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ORIGINAL ARTICLE

Negative pretransplant serostatus for *Toxoplasma gondii* is associated with impaired survival after heart transplantation

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Summary

Chronic *Toxoplasma gondii* infection is known to trigger potentially adverse immunoregulatory changes, but limited data exist on long-term implications for heart transplant (HTX) recipients. We evaluated the risk of all cause mortality regarding *T. gondii* serostatus prior to HTX. Pre-HTX *T. gondii* serostatus was obtained in 344 recipients and 294 donors. Mean age was 52.1 ± 10.2 years and mean follow-up time after HTX was 5.7 (±5.5, median 3.5) years. All seronegative patients received prophylaxis with pyrimethamine/sulfomethoxazole or cotrimoxazol for 6 months after transplantation. Multivariate survival analysis adjusted for diabetes mellitus, pre-HTX renal function, recipient age, type of primary immunosuppression (i.e. HTX before 2001), cytomegalovirus (CMV) high-risk status, ischemic time, and number of treated rejection episodes was performed. Overall, 190 recipients (55.2% of total) were seronegative and 154 (44.8% of total) were seropositive for *T. gondii* prior to HTX. One hundred and fifty-two recipients died during follow-up (44.2% of total). Negative recipient Toxoplasma serostatus was associated with a significantly higher risk of all-cause mortality ($P = 0.0213$). Recipient *T. gondii* serostatus did not influence the number of cellular or humoral rejection episodes. Analyses of specific causes of death showed a trend toward a higher number of infection-related deaths in the seronegative subgroup ($P = 0.13$). No statistically significant effects of *T. gondii* donor/recipient seropairing, or seroconversion were observed. Negative preoperative serostatus for *T. gondii* in HTX recipients appears to be an independent risk factor associated with increased all-cause mortality. The cause of impaired survival in Toxoplasma seronegative recipients is currently unclear; possible explanations include an alteration of immunereactivity/-regulation or adverse effects of prophylactic medication.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite causing a lifelong infection. In immunocompetent subjects, Toxoplasmosis is usually benign or asymptomatic, but it may be associated with severe sequelae in immunocompromised subjects. Thus, Toxoplasmosis has been recognized as a clinical infectious complication in heart transplant (HTX) recipients (R), mandating prophylactic treatment in seronegative graft recipients, especially when receiving an organ from a seropositive donor, as this is generally followed by obligate infection of the host [1,2]. However, limited data exist on the implication of chronic infection and associated immunoregulatory changes for prognosis in HTX recipients. A balanced immune response is required to control *T. gondii* reactivation. Chronic parasite latency is maintained by an adaptive T-cell response involving interleukin

(IL)-12-driven interferon (IFN)-γ responses [3]. However, an excessively vigorous response can lead to pathologic effects, including endothelial cell activation, which is especially important in immunosuppressed patients after HTX [4]. Therefore, we examined the association between recipient *T. gondii* serostatus prior to HTX and mortality, development of cardiac allograft vasculopathy (CAV), and acute cellular and humoral rejection among HTX patients. We also determined whether donor serostatus and donor/recipient *T. gondii* seropairing were related to the occurrence of these endpoints.

Methods

Patient characteristics

Heart transplant was performed between July 1989 and May 2008. Detailed baseline characteristics for all 344 patients are shown in Table 1. Mean age was 52.1 years

Table 1. Baseline characteristics (n = 344).

