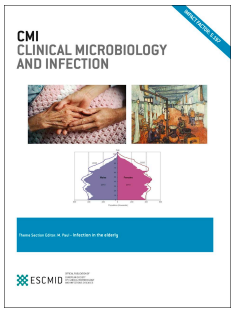

Toxoplasmosis after allogeneic hematopoietic cell transplantation: experience using a PCR-guided pre-emptive approach

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Original Article:

Toxoplasmosis after allogeneic hematopoietic cell transplantation: experience using a PCR-guided pre-emptive approach

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Abstract

Objectives: Prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMZ) is recommended in Toxoplasma seropositive allogeneic cell transplant (HCT) recipients to prevent reactivation but is associated with numerous side effects. We report our experience of a polymerase chain reaction (PCR)-guided pre-emptive approach in patients not receiving prophylaxis.

Methods: In this retrospective single center experience, seropositive recipients and seronegative recipients receiving a graft from a seropositive donor were screened by PCR for the presence of *T. gondii* DNA in peripheral blood till at least 6 months after transplantation. Confirmed PCR-positivity triggered a pre-emptive anti-Toxoplasma therapy. Cases of Toxoplasma reactivation (using the European Society for Blood and Marrow Transplantation definitions) were compared with 4 controls (without reactivation) - matched in time and recipient serostatus - to identify risk factors for reactivation by multivariate analysis.

Results: From November 2001 to August 2020, 1455 consecutive adult patients (59 cases and 1396 controls) were screened. The overall 1-year cumulative incidence of toxoplasmosis was 4.1% and the 1-year cumulative incidence in the seropositive recipients was 8.5%. Reactivation was associated with second transplant (OR 2.51; 95% CI 1.28-4.94: p=0.011), myeloablative conditioning (OR 2.24; 95% CI 1.17-4.41: p=0.011), total body irradiation (OR 2.29; 95% CI 1.17-4.44: p=0.010), acute graft-versus-host disease (GvHD) (OR 2.27; 95% CI 1.26-4.08: p=0.008) and use of high dose corticosteroids (OR 2.08; 95% CI 1.14-3.78: p=0.018). In multivariate analysis only acute GvHD remained significant (adjusted OR 2.54; 95% CI 1.16-5.71: p=0.021).

Conclusions: A PCR-based pre-emptive approach might serve as an acceptable alternative for patients unable to start with or to continue TMP-SMZ prophylaxis. Acute GvHD was identified as the single independent predictor for reactivation.

Introduction

Toxoplasmosis following allogeneic hematopoietic cell transplantation (allo-HCT) results from reactivation of latent *Toxoplasma* cysts. Although rare, the overall mortality rate is high due to late or missed diagnosis [1]. The probability of being *Toxoplasma* seropositive pre-transplant increases with age and ranges from 5% to 90% depending on geographic area [2]. The American Society for Blood and Marrow Transplantation guidelines recommend prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMZ) for patients at high risk [3]. However, breakthrough infections occur, prophylaxis may cause gastrointestinal intolerance and skin rashes, and the absorption is reduced in patients with intestinal GvHD. Also, given its myelotoxic effect, TMP-SMX is preferentially started after stable engraftment, although early *Toxoplasma* reactivation occurs [4].

Testing of body fluids or tissue by polymerase chain reaction (PCR) techniques has been used for the diagnosis of toxoplasmosis in allo-HCT recipients [1,5–8]. Since November 2001, we have implemented the routine monitoring by PCR of *Toxoplasma* seropositive recipients while abandoning the routine use of TMP-SMZ. In cases of confirmed PCR-positivity, pre-emptive therapy was started.

The primary objective of this observational single center study was to determine the feasibility and outcomes of a PCR-based approach. Incidences of *Toxoplasma* infection and disease and the identification of risk factors were secondary objectives.

Materials and methods

Study design and participants. This is a retrospective study of *Toxoplasma*-seropositive allo-HCT recipients at the University Hospitals Leuven, Belgium (November 2001 to August 2020). Consecutive adult (≥18 years of age) patients were included. Institutional databases were accessed to determine *Toxoplasma* infection and disease, survival, causes of death, patient characteristics and transplant-related variables (Table 1 and Table 2). Each case of toxoplasmosis was compared with four *Toxoplasma*-seropositive controls (matched for date of transplantation), excluding recipients who received TMP-SMZ for any reason. This study was approved by the Ethics Committee Research of University hospitals Leuven (S64800-December 28, 2020). Given the retrospective and non-interventional nature of the study, the need for informed consent was waived.

