. Furthermore, physicians may consider the rare possibility of false-negative pre-transplant serology in AHSCT candidates.

The finding that late-onset TXP is associated with etanercept is part of the problematic of steroid-refractory aGvHD requiring immunosuppressive regimen intensification. Tumour necrosis factor-α is essential for the control of *T. gondii* in the central nervous system during *in vivo* primary infection and etanercept treatment has been shown to facilitate experimental *T. gondii* reactivation [22,23].

Finally, respiratory manifestations are prominent features of TXP, as formerly reported [24,25]. In case of haemophagocytic syndrome after AHSCT, *Toxoplasma* should be considered as an aetiological agent, as previously noted in cases of *Toxoplasma* disseminated diseases [26–28].

Our study has strengths and limitations. The strength is the relatively large case cohort and exhaustive data collection. The control population was selected from patients in concurrent and previous years to minimize bias related to practice shifts and matched with respect to donor relationship. We were unable to match cases using the number of available control subjects from the study period with age, sex and stem cell source variables. We acknowledge the bias due to donor type matching, which may affect the outcome (delay in engraftment and/or aGvHD occurrence) but otherwise benefited the purpose of the study by linking risk factors and TXP outcome. Although our choice of control subjects provides a rational comparator group to evaluate risk factors, some previously reported post-AHSCT risk factors were not found, probably because of underpowering. We also acknowledge that the risk associated with absence of effective prophylaxis may be overestimated due to the higher proportion of R+ among cases than among controls. The number of cases was too low to perform accurate multivariate testing. Finally, the retrospective design precluded the collection of all variables of interest (particularly, immune reconstitution data).

In summary, *Toxoplasma*-risk management after AHSCT in high seroprevalence settings relies on: (i) awareness of R/D serostatus, with a specific focus on R+; (ii) in R+, initiation of a prophylaxis as early as possible. The PCR-driven pre-emptive monitoring in R+ high-risk patients, especially if not on prophylaxis, appears to be crucial but needs to be standardized. Studies are required to evaluate the efficacy and safety of the three times per week trimethoprim-sulfamethoxazole regimen or atovaquone, particularly in the pre-engraftment period after AHSCT.

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Appendix A. Supplementary data

Additional Supporting Information may be found in the online version of this article can be found at http://dx.doi.org/10.1016/j.cmi.2016.04.025.

Transparency Declaration

All authors declare that they have no conflict of interest.

Authors' Contributions

AC contributed to conception and design of the study, acquisition of the data, interpretation of the data, drafted the manuscript and approved the final version; MLM participated in the design of the study and performed statistical analysis, revision of the paper

for important intellectual content, and approved the final version; DD and MR contributed to acquisition of the parasitological data, revision of the paper for important intellectual content, and approved the final version; SDL, MB, HLW, FB, FEN, XT, LG, CC, TF, FW, GS, MM contributed to acquisition of the data, interpretation of the results, revision of the paper for important intellectual content, and all approved the final version; FA contributed to conception and design of the study, analyses and interpretation of the data, drafted the manuscript and approved the final version. All authors read and approved the final manuscript.

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Lyon HEMINF study group members

F. Ader, E. Bachy, M. Balsat, F. Barraco, N. Benech, A-L. Bienvenu, G. Billaud, F. Biron, A. Boibieux, C. Chidiac, A. Conrad, S. Ducastelle-Leprêtre, O. Dumitrescu, D. Dupont, V. Escuret, T. Ferry, G. Fossard, E. Frobert, L. Gilis, S. Goutelle, A. Grateau, Y. Guillermin, M. Heiblig, H. Labussière-Wallet, M. Le Maréchal, L. Lebras, B. Lina, G. Lina, P. Miailhes, A-S. Michallet, M. Michallet, M-C. Michallet, G. Monneret, F. Morfin-Sherpa, F-E. Nicolini, T. Perpoint, M. Peyrouse de Montclos, S. Picot, F. Poitevin-Later, A. Quintela, M. Rabodonirina, S. Roux, J. Saison, G. Salles, C. Sarkozy, A. Sénéchal, M. Sobh, X. Thomas, F. Valour, F. Wallet, M. Wallon, E. Wattel.

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