Characteristics	Overall (n = 344)	Recipient *T. gondii* seronegative (n = 190)	Recipient *T. gondii* seropositive (n = 154)	P-value
Demographics (recipient)				
Recipient age (years ± SD)	52.1, 10.2	50.6, 10.6	53.9, 9.3	P = 0.02
Male recipient (% of group)	267 (77.6)	153 (80.5)	114 (74.0)	P = ns
Etiology for HTX				
Ischemic heart disease (% of group)	114 (33.1)	55 (28.9)	59 (38.3)	P = ns
Cardiomyopathy (% of group)	182 (52.9)	101 (53.2)	81 (52.6)	P = ns
Other (% of group)	48 (14.0)	34 (17.9)	14 (9.1)	P = ns
CMV serostatus				
Recipient CMV seropositivity (% of group)	176 (51.1)	90 (47.3)	86 (55.8)	P = ns
CMV mismatch (R–/D+) (% of group)	78 (22.7)	48 (25.2)	30 (19.5)	P = ns
Immunosuppressive therapy				
Cyclosporine A (% of group)	148 (43.0)	82 (43.2)	66 (42.9)	P = ns
Tacrolimus (% of group)	61 (17.7)	40 (21.1)	21 (13.6)	P = ns
Steroids (% of group)	135 (39.2)	82 (43.2)	53 (34.4)	P = ns
Azathioprine (% of group)	62 (18.0)	34 (17.9)	28 (18.2)	P = ns
Mycophenolate mofetil (% of group)	132 (38.4)	76 (40.0)	56 (36.4)	P = ns
Myfortic (% of group)	17 (4.9)	9 (4.7)	8 (5.2)	P = ns
Sirolimus (% of group)	10 (2.9)	3 (1.6)	7 (4.5)	P = ns
Everolimus (% of group)	44 (12.8)	20 (10.5)	24 (15.6)	P = ns
Cardiovascular medication				
Angiotensin-converting enzyme inhibitor/receptor antagonist (% of group)	336 (97.7)	185 (97.4)	151 (98.1)	P = ns
Statin (% of group)	340 (98.8)	188 (98.9)	152 (98.7)	P = ns
Cardiovascular risk factors				
Arterial hypertension (% of group)	196 (57.0)	106 (55.8)	90 (58.4)	P = ns
Diabetes mellitus (% of group)	71 (20.6)	40 (21.1)	31 (20.1)	P = ns
Current smoker (% of group)	0 (0.0)	0 (0.0)	0 (0.0)	P = ns
Biochemical data				
Total cholesterol (mg/dl ± SD)	183.9, 47.5	183.3, 45.0	184.6, 50.4	P = ns
HDL (mg/dl ± SD)	46.9, 15.6	47.8, 16.7	45.9, 14.1	P = ns
LDL (mg/dl ± SD)	101.4, 33.7	100.1, 29.0	103.0, 38.8	P = ns
Triglycerides (mg/dl ± SD)	155.8, 95.5	149.4, 94.5	163.8, 96.3	P = ns
Serum creatinine (mg/dl ± SD)	1.3, 0.5	1.3, 0.5	1.3, 0.5	P = ns
Hemoglobin (g/dl ± SD)	12.5, 2.0	12.6, 2.0	12.5, 1.9	P = ns
Leukocytes (/nl ± SD)	7.5, 4.5	7.5, 4.6	7.5, 4.4	P = ns
Thrombocytes (/nl ± SD)	242.8, 89.6	244.2, 95.2	241.0, 82.0	P = ns
Donor characteristics				
Gonor age (all donors) (years ± SD)	37.9, 16.5	37.8, 18.9	37.9, 13.0	P = ns
Male donor (% of group)	174 (50.6)	94 (49.5)	80 (51.9)	P = ns
Ischemic time (hours ± SD)	3.2, 1.0	3.2, 1.1	3.2, 0.9	P = ns

Data are reported as mean ± SD or n (% of group), as appropriate.
CMV, cytomegalovirus; HTX, heart transplantation; HDL, high density lipoproteins; LDL, low density lipoproteins; R/D, recipient/donor; SD, standard deviation.

(±10.2 years) and mean follow-up time for mortality was 5.7 years (range 0.0–18.9 ± 5.5, median 3.5 years). Mean donor age was 37.9 years (±16.5, median 39.0 years), 174 donors (D) were women (49.4% of total). Compared with recipients, donors were significantly younger ($P < 0.0001$). Mean ischemic time was 3.2 h (±1.0 h). Beside recipients' age ($P = 0.02$), there were no significant differences in demographics, immunosuppressive therapy and serological parameters between *T. gondii* seropositive and seronegative recipients (Table 1). An azathioprine (AZA) containing immunosuppression was replaced by a mycophenolate mofetil (MMF) based immunosuppressive regimen at the Heidelberg HTX center in 2001.