Transplantation protocol. The conditioning regimen was defined as myeloablative (MAC) or reduced intensity (RIC) as previously published [9]. Standard antimicrobial prophylaxis included oral levofloxacin 500 mg/day from the start of conditioning till recovery of neutrophils (≥0.5 x10^9/L), fluconazole 400 mg/day (until immune suppression was weaned), and monthly inhaled pentamidine (300 milligram) to prevent *Pneumocystis* infections (until > 200 CD4-cells/µL). Patients intolerant of pentamidine received trimethoprim-sulfamethoxazole (160/800 mg thrice weekly) and were excluded from this analysis. Meropenem or cefepime was started empirically in case of neutropenic fever till neutrophil recovery. Broad-spectrum antifungals were not given empirically; their initiation was triggered by imaging (e.g. chest CT-scan) and screening for serum galactomannan, as previously described [10].

***Toxoplasma* serostatus and monitoring**. Allo-HCT recipients and their donors were screened pre-transplant for the presence of *Toxoplasma* IgG antibodies [2001-2009: AxSYM (Abbott), since 2009: ARCHITECT (Abbott)]. The presence of *T. gondii* DNA in peripheral blood in (a) seropositive recipients (R^+) and (b) seronegative recipients (R^-) receiving a graft from a seropositive donor (D^+) was monitored prospectively using an in-house developed real-time quantitative PCR (qPCR) targeting the multicopy 18S ribosomal DNA gene (110 copies/genome). PCR screening was performed weekly from day -7 to

day +100 post-transplant; thereafter every 1 to 2 weeks until 6 months after transplant (or longer in case of ongoing immunosuppression. PCR was also applied to bronchoalveolar lavage (BAL) fluid, cerebrospinal fluid, vitreal fluid and tissue to diagnose toxoplasmosis.

***Toxoplasma* definitions**. Toxoplasmosis was defined according to the European Society for Blood and Marrow Transplantation (EBMT) definitions [11]. Briefly, *Toxoplasma* infection implied a positive blood PCR (with or without fever) but without evidence of organ involvement. Two consecutive positive blood PCRs were defined as "clinically significant" (as opposed to a single positive test followed by spontaneous clearance). *Toxoplasma* disease implied evidence of organ involvement. Proven disease implied histologic or cytological demonstration of tachyzoites in tissue samples obtained by an invasive procedure. Patients with clinical and radiological features of organ involvement plus at least one positive PCR result from blood, CSF, or BAL fluid and absence of other etiological pathogens had probable disease. Possible disease was defined by highly suggestive imaging features (cranial CT and/or MRI) of central nervous system (CNS) toxoplasmosis and response to anti-*Toxoplasma* therapy, but without supportive microbiological evidence. Cases were considered 'early' before day +30. Death was attributed to toxoplasmosis (1) in patients who did not respond to therapy (i.e., who had stable disease or disease progression) at time of death; (2) in patients with a partial response to therapy who died as the result of an acute event involving any of the sites of infection or of an unknown cause; and (3) in patients who died as a result of the toxicity of anti-*Toxoplasma* therapy.

Anti-*Toxoplasma* approach. Prophylaxis with TMP-SMZ was not given. In case of two consecutive positive blood PCRs, pre-emptive therapy with oral pyrimethamine (75-100 mg/day following a loading dose of 200 mg on the first day) and clindamycin (oral or parenteral; 600 mg 3 time daily) was started until 2 consecutive negative PCR tests (at least 5 days apart) were obtained (as per reference: [7]). Additional diagnostic work-up was requested as per clinical presentation. *Toxoplasma* disease was treated similarly until clinico-radiological resolution (minimum duration 6 weeks). Secondary prophylaxis with TMP-SMZ was not used in cases of resolved *Toxoplasma* infection. However, following

successful treatment of *Toxoplasma* disease, patients were started on TPM-SMZ 160/800 mg thrice weekly for as long as immunosuppression persisted and for as long as TMP-SMZ was tolerated.

