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Preoperative *Toxoplasma gondii* serostatus does not affect long-term survival of cardiac transplant recipients. Analysis of the Spanish Heart Transplantation Registry

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ABSTRACT

Background: It's unclear whether pre-transplant *T. gondii* seropositivity is associated with impaired survival in heart transplant recipients.

Objectives: To test the above-mentioned hypothesis in the Spanish Heart Transplantation Registry.

Methods: Post-transplant outcomes of 4048 patients aged > 16 years who underwent first, single-organ heart transplantation in 17 Spanish institutions from 1984 to 2014 were studied. Long-term post-transplant survival and survival free of cardiac death or retransplantation of 2434 (60%) *T. gondii* seropositive recipients and 1614 (40%) *T. gondii* seronegative recipients were compared.

Results: *T. gondii* seropositive recipients were older, had higher body mass index, and presented higher prevalence of hypertension, hypercholesterolemia, COPD and Cytomegalovirus seropositivity than *T. gondii* seronegative recipients. In univariable analysis, pre-transplant *T. gondii* seropositivity was associated with increased post-transplant all-cause mortality (non-adjusted HR 1.15; 95% CI 1.04–1.26). However, this effect was no longer statistically significant after multivariable adjustment by recipient's age and sex (adjusted HR 1.01, 95% CI 0.92–1.11). Extended multivariable adjustment by other potential confounders showed similar results (adjusted HR 0.99, 95% CI 0.89–1.11). *T. gondii* seropositivity had no significant effect on the composite outcome cardiac death or retransplantation (non-adjusted HR 1.08, 95% CI 0.95–1.24, $p = 0.235$). The distribution of the causes of death was comparable in *T. gondii* seropositive and *T. gondii* seronegative recipients. No statistically significant impact of donor's *T. gondii* serostatus or donor-recipient *T. gondii* serostatus matching on post-transplant survival was observed.

Abbreviations: CAV, coronary allograft vasculopathy; CI, confidence interval; HR, hazard ratio; HT, heart transplantation; TG, *Toxoplasma gondii*.

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Conclusions: Our analysis did not show a significant independent effect of preoperative *T. gondii* serostatus on long-term outcomes after heart transplantation.

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1. Background

Over the past 10 years, there has been an unsolved controversy about the potential influence of *Toxoplasma gondii* (TG) serostatus of heart transplantation (HT) recipients on their long-term outcomes after the operation [1]. A pioneer retrospective report from a Norwegian center [2] showed a striking reduction of late post-transplant survival among TG seropositive recipients as compared with seronegative ones, which was attributed to an increased incidence of advanced coronary allograft vasculopathy (CAV). Enhancement of several immune responses triggered by chronic TG infection was proposed as a hypothetical explanation for these intriguing findings.

The validity of the Norwegian analysis was openly questioned due to inconsistencies in the statistical methodology used to assess confusion bias [3]. Later on, three subsequent single-center studies failed to confirm an independent effect of TG serostatus on post-transplant outcomes [4–6]; moreover, one study reported increased early post-transplant mortality among TG seronegative recipients [7]. Until now, no significant influence of either donor's TG serostatus or donor-recipient TG serostatus matching on post-transplant outcomes has been proven [2,4–6].

Given the discrepancies among previously published single-center studies, we aimed to investigate a potential impact of recipient's TG serostatus on long-term post-transplant survival in the multi-institutional cohort of the Spanish Heart Transplantation Registry [8].

2. Methods

2.1. Study description

The Spanish Heart Transplantation Registry is a prospective database promoted by the Working Group in Heart Failure of the Spanish Society of Cardiology that contains detailed clinical information about all HT procedures performed in our nation since 1984 to present. The registry is updated in a yearly basis with data supplied by all transplant centers of the country. This database has been described elsewhere [8].

For the present study, we selected from the Spanish Heart Transplantation Registry database all patients aged >16 years who underwent first, single-organ, orthotopic HT in Spain until December 31st, 2014, for whom reliable information about their pre-transplant TG serostatus was available. Vital status as of December 31st, 2015 was known for all subjects.

Baseline clinical characteristics and long-term post-transplant survival of TG seropositive and TG seronegative recipients were compared. Pre-transplant TG seropositivity was defined by the detection of specific immunoglobulin G against the parasite, according to local diagnostic protocols of participating hospitals. This investigation was approved by the Local Committee for Ethics in Clinical Research of A Coruña-Ferrol, Department of Healthcare, Autonomous Community of Galicia (Spain).