Patient serologic testing

Three hundred and forty-four HTX recipients and 294 donors at our center underwent evaluation for *T. gondii* serostatus, using an inhibition-competition enzyme immunoassay with a final fluorescent detection as a screening test [IgM and IgG: enzyme-linked fluorescent assay (ELFA): Vidas™ Toxo Compétition (TXC), bioMérieux SA, Lyon, France]. In case of a positive screening result, ELFAs for specific immunoglobulin detection [IgM: Vidas™ Toxo IgM (TXM), bioMérieux SA, Lyon, France; IgA: ETI-TOXOK-A reverse PLUS, DiaSorin S.p.A., Saluggia (Vercelli), Italy] and indirect immunofluorescence tests (IIF, Toxo-Spot IF, bioMérieux SA, Lyon, France) for determination of IgG titre were used. All tests were performed according to previously published methods [5] according to the manufacturers' instructions. During the late phase of the observation period (beginning in 2004), toxoplasma PCR was performed in cases of suspected opportunistic infection ($n \approx 20$), which were always negative.

Prophylaxis regimen

All seronegative patients received prophylactic treatment for 6 months after transplantation with pyrimethamine (50 mg/day)/sulfomethoxazole [4 g/day, plus folate supplementation (1989–2005)] or cotrimoxazol (800 mg/day)/trimethoprim (160 mg/day) from 2005 until the end of the observation period. No alternative prophylactic strategies were used.

Definition of endpoints

Mortality

Survival data were available from the local HTX database in all patients. As described previously [1], cause of death was considered to be CAV based on clinical history, examination, and investigation findings consistent with the diagnosis of myocardial infarction/heart failure as cause of death in a patient known to have significant CAV, sudden death occurring in a patient known to have significant CAV or autopsy findings confirming CAV as cause of death.

Cardiac allograft vasculopathy

Patients were evaluated annually by coronary angiography during the first 2 years post HTX and then annually in case of manifest CAV and 5 years post HTX if no CAV was detected during the first 2 years. The classification used by Costanzo *et al.* [6] was applied to grade the CAV as mild, moderate, or severe on the basis of left main stem involvement, primary vessel stenoses, and branch stenoses.

Acute cellular and antibody-mediated rejection

Results of endomyocardial biopsies were obtained for all HTX-patients. Biopsies were performed upon clinical indication and by protocol at the following intervals: weekly during the first month after HTX, once a month from months 2–6, every other month from months 6–12, every 6 months in the second year, and then during routine examinations at years 3, 5, and 10. Starting in 2005, in case of hemodynamic changes (deterioration of left ventricular ejection fraction, dyspnea, deterioration of echocardiographic tissue doppler-parameters, new bundle branch block) in the absence of cellular rejection, biopsies were analyzed for detection of antibody-mediated rejection (including endothelial swelling, CD68-positive monocytes in the vascular wall, and endothelial C4d and complement deposits). Older samples were not retrospectively analyzed.

Statistical analysis

Analysis was performed using the SPSS statistical software (version 14.0, SPSS Inc., Chicago, IL, USA), and a two-sided P value of <0.05 was considered statistically significant. Student t-test was used for normally distributed variables and the Mann–Whitney test for other variables. Categorical variables were compared using the chi-square test. Separate Kaplan–Meier analyses with multivariate COX-regression analyses were performed for the survival.

Results

Toxoplasma serostatus

Overall, 190 recipients (55.2% of total) were seronegative and 154 (44.8% of total) were seropositive for *T. gondii* prior to HTX. No significant changes were observed in the proportion of Toxoplasma seronegative recipients in different eras of transplantation (1989–2000: 52.2% of

subgroup, vs. 2001–2008: 59.6% of subgroup, $P = 0.18$). Regarding donor *T. gondii* serostatus, an era effect was seen (1989–2000: 4.5% of subgroup seropositive, vs. 2001–2008: 36.2% of subgroup seropositive, $P < 0.0001$). Additionally, donors were statistically significantly younger before 2001 (1989–1999: mean 34.7 ± 12.7 years, median 37.9 years vs. 2001–2008: mean 42.3 ± 19.9 years, median 42.3 years, $P < 0.0001$). A statistically significant correlation of Toxoplasma serostatus with recipient age was found (mean recipient age Toxoplasma positive 53.9 ± 9.3 years, mean recipient age Toxoplasma negative 50.6 ± 10.6 years, $P = 0.002$).

