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Predictors of Quilty Effect in a Population Bridged to Orthotropic Heart Transplantation with a Mechanical Circulatory Support Device

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Predictors of Quilty Effect in a Population Bridged to Orthotropic Heart Transplantation with a Mechanical Circulatory Support Device

A thesis submitted in partial satisfaction of the requirements

for the degree Master of Science in Clinical Research

by

Seema Arun Mehta

2014

ABSTRACT OF THE THESIS

Predictors of Quilty Effect in a Population Bridged to Orthotropic Heart Transplantation with a Mechanical Circulatory Support Device

by

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Masters of Science in Clinical Research

University of California, Los Angeles, 2014

Professor Robert M. Elashoff, Chair

Background: Quilty effect (QE), an infiltrate consisting of small vessels, B-cells, and T-cells is found exclusively in endomyocardial biopsies from heart transplant recipients. We hypothesize that mechanical circulatory support devices (MCSD) used as a bridge to transplantation (BTT) predispose orthotropic heart transplant (OHT) recipients to developing QE. We sought to 1) define the predictors of QE in those BTT with MCSD, and 2) determine the impact of QE on post-OHT outcomes.

Methods: A retrospective chart review was performed of all adult patients with MCSD, implanted for BTT, and successfully transplanted between 1996 and 2012 (N = 115). Using logistic regression, time-to-event curves, and Cox Proportional Hazards, clinical parameters

were evaluated as predictors of the primary outcome, QE, and secondary outcomes of acute cellular rejection (ACR), pathologic antibody mediated rejection (pAMR), and death.

Results: 100 subjects developed QE (86.9%); 49 (43%) developed QE within the first 15 days post-OHT (early QE). Cyclosporine (CsA) use was protective of early QE (adjusted OR 0.29, p=0.02). Donor age decreased odds of ever developing QE (adjusted OR 0.94, p=0.03). QE increased one's odds of developing biopsy-proven ACR (adjusted OR 13.2, p=0.02). Cox Regression model revealed an adjusted HR of 0.61 (p=0.0007) for every 10-years of donor age.

Conclusions: QE incidence in this population was higher than prior studies have found. CsA use and donor age were associated with QE development, consistent with previously published reports. Studies comparing a BTT population with those who proceed directly to OHT, and assessing the recurrent nature of QE are necessary to better understand QE in this cohort.

The thesis of Seema Arun Mehta is approved.

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Table of Contents

List of Figures and Tables

CHAPTER 1. MANUSCRIPT

ABSTRACT

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INTRODUCTION

Quilty effect is a phenomenon described as found exclusively on heart biopsies from heart transplant recipients (1-3). This biopsy finding is an endocardial infiltrate consisting of vascular channels, lymphocytes, histiocytes, dendritic cells, and scattered plasma cells. It was first described in 1981 by Billingham in a patient for whom the lesion was named (1, 2, 4, 5). Since then, more than 30 years later, our knowledge of what causes QE, as well as its clinical implications, remain limited and controversial.

The incidence of QE has been reported as being anywhere from 8 – 78.6% (4, 6), with a higher incidence amongst pediatric transplant recipients (2, 7). Furthermore, the incidence appears to be the highest in the first year post-orthotropic heart transplantation (OHT) (2, 4, 7). As noted above, QE lesions are dense nodules composed of capillary-like vessels, a central core of B-cells, and a peripheral margin of T-cells (8, 9). These nodular infiltrates are connected to the endocardium, and historically were classified into two groups based upon their relationship to the myocardium: 1) Quilty A, a non-invasive QE that lies superficial to the myocardium and 2) Quilty B, an invasive QE that encroaches upon the subadjacent myocardium (6, 9, 10). QE has previously caused significant variability in the diagnosis of International Society for Heart and Lung Trasnplantation (ISHLT) Grade 2 and 3A cellular rejection on endomyocardial biopsies (EMBs) (9, 11-13). Because of its appearance when infiltrating the myocardium, QE was often mistaken as histopathologic evidence of cellular rejection (9). Consequently, in 2005 ISHLT revised their pathology grading system to better distinguish between QE lesions and cellular rejection, and the categorization of QE into A and B has since been eliminated (14).

Past studies performed to assess the cause and clinical relevance of QE have revealed conflicting results. Many have found that QE is associated with cyclosporine use, a calcineurin inhibitor used to prevent rejection after heart transplantation (2, 11, 12, 15, 16), while some

have proposed that it is a herald of allograft rejection (7, 11, 17-19) or a possible localized reaction to a cardiotropic virus (such as Epstein-Barr virus or Cytomegalovirus - CMV) (3, 6, 20). Although many of these studies did include those who were bridged to OHT with a mechanical circulatory support device (MCSD), to date there has not been a study looking solely at the patients who have sustained the immunomodulatory effects of MCSD and the impact that may have on the development of QE.

It is well known that the immune phenomena occurring after MCSD placement includes that of systemic T-cell activation, apoptosis, cell-mediated immune defects, increased activity of polyclonal B-cells, and allosensitization – an immune alteration characteristic of both systemic lupus erythematosus and human immunodeficiency virus-1 infection (21-29). The impact that these immune changes have on the post-transplant course has been studied and reveal a spectrum of findings including: improved benefit and survival, decreased survival and worsened outcomes, and no impact on the post-transplant course (30-37).

MCSD pumps are continuous flow pumps, and have been categorized into two types: non-pulsatile (axial) and pulsatile (centrifugal). These two pump-types are distinguished based upon their rotating elements. The non-pulsatile pump provides support in a volume-displacement manner, and has a rotating element similar to a propeller within a pipe, or an auger. It is often thought of as a “pusher” of volume (38). Where as, the pulsatile pump provides support by “throwing” fluid using a spinning disk with blades (38). It was not until 2008, when non-pulsatile pumps were the preferred MCSD. Non-pulsatile pump types generally have better outcomes and are associated with less adverse events (39, 40). Additionally, data has shown that those supported with non-pulsatile pumps have improved post-OHT outcomes, when compared to those who have received pulsatile pumps (39, 40). Furthermore, it has been shown that the pulsatile MCSDs are more immunostimulatory than the non-pulsatile MCSDs (41). Additionally,

regardless of pump type, the duration of support on the device also plays a role in the immunomodulating effects from the MCSD (41); the longer the device support is needed, the greater the immunomodulating effects.

Another piece of information that is necessary to risk stratify a patient's immunologic risks post-OHT is their CMV serostatus. CMV is one of the most important viruses to affect solid organ transplant recipients. It is well known that CMV can lead to both direct and indirect physiologic consequences when present. Direct effects of CMV disease include end-organ damage, tissue invasive disease, and increased morbidity and mortality, but the indirect effects are less conspicuous with a smoldering immunomodulatory effect. Immune modifying effects from CMV can include acute and chronic allograft rejection, as well as transplant cardiac vasculopathy (42-61).

The treatment approach to CMV is to not only treat the virus, but in many instances also includes decreasing immunosuppressive therapies the patient is receiving with the goal of allowing host immune defenses to decrease and/or eradicate viral activity. Although, decreasing immunosuppressive therapies may aid in viral quiescence, it may also allow for allograft rejection to occur. On the other hand, if viral dormancy is not obtained, the immunomodulatory effects of CMV are allowed to exponentiate, hence increasing potential for allograft rejection. There is a fine balance between these two.