Statistical analysis. Comparison between the reactivating (case) and non-reactivating (control) group was done using the chi-squared or Fisher test for categorical variables, and the t-test or Mann–Whitney U-test for continuous data. All calculated p-values are two-sided, and p-values less than 0.05 are considered statistically significant. The association between the suspected variables of interest were interpreted based on calculating an odds ratio. Logistic regression models were fit to calculate the odds ratios (OR) and the corresponding 95% CIs. A multiple logistic regression model was used for the multivariate analysis, including as covariates all variables associated with the dependent variable in univariate testing with a p-value of <0.05. Survival curves were made by Kaplan-Meier method, compared using the log-rank test. Landmark Cox proportional hazards regression models were used to determine overall survival of cases versus controls, to reduce the propensity for immortal time bias. The mean of the median onset of *Toxoplasma* disease and of *Toxoplasma* infection in days after transplantation, 59 days, was chosen as landmark. We also performed time-dependent Cox regression analysis to assess reproducibility of the results. Adjusted survival analysis was done with Cox proportional hazard regression, calculating hazard ratios (HR) and the corresponding 95% CIs. We adjusted for all covariates that came forward as significant in univariate analysis of the risk factors. Analyses were performed with the statistical software R (Version 1.3.1056). For the case-control study, possible cases were excluded given the uncertainty of the diagnosis of *Toxoplasma*-related diseases.

Results

T. gondii reactivation. We performed 1455 consecutive allo-HCTs, of which 680 (46.7%) were R^-/D^-. PCR monitoring was performed in the remaining 775, including 117 R^-/D^+ (8%) and 658 $R^+/D^{+/-}$ (45.2%) ones (Figure 1). A mean of 1.09 PCRs were performed weekly per case from transplantation until the first positive PCR, and a mean of 1.18 PCRs were performed weekly from transplantation until 6 months post-transplant or until death, whatever came first. The overall 1-year cumulative incidence of toxoplasmosis was 4.1% (59/1455). No cases were observed in the R^-/D^+ subgroup, whereas the 1-year incidence in the $R^+/D^{+/-}$ group was 8.5% (58/685, p-value=0.0016). Cumulative incidence in the $R^+/D^{+/-}$ group at day +30 was 2.2% (95% CI 1.1-3.3), at day +100 6.0% (95% CI 4.2-7.7) and at day +200 8.0% (95% CI 6.0-10.0). One patient of the R^-/D^- group was diagnosed with proven cerebral toxoplasmosis.

The median duration from transplantation to diagnosis of first episode of infection and disease was 40 days (range: 13-246 days) and 78 days (range: 25-382 days), respectively. At diagnosis, blood PCR was negative in 8 patients (13.6%), including 7 subjects with central nervous system involvement and 1 patient with chorioretinitis. Six of these 8 patients presented more than 3 months posttransplant. Toxoplasmosis beyond 6 months post-transplantation was observed in 5 patients.

First and subsequent episodes of *Toxoplasma* infection and disease. The characteristics of the 59 patients with toxoplasmosis are summarized in Table 2 (details are provided in the Supplement and Supplementary Tables 1 and 2).

Post-transplant toxoplasmosis in *Toxoplasma* seropositive recipients. On univariate analysis, toxoplasmosis was associated with second transplant (OR 2.51; 95% CI 1.28-4.94: p=0.011), MAC (OR 2.24; 95% CI 1.17-4.41: p=0.011), use of 10-12 Gy TBI (OR 2.29; 95% CI 1.17-4.44: p=0.010), acute GvHD (OR 2.27; 95% CI 1.26-4.08: p=0.008) and high dose corticosteroids (equivalent ≥16mg methylprednisolone during >2 weeks) (OR 2.08; 95% CI 1.14-3.78: p=0.018). On multivariate analysis only acute GvHD remained significant (p=0.021, adjusted OR 2.54; 95% CI 1.16-5.71).