Recipient *Toxoplasma gondii* serostatus and mortality

In total, 152 recipients died during follow-up (44.2% of total). Actuarial survival analysis (Kaplan–Meyer plot) confirmed impaired survival in the *T. gondii* seronegative patient cohort (Fig. 1a). Relevant demographic, clinical and serological risk factors for post-transplant survival (from previous own analyses and literature search) were tested by univariate analysis: diabetes mellitus ($P = 0.011$), ischemic cardiomyopathy versus nonischemic cardiomyopathy (P = ns), pre-HTX renal function assessed by MDRD below 62.9 ml/min × 1.73 m^2 (median, $P = 0.1011$), recipient/donor age ($P = 0.0086$/P = ns) and gender (P = ns/P = ns), racial and gender mismatch (P = ns/P = ns), type of primary immunosuppression (AZA/MMF based, i.e. HTX before 2001, $P < 0.0001$), Panel Reactive Antibodies in percent (P = ns), human leukocyte antigen mismatch (P = ns), ischemic time ($P = 0.0123$), cytomegalovirus (CMV) (D+/R−) ($P = 0.0042$), pulmonary artery resistance (P = ns), number of rejections requiring treatment ($P = 0.0863$). Risk

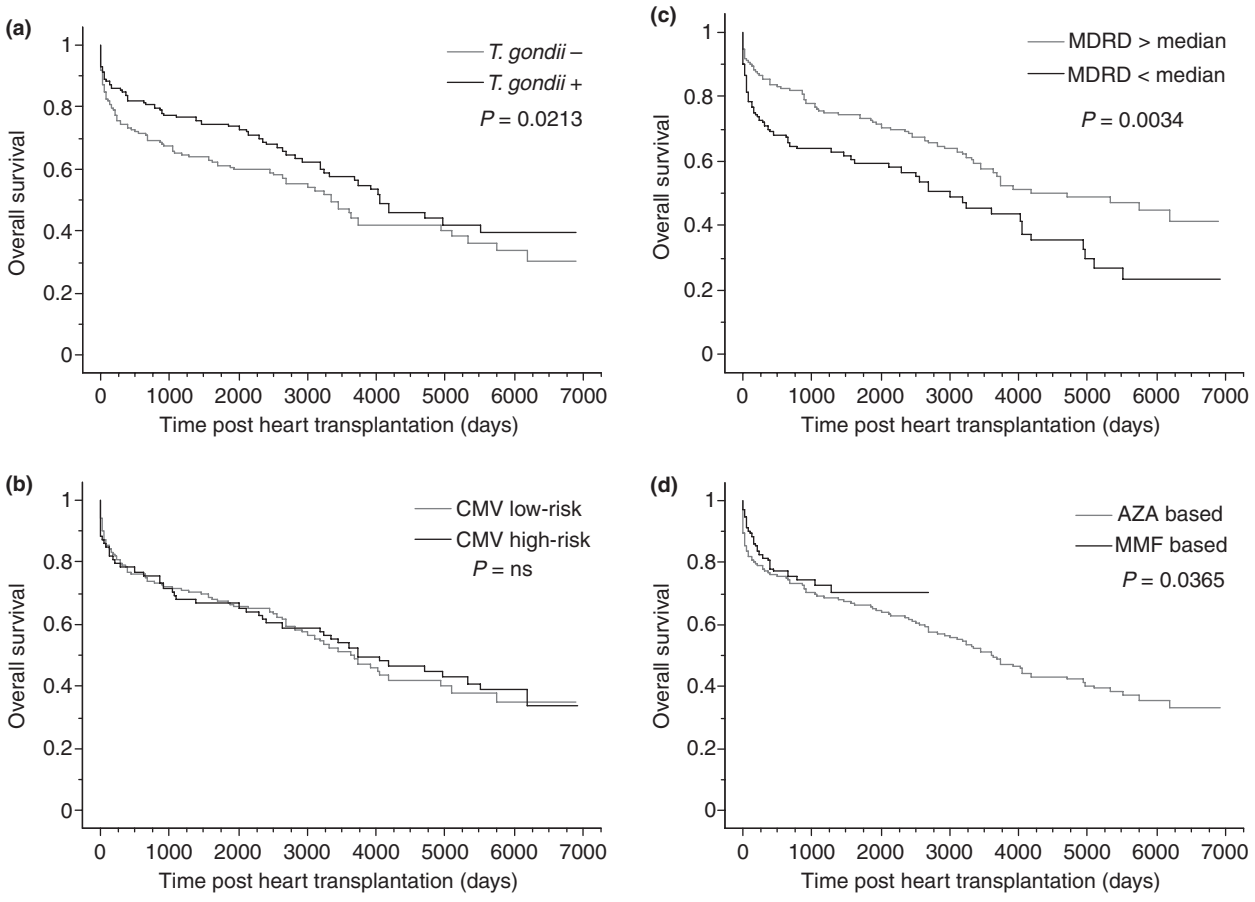

Figure 1 (a) Survival according to recipient *Toxoplasma gondii* serostatus at time of heart transplantation: seronegative recipients are shown in grey and seropositive recipients in black. (b) Survival according to cytomegalovirus (CMV) risk status at time of heart transplantation: low-risk patients are shown in grey and CMV D+/R− patients in black (P = ns). (c) Survival according to Modification of Diet in Renal Disease (MDRD) above/below median at time of heart transplantation: recipients with an MDRD above median are shown in grey and recipients with an MDRD below median in black ($P = 0.0034$). (d) Survival according to primary immunosuppression [mycophenolate mofetil (MMF)/azathioprine (AZA) based, $P = 0.0365$].

Table 2. Multivariate regression analysis (Cox-proportional hazard analysis) of survival risk in all study patients including parameters with $P \leq 0.2$ in univariate analysis.

Variable	*P*-value
MDRD <60 ml/min/1.73 m^2	0.0034
Recipient *T. gondii* serostatus	0.0213
Recipient age	0.0224
Diabetes mellitus	0.0240
AZA versus MMF based immunosuppression	0.0365