Therefore, CMV serostatus of the recipient as well as the donor are taken into account when managing patients. When risk stratifying the patients, they are usually thought of as donor-recipient pairs: D+/R- (donor CMV seropositive/recipient seronegative), D-/R+, D+/R+, and D-/R-. Of the four seropair groups, D+/R- portends the highest risk of CMV disease and immunomodulatory effect. D+/R+ and D-/R+ carry an intermediate risk, and D-/R- carries the

lowest risk. Furthermore, depending on the donor-recipient seropair, a patient is usually given a standardized course of anti-CMV prophylactic therapy early in the post-OHT period (3 – 12 months), when the net state of immunosuppression tends to be at its greatest (62, 63).

Hence, there are numerous immumodulatory forces are play that can impact post-transplant outcomes. Given the significant immune impact of the MCSD and the known immunologic findings of QE, we hypothesize that MCSD use may predispose OHT recipients to developing QE. Therefore, we sought to define the clinical parameters in a population bridged to transplantation (BTT) using a MCSD that may predict the development of QE, and to determine what impact QE may have on post-OHT outcomes, including acute cellular rejection, antibody mediated rejection, and death.

METHODS

Study design: This was a retrospective chart review of all adult patients (18 years or older) who underwent MCSD placement and were successfully bridged to transplantation at a large transplant center between January 1, 1996 and December 31, 2012. Any patient who had previously received an OHT was excluded. All patient follow up data was included up through August 31, 2013.

Clinical Data Collection: Recipient and donor data was collected. Each recipient's electronic medical record (EMR) was reviewed to obtain all predictor variables. Donor specific data was obtained by performing electronic data queries of the transplant database, which has been created and maintained at the transplant center. Any recipient or donor information that was unavailable in the EMR or the institutional transplant database was obtained through a formally requested United Network for Organ Sharing (UNOS) data query.

The independent recipient variables included the following: recipient age at time of MCSD placement; recipient gender; etiology of heart failure; history of tobacco use; weight (prior to MCSD placement; kilograms); height (in meters); body mass index (kg/m^2); type of MCSD pump used (pulsatile or non-pulsatile); duration of MCSD use (days); recipient CMV IgG serostatus; and immunosuppressive therapies received (specifically, cyclosporine (CsA) and tacrolimus). Of note, the etiology of heart failure variable was dichotomized into either ischemic (cardiomyopathies due to ischemia, coronary artery disease, and hypertension) or non-ischemic (including congenital, sarcoidosis, amyloidosis, autoimmune and various causes of dilated cardiomyopathies, such as postpartum, alcohol, valvular, viral, or toxin-mediated).

The independent donor variables collected were age, gender, and CMV IgG serostatus.

Dependent variable information was acquired by performing data queries of the transplant center's pathology database: Sunquest PowerPath ®. Additionally, the institutional transplant database was queried to obtain date of last follow up, as well as date of death, when applicable. Any missing data with regards to episodes of rejection, date of death and date of last follow up, was then obtained through a formally requested UNOS data query. The data collected included the following: EMB results; date of last follow up at the transplant center; and date of death.

EMBs were generally performed following a typical surveillance schedule (once a week for the first 4 weeks post-OHT, followed by every two weeks for the subsequent 6 weeks, then every three months for the remainder of the first year and three to four times in the second year and annually thereafter). EMBs performed outside of this routine were done so in the event of clinical suspicion for graft dysfunction and/or rejection, as well as after completion of therapy for an acute rejection episode. The date of each EMB for each patient along with the EMB pathology results – no pathology, acute cellular rejection (ACR; stages 1R – 3R), pathologic

antibody mediated rejection (pAMR; stages 1-3), QE (types A and B), or ischemic changes (early and late) – were recorded. Only the biopsies performed at our institution were included in this data, as well as in the analyses.

Outcomes: The primary outcome of interest was predictors of ever developing QE post-OHT. Secondary outcomes are as follows:

1) QE within the first 15 days post-OHT (early QE);
2) Acute cellular rejection;
3) Pathologic antibody mediated rejection; and
4) Death.

Of note, any possible relationship between the development of QE and the development of future rejection episodes (ACR and/or pAMR), as well as death were investigated.

Data Analysis: Summary statistics for baseline characteristics were computed for the cohort in its entirety, as well as for the groups stratified upon the CMV donor-recipient serostatus pair. CMV serostatus data was incomplete in five subjects (either the donor (4) or the recipient (1) status was unknown). These subjects were included in assessing the baseline characteristics and any analyses executed on the entire cohort. However, these five subjects were excluded for any analyses performed on the CMV donor-recipient serostatus stratified cohort. All analyses, except Cox regression models, were performed using JMP ® Pro 11. Cox regression analyses were performed using Statistical Analysis System (SAS) Version 9.3 ®.

Subsequently, the entire cohort was divided into two groups: those who developed their first Quilty lesion in less than or equal to 15 days post-OHT (early QE) and those who developed their first Quilty lesion after 15 days post-OHT. The median number of patients who ever developed QE, did so within the first 15 days, and therefore, this 15-day time point was

selected. Subject characteristics were compared between groups using a Fisher's exact test for categorical variables and a Wilcoxon rank sum test for continuous variables. A p-value of ≤ 0.05 was considered statistically significant.

Univariate logistic regression analyses were performed to evaluate factors correlating with the primary and secondary outcomes. Variables that were statistically significantly associated (p-value ≤ 0.05) with the outcome(s) were then combined in multivariate logistic regression models. In the multivariate logistic analyses, a backward stepwise regression using minimum BIC criteria was used to create the final model. All interactions were evaluated for in the univariate model and were only included in tables if statistically significant.

Additionally, Kaplan-Meier curves were constructed for the following time to event outcomes: time to development of the first Quilty lesion, time to first biopsy-proven ACR, time to first biopsy-proven AMR event, and time to death. Several sets of curves were created for each outcome by stratifying among the CMV donor-recipient groups, CMV discordant and concordant serostatus, and cyclosporine use. The CMV discordant group included D+/R- and D-/R+, and the concordant group included D+/R+. The D-/R- group was not included because of their lack of CMV at time of transplantation. For all time-to-event curves, a Log-rank test was used to compare survival curves between the groups.

Lastly, Cox Proportional Hazards univariate and multivariate regression models were created specifically to look at the impact of certain covariates on the development of the first QE event. A Log-rank test was used to compare the groups.

RESULTS

Cohort characteristics: In total there 118 patients were identified as having received a MCSD as a BTT. However, 3 of these patients were undergoing a second heart transplant after receiving a MCSD, and consequently were excluded from this study. Therefore, the cohort included a total of 115 subjects. As detailed in the Methods, in performing the analyses stratified by CMV sero-pairs, any pair with an unknown serostatus was excluded, and therefore 110 patients were used for these analyses (Figure 1).

Table 1 summarizes all baseline characteristics. In the overall cohort, the median recipient age was 51 years (IQR of 42 - 59) and the median BMI was 24.9 kg/m^2 (IQR 22.2 – 29). The youngest recipient was 18 years old, and the oldest recipient was 72 years old. The majority of the patients were men (86.1%), had a non-ischemic etiology of their heart disease (60%), and received a pulsatile MCSD pump type (78.3%). Almost half of the patients (45.2%) had a prior history of tobacco use. Most patients required the use of a MCSD for a median time of 76 days before being successfully transplanted. With regards to the donor data, the majority of the donors were men (81.7%), with a median age of 26 years (IQR 20.3 – 34). The youngest donor was 11 years old, and the oldest donor was 58 year old. The median allograft ischemia time was 246 minutes.

