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Toxoplasma gondii Serostatus Is Not Associated With Impaired Long-Term Survival after Heart Transplantation

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Background. Conflicting data have been reported about the effect of *Toxoplasma* serostatus on mortality after heart transplantation. Either a positive or a negative recipient *Toxoplasma* serostatus was found to be associated with increased mortality.
Methods. We evaluated the effects of *T. gondii* infection on survival of our 582 cardiac allograft recipients operated upon between June 1984 and July 2011.
Results. The 258 *Toxoplasma* seronegative and 324 seropositive recipients differed in age, pretransplantation diagnosis, ischemia time, renal function, donor *Toxoplasma* serology, and maintenance immunosuppression. After a median follow-up time of 8.3 years (range, 0–26 years), 117 (45%) seronegative and 219 (67%) seropositive patients died. No difference was found in deaths due to cardiac allograft vasculopathy. After adjustment for all relevant clinical characteristics, the recipient *Toxoplasma* serostatus was not associated with mortality (hazard ratio, 1.21; 95% confidence interval [CI], 0.95–1.54). With the *Toxoplasma* serostatus combination donor negative/recipient negative as a reference, univariate hazard ratios for the *Toxoplasma* serostatus combinations were D+/R- 0.52 (95% CI, 0.37–0.73), D-/R+ 0.65 (95% CI, 0.40–1.05), and D+/R+ 0.78 (95% CI, 0.57–1.07). Multivariate analysis, however, showed that donor *Toxoplasma* serostatus was not independently associated with mortality.
Conclusions. The *Toxoplasma* serostatus of both the recipient and donor appeared not to be independent risk factors for mortality after heart transplantation.

Keywords: Heart transplantation, Toxoplasmosis, Cardiac allograft vasculopathy, Mortality.

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Until now, two studies investigated whether the long-term survival after heart transplantation is affected by the *Toxoplasma* serostatus of the recipient and the donor (*1, 2*). These studies, however, yielded conflicting results as to the recipient serostatus. Arora et al. reported that a *Toxoplasma* seropositive status in heart transplant recipients was associated with an increased risk of all-cause mortality as well as risk of death by cardiac allograft vasculopathy and Doesch et al. reported that a *Toxoplasma* seronegative status was associated with an increased mortality risk (*1, 2*). In both studies, no association of donor serostatus and mortality was found.

Toxoplasma gondii is an obligate intracellular protozoan parasite with a worldwide distribution that can invade and replicate in almost all nucleated cells of warm-blooded mammals. After the primary acute infection, the parasite remains in the body in a quiescent state by the formation of tissue cysts in predominantly muscle and brain tissue. Therefore, toxoplasmosis is a lifelong infection and the prevalence of human *T. gondii* infections thus increases with age. Its prevalence is reported to be more than 50% in many parts of the world (*3*). In immunocompetent individuals, the infection is usually asymptomatic, although cases of severe disease due to unusual *T. gondii* genotypes from South and Central America have

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TABLE 1. Characteristics of all 582 cardiac allograft recipients