No. rejection episodes requiring therapy	0.0400
Ischemic time	0.1965
CMV high-risk	0.8107

AZA, azathioprine; MMF, mycophenolate mofetil.

factors showing an impact on post-HTX survival by univariate analysis or at least a strong trend ($P \leq 0.2$) were subsequently included in multivariate analysis.

Negative recipient Toxoplasma serostatus was an independent risk factor associated with a significantly higher all-cause mortality ($P = 0.0213$) (Fig. 1a, Table 2). Conditional survival analysis (including only patients surviving to >30 days post HTX) revealed similar results ($P = 0.02$). Further analysis revealed that CMV high-risk status did not influence overall survival ($P = 0.8107$, Fig. 1b), whereas MDRD below median ($P = 0.0034$, Fig. 1c), diabetes ($P = 0.0240$), recipient age ($P = 0.0224$), and number of previous treated rejection episodes ($P = 0.0400$) were independent risk factors associated with a significantly impaired overall survival. Additionally, a primarily AZA based immunosuppression (i.e. HTX before 2001, $P = 0.0365$, Fig. 1d) was an independent risk factor regarding overall survival. Detailed analysis regarding specific causes of death showed a trend toward a higher number of infection-related deaths in the seronegative subgroup ($P = 0.13$, Logrank test) (Table 3). In 14 patients (4.1% of total), death was attributed to CAV; when considering CAV mortality only, recipient *T. gondii* serostatus was no longer an independent risk factor ($P = 0.88$, Logrank test).

Toxoplasma gondii seropositivity and CAV

Data regarding development of CAV were available in 270 patients. In this subgroup, median follow-up time until initial diagnosis was 4.8 years (range: 0.2–17.7 years, mean 5.6 ± 4.7 years). Forty-seven patients (17.4% of subgroup) developed CAV with a stenosis of at least 25% of vessel diameter. *Toxoplasma gondii* seronegative recipients ($P = 0.11$) and patients with seropositive donors ($P = 0.99$) were not at significantly higher risk of CAV development. There was no significant difference regarding CAV incidence among the 4 D/R seropairing groups. Similarly, CMV infection did not influence the risk of CAV development (all P = ns).