The majority of the donors were CMV seropositive (73%). Similarly, the majority of the recipients were CMV seropositive (73%). When stratified into CMV donor-recipient (D/R) serostatus pairs, 22 were D+/R-, 23 were D-/R+, 60 were D+/R+, and 5 were D-/R-.

After successful transplantation, each patient was followed at our institution for median time of 289 days (approximately 10 months; range 0 – 2,888 days). During this time, a total of 1,337 biopsies were performed at our institution. The median number of biopsies performed per patient was 12, with a range of 0 – 29. Total follow up time, which frequently differed from that of institutional follow up time, was obtained through UNOS. The median follow up time in total was 1,362 days (just below 4 years; range 5 – 5,148 days).

Transplantation and immunosuppression: Successful BTT per year is shown in Figure 2A. Each percent represents the proportion of patients in this cohort transplanted each year. The majority these patients were transplanted in the more recent years: 2007 to 2012. Interestingly, the distribution of primary calcineurin inhibitor used by year, as demonstrated in Figure 2B, reveals that cyclosporine was used routinely until 2001. In 2001, there is a marked increase in tacrolimus use, as compared to CsA. In 2006 - 2007, there is a peak in CsA use. This is attributed to a clinical trial that our institution participated in at that time. In this trial, some subjects from this cohort were randomized to receive either lower-dose cyclosporine plus everolimus (a mammalian target of rapamycin –mTOR – inhibitor which is often used in part of an immunosuppression in solid organ transplant recipients) or standard dose CsA with mycophenolate mofetil (also a commonly used immunosuppressive agent in solid organ transplant recipients) (64). After completion of our institution's participation in this study, it appears that cyclosporine is still used, but rather sparingly.

Outcomes: In the overall cohort (n=115), 100 (86.9%) had QE on at least one biopsy; 89 (77.4%) had at least one biopsy-proven ACR, and 14 (12.2%) had at least one biopsy-proven pAMR. In total, there were 24 (20.8%) dead and 3 (2.6%) who were lost to follow up. Of the

remaining 88 who are known to be alive, 5 required re-transplantation. Table 2 summarizes the outcomes for the overall cohort as well as the stratified cohort.

Since the majority had experienced both QE and ACR at some point post-OHT, this was further assessed. Table 3 reveals that of the 100 who had QE, 85 also had ACR.

Immunosuppressive regimen and outcomes: Table 4 demonstrates the distribution of primary calcineurin inhibitor (CsA versus tacrolimus) by QE or ACR. Of the 100 who ever had QE, 23 received CsA and 82 received tacrolimus. Of the 89 who had ACR, 21 received CsA and 73 received tacrolimus.

In total, 25 ever received CsA, and 95 ever received tacrolimus. Five had started one regimen and changed to another: 4 started CsA and changed to tacrolimus and 1 changed from tacrolimus to CsA. Of the 4 who started CsA and changed to tacrolimus – 3 did so because of persistent ACR or pAMR and 1 did so for unknown reasons. The one subject who changed from tacrolimus to CsA did so because of severe tremor associated with tacrolimus use.

To note, of the 25 who ever received CsA, 11 had received as part of the clinical trial that was ongoing during 2006 - 2007. Three of the 11 had received a slightly decreased CsA dose with everolimus and the other 8 received standard CsA dosing with mycophenolate mofetil.

Furthermore, it was pertinent to determine which calcineurin inhibitor was in use prior to the onset of the first biopsy proven QE and first biopsy proven ACR. Twenty-two had started CsA before the onset of their first QE lesion (one started after the first biopsy-proven QE), and 78 started tacrolimus before their first QE lesion (4 started tacrolimus afterwards). For ACR, 20 started CsA before the first biopsy proven ACR (1 started after), and 69 started tacrolimus before their first ACR event (where as 4 started afterwards).

Univariate analyses: The overall cohort was first evaluated by dividing it into two groups: whether they experienced their first biopsy-proven QE in the first 15 days post-OHT (early QE) or beyond the first 15 days. Forty-nine (43%) developed early QE. By 3 months, 90 developed QE (86%); and by 6 months, 96 (92%) developed QE (Figure 3). Univariate analyses revealed that there were not any statistically significant differences between these two groups (Table 5).

Univariate logistic regression analyses were performed to determine the predictors of the primary and secondary outcomes (Table 6).

Quilty Effect within first 15 days (Early QE): In patients who developed early QE, CsA and tacrolimus use were statistically significant. It appears that CsA use early on decreases the odds of having early QE (OR 0.29, 95% CI 0.09 – 0.8), where as tacrolimus use increases the odds of having early QE (OR 5, 95% CI 1.5 -22.5).

QE Ever: Donor age appears to be the only statistically significant variable, revealing an OR of 0.94 (p = 0.02).

Rejection and Death: Ever having QE was statistically significantly associated with ACR development (14.2, 95% CI: 4.2 – 57.4). Otherwise, there were no other statistically significant predictor variables within this cohort for ACR, AMR, or death.

Multivariate analyses: The variables that were deemed statistically significant in univariate analyses were included in the multivariate model. However, some of the univariate models did not have any variables that were statistically significant and/or only had one variable that was significant. Therefore, the following multivariate models were assessed:

1) Early QE, 2 models were evaluated.
    a. CsA use and donor gender
    b. Tacrolimus use and donor gender

These variables were chosen because CsA and tacrolimus were statitistically significant in the univariate model, but also based on the fact that generally female donor gender portends a poorer prognosis overall.

2) QE ever, 2 models were evaluated.
    a. CsA use and donor age
    b. Tacrolimus use and donor age.

These variables were chosen because donor age is statistically significant in the univariate model for QE ever, but CsA and tacrolimus were selected based upon previously published studies that the immunosuppressive regimen may play a role in QE development.

3) ACR ever, 2 models were evaluated.
    a. QE ever and CsA use
    b. QE ever and tacrolimus use

Similar to the QE models, QE ever was chosen in the ACR multivariate model because it was statistically significant in the univariate model, and CsA and tacrolimus were chosen based upon prior knowledge that CsA tends to be associated with worse outcomes with regards to ACR development.

4) pAMR – the multivariate model created included QE and CMV seroconcordance versus discordance.
5) Death – the multivariate model created included recipient gender and QE.

Recipient gender was included based upon prior knowledge that recipient gender being female generally have worse outcomes when compared to men.

Of note, for the outcomes where both CsA and tacrolimus were of interest as predictors, these were split into two models because of the colinearity that likely exists between CsA use and tacrolimus use.

Early QE and QE ever: In early QE, CsA and tacrolimus use remained statistically significant. The adjusted OR for CsA use was 0.33 (p=0.03), and the adjusted OR for tacrolimus use was 4.36 (p=0.02).

In QE ever, donor age remained statistically significant. When evaluated in the model with CsA, the adjusted OR for donor age was 0.94 (p=0.03), and in the model with tacrolimus, the adjusted OR for donor age was 0.95 (p=0.03). In other words, for every year older the donor, the decrease in odds there was of ever developing QE.