2.2. End-points

Long-term survival after HT was the primary end-point of the study. The composite outcome cardiac death or cardiac re-transplantation was a secondary end-point.

Cardiac death was defined as any death attributable to heart failure, myocardial ischemia or arrhythmia, including deaths secondary to acute rejection, CAV, or primary or unexplained graft dysfunction. For survival analysis, sudden deaths and deaths from an unknown origin were also assumed as cardiac deaths.

2.3. Statistical analysis

In this manuscript, continuous variables are presented as mean \pm standard deviation or median (interquartile rank), depending on whether they follow a normal distribution, and categorical variables are presented as proportions. Baseline clinical characteristics of TG seropositive and TG seronegative recipients were compared by means of the chi-squared test, the T-student test or the Mann-Whitney test, as required.

Non-adjusted long-term post-transplant survival curves of TG seropositive and TG seronegative recipients were constructed with the Kaplan-Meier method and compared with the log rank test.

Cox's multivariable models were used to control the influence of confusion bias on the statistical effect of recipient's TG serostatus on long-term post-transplant survival. A first

multivariable model (*Model 1*) including recipient's age, recipient's gender and recipient's TG serostatus was constructed in order to adjust the statistical effect of TG seropositivity on survival by demographic factors. Further adjustment was performed by means of an extended multivariable model (*Model 2*), which included several baseline variables additional to demographic factors, which were considered by the investigators as potential confounders. A confounder was defined as a baseline variable showing an asymmetric distribution between TG seropositive and TG seronegative patients, together with either a statistical significant association with post-transplant survival in the study population or a consistent association with post-transplant survival in previous literature. Covariables selected for extended multivariable adjustment (*Model 2*) were age of the recipient, gender of the recipient, body mass index, diabetes, hypertension, ischemic heart disease, Cytomegalovirus serostatus, age of the donor, mechanical circulatory support, mechanical ventilation, tacrolimus use, mophetil mycophenolate use, transplant era, and cold ischemic time. Adjusted long-term post-transplant survival curves for TG seropositive and TG seronegative recipients were estimated by means of both multivariable models [1 and 2].

In the subpopulation of subjects for whom both recipient's and donor's TG serostatus were known, we performed an exploratory analysis about a potential influence of donor-recipient TG serostatus matching on long-term post-transplant survival, by using the Kaplan-Meier method and multivariable Cox's regression. For this analysis, patients were divided in four categories according to donor-recipient TG serostatus matching, as follows: donor (+)/recipient (+), donor (+)/recipient (–), donor (–)/recipient (+), and donor (–)/recipient (–). The donor (+)/recipient (+) subgroup was considered as the reference category for statistical comparisons.

Statistical analysis was performed with Stata 12. Statistical significance was set as a *p* value <0.05 for all tests.

3. Results

3.1. Patients

From 1984 to 2014, 7277 patients underwent first, single-organ, orthotopic HT in 17 Spanish institutions, 4048 of which constituted the study population. Reasons for exclusion were age < 16 years (*n* = 390), re-transplantation (*n* = 164), multi-organ transplantation (*n* = 124) and the lack of information about recipient's pre-transplant TG serostatus (*n* = 2533). Before HT, 2434 (60%) study patients were TG seropositive and 1614 (40%) were TG seronegative.

3.2. Baseline clinical characteristics

The *Table 1* shows a comparison of baseline clinical characteristics of TG seropositive and TG seronegative recipients. At the time of HT, TG seropositive recipients were significantly older, had higher body mass index and higher prevalence of cardiovascular risk factors like hypertension and hypercholesterolemia, and more frequently presented chronic obstructive pulmonary disease and Cytomegalovirus seropositivity than TG seronegative recipients.

The need for preoperative mechanical ventilation and mechanical circulatory support was more frequent among TG seronegative recipients; cold ischemic times were also longer in these individuals.

The use of tacrolimus and mycophenolate mophetil as part of the initial immunosuppressive regimen was less frequent among TG seropositive recipients, which in a higher proportion were transplanted during the older era (1984–2000).

3.3. Causes of death

Median follow-up after HT was 2103 days (interquartile rank 568 to 4153 days). Over this period, 1903 (47%) patients died, of which 1215 were TG seropositive and 688 were TG seronegative before HT. Also, 79 (2%) patients underwent cardiac re-transplantation, of which 48 were TG seropositive and 31 were TG seronegative before their first HT.