Toxoplasma gondii serostatus and acute cellular and humoral rejection

During follow-up after HTX, 0.28 ± 0.58 treated cellular rejections per year were observed. No statistically significant differences in *T. gondii* seropositive versus seronegative recipients were seen (0.27 ± 0.52 vs. 0.29 ± 0.62 rejections per year, P = ns). Accordingly, during the first 2 years after HTX, 0.43 ± 0.56 vs. 0.41 ± 0.52 rejections per year were observed (P = ns). The number of humoral rejections (analyzed since 2005) showed no statistically significant differences (P = ns). Similarly, there was no significant difference in the number of treated rejection episodes among the 4 D/R seropairing groups (P = ns).

Table 3. Specific causes of death.

Specific cause of death	overall (n = 344, % of subgroup)	Recipient *T. gondii* seronegative (n = 190, % of subgroup)	Recipient *T. gondii* seropositive (n = 154, % of subgroup)	*P*-value (Logrank)
Early graft loss	24 (7.0)	14 (7.4)	10 (6.5)	0.75
Infection	37 (10.8)	24 (12.6)	13 (8.4)	0.13
Acute rejection	3 (0.9)	3 (1.6)	0 (0.0)	n.a.
CAV	14 (4.1)	7 (3.7)	7 (4.5)	0.94
Lymphoma	11 (3.2)	6 (3.2)	5 (3.2)	0.73
Malignancy	13 (3.8)	5 (2.6)	8 (5.2)	0.43
Other causes (neurologic, renal failure, hemorrhage, respiratory failure, multiple organ failure)	33 (9.6)	21 (11.1)	12 (7.8)	0.17
Unknown	17 (4.9)	9 (4.7)	8 (5.2)	0.84

CAV, cardiac allograft vasculopathy.

Donor *Toxoplasma gondii* serostatus and mortality

Donor *T. gondii* serostatus was available in 294 patients. Of 294 donors, 244 (83.0% of subgroup) were *T. gondii* seronegative and 50 (17.0% of subgroup) were seropositive. There were 121 (49.6% of subgroup) and 10 (20.0% of subgroup) deaths in these two groups respectively ($P = 0.16$, Logrank-test, Fig. 2a). In multivariate analysis including donor and recipient serostatus, donor serostatus was not an independent risk factor for overall survival ($P = 0.15$). In donors with available *T. gondii* serostatus, mean donor age was 37.2 ± 14.8 years. A strong trend toward a higher mean age in seropositive donors was observed (seropositive: 39.9 ± 12.4 years, seronegative: 36.6 ± 15.2 years, $P = 0.06$). When considering donor and recipient seropairing status, 20 HTX-patients (6.8%) were D+/R+, 30 (10.2%) D+/R−, 115 (39.1%) D−/R+, and 129 (43.9%) D−/R−. Kaplan–Meier analysis showed no statistically significant difference in survival between the four donor/recipient seropairing groups ($P = 0.34$, Logrank-test, Fig. 2b).

Effects of Toxoplasma Seroconversion after HTX

In all, 190 patients were tested seronegative for *T. gondii* prior to HTX. Out of these patients, follow-up tests regarding Toxoplasma serostatus were available in 125 patients. Seventy-nine patients (63.2% of subgroup) remained Toxoplasma seronegative and 46 (36.8% of subgroup) patients became Toxoplasma seropositive during follow-up in the presence of prophylaxis. No clinically overt Toxoplasma infection occurred in any of the patients who were seronegative prior to HTX. Out of these patients, 61 patients (48.8% of subgroup) died during follow-up. In patients remaining *T. gondii* seronegative, 37 of 79 patients died (46.8% of subgroup), and in seroconverting patients 24 of 46 patients died (52.2% of subgroup) (log rank test: $P = 0.92$, Fig. 2c, Table 4). In a further multivariate analysis including diabetes, recipient age, primary immunosuppression (AZA/MMF), MDRD 62.9 ml/min/1.73 m^2 or below, CMV high-risk status, ischemic time, and number of rejections episodes requiring therapy, Toxoplasma seroconversion after HTX was not an independent risk factor ($P = 0.89$).