At the time of toxoplasmosis, the median CD4-positive cell count of cases was 0.0460 x 10^9 cells/L (0-1.4). Median CD4-cell count of controls measured at the same time point after transplantation as the time of first PCR positivity in cases, was significantly higher: 0.13 x 10^9 cells/L (0-2.6) ($p<0.0001$) in univariate analysis. In multivariate analysis p-value was 0.41. CD8-positive cells were lower in cases (0.14 x 10^9/L (0-3.8)) versus controls (0.25 x 10^9/L (0-3.4)) (p=0.0059 in univariate analysis, p=0.44 in multivariate analysis). Differences in the levels of immunoglobulins in cases at the time of diagnosis compared to the same time point after transplantation in controls were not significant.

Probability of survival. The overall survival for cases at 1, 3, and 6 months after landmark was 85.5% (95% CI, 76.6%-95.3%), 76.2% (95% CI, 65.8%-88.4%) and 57.6% (95% CI, 45.9%-72.4%), respectively, and was significantly worse compared to controls (Figure 2, log-rank test, p=0.00067). The non-relapse mortality rate for cases was 43.9% versus 28.1% for controls (OR 2.0; 95% CI 1.047-3.785: p=0.026). After adjusting for covariates, a trend for toxoplasmosis as an independent risk factor for mortality was seen (adjusted HR 1.51; 0.065). The attributable mortality rate was estimated at 21.1% (12 cases). Functional sequelae in patients that survived *Toxoplasma* reactivation are summarized in Supplementary Table 3.

Discussion

The incidence of toxoplasmosis in allo-HCT recipients ranges from 6% in Europe to <0.5% in USA or Japan [12]. In one prospective study, 16% reactivated the infection over the first 6 months posttransplant and 6% developed disease [7]. Our overall incidence of 4.1% is in line with incidences reported by centres with a similar high seroprevalence while using TMP-SMZ prophylaxis [2,4,13,14]. This is remarkable since the absence of TMP-SMZ prophylaxis has previously been identified as a risk factor [14]. This raises the question whether TMP-SMZ regimens used to prevent *Pneumocystis* infections also effectively prevent toxoplasmosis. Indeed, various intermittent dosing regimens for *Pneumocystis* prevention have been studied; however, in countries with a high *T. gondii* seroprevalence, experts suggest daily dosing [15].

Despite the virtual absence of cases in the seronegative group, the rate of toxoplasmosis was almost 9% in the seropositive recipients. Should prophylaxis be given to all seropositive recipients, given a reported 11.6% case rate in seropositive recipients using TMP-SMZ prophylaxis [13]? At least the following questions should be addressed [16]. First, is toxoplasmosis a serious event? Yes, it is, given the related morbidity and the high attributable mortality rate. Second, is toxoplasmosis difficult to treat? Probably not, provided the disease is detected sufficiently early. Indeed, many fatal cases have been diagnosed too late or remained undetected pre-mortem. Third, is prophylaxis safe and well tolerated? Side effects of TMP-SMZ are manifold, including drug sensitivity rashes (mimicking cutaneous GvHD), gastro-intestinal intolerance, and myelosuppression. In one study, 49% of patients discontinued TMP-SMZ due to toxicity [17]. However, side effects largely depend on dose and duration, both of which are unknown with respect to an optimal prevention of toxoplasmosis. Fourth, is prophylaxis effective? The answer to that question remains largely unknown, as discussed before. In addition, its use is contra-indicated before marrow engraftment and alternative drugs are less effective [5].

Similar to cytomegalovirus (CMV) reactivation post-transplant, a PCR-based pre-emptive treatment has been suggested as an alternative approach. Indeed, blood usually becomes positive at

or before the onset of tissue invasive disease, allowing earlier therapy, and increasing parasite load appears to correlate with development of disease [7]. However, as also seen in our series, blood samples may test repeatedly negative, especially in cases of brain and/or eye involvement. Whether more sensitive assays can solve the problem of false-negativity remains to be investigated.

Although the overall survival of controls was significantly better than that of cases, 73% of cases – many with CNS involvement – was still alive at day +150, which is better than previously published [13,14,18,19]. This outcome may be party explained by the large number of patients with infection, not disease. Our study (and others [20]) also showed that pre-emptive therapy till two consecutive negative PCR results was not always sufficient. Indeed, 8 patients experienced a second episode, especially while receiving corticosteroids. At present, the optimal treatment duration for infection and disease, the need for secondary prophylaxis as well as the kinetics of immune reconstitution to *T. gondii* after allo-HCT are areas for future research and should consider individual components of the cellular immune system or treatment of GvHD.