Acute Cellular Rejection: In both multivariate models assessed, QE ever remained statistically significant. In the model with CsA, QE ever had an adjusted OR of 13.2 (p <0.0001), and in the model with tacrolimus, QE ever had an adjusted OR of 13 (p <0.0001).

Pathologic Antibody Mediated Rejection: Neither variable was statistically significant, but the adjusted OR for QE ever was 1.8 (95% CI of 0.3 – 34.3), and the adjusted OR for CMV discordant versus concordant was 1.08 (95% CI of 0.32 – 3.6).

Death: Similarly, neither variable was statistically significant, but the adjusted OR for recipient gender was 2.3 (95% CI 0.58 – 15.2), and the adjusted OR for QE ever was 1.9 (95% CI 0.5 - 12.8).

Time to Event Analyses: All time to outcome events were assessed. Figures 4, 5, and 6 demonstrate time to first biopsy-proven QE, ACR, pAMR and time to death. These were

modeled within the stratified cohort (Figure 4), by CsA use (Figure 5), and by CMV serodiscordant versus CMV seroconcordant (Figure 6). None of these were statistically significant.

Cox Regression Model: Cox proportional hazards regression model was utilized to assess the impact of specific covariates on the risk of development of the first biopsy-proven QE event. In both univariate and multivariate models, donor age was statistically significant. The adjusted hazards ratio was for every 10-increase in donor age was 0.61 ($p = 0.0007$) (Table 7).

DISCUSSION

QE Incidence: Interestingly, the incidence of QE is higher in our cohort as compared to previously published reports (86.9% versus 78.6%). At our institution, a total of 3 pathologists read all EMBs for this cohort, one of whom has read the majority. This results in decreased inter-reader variability, and may potentially reflect a more accurate incidence of QE. Furthermore, this may also be consistent with our hypothesis that MCSD use predisposes to QE development, and this higher incidence may be a reflection of this cohort all receiving MCSD.

QE and CsA and Tacrolimus use: In this cohort, CsA use was an important predictor of early QE. In this early post-OHT period, CsA use was associated with decreased odds of developing early QE. However, CsA appears to increase the odds of ver QE development, though this was not statistically significant. This may represent a time-related association with CsA use and it's serum concentration and/or myocardial concentration. Our study revealed findings consistent with that of the prior studies (2, 11, 12, 15, 16, 65) – that CsA use increases one's propensity to

develop QE. The pathophysiology of this is still poorly understood, and may either reflect poor CsA levels within the myocardium or may be due to an idiosyncratic reaction (2, 11, 12, 15, 16, 65). Another possibility is that the use of CsA is confounded by an unidentified patient factor that has not been taken into account. This is not entirely clear based on this cohort, and further investigation would be necessary to better understand this. In general, tacrolimus is associated with improved outcomes and less adverse events, when compared to CsA use (66-70). Furthermore, in its early days, tacrolimus had also been used as salvage therapy for patients who developed refractory and/or recurrent cardiac allograft cellular mediated rejection while on CsA (71-74). Thus, tacrolimus is a superior agent when compared to CsA. Consequently, since the early 2000s, tacrolimus use is the standard of care, therefore making further investigations into CsA challenging.

In this cohort, one confounder that was difficult to adjust for was that close to half of those who received CsA, did so in the context of a clinical trial that was ongoing between 2006 and 2007 at our institution. In this small group of subjects, some received a low-dose of CsA with everolimus – an uncommonly used alternative anti-rejection regimen. Since so few received this regimen it likely had little impact on the overall analyses, but the effect of everolimus is difficult to separate from the effect of CsA.

Given the improved outcomes and safety profile with tacrolimus (66-74), it was interesting to find that tacrolimus use increases one's likelihood of developing early QE. Similar to CsA use, this also appears to have a time-dependent effect. When evaluating the relationship of tacrolimus use, there was a trend towards decreased odds of QE ever, although this did not reach statistical significance (OR 0.33, 95% CI of 0.02 – 1.85). These findings are consistent

with a prior study by Sgrosso, et al (65). Perhaps this is due to enhanced concentrations of tacrolimus within the myocardium, or secondary to an inherent property of the drug that makes it intrinsically different when compared to CsA in its effects to protect from QE development.

QE and Age: In this cohort, we found that recipient age had no impact on the development of early QE or QE ever. However, donor age, was statistically significant for both early and ever QE ever. In the logistic regression, donor age was slightly protective in the development of QE, in other words, with older donors, there is decreased odds of developing QE ever. Furthermore, in the Cox Proportional Hazards model it appears that this protective effect is also seen. This may be explained by immunosenescence due to aging (75), and supports the prior findings that there is a lower incidence of QE among older patients as compared to pediatric transplant recipients (2, 7).

QE and CMV serostatus: In this cohort, it appears that donor CMV seropositivity appears to decrease the odds of early QE (OR 0.62, 95% CI 0.25 – 1.55) and ever QE (OR 0.48, 95% CI 0.07 – 1.93). However, when the D/R seropairs were evaluated, D+/R- an D+/R+ decreased the odds of early QE, but increased the odds of QE ever. Furthermore, when serodiscordance (D+/R- or D-/R+) is compared to seroconcordance (D+/R+), it appears that serodiscordance is increases the odds of developing early QE and QE ever. Perhaps these findings are due to the aggressive CMV-prophylactic measures that are taken in these patients, as the majority of this cohort would have received prophylaxis for 3 to 12 months, per older guidelines (62, 63). This raises the possibility that the length of CMV prophylaxis may impact the likelihood of QE development, an element that is difficult to analyze in a retrospective review. Further studies, such as a randomized controlled trial comparing various durations of CMV prophylaxis and the

time to developing QE and the frequency of QE would be necessary to further assess the impact of CMV prophylaxis on this outcome.

Another possible theory to explain the decreased odds in early QE among the D+/R- and D+/R+ subjects, and their progression to increased odds to QE ever is if the recipient immune system is primed to create an immune response to CMV presence in comparison to those who are D-/R+ and D-/R-. In other words, if a donor heart has CMV antigen present (D+), and is given to a recipient that also has CMV antibody present (R+), the normal immune response would be to react to the CMV antigen present in the donor heart. However, since the recipient, at time of transplantation, is given a large load of immunosuppression and since they are also started on anti-CMV prophylaxis in the immediate post-OHT period, this immune response to the donor CMV antigen is blunted. Thus, if QE is associated with CMV, this development may be hampered early post-OHT due to the rationale as described. However, as time progresses, and immunosuppression slightly decreases and CMV prophylaxis is no longer administered, this immune reaction is allowed to occur, and may consequently allow for QE to develop. A schematic to illustrate this theory is proposed in Figure 7A-D. To further test this possibility, a study using immunohistochemistry staining on EMB specimens that are positive for QE, may be able to assist in determining what immune interactions are occurring and if CMV positive cells are present at the QE site.

In the Cox model, recipient CMV seropositivity and donor CMV seropositivity were also found to be protective of first QE event. Given that there is significantly less risk of CMV-associated disease in the seroconcordant groups, there is likely less immune phenomena attributable to CMV occurring, therefore, decreasing their risk of developing immune mediated findings, including QE.