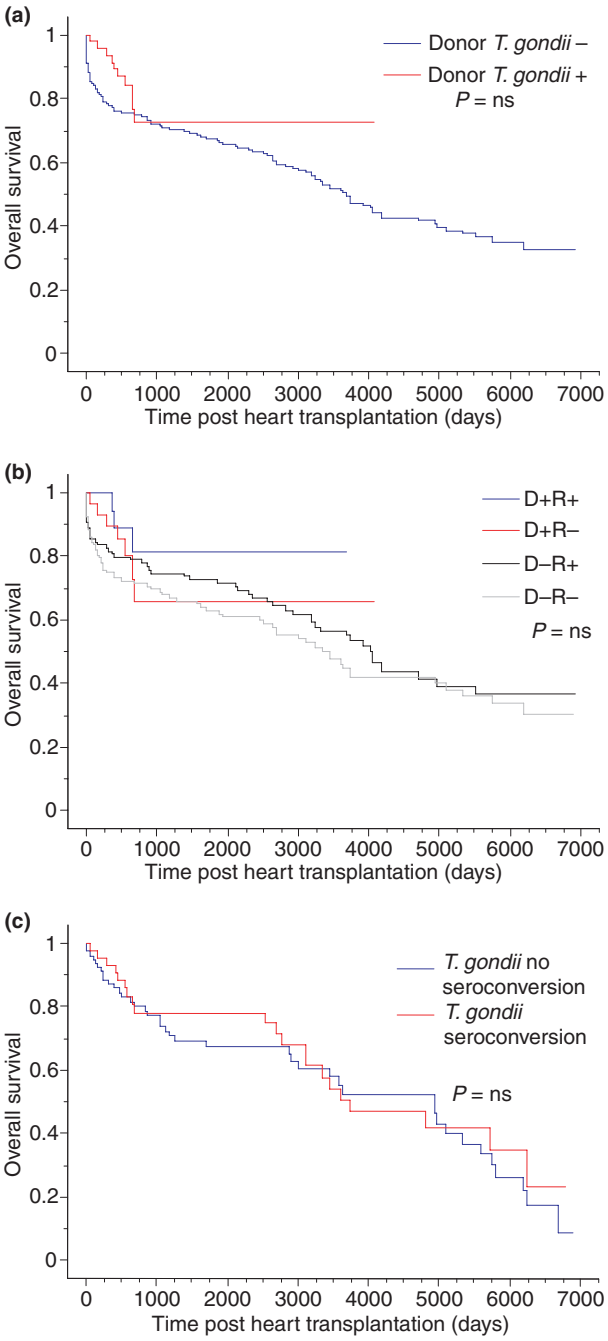

Figure 2 (a) Survival according to donor *Toxoplasma gondii* serostatus at time of heart transplantation: seronegative recipients are shown in blue and seropositive recipients in red. (b) Survival according to status of donor (D)/recipient (R) *Toxoplasma gondii* seropairing at time of heart transplantation: D+R+ blue, D+R− red, D−R+ black, D−R− grey. (c) Survival according to recipient *Toxoplasma gondii* seroconversion (in initially seronegative patients with available follow-up testing, $n = 125$): not seroconverting recipients are shown in blue and seroconverting recipients in red.

Discussion

The present study demonstrates that negative pretransplantation serostatus for *T. gondii* in heart allograft recipients is associated with an adverse outcome after HTX. In contrast to previously published data [1], a significantly impaired overall survival was observed in seronegative recipients, with a trend toward a higher risk of infection-

Table 4. Specific causes of death in seronegative follow-up patients (n = 125).