The presumed cause of death was cardiac in 616 (32%) deceased patients and non-cardiac in 1038 (55%) deceased patients; in the

Table 1

Baseline clinical characteristics of *T. gondii* seronegative recipients and *T. gondii* seropositive recipients.

	<i>Toxoplasma gondii</i> seropositive recipients N = 2434	<i>Toxoplasma gondii</i> seronegative recipients N = 1614	p
Recipients			
Age (years), mean \pm standard deviation	54.9 \pm 9.6	48.7 \pm 12.9	<0.001
Body mass index	26 \pm 3.9	25 \pm 4.1	<0.001
Women	18.1%	22.7%	<0.001
Transplant era			<0.001
1984–2000	30.2%	24.2%	
2001–2014	69.8%	75.8%	
Ischemic heart disease	32.9%	30.1%	0.065
History of smoking	20.2%	19.5%	0.638
Diabetes mellitus	16.2%	14.3%	0.112
Hypertension	34.4%	28.1%	<0.001
Hypercholesterolemia	41.2%	34.3%	<0.001
Previous cardiac surgery	23.5%	21.6%	0.166
<i>Cytomegalovirus</i> seropositive recipient	86.1%	74.2%	<0.001
Previous malignancy	3.5%	4.4%	0.153
Chronic obstructive pulmonary disease	12.5%	8.3%	<0.001
Inotropic therapy	29.6%	34.7%	0.001
Mechanical circulatory support	17.6%	22.5%	0.003
Dialysis	8.3%	9.1%	0.435
Mechanical ventilation	9.5%	12.5%	0.004
Renal dysfunction ^a	16%	17.2%	0.329
Liver dysfunction ^b	17.3%	16.8%	0.690
Mean pulmonary pressure (mm Hg)	30.1 \pm 10.6	30.3 \pm 10.6	0.371
Pulmonary vascular resistance (Wood units)	2.4 \pm 2.2	2.2 \pm 1.3	0.066
Donors			
Cold ischemic time (min)	184.8 \pm 64.7	191.0 \pm 65.8	0.009
Women	30.4%	33.6%	0.034
Body mass index (kg/m ²)	25.2 \pm 3.6	25.1 \pm 3.6	0.289
Age (years)	35.9 \pm 13.3	35.8 \pm 13.2	0.719
Baseline immunosuppression			
Corticosteroids	98%	97.1%	0.506
Cyclosporine A	63.6%	55.6%	<0.001
Tacrolimus	31.9%	39.8%	<0.001
Mycophenolate mophetil	64.5%	70.4%	<0.001
Azathioprine	32.8%	25.5%	<0.001
Sirolimus	1.4%	1.2%	0.575
Everolimus	1.8%	2.4%	0.224

^a Serum creatinin >2 mg/dl.

^b Serum bilirubin >2 mg/dl.

remaining 249 (13%) deceased patients, it was unknown. The distribution of the causes of death among TG seronegative recipients and TG seropositive recipients showed no relevant differences, as detailed in the Table 2.

3.4. Primary end-point: all-cause death

Kaplan-Meier long-term post-transplant survival curves of TG seropositive and TG seronegative recipients are depicted in Fig. 1. TG seropositive recipients had shorter non-adjusted survival than seronegative ones (non-adjusted hazard ratio (HR) 1.15; 95% Confidence Interval (CI) 1.04–1.26; log rank p < 0.001). Non-adjusted post-transplant all-cause mortality rates were 62.6 (95% CI 58.1–67.5) deaths per 1000 patients-year among TG seronegative recipients and 70.2 (95% CI 66.4–74.3) deaths per 1000 patients-year among TG seropositive ones.

The statistical effect of TG serostatus on long-term post-transplant survival was no longer statistically significant after multivariable adjustment (Supplementary Table 1). By means of the multivariable model 1, which included the demographic factors age and sex of the recipient as co-variables, the estimated HR for death for TG seropositive recipients vs. TG seronegative ones was 1.01 (95% CI 0.92–1.11, p = 0.842).

Table 2

Distribution of the causes of death of *T. gondii* seropositive recipients and *T. gondii* seronegative recipients.

	<i>T. gondii</i> seronegative recipients N = 688	<i>T. gondii</i> seropositive recipients N = 1215
Cardiac	233 (34%)	383 (32%)
Graft failure, postoperative	76	119
Graft failure, acute rejection	29	37
Graft failure, coronary allograft vasculopathy	26	62
Graft failure, non-specified	36	58
Sudden death	66	107
Non-cardiac	364 (53%)	674 (55%)
Multi-organ failure	109	128
Infection	65	162
Malignancy	47	117
Other	143	267
Unknown	91 (13%)	158 (13%)

Extended multivariable adjustment was conducted by means of multivariable model 2, which included several potential confounders additionally to demographic factors, but no significant change of the results was obtained. By means of the model 2, the estimated HR for death for TG seropositive recipients vs. TG seronegative ones was 0.99 (95% CI 0.89–1.11, p = 0.925).