	Toxoplasma serostatus		
	Negative	Positive	*P*
Number of patients, n (%)	258 (44)	324 (56)	
Recipient			
Female gender, n (%)	81 (31)	61 (19)	0.0001
Age (years; mean±SD)	41±16	51±10	<0.0001
<19, n (%)	37 (14)	5 (1.5)	<0.0001
19–59, n (%)	204 (79)	268 (83)	0.26
>59, n (%)	17 (7)	51 (16)	0.0006
Pretransplantation diagnosis			
Cardiomyopathy, n (%)	142 (55)	131(40)	0.0006
Ischemic heart disease	103 (40)	179 (55)	0.0002
Failure of first graft	2	3	NS
Other	11 (4)	11 (3)	NS
Prior cardiac surgery	67 (26)	77 (24)	0.30
PRA >0 (number of patients)	76 (31)	99 (32)	0.87
Long-term LVAD	9 (3)	5 (2)	NS
CMV seronegative	121 (47)	140 (43)	0.39
CMV mismatch (D+/R-)	58 (22)	69 (21)	0.76
Serum creatinine pre-Tx (L; µmol/L mean±SD)	110±42	125±52	<0.0001
Diabetes mellitus pre-Tx, n (%)	17 (6)	16 (5)	0.30
Donor			
Female gender, n (%)	129 (51)	144 (44)	0.17
Age (years; mean±SD)	32±14	33±13	0.48
<51, n (%)	230 (91)	282 (88)	0.46
>50, n (%)	24 (9)	37 (12)	0.42
Cause of death, n (%)			
Trauma	94 (41)	138 (48)	0.11
Hemorrhagic stroke	116 (51)	139 (48)	0.63
MM AB+DR >4, n (%) of patients	121 (48)	167 (52)	0.26
Toxoplasma seropositive, n (%)	105 (41)	85 (26)	0.003
Toxoplasma serostatus unknown	29 (11)	126 (38)	<0.0001
Operation			
Ischemia time (min; mean±SD)	180±44	171±43	0.02
Postoperative course			
Induction therapy, n (%)	202 (78)	244 (76)	0.52
Maintenance immunosuppression, n (%)			
Cyclosporine based	117 (48)	203 (68)	<0.0001
Tacrolimus based	133 (52)	99 (32)	<0.0001
+MMF	64 (26)	54 (18)	0.02
Statin started immediately post-Tx	123 (48)	123 (38)	0.05
Acute rejection			
0 episodes, n (%) of patients	58 (22)	79 (25)	0.59
>2 episodes, n (%) of patients	55 (21)	82 (25)	0.26
CMV disease, n (%)	33 (14)	60 (20)	0.06
Treatment for hypertension at 1 year	160 (62)	228 (70)	0.09
Diabetes mellitus post-TX	59 (23)	80 (25)	0.84
Serum creatinine at 1 year (µmol/L; mean±SD)	127±66	145±67	0.003
Cholesterol at 1 year (mmol/L; mean±SD)	6.4±5.5	6.4±2.3	0.82
Triglycerides at 1 year (mmol/L, mean±SD)	3.6±1.4	2.3±1.2	0.12
CAV at 1 year n (%) of resp. 230/280 CAG	19 (8)	26 (9)	0.75
CAV at 4 years n (%) of resp. 171/218 CAG	25 (15)	36 (17)	0.78

been reported (*4*). In immunocompromised patients, however, toxoplasmosis can be a severe disease (*3*).

Solid-organ transplant recipients are immunocompromised due to the required high dose of immunosuppressive medication. Heart transplant recipients are known to be at risk for toxoplasmosis, especially when recipients are seronegative (and thus naïve for *T. gondii*) and receive a heart transplant, containing tissue cysts, from a *T. gondii*–infected donor (*5*). In these cases, prophylactic treatment is indicated to prevent toxoplasmic myocarditis and fatal disseminated toxoplasmosis (*5, 6*). Also, antibiotic drugs, administered prophylactically to *Toxoplasma* mismatched recipients, could have a direct or indirect immunomodulatory effect and could negatively influence patient outcome by their side effects (*7*).

Cardiac allograft vasculopathy, a process in which chronic inflammation and immune activation play an important role, is a major cause of death after heart transplantation (*8, 9*). A *T. gondii* infection might influence the host immunologic status and thereby affect the risk for cardiac allograft vasculopathy. Therefore, the effects of a *T. gondii* infection on the survival after heart transplantation have been subject of study. Because of the earlier conflicting results of studies, which involved rather small numbers of patients and a limited follow-up duration, we performed a study on our whole population of heart transplant recipients to evaluate the effects of a *T. gondii* infection in the recipient and/or the donor on survival after heart transplantation.

RESULTS

Between June 1984 and July 2011, a total of 582 heart transplants have been performed in 577 patients. Recipients with a retransplant have been assessed as separate cases. Follow-up was complete in all cases. The *Toxoplasma* serostatus of the 582 cases was determined and Table 1 shows the characteristics of cardiac allograft recipients according to their pretransplantation *Toxoplasma* serostatus. The 324 *Toxoplasma* seropositive cases were 10 years older than the 258 seronegative cases, suffered more often from ischemic heart disease, and had worse renal function. Due to missing serum samples, the serostatus of 155 donors was unknown. One hundred five (41%) of the *Toxoplasma*

seronegative recipients and 85 (26%) of the *Toxoplasma* seropositive recipients received the heart of a seropositive donor.