QE and Gender: Being a male recipient was associated with increased odds of developing early QE (OR 1.26, 95% CI 0.48 – 3.96), though not statistically significant. Furthermore, this effect was reversed in evaluating ever developing QE (OR 0.96, 95% CI 0.14 – 4.06), but this was also not statistically significant. Generally, females have a greater risk of allograft rejection (76, 77), likely due to a greater degree of allosensitization amongst other reasons, and given the pathologic findings of B- and T-cells in QE, one would expect women in this cohort to have more QE. One reason for our finding of men having a higher odds of developing early QE, may be due to the fact that this cohort is largely comprised of men (86.1%), and therefore we may not have not adequately captured the odds associated with being female and developing QE. Further studies with a larger cohort would be necessary to more accurately assess the impact of gender on QE.

QE and MCSD Parameters: Non-pulsatile MCSD pump types generally have better outcomes and less adverse events while on the MCSD, as well as post-OHT (39, 40). Furthermore, it has been shown that the pulsatile MCSDs are more immunostimulatory than the pulsatile MCSDs (41). In this cohort, however, the non-pulsatile pump type increased the odds of ever developing QE (OR 1.81; 0.45 – 12.2), though this was not statistically significant. Additionally, time spent on the MCSD had no impact whatsoever on QE development. Thus, MCSD use, regardless of type of pump or duration of support, does not significantly contribute to developing early QE or QE ever, although this question should be addressed again as the filed of MCSD continues to evolve, with the introduction and growing use of both the total artificial heart and smaller pulsatile devices.

Rejection and Death: Similar to Zakliczynski et al (19), we found that QE ever is associated with biopsy-proven ACR development. Additionally, like other studies (10, 18), we found a trend towards increased odds of pAMR and death, though not statistically significant (OR of pAMR 1.88, 0.33 – 35.66; OR of death 1.69, 0.41 – 11.2). If this cohort were larger, we may have been able to detect a statistically significant difference.

Furthermore, univariate analyses revealed some additional interesting trends. It appears that non-pulsatile MCSD pump type, recipient CMV seropositivity, donor CMV seropositivity, and tacrolimus use revealed a trend of decreasing the odds of developing ACR, with ORs all less than 1, though none were statistically significant. Additionally, donor male gender and post-OHT cyclosporine use may increase one's odds of developing ACR, though these variables were not statistically significant, as well.

In evaluating predictors of pAMR, none of the variables in the univariate analyses were statistically significant. However, as noted with the other outcomes, some interesting tendencies were observed. Recipient gender being male, donor CMV seropositivity, and cyclosporine use revealed decreased odds of developing pAMR. Wehre as, ischemic etiology, recipient CMV seropositivity, donor male gender, tacrolimus use, and developing QE ever had ORs greater than 1, therefore increasing the odds of pAMR.

Similarly, in the univariate analyses looking at death, none of the variable were statistically significantly associated with death, though there were some covariates with notable propensities. Non-pulsatile MCSD pump type and donor CMV seropositivity had ORs that were

less than 1, indicative of a possible protective effect in this cohort. However, recipient male gender, donor male gender, cyclosporine use, and QE ever appear to have an adverse impact on this cohort, increasing the odds of death.

Strengths and Limitations: A strength of this study is that it evaluates QE solely in a population that is BTT using an MCSD; to date, this has not been examined before.

Although this cohort is uniquely heterogeneous spanning a large time frame, in which there was marked evolution in regards to MCSD pump type, immunosuppressive regimens, and advancements in histopathologic definitions of EMB findings, this is poses as a limitation of this study as it adds confounders related to changes in practice and protocol that cannot be accounted for in this retrospective analysis. Furthermore, the analyses presented do not compare a population that has undergone MCSD to a population that undergoes OHT without MCSD assistance. To fully comprehend the effect of the MCSD as well as other predictors, on QE, comparison with a group that proceeds directly to OHT is necessary. Additionally, assessing QE over time, as a repeated outcome variable, would be beneficial in better understanding the recurrent nature of QE and it's impact on ACR, pAMR, and death.

Future Directions: Expanding upon these analyses by using a comparison group (those who directly underwent OHT without a MCSD bridge), as well as taking into account the type and duration of CMV prophylactic regimens may better elucidate the role that MCSD and CMV may be playing in QE development. Reaching a better understanding of the factors influencing progression to rejection after heart transplant can allow for more precise patient risk

stratification and individualization of immunosuppression regimens, antiviral prophylaxis, and patient monitoring, improving patient outcomes.

Figure 1. Schematic demonstrating patient selection.