Adjusted long-term post-transplant survival functions of TG seropositive recipients and TG seronegative recipients, as estimated by means of Cox's multivariable models 1 and 2, are represented graphically in Fig. 2.

3.5. Secondary end-point: cardiac death or cardiac re-transplantation

Univariable analysis did not reveal a statistically significant association between TG seropositivity and the risk of the composite outcome cardiac death or cardiac re-transplantation (non-adjusted HR 1.08, 95% CI 0.95–1.24; p = 0.235). Non-adjusted incidence rates of the secondary end-point were 32.1 (95% CI 29.0–35.7) events per 1000 patients-year among TG seronegative recipients and 34.1 (95% CI 31.4–36.9) events per 1000 patients-year among TG seronegative ones.

Neither multivariable adjustment by age and sex of the recipient (Model 1: adjusted HR 1.08, 95% CI 0.95–1.24; p = 0.231) nor by other potential confounders (Model 2: adjusted HR 1.04, 95% CI 0.88–1.22; p = 0.650) led to a significant change of the result.

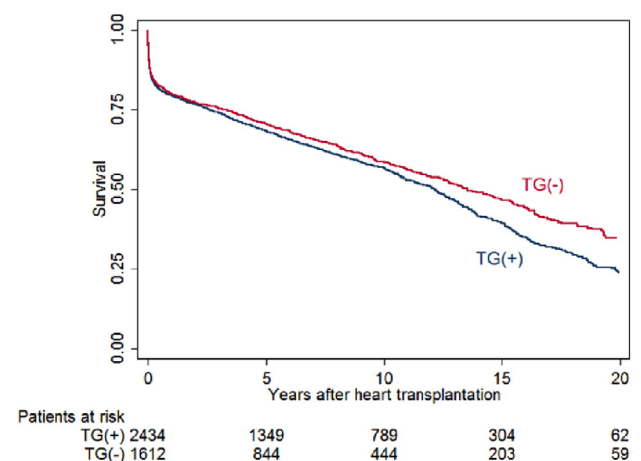


Fig. 1. Kaplan-Meier analysis of long-term post-transplant survival of *T. gondii* seropositive recipients (blue line) and *T. gondii* seronegative recipients (red line). TG, *Toxoplasma gondii*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

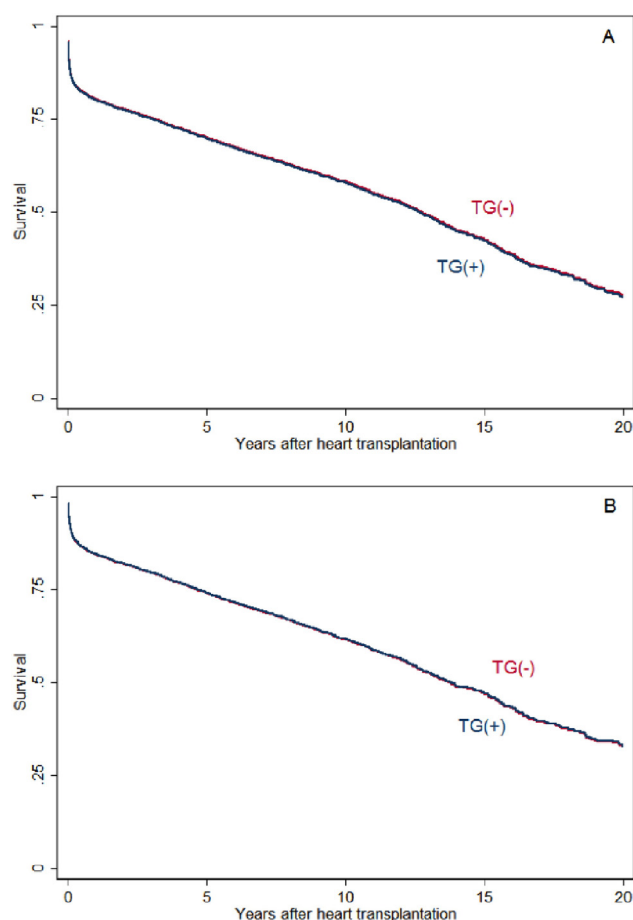


Fig. 2. Adjusted survival functions of *T. gondii* seropositive recipients (blue line) and *T. gondii* seronegative recipients (red line), as estimated by means of Cox's proportional hazards regression. Panel A, Multivariable model 1 (age- and sex-adjusted survival). Panel B, Multivariable model 2 (extended multivariable adjustment). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.6. Donor-recipient *T. gondii* serostatus matching