The use of induction immunosuppression was not different between *Toxoplasma* seropositive and seronegative cases, but the *Toxoplasma* seropositive recipients received more often cyclosporine instead of tacrolimus for maintenance immunosuppression (Table 1). Early statins were more often administered to *Toxoplasma* seronegative patients. During follow-up, there were no differences in the number of rejection episodes, in the occurrence of cytomegalovirus (CMV) disease, or in treatment of hypertension and diabetes mellitus. The prevalence of cardiac allograft vasculopathy, shown at routine angiography after 1 and 4 years, was similar in both groups.

Mortality and *T. gondii* Seropositivity

During a median follow-up time of 8.3 years (range, 0–26 years) 336 transplant recipients died, of whom 219 (65%) were *Toxoplasma* seropositive and 117 (35%) were *Toxoplasma* seronegative. Long-term cumulative survival of the (unadjusted) *Toxoplasma* seropositive patients appeared worse than that of the *Toxoplasma* seronegative patients (Fig. 1). Causes of death were comparable between the *Toxoplasma* seropositive and seronegative recipients (Table 2). Especially, no difference was found in deaths due to cardiac allograft vasculopathy defined as late cardiac death. (Table 2). This applied to all recipients as well as to the 4-year survivors.

Univariate analysis of all-cause mortality in all 582 cases showed that, besides recipient *Toxoplasma* seropositivity, recipient age, ischemic heart disease, pretransplantation diabetes mellitus, reoperation, pretransplantation and 1-year posttransplantation renal function, and cardiac allograft vasculopathy at 1 and 4 years were risk factors of mortality, whereas tacrolimus (instead of cyclosporine)–based immunosuppression and early statins appeared to be protective (Table 3). After adjustment for all relevant clinical characteristics independent of the *P* value, as described in Table 3, the recipient *Toxoplasma* serostatus was not associated with higher mortality (hazard ratio [HR], 1.21; 95% confidence interval [CI], 0.95–1.54; *P*=0.18).

TABLE 2. Causes of death in all patients

	Recipient *Toxoplasma* serostatus		
	Negative	Positive	*P*
Patients	258	324	
Deaths, n (%)	117 (45)	219 (67)	<0.0001
Cause of death			
Early cardiac	11 (9)	22 (10)	1.00
Acute rejection	8 (7)	9 (4)	0.30
Infection	10 (9)	19 (9)	1.00
Malignancy	20 (17)	40 (18)	0.88
Vascular disease	9 (8)	28 (13)	0.20
Late cardiac	33 (28)	53 (24)	0.43
Other	26 (22)	48 (22)	1.00

Multivariate analysis of the *Toxoplasma* serostatus combinations shown in Table 3 showed that donor *Toxoplasma* serostatus was not independently associated with mortality (HR [95% CI], D+/R- 0.74 [0.52–1.06], D-/R+ 0.88 [0.53–1.46], and D+/R+ 0.82 [0.56–1.20]). Analysis of the 510 one-year survivors showed that female gender of the recipient was an extra univariate risk factor of mortality (not shown). Multivariate analysis confirmed characteristics as recipient age and cyclosporine-based immunosuppression (vs. tacrolimus) as independent risk factors of mortality and the protective effect of early statins (Table 4). Pretransplantation diabetes mellitus was an independent risk factor in all patients and in the 1-year survivors.