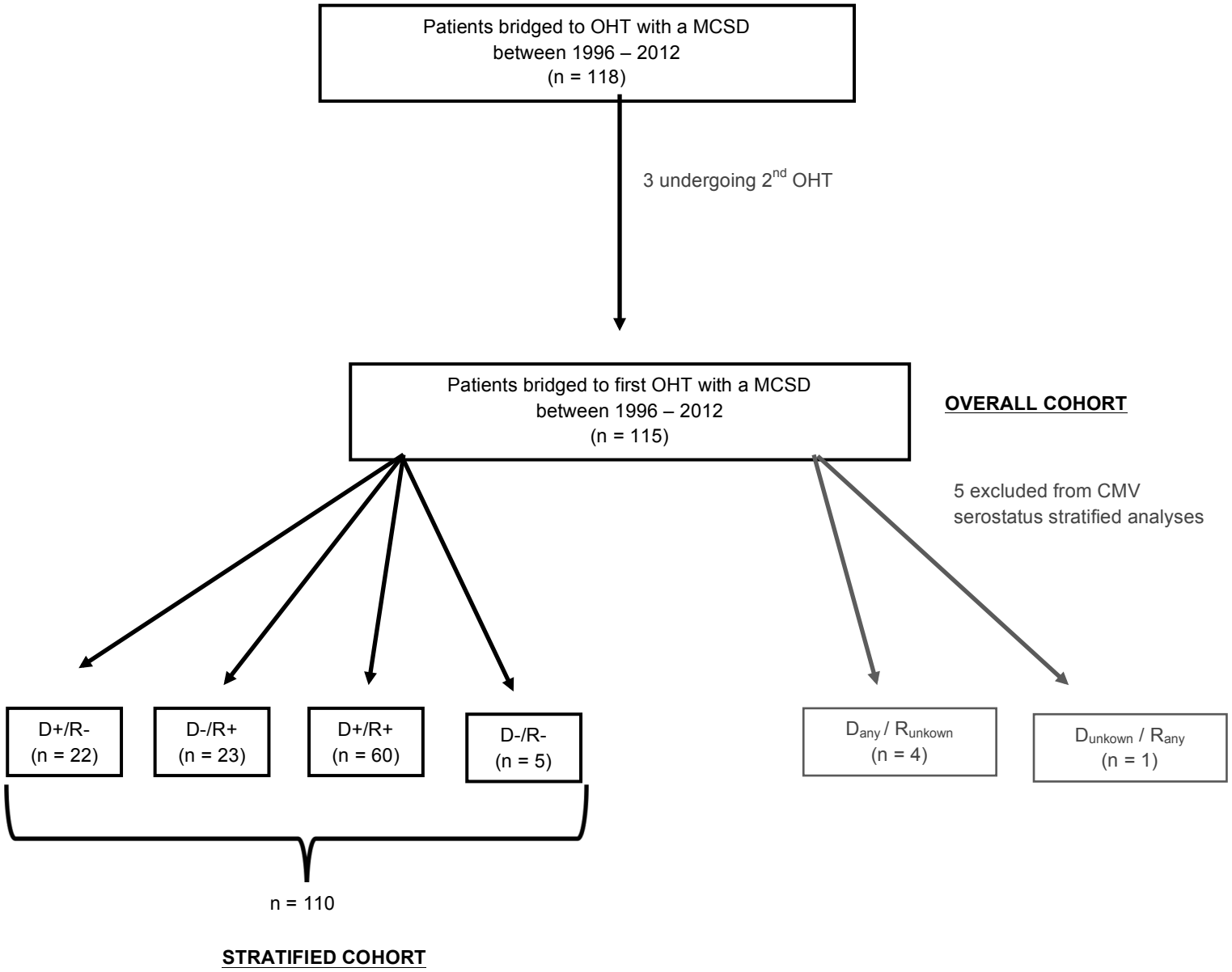
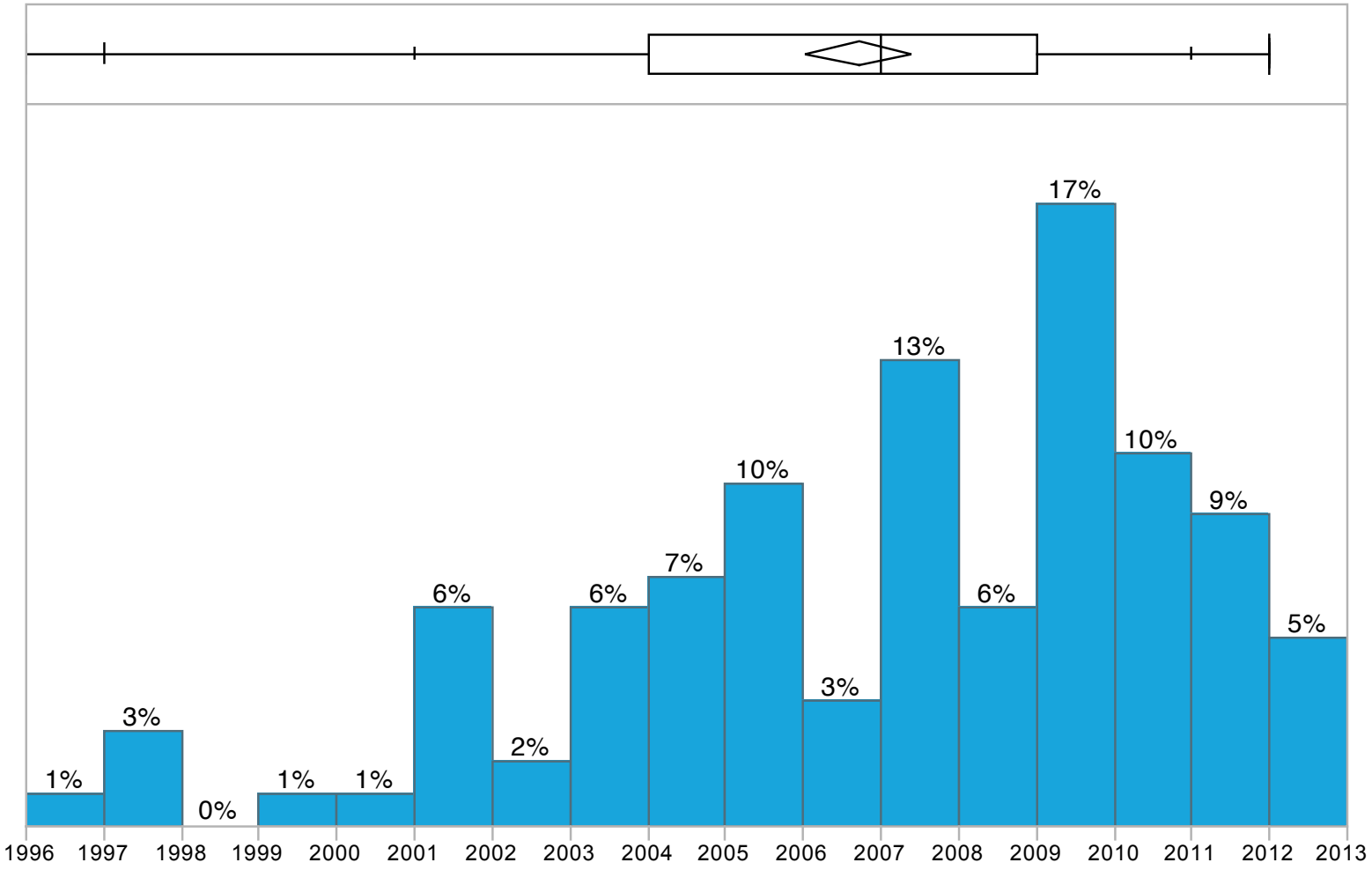


Table 1. Characteristics of cohort: overall and by CMV serostatus pairs					
	Overall Cohort (n = 115)	**Stratified Cohort (n = 110)**			
		D+/R- (n = 22)	D-/R+ (n = 23)	D+/R+ (n = 60)	D-/R- (n = 5)
Recipient Age, median (IQR)	51 (42 – 59)	50 (40.3- 59.3)	50 (36- 55)	52 (42.3- 60.8)	52 (49.5- 63)
Recipient Gender, n (%)					
Male	99 (86.1%)	17 (77.3%)	19 (82.6%)	54 (90%)	4 (80%)
Female	16 (13.9%)	5 (22.7%)	4 (17.4%)	6 (10%)	1 (20%)
Etiology of HD, n (%)					
Non-ischemic	69 (60%)	15 (68.2%)	15 (65.2%)	29 (48.3%)	5 (100%)
Ischemic	46 (40%)	7 (31.8%)	8 (34.8%)	31 (51.7%)	0
Prior tobacco use, n (%)	52 (45.2%)	7 (31.8%)	10 (43.5%)	31 (51.7%)	3 (60%)
BMI (kg/m^2), median (IQR)	24.9 (22.2 – 29)	24.3 (22.7- 31.7)	26.85 (24.1- 29.7)	24.2 (21.4- 28.5)	27.6 (25.3- 30.7)
MCSD pump type, n (%)					
Non-pulsatile	25 (21.7%)	6 (27.3%)	8 (34.8%)	10 (16.7%)	1 (20%)
Pulsatile	90 (78.3%)	16 (72.7%)	15 (65.2%)	50 (83.3%)	4 80%)
Duration of MCSD support (days), median (IQR)	76 (45 – 124)	77.5 (47.5- 139.8)	84 (50- 186)	65 (44 – 110.8)	84 (51.5- 123.5)
Donor gender, n (%)					
Male	94 (81.7%)	19 (86.4%)	18 (78.3%)	47 (78.3%)	5 (100%)
Female	18 (15.7%)	2 (9.1%)	4 (17.4%)	12 (20%)	0
Unknown	3 (2.6%)	1 (4.5%)	1 (4.3%)	1 (1.7%)	0
Donor age, median (IQR)	26 (20.3 – 34)	27 (20.5 – 39.5)	22.5 (18 -31)	26 (21 -33)	38 (23.5 – 40.5)
Post-OHT CsA use, n (%)					
Yes	25 (21.7%)	6 (27.3%)	4 (17.4%)	13 (21.7%)	1 (20%)
No	90 (78.3%)	16 (72.7%)	19 82.6%)	47 (78.3%)	4 (80%)
Recipient CMV serostatus, n (%)					
Positive	84 (73%)	---	---	---	---
Negative	27 (23.5%)				
Unknown	4 (3.5%)				
Donor CMV serostatus, n (%)					
Positive	84 (73%)	---	---	---	---
Negative	28 (24.3%)				
Unknown	3 (2.6%)				

Figure 2A. Distribution of those bridged with MCSD and successfully transplanted by year (1996 – 2012).