Specific cause of death	Seroconverting recipients (n = 46, % of subgroup)	Nonseroconverting recipients (n = 79, % of subgroup)
Early graft loss	3 (6.5)	4 (5.1)*
Infection	6 (13.0)	13 (16.5)*
Acute rejection	1 (2.2)	1 (1.3)*
CAV	3 (6.5)	1 (1.3)*
Lymphoma	2 (4.4)	2 (2.5)*
Malignancy	0 (0)	2 (2.5)*
Other causes (neurologic, renal failure, hemorrhage, respiratory failure, multiple organ failure)	7 (15.2)	8 (10.1)*
Unknown	2 (4.4)	6 (7.6)*

*All P = ns (Logrank).
CAV, cardiac allograft vasculopathy.

related deaths. The relationship between recipient *T. gondii* seronegativity and all-cause mortality may have several explanations. Recipient seronegativity against *T. gondii* may be attributed to a generally impaired immune status in transplant recipients with heart failure, manifested by loss of protective antibodies against *T. gondii*. In these already immunocompromised patients, additional therapeutic immunosuppression given after transplantation might be excessive, and could explain the trend toward a higher number of infection-related deaths. Additionally, anti-Toxoplasma prophylaxis given in seronegative recipients might negatively influence renal function or hematologic parameters; however, no deleterious effects of prophylaxis on these parameters were observed in another currently published study [1].

Alternatively, impaired survival in seronegative recipients may be caused by adverse immunologic effects of seroconversion despite prophylaxis. However, further subgroup analysis did not demonstrate a statistically significant negative effect of Toxoplasma seroconversion after HTX. Multivariate analysis revealed other parameters like MDRD below median, recipient age, diabetes mellitus, number of treated rejection episodes, and a primarily AZA based immunosuppression (i.e. HTX before 2001) as further independent risk factors for actuarial survival. Interestingly, impaired overall survival in seronegative patients cannot be attributed to infection with *T. gondii*, as no overt Toxoplasma infection was observed in any of the patients that were seronegative prior to HTX, and in the majority of *T. gondii* seronegative patients, no seroconversion was seen. Additionally, our data show no association between *T. gondii* serostatus and renal function, diabetes mellitus, arterial hypertension and preoperative diagnosis. Therefore, the importance of recipient *T. gondii* serostatus regarding long-term outcome is still unclear, warranting future large multi-center analyses.

The present study shows that in contrast to recipient *T. gondii* serostatus, donor *T. gondii* serostatus had no effect on overall survival. The lower percentage of seropositive donors, especially until 2001, may be attributed to the generally younger donor age in this era. In addition, the sensitivity and completeness of testing for *T. gondii* serostatus in donors are difficult to ascertain in earlier transplants (before 1996); therefore, the low percentage of positive donors has to be analyzed with caution.

For reasons of the therapeutic immunosuppression after HTX, we also speculated whether D+/R− patients are at an additional risk of mortality, but in-line with currently published data by Arora *et al.* [1], analysis of mortality according to D/R seropairing status demonstrated no significantly higher risk of all-cause death in any seropairing group. Furthermore, our data show that donor and recipient seropairing status is no risk factor for cellular and humoral rejection episodes, or development of CAV. Additionally and in contrast to previously published data [1], the present study did not demonstrate an association between recipient serostatus and development of advanced CAV. This may be because of exclusive angiographic assessment of CAV; intravascular ultrasound would have allowed a more detailed assessment of CAV and could be used in future studies measuring the inflammatory response in *T. gondii* seropositive and seronegative recipients.

Conclusions

These findings suggest that *T. gondii* seronegativity among HTX recipients is associated with a significantly increased risk of all-cause mortality with a trend toward a higher number of infection related deaths. The underlying reason for the observed higher all-cause mortality among seronegative recipients is currently unclear, warranting more detailed, multi-center analyses of the usefulness of *T. gondii* prophylaxis in HTX recipients.

Authorship

Andreas O. Doesch, MD: Designed the study, collected and analyzed data, drafted the manuscript; Kerstin Ammon: Collected data; Mathias Konstandin, MD: Collection/interpretation of data; Sultan Celik, MD, Arnt Kristen, MD, Lutz Frankenstein, MD and Susanne Müller: Collected data; Falk-Udo Sack, MD: Data interpretation; Hugo A. Katus, MD: Final approval; and Thomas J. Dengler, MD: Designed the study, data interpretation, final approval.

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