Our approach relies on an in-house developed PCR assay targeting the 18S ribosomal DNA gene. As with other PCR-based pre-emptive strategies (e.g. CMV), diagnostic performance, sensitivity as well as specificity, may vary according to the techniques used for DNA extraction and amplification. So, pending standardization (and commercialization) of protocols for molecular diagnosis of toxoplasmosis in immunocompromised individuals and given the high related mortality rate, centers may prefer to continue prophylaxis with TMP-SMZ, especially in the highest risk groups (R^+). However, our data also show that a PCR-based pre-emptive approach can be used in patients unable to tolerate or to continue TMP-SMX.

Obviously, our study has limitations. Its retrospective design only allows us to make associations. In addition, many co-variables have changed over those 20 years, including the seroprevalence of the transplant population. However, although imperfect, we tried to intercept this

by using controls matched in time. Multiple testing in this case-control study could have caused a higher probability of type I error [21].

Given the rarity of the complication, it is unlikely that a strategic study will ever be done. However, novel therapeutic agents with excellent safety profiles and covering *Pneumocystis* though not *Toxoplasma* are potentially on the way. Rezafungin is currently being tested against an azole/TMP-SMZ combination for primary prophylaxis in allo-HCT [22]. If proven effective, approaches towards early detection and management of toxoplasmosis will be needed and PCR monitoring of seropositive recipients with pre-emptive therapy might be an attractive alternative.

In conclusion**,** toxoplasmosis after allo-HCT is an early complication of seropositive recipients only, especially when treated for acute GvHD. Although prophylaxis with TMP-SMZ has been recommended, questions remain regarding optimal dose and frequency, time of administration, duration of prophylaxis, and management of adverse events. A PCR-based approach allows for early diagnosis and treatment, which is likely to improve outcomes.

Contributors

Each author has made substantial contributions to the conception or acquisition of the study or to the analysis or interpretation of the data and substantively revised the work. We describe contributions to the paper using the CRediT taxonomy [23]. Writing & Original Draft: R.A. and J.M; Review & Editing: T.M., M.B., H.S. and K.L.; Conceptualization: J.M.; Investigation: R.A.; Methodology: R.A., K.L. and J.M.; Formal Analysis: R.A. and J.M. We wish to acknowledge the help provided by Margaux Delporte and Thomas Neyens from the Leuven Biostatistics and Statistical Bioinformatics Centre (L-BioStat) on consulting on our methods and analyses.

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Conflicts of interest

The Authors declare that there are no conflicts of interest.

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Table 1

Table 1: Characteristics of cases and controls

Characteristic	Toxo Cases (n=57)	Controls (n=228)	Univariate analysis p-value	Multivariate analysis p-value
Sex			0.88	
Male	35 (61.4%)	137 (60.1%)		
Female	22 (38.6%)	91 (39.9%)		
Age at Tx	Median 54.7 (18.5-76.9)	Median 56.8 (18.4-74.1)	0.30	
Number of CD34+ cells /kg (10^6) in graft	Mean 5.9 ± 2.5	Mean 6.7 ± 3.1	0.054	
Karnofsky score at Tx			0.42	
≥ 80	54 (94.7%)	221 (96.9%)		
Disease status at Tx				
CR	36 (63.2%)	120 (52.6%)	0.18	
PR	4 (7.0%)	21 (9.2%)	0.79	
Refractory	0	21 (9.2%)	*0.010*	
Relapse	10 (17.5%)	24 (10.5%)	0.17	
Previously untreated	7 (12.3%)	42 (18.4%)	0.33	
Underlying disease				
Acute leukemia	33	109	0.19	
Myelodysplastic syndrome	7	37	0.54	
Myeloproliferative disorder	3	22	0.43	
Lymphoproliferative disorder	10	30	0.40	
Multiple myeloma	4	11	0.51	
Aplastic anemia	0	6	0.60	
Other	0	13	0.078	
Previous HCT (allo-HCT + auto-HCT)			*0.011*	0.19
Yes	17 (29.8%)	33 (14.5%)		
Conditioning regimen			*0.011*	0.47
MAC	39 (68.4%)	112 (49.1%)		
TBI-containing	22 (38.6%)	49 (21.5%)	*0.010,*	0.21
GvHD prophylaxis				
Calcineurin inhibitor	51 (89.5%)	220 (96.5%)	*0.040*	0.3382
MMF	19 (33.3%)	132 (57.9%)	*0.0010*	0.1067