DISCUSSION

We showed that the long-term survival of heart transplant recipients is not associated with the *Toxoplasma*

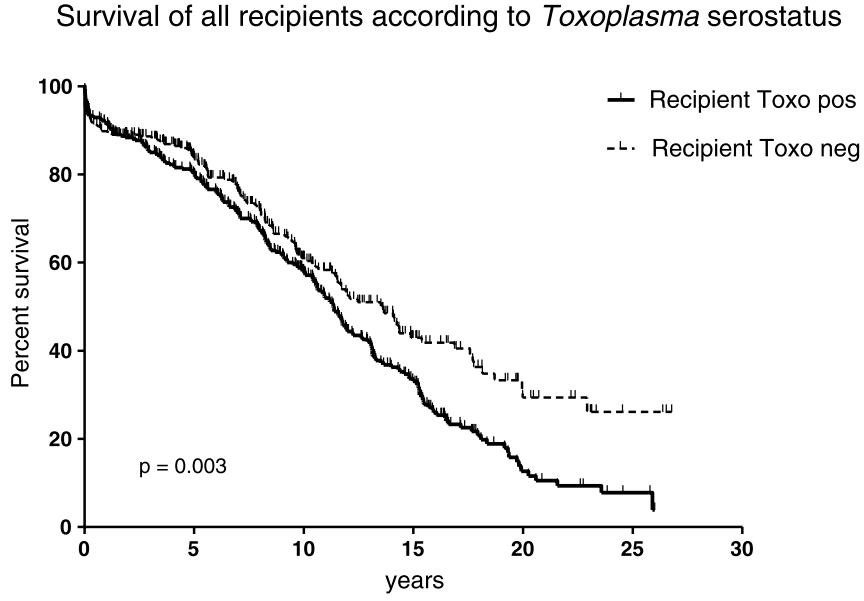

FIGURE 1. Survival of all recipients according to *Toxoplasma* serostatus.

TABLE 3. Univariate predictors of all-cause mortality in all patients

	HR	95% CI	P
Recipient			
Female gender	1.174	0.897–1.538	0.24
Age	1.027	1.017–1.037[a]	<0.001
Ischemic heart disease	1.528	1.230–1.899[a]	<0.001
PRA > 0	1.184	0.948–1.480	0.147
CMV seropositive	1.174	0.946–1.459	0.15
CMV mismatch (D+/R-)	0.833	0.635–1.094	0.19
Toxoplasma seropositive	1.496	1.193–1.875[a]	<0.001
Serum creatinine pre-Tx	1.002	1.000–1.004[a]	0.03
Diabetes mellitus pre-Tx	1.216	1.024–1.444[a]	0.03
Donor			
Female gender	1.001	0.806–1.245	0.99
Age	1.006	0.996–1.015	0.25
Trauma cause of death	1.078	0.851–1.364	0.53
MM AB+DR > 4	1.025	0.827–1.270	0.82
Donor/recipient Toxoplasma serostatus combinations			
Donor/recipient negative/negative (reference)	1.00		
Donor/recipient positive/negative	0.524	0.037–0.73	<0.001
Donor/recipient negative/positive	0.65	0.40–1.05	0.08
Donor/recipient positive/positive	0.78	0.57–1.07	0.12
Operation			
Reoperation	1.224	1.022–1.466[a]	0.03
Ischemia time	1.000	0.998–1.003	0.75
Postoperative course			
Induction therapy	0.942	0.757–1.173	0.59
Maintenance immunosuppression			
Cyclosporine based	2.830	2.111–3.794[a]	<0.001
Tacrolimus based	0.353	0.264–0.474[a]	<0.001
Early statin per protocol	0.485	0.367–0.640[a]	<0.001
Acute rejection			
Number of episodes 0	0.805	0.623–1.041	0.10
Number of episodes >2	0.881	0.652–1.191	0.41
CMV disease	0.989	0.738–1.326	0.94
Treated hypertension at 1 year post-Tx	0.672	0.530–0.852[a]	0.001
Diabetes mellitus post-Tx	0.986	0.791–1.228	0.90
Serum creatinine at 1 year post-Tx	1.003	1.001–1.004[a]	<0.001
Total cholesterol at 1 year post-Tx	0.937	0.884–0.994[a]	0.03
Triglycerides at 1 year post-Tx	0.923	0.838–1.077	0.115
CAV at 1 year post-Tx	1.097	1.026–1.173[a]	0.01
CAV at 4 years post-Tx	1.116	1.043–1.194[a]	0.002

[a] Significant difference.

serostatus of neither the recipient nor the donor. By inclusion of more patients and a longer follow-up period, our results may partly solve the disagreement between earlier findings (*1, 2*).