% Successfully transplanted after bridge with MCSD by year

Figure 2B. Distribution of cyclosporine and tacrolimus use by year of OHT.

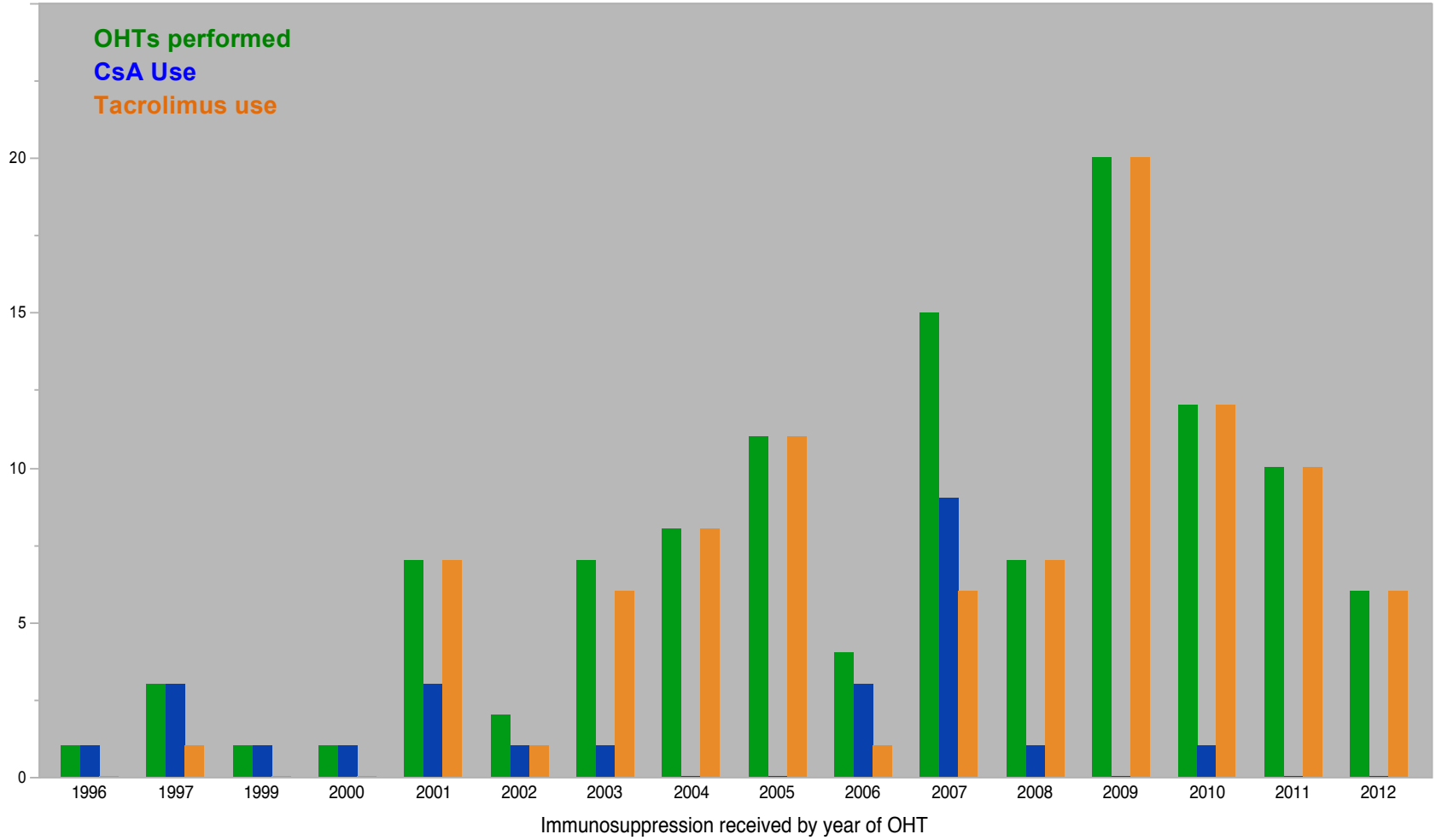


Table 2. Distribution of outcomes by CMV seropairs					
	Overall cohort (n = 115)	**D+/R- (n = 22)**	**D-/R+ (n = 23)**	**D+/R+ (n = 60)**	**D-/R- (n = 5)**
QE +, n (%)	100 (86.9%)	20 (90.9%)	22 (95.6%)	49 (81.7%)	4 (80%)
ACR +, n (%)	89 (77.4%)	19 (86.4%)	20 (86.9%)	43 (71.7%)	5 (100%)
pAMR +, n (%)	14 (12.2%)	1 (4.5%)	5 (21.7%)	7 (11.7%)	0
Death*, n (%)	24 (20.8%)	5 (22.7%)	6 (26.1%)	12 (20%)	1 (20%)

Table 3. Distribution of QE by ACR			
	ACR Ever		
		Yes	No
QE Ever	Yes	85	15
	No	4	10

Table 4. Primary calcineurin inhibitor use by QE ever and ACR Ever					
		QE Ever		**ACR Ever**	
		Yes	No	Yes	No
Cyclosporine Use	Yes	23	1	21	3
	No	77	13	68	22
Tacrolimus Use	Yes	82	13	73	22
	No	18	1	16	3

Figure 3. Development of first QE within 15 days post-OHT, 3 months post-OHT, and 6 months post-OHT