GvHD	34 (59.6%)	115 (40.4%)	0.24	
Acute	30 (52.6%)	75 (32.9%)	*0.0087*	*0.021*
Grade I-II	19 (33.3%)	50 (21.9%)		
Grade III-IV	11 (19.3%)	25 (11.0%)		
Chronic	4 (7.0%)	40 (17.5%)	0.063	
Chronic extensive	3 (5.3%)	28 (12.3%)		
Chronic limited	1 (1.8%)	12 (5.3%)		
High dose corticosteroids	36 (63.2%)	103 (45.2%)	*0.018*	0.62
Donor Toxo serostatus			0.75	
D-	31 (62.0%)	106 (58.6%)		
D+	19 (38.0%)	75 (41.4%)		
Unknown	7	47		
Donor type				
Haplo-identical	12 (21.1%)	21 (9.2%)	*0.019*	
HLA-identical	12 (21.1%)	84 (36.8%)	*0.028*	
Syngeneic	0	2		
Matched Unrelated	32 (56.1%)	116 (50.9%)	0.55	
Mismatched Unrelated	1 (1.8%)	7 (3.1%)	1	
Stem cell source				
Cord Blood	1 (1.8%)	4 (1.8%)	1	
Lymphocyte count day 30 ($x10^9$/L)	Median 0.4 (IQR 0.4)	Median 0.55 (IQR 0.6)	*0.0028*	0.62
Leukocyte engraftment (days)	Mean 16.5 ± 5.7	Mean 15.1 ± 6.0	0.11	
Neutrophil Engraftment (days)	Mean 15.8 ± 5.9	Mean 17.4 ± 7.8	0.16	
Platelet Engraftment (days)	Median 15.0 (range 0-216)	Median 15.0 (range 0-289)	0.41	
T-cell count at diagnosis + at the same time post-Tx ($x10^9$/L)				
CD4+	Median 0.046 (range 0-1.4)	Median 0.13 (range 0-2.6)	*<0.0001*	0.41
CD8+	Median 0.14 (range 0-3.8)	Median 0.25 (range 0-3.4)	*0.0059*	0.44
CMV reactivation	25 (43.9%)	76 (33.3%)	0.016	
CMV serostatus pre-Tx	25 negative	87 negative	0.4511	
EBV reactivation	29 (50.9%)	64 (28.1%)	*0.0015*	0.060
EBV serostatus pre-Tx	1 negative	6 negative	1	

Data are n (%) of patients, unless otherwise indicated. Variables were compared using Mann-Whitney U-test, chi-squared test or the Fisher exact test, where appropriate.

Toxo = toxoplasmosis, Tx = transplantation, CR = complete remission, PR = partial remission, allo-HCT = allogeneic hematopoietic cell transplantation, auto-HCT = autologous hematopoietic cell transplantation, MAC = myeloablative conditioning regimen, TBI = total body irradiation, GvHD = graft-versus-host disease, MTX = methotrexate, MMF = mycophenolate, high dose steroids = corticosteroids equivalent to >= 16mg methylprednisolon during >2 weeks, D- = seronegative donor, D+ seropositive donor, CD4+ = CD4 T-helper cells, CD8+ = CD8 T-helper cells, CMV = cytomegalovirus, EBV = Epstein-Barr virus