Beneficial effects of the early use of statins and tacrolimus-based maintenance immunosuppression have been reported earlier from the U.S. and European centers (*10–13*). Therefore, an imbalance of beneficial demographics in our *Toxoplasma* seronegative recipients (younger, less ischemic heart disease, better renal function, more tacrolimus maintenance immunosuppression, and more early statin use) may have masked a true mortality risk. We assume, however, that this has been overcome by putting all known risk factors into the multivariate model.

Searching for an explanation of the contradictory results of the studies of Arora et al. (*1*) and Doesch et al. (*2*) and our study, we demonstrated several differences between the studies. A summary is presented in Table 5. Populations differ not only in patient demographics but also in percentages of *Toxoplasma* seronegative recipients and in percentages of donors with unknown *Toxoplasma* serostatus. No *Toxoplasma* prophylactic treatment was used in the study of Arora et al. (*1*). The most obvious difference, however, is the time period covered by the studies. Time as a confounding factor applies above all to our study, which includes almost the whole history of calcineurin inhibitor–based heart transplantation. Multivariate analysis of all known risk factors will not completely have corrected for this issue. We refrained from adding "era" effect as a risk factor in to the analysis because of too much overlap of the different eras at different time points in diagnostic procedures (for CMV, toxoplasmosis, acute rejection, and cardiac allograft vasculopathy) as well as in therapeutic strategies (CMV prophylaxis, *Toxoplasma* prophylaxis, induction and maintenance immunosuppression, use of statins, etc.). When we included "year of transplant" as a continuous variable the HR (95% CI) was 0.80 (0.79–0.82) but in the multivariate model, there was large confounding with several other variables such as "early statin," which was given only after 1995. Therefore, we had to remove "year of transplant"' from the model.

Others demonstrated an association of recipient *Toxoplasma* seropositivity with especially mortality due to cardiac allograft vasculopathy (*1*). The background of such

TABLE 4. Multivariate predictors of all-cause mortality

	HR	95% CI	P
All patients			
Recipient age	1.028	1.011–1.046	0.001
Diabetes mellitus pretransplantation	2.267	1.027–5.004	0.04
Cyclosporine-based maintenance immunosuppression	2.564	1.611–4.080	<0.001
Early statin per protocol	0.518	0.305–0.879	0.02
1-year survivors			
Recipient age	1.029	1.012–1.047	0.001
Diabetes mellitus pretransplantation	2.344	1.061–5.179	0.035
Cyclosporine-based maintenance immunosuppression	2.655	1.656–4.255	<0.001
Early statin per protocol	0.488	0.283–0.839	<0.001
4-year survivors			
Recipient age	1.037	1.011–1.063	0.005
Cyclosporine-based maintenance immunosuppression	2.251	1.289–3.930	0.004
Early statin per protocol	0.319	0.143–0.712	<0.001

TABLE 5. Comparison between studies on *Toxoplasma* serostatus and survival after heart transplantation

	Arora et al. (*1*)	Doesch et al. (*2*)	Current study
Number of patients	288	344	582
Recipient age (years, mean)	54	52	47
Donor age (years, mean)	36	38	32
Toxoplasma seronegative recipients (%)	73	55	44
Unknown donor *Toxoplasma* serology (%)	15	?	27
CMV mismatch D+/R- (%)	21	23	22
Time period of study	1994–2005	1989–2008	1984–2012
Inclusion of >1 era			
For donor age	?	+	+
For diagnostic strategies			
Toxoplasma serology	−	±	+
CMV	?	?	++
AR	−	+	+
CAV	−	−	+
For therapeutic strategies			
Toxoplasma prophylaxis	−	+	++
CMV prophylaxis	?	?	++
CAV (e.g., PCI)	?	?	+
Induction immunosuppression	?	?	+
Maintenance immunosuppression	−	+	+
Early use of statins	+	−	+

?, no information available; −, only one modification during the study; ±, only one criterion with minor modifications; +, two modifications; ++, more than two modifications.