Donor's TG serostatus was known in 1975 (49%) recipients. Among them, 559 (28%) were donor (+)/recipient (+), 302 (15%) were donor (+)/recipient (−), 620 (32%) were donor (−)/recipient (+) and 493 (25%) were donor (−)/recipient (−).

Regardless donor's TG serostatus, TG seronegative recipients showed a trend to higher non-adjusted post-transplant survival than seronegative ones; however, differences across four categories of donor-recipient TG serostatus matching did not reach statistical significance (p log rank = 0.083, Supplementary Fig. 1).

Considering the donor (+)/recipient (+) subgroup as the reference category, age- and sex-adjusted HR (Model 1) for post-transplant all-cause mortality in the donor (+)/recipient (−), donor (−)/recipient (+) and donor (−)/recipient (−) subgroups were 0.92 (95% CI 0.73–1.15; p = 0.448), 1.04 (95% CI 0.87–1.23; p = 0.676), and 1.02 (95% CI 0.84–1.24; p = 0.835), respectively. Extended multivariable adjustment (Model 2) showed similar results.

4. Discussion

To the best of our knowledge, this is the first multi-institutional study to address the hypothesis that pre-transplant TG seropositivity is a risk factor for mortality in HT recipients. By using a previously collected dataset of >4000 patients who received a heart graft in Spain

over the last 30 years, we failed to demonstrate an independent impact of pre-transplant TG serostatus on post-transplant survival. Neither a significant effect of donor-recipient TG serostatus matching on post-transplant outcomes was observed.

Since the early eras of transplantation, doctors taking care of transplant recipients are concerned about the possibility of opportunistic infections in such patients. *T. gondii* is an intracellular parasite, for which the human body constitutes a secondary host. In immune competent subjects, the clinical course of TG infection is usually oligosymptomatic, as the parasite is efficiently cleared by cellular immunity. However, in the setting of cellular immunodeficiency, TG infection may lead to severe clinical consequences, mainly at the central nervous system. Primary infection of a never before exposed subject carries the highest risk of complications; this is the reason why TG serostatus is routinely tested during the selection process of HT candidates, and why primary chemoprophylaxis against TG is usually administered to TG seronegative patients who receive a graft from a TG seropositive donor [9].

Approximately 10 years ago, there was a change in how transplant doctors regard the clinical implications of TG exposure in HT recipients. A striking study based on 288 individuals who underwent HT at a single Norwegian institution reported a strong increase of late post-transplant mortality among TG seropositive recipients in comparison with TG seronegative ones [2]. This finding was attributed to an increased incidence of advanced CAV and death due to this condition. To explain their results, the authors hypothesized that chronic TG infection might contribute to the development of CAV and graft dysfunction through the enhancement of several immunological responses, as endothelial cell activation, cytokine overexpression and T-helper cell activation [2,10–11]. Until now, these pathophysiological mechanisms remain essentially speculative, as no consistent proofs of a causal association between TG exposure and the development of CAV have been presented.

Later on, a few subsequent single-center studies addressed this intriguing topic, but all failed to reproduce earlier findings [4–6]. In their univariable analysis based on 582 patients who underwent cardiac transplantation since 1984 to 2011 in a Dutch center, Van Hellemond et al. [4] also reported reduced non-adjusted long-term post-transplant survival in TG seropositive recipients; however, after multivariable adjustment, the effect of TG serostatus on survival was no longer statistically significant. A Spanish group [5] reached similar conclusions by analyzing the long-term post-transplant outcomes of 657 patients who received a heart graft since 1991 to 2014; in this study, a moderately increased risk of acute rejection was observed among TG seropositive recipients, but this fact did not carry a significant impact on survival. Another single-centre American study focused on 785 patients who underwent HT between 1995 and 2012, showing no significant impact of pre-transplant TG serostatus on post-transplant survival [6]. More conflicting results were reported by Doesch et al. [7], who described increased early postoperative mortality among TG seronegative recipients in their analysis based on 344 patients transplanted since 1989 to 2008 in a German institution.