Table 2

Table 2: Aggregate data of all 59 cases

	Cases (n = 59)
Male Female	36 (61%) 23 (39%)
Age at transplantation (Tx) (years)	Median 55 (IQR 22)
BMI (kg/m²)	Mean 25 (SD 4.6)
Karnofsky score at Tx ≥ 80	56 (95%)
Underlying disease	
Acute leukemia	34 (58%)
Myelodysplastic syndrome	7 (11%)
Myeloproliferative disorder	4 (7%)
Lymphoproliferative disorder	10 (17%)
Multiple myeloma	4 (7%)
Disease status at Tx	
CR	37 (63%)
PR	4 (7%)
Relapse	10 (17%)
Refractory	1 (2%)
Untreated	7 (11%)
Previous Tx	18 (31%)
Blood group	
A-	4 (7%)
A+	23 (39%)
AB+	1 (2%)
B-	2 (3%)
B+	3 (5%)
O-	6 (10%)
O+	20 (34%)
Donor type	
Haplo-identical	13 (22%)
HLA-identical sibling	12 (20%)
Matched unrelated	33 (56%)
Mismatched unrelated	1 (2%)
CD34+ graft cell count	Mean 5.9 (10^6) /kg (SD 2.5)
Conditioning regimen	
Myeloablative	39 (66%)
Non-myeloablative	20 (34%)

Tx = transplantation, BMI = Body Mass Index, CR = complete remission, PR = partial remission, MAC = myeloablative conditioning regimen, NMAC = non-myeloablative conditioning regimen

Figure1

Figure legend

Figure 1: Overview of the incidence of post-transplant toxoplasmosis;

allo-HCT = allogeneic hematopoietic cell transplantation, toxo = toxoplasmosis;

* Prevalence of *Toxoplasma* in Belgium is estimated around 49% *[2]*.