association is based on the fact that cardiac allograft vasculopathy is a process in which chronic inflammation and immune activation play an important role (*8, 9*). Upon infection by *T. gondii*, the host immune system is manipulated by parasite secreted factors that induce a proinflammatory response resulting in the production of interleukin-12 by monocytes, neutrophils, and dendritic cells and interferon-γ by natural killer cells and T-cells (*14*). Subsequently, a delicate balance between proinflammatory and anti-inflammatory signals is required to convert the active primary infection to its chronic and latent phase that allows both host and parasite survival (*15*). For these reasons, the immunologic effects of a *T. gondii* infection might influence the risk for cardiac allograft vasculopathy. However, we could not confirm the association of recipient *Toxoplasma* seropositivity and the occurrence of cardiac death.

The antibiotic prophylactic drugs given to *Toxoplasma* mismatch recipients (D+/R-) could also have a direct or indirect immunomodulatory effect. Although such a phenomenon has not been reported for pyrimethamine and sulfadiazine, spiramycin induces increased interleukin-6 production by monocytes in vitro (*7*). In addition, patient outcome could be negatively influenced by side effects of the antibiotic prophylactic drugs. Spiramycine is hepatotoxic, pyrimethamine can induce bone marrow suppression, and sulfadiazine is hepatotoxic and nephrotoxic. However, oral folinic acid administration prevents the negative side effects of a pyrimethamine-sulfadiazine regimen in most cases. This study, as well as the studies by Arora et al. (*1*) and Doesch et al. (*2*), did not identify a difference in mortality between *Toxoplasma* seronegative recipients that received the heart of a seropositive versus a seronegative donor, suggesting that the antibiotic prophylactic drugs given to *Toxoplasma* mismatch recipients (D+/R-) do not negatively affect outcome.

Our analyses confirmed recipient age and pretransplantation diabetes mellitus as independent risk factors for mortality. Our finding that cyclosporine (vs. tacrolimus) for maintenance imunosuppression appeared to be an independent risk factor of all-cause mortality confirms the results of Guethoff et al., who recently showed a survival benefit of tacrolimus versus cyclosporine in the 10-year follow-up of a randomized trial (*12*). Early prescription of statins had a protective effect as has been demonstrated by others in short-term as well as in long-term studies (*10, 11*).

In conclusion, we found that neither the *Toxoplasma* serostatus of the transplant recipient nor the serostatus of the donor is an independent risk factor for mortality after heart transplantation. Time bias, however, may have acted as a confounding factor for our results, but long term studies in a field with frequent changes in diagnostic and therapeutic strategies will always be harmed by the effects of time.

Limitations of the Study

Our analysis involved all heart transplant recipients, from the start of the program in 1984. This resulted in a study population that embraced the whole evolution of heart transplantation with increasing recipient and donor ages, different diagnostic laboratory methods, different immunosuppression protocols, and different therapies for infection prophylaxis. In addition, statins early after transplantation were introduced only from 2000. Another limitation is the lack of donor *Toxoplasma* serostatus in a considerable number of transplant recipients. The larger percentage of unknown donor *Toxoplasma* serostatus in the seropositive recipients can be explained by the fact that more effort was put in finding out the donor serostatus in seronegative recipients because of the required prophylaxis in case of a mismatch. In this respect, however, it may be important that *Toxoplasma* seronegative recipients of a donor with unknown serostatus have received the, for that time, usual prophylactic treatment.

MATERIALS AND METHODS

Patients

From the start of the heart transplant program in 1984, clinical data of all heart transplant recipients have been prospectively collected. Patients consented to use deidentified data for research purposes and the institutional review board of the Erasmus MC approved the present study. Enrolled were all cases who underwent heart transplantation between June 1984 and July 2011. For the study, the database was closed on July 15, 2012.

Patients with preformed donor-specific anti–human leukocyte antigen antibodies (panel-reactive antibody [PRA]>4%) were only transplanted after a negative crossmatch. During the early years of the program, only donors under age 36 were accepted for transplantation. Later on, the upper

age limit of donors has gradually shifted to 65 years with a median donor age of 46 years during the last 10 years.