Similarly to described in other studies [4,5], TG seropositive recipients of our multi-institutional cohort presented shorter non-adjusted long-term post-transplant survival than TG seronegative recipients in univariable analysis. However, this statistically significant, negative effect of pre-transplant TG seropositivity on post-transplant survival was lost after a simple multivariable adjustment by baseline demographic factors –age and sex of the recipient–. A more complex multivariable adjustment, which included several additional covariables selected by study investigators as potential confounders, did not result in a relevant change in the sense of the result.

Our findings suggest that confusion bias is the most probable explanation for the hypothesized association between pre-transplant TG serostatus and post-transplant outcomes. The prevalence of TG exposure, and so, TG seropositivity, is directly related to age [12]. In our series, TG seropositive recipients were, as an average, >6 years older

than TG seronegative ones at the time of HT. As a result, TG seropositive recipients presented a higher prevalence of adverse clinical conditions related to age, as cardiovascular risk factors, chronic obstructive pulmonary disease and CMV seropositivity. This reality was similar in previous studies [2,4,5].

A glance to the Kaplan-Meier long-term post-transplant survival curves of TG seropositive and TG seronegative recipients only reveals a clear separation between them at late follow-up. In our opinion, this fact reflects the impact of the unbalanced baseline distribution of age and co-morbidities in both study groups on long-term post-transplant survival. Interestingly, adjusted HR for all-cause mortality estimated by two different multivariable models were very close to 1, indicating a consistent neutral effect of TG serostatus on survival. This constitutes a strong argument to affirm that the proposed association between TG serostatus and post-transplant outcomes is probably spurious, rather than causal.

Due to the lack of data, we could not compare the incidence of new-onset CAV or acute rejection in TG seropositive and TG seronegative recipients. Nevertheless, neither univariable nor multivariable analyses revealed a statistically significant difference between both groups with regard to the risk of the composite end-point cardiac death or cardiac re-transplantation, which is an appropriate surrogate for those graft-related adverse clinical events. To date, only one study described an excess of CAV-related mortality in TG seropositive recipients [2]; other authors [4–5,7], like us, did not observe any relevant difference with regard to the distribution of the causes of death according to pre-transplant TG serostatus.

Our analysis did not show a significant impact of donor's TG serostatus or donor-recipient TG serostatus matching on post-transplant outcomes. This conclusion may be regarded with caution, given that recipient's TG serostatus was only known in 49% patients of our population. Nonetheless, this finding is also consistent with previous studies [2,4–7]. The lack of a negative prognostic effect of a TG seropositive donor in a TG seronegative recipient has been proposed as an argument against a causal association between TG serostatus and post-cardiac transplant outcomes [1].

Our study has a few limitations. As a retrospective one, it is exposed to potential selection and information biases inherent to this type of investigation. Comprehensive multivariable adjustment was conducted in order to control the effect of potential confounders; however, we cannot rule out categorically that any other non-tested variable may have influenced observed statistical associations. The study addressed a heterogeneous population, as it involved several hospitals across Spain, with different center-specific protocols regarding post-transplant chemoprophylaxis and infection surveillance. It is remarkable that our analysis excluded near 45% HT recipients enrolled in the Spanish Heart Transplant Registry; the lack of reliable information about their preoperative TG serology was the reason for exclusion in most cases. The lack of information about evolutionary TG serology prevented us to analyze the incidence of TG new infection or reactivation over post-transplant follow-up. Specific information about the type, duration and compliance of TG chemoprophylaxis was not available. Given the long period studied, a significant era effect cannot be excluded; notwithstanding this, no relevant changes in the association between TG serostatus and post-transplant outcomes was observed when transplant era was entered as a covariable in multivariable models constructed for statistical adjustment.

5. Conclusions

In conclusion, our study showed a statistically significant, moderate reduction of non-adjusted long-term survival after orthotopic HT among recipients who showed a positive serostatus against TG before the intervention, as compared with seronegative ones. However, the effect of preoperative TG seropositivity on post-transplant survival remained neutral after multivariable adjustment, both in a simple

model which only included recipient's age and sex as co-variables, and also in an extended model which added several other potential confounders. Our data suggest that the hypothesized association between recipient's TG serostatus and long-term post-transplant survival is, rather than causal, more probably explained by confusion bias derived by an older age at the time of HT and a higher prevalence of comorbidities among seropositive recipients. Therefore, this study does not support any specific change in the standard clinical management of TG seropositive individuals undergoing orthotopic HT.

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Disclosures

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijcard.2017.09.215>.

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