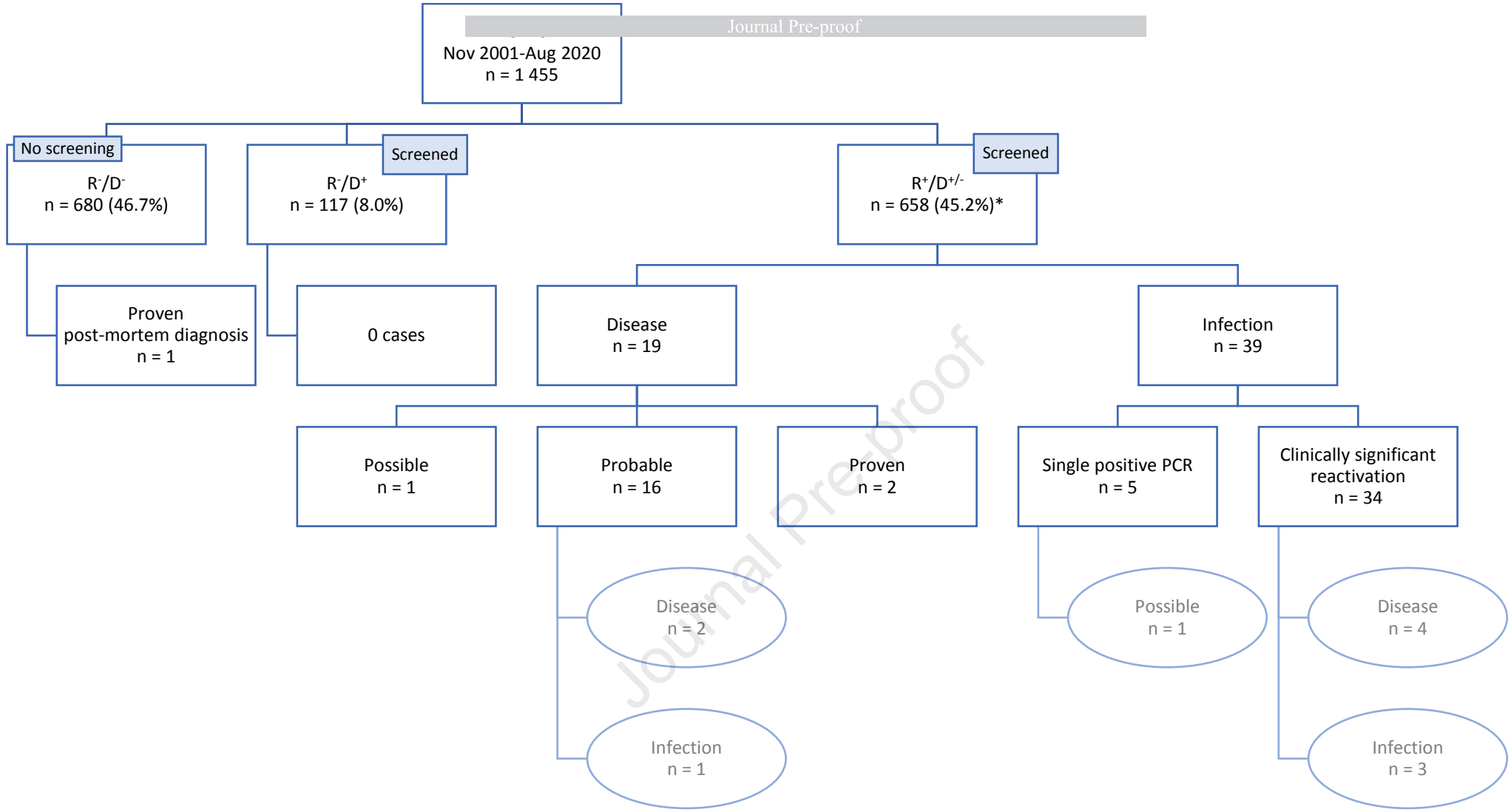

Figure 1

* Prevalence of *Toxoplasma* in Belgium is estimated around 49% *[2]*.

= Relapse case

Figure 2

Figure legend

Figure 2: Kaplan Meier Survival curve, based on Landmark Cox proportional hazards regression model: The overall survival after allo-HCT was significantly worse for cases compared to controls (p=0.00067). After adjusting for covariates, a trend for toxoplasmosis as an independent risk factor for mortality was seen (0.065).

allo-HCT = allogeneic hematopoietic cell transplantation.

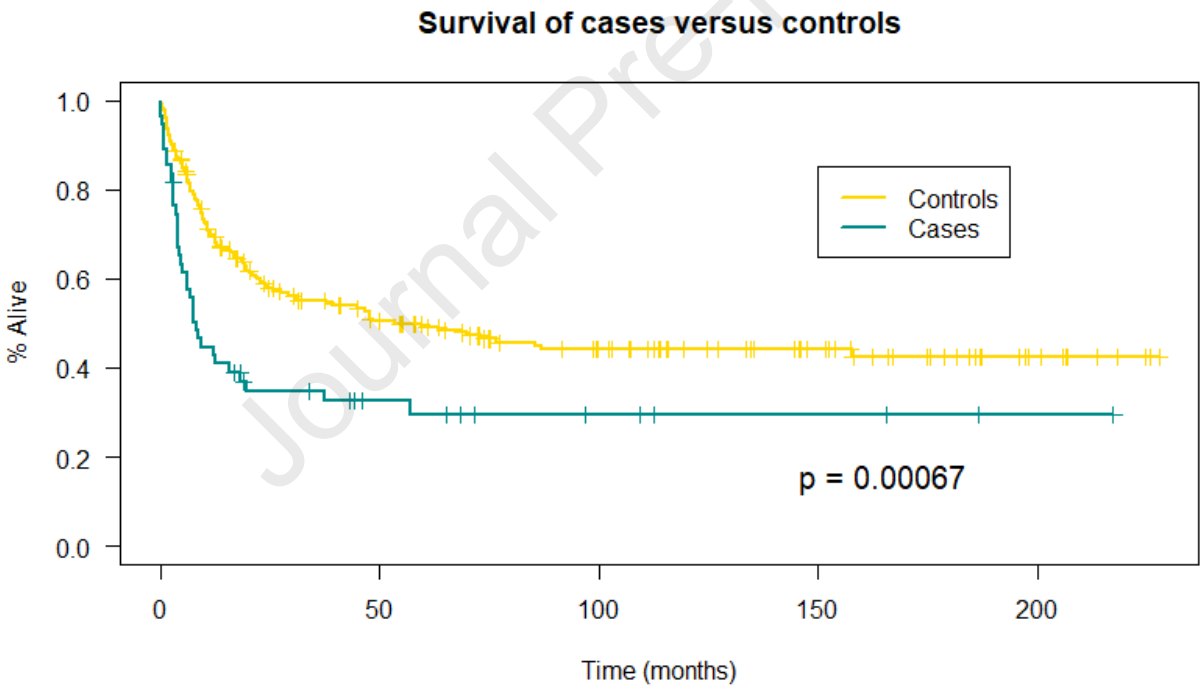

Figure 2