Immunosuppression and Rejection

Over the years, several regimens of induction therapy have been used, initially within sequential randomized trials: ALG (lymphoglobulin; Institut Merieux, Lyon, France), OKT3 (Ortho Pharmaceutical, Raritan, NJ), and BT563 (daclizumab, Zenapax; Roche, Basel, Switzerland) (*16–18*). Later on, ALG (lymphoglobulin) was used, and during recent years, rabbit antithymocyte globulin (ATG; Fresenius, Kabi, The Netherlands) was administered.

From 1984 to 1999, maintenance immunosuppression consisted of cyclosporine and steroids complemented with azathioprine or mycophenolate mofetil (MMF) when two or more rejection episodes had occurred. From 2000 onwards, the regimen consisted of a combination of tacrolimus, steroids, and MMF. Rejection episodes were treated with pulsed high doses of steroids or rabbit ATG (initially rabbit ATG; National Institute for Public Health, Bilthoven, The Netherlands and later on with rabbit ATG (Genzyme Polyclonals, Marcy-l'Étoile, France) in cases of steroid-resistant rejection.

From 1984 to 1990, grading of acute rejection was according to Billinghams' original criteria, from 1990 to 2005 according to the International Society for Heart and Lung Transplantation (ISHLT) working formulation for the standardization of grading, and from 2005 onwards according to the revised ISHLT working formulation (*19–22*). Rejection was treated when "moderate rejection" or more, "grade 3A" or more, or "grade 2R" or more were diagnosed, respectively.

T. gondii Serology

All recipients were evaluated for specific IgG and IgM antibodies against *T. gondii* by enzyme immunoassays (EIAs). In addition, donors of whom serum samples were available were evaluated. Initially, *T. gondii*–specific antibodies were tested by a homemade EIA method as described before (*23*). From 2000 onwards, a commercially available EIA method (Vidas TOXO IgG and IgM; bioMerieux, Lyon, France) was used according to the manufacturer's instructions. When the EIA method resulted in equivocal test results, the *Toxoplasma* serostatus was determined by examination of follow-up sera and/or by Western blot analysis as described before (*23*).

Infection Prophylaxis

Prophylaxis against *T. gondii* was applied in *Toxoplasma* seronegative recipients of the heart of a *Toxoplasma* seropositive donor (or of the heart of a donor of whom the serostatus was not known) according to the knowledge at the time and sequentially consisted of a 6-month course of spiramycin (1985–1988) or pyrimethamine (1989–1994). From 1995 to July 2011, the combination of pyrimethamine and sulfadiazine was used. The administration of pyrimethamine was always combined with folinic acid.

In the early phase of the program, CMV seronegative recipients of the heart of a seropositive donor received passive immunization with anti-CMV immunoglobulins (Cytotect; Biotest Pharma, Dreieich, Germany) during the first 72 days after transplantation (*24*). Later on, oral valganciclovir was prophylactically administered for 6 months (or longer in case of rejection treatment) in these CMV mismatched patients.

Assessment of Cardiac Allograft Vasculopathy

Annual coronary arteriography was performed during the first 7 years of the program (*25*). Later on, coronary angiography (CAG) was performed per protocol after 1 year and repeated only after 4 years when no disease other than wall irregularities was shown. From year 5, CAG was performed when annual myocardial perfusion imaging showed perfusion defects or when electrocardiogram changes or other clinical signs of coronary syndromes occurred (*26*). The use of statins (pravastatin) per protocol early after transplantation was introduced in 1996.

Definitions

The presence of cardiac allograft vasculopathy was defined positive when at least ISHLT- CAV1 was diagnosed by CAG (*27*). Early cardiac death was defined as death caused by primary graft failure. Late cardiac death was defined as death caused by arrhythmia, conduction disorder, unwitnessed sudden death, or heart failure with angiographically or by postmortem examination-proven allograft vasculopathy.

Statistical Analysis

Categorical variables were compared by chi-square tests and continuous variables by Student's *t* test. Cumulative survival analyses were constructed using the Kaplan–Meier method. Among patient subgroups, the Mantel–Haenszel log-rank test was used to compare survival. The Cox proportional hazards model was used to identify independent risk factors for mortality. Preselected patient characteristics were all variables described in Table 3, independent of the *P* values.

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