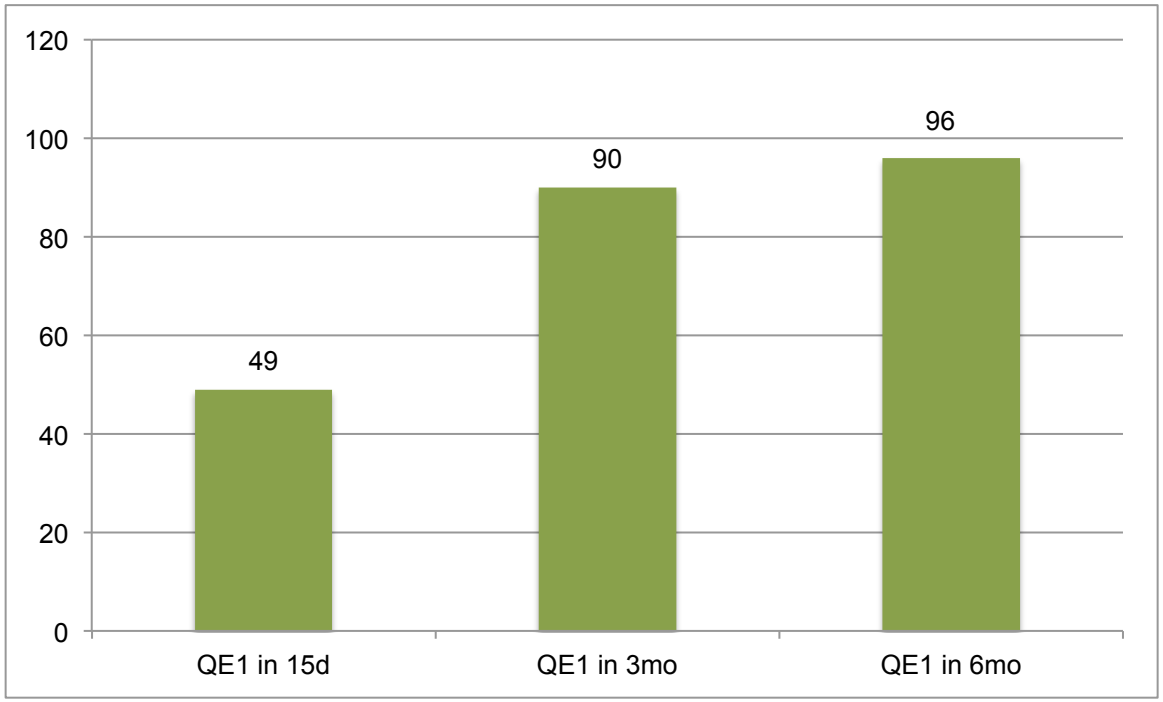


Table 5. Characteristics of patients by QE development within first 15 days post-OHT versus those who develop QE beyond the first 15 days			
	QE in ≤ 15 days post-OHT (n = 46)	**QE >15 days post-OHT (n = 54)**	**p-value**
Recipient Age, median (IQR)	51 (41.5 – 59.25)	52 (35.25 – 60)	0.86
Recipient Gender, n (%)			1.00
Male	40 (86.9%)	46 (85.2%)	
Female	6 (13%)	10 (14.8%)	
Etiology of HD, n (%)			0.54
Non-ischemic	29 (63%)	30 (55.6%)	
Ischemic	17 (37%)	24 (44.4%)	
Prior tobacco use, n (%)	21 (46%)	23 (42.6%)	0.69
BMI (kg/m^2), median (IQR)	25.6 (20 – 29.3)	26.1 (22.7 – 29.2)	0.48
MCSD pump type, n (%)			1.00
Non-pulsatile	10 (21.7%)	14 (22.2%)	
Pulsatile	39 (78.3%)	51 (77.8%)	
Duration of MCSD support (days), median (IQR)	70 (42.3 – 129.3)	74.5 (39 – 111)	0.48
Recipient CMV serostatus, n (%)			0.81
Positive	34 (73.9%)	38 (70.3%)	
Negative	10 (21.7%)	14 (25.9%)	
Unknown	2 (4.3%)	2 (3.7%)	
Donor gender, n (%)			0.39
Male	36 (78.3%)	47 (87%)	
Female	8 (17.4%)	6 (11.1%)	
Unknown	2 (4.3%)	1 (1.9%)	
Donor age, median (IQR)	24.5 (19.3 – 31)	26 (21 – 34)	0.29
Donor CMV serostatus, n (%)			0.36
Positive	30 (65.2%)	41 (75.9%)	
Negative	14 (30.4%)	12 (22.2%)	
Unknown	2 (4.3%)	1 (1.9%)	
CsA use prior to QE, n (%)	5 (10.9%)	17 (31.5%)	1.00
Tacrolimus use prior to QE, n (%)	37 (80.4%)	41 (75.9%)	1.00

Table 6. Predictors of QE, ACR, pAMR, and death; OR (95% CI)						
	QE in ≤ 15 days post-OHT			**QE Ever**		
	Univariate	**Multivariate**		**Univariate**	**Multivariate**	
		Model 1	Model 2		Model 1	Model 2
Recipient Age	1.00 (0.98 – 1.03)			0.99 (0.95 – 1.03)		
Recipient Gender (female)	1.26 (0.43 – 3.96)			0.96 (0.14 – 4.06)		
BMI	0.99 (0.93 – 1.06)			1.03 (0.94 – 1.15)		
MCSD pump type (pulsatile)	0.97 (0.38 – 2.42)			1.81 (0.45 – 12.2)		
Duration of MCSD support	1.00 (1.00 – 1.01)			1.00 (1.0 – 1.01)		
Recipient CMV serostatus (negative)	1.25 (0.49 – 3.25)			0.81 (0.17 – 2.85)		
Donor gender (female)	0.66 (0.24 – 1.85)*	0.78 (0.27 – 2.21)	0.87 (0.29 – 2.6)	2.23 (0.55 – 7.76)		
Donor age	0.97 (0.93 – 1.01)			**0.94 (0.89 – 0.99)***	**0.94 (0.9 – 0.99)**	**0.95 (0.9 – 0.99)**
Donor CMV serostatus (negative)	0.62 (0.25 – 1.55)			0.48 (0.07 – 1.93)		
CsA use (no)	**0.29 (0.09 – 0.80)***	**0.33 (0.1 – 0.92)**		3.71 (0.68 – 69.3)*	3.63 (0.64 – 68.8)	
Tacrolimus use (no)	**5 (1.5 – 22.5)***		**4.36 (1.3 – 20)**	0.33 (0.02 – 1.85)*		0.33 (0.02 – 1.9)
CMV Seropair (D+/R- vs. D-/R-)	0.67 (0.07 – 6.5)			2.5 (0.11 – 33.4)		
CMV Seropair (D+/R+ vs. D-/R-)	0.75 (0.084 – 6.66)			1.2 (0.06 – 9.4)		
CMV Seropair (D-/R+ vs. D-/R-)	1.2 (0.13 – 11.5)			5.5 (0.19 – 160.7)		
CMV Seroconcordance (vs. discordance)	1.21 (0.53 – 2.8)			2.86 (0.8 – 1.19)		

* Variables included in the multivariate model

Table 6 (continued). Predictors of QE, ACR, AMR, and death; OR (95% CI)							
	ACR			pAMR		Death	
	Univariate	Multivariate		Univariate	Multivariate	Univariate	Multivariate
		Model 1	Model 2				
Recipient Age	0.99 (0.95 – 1.02)			0.97 (094 – 1.01)		0.98 (0.95 – 1.02)*	2.3 (0.58 – 15.2)
Recipient Gender (female)	---			0.32 (0.09 – 1.33)		2.27 (0.58 – 15.12)	
BMI	1.01 (0.94 – 1.10)			1.04 (0.95 – 1.13)		1.05 (0.98 – 1.12)	
MCSD pump type (pulsatile)	0.70 (0.25 – 2.16)			1.07 (0.23 – 3.89)		0.81 (0.25 – 2.31)	
Duration of MCSD support	1.00 (0.995 – 1.00)			1.00 (1.00 – 1.01)		1.00 (0.99 – 1.00)	
Recipient CMV serostatus (negative)	0.41 (0.09 – 1.36)			4.46 (0.81 – 83.2)		1.04 (0.38 – 3.16)	
Donor gender (female)	1.6 (0.47 – 4.93)			1.14 (0.27 – 7.84)		2.8 (0.72 – 18.49)	
Donor age	0.99 (0.95 – 1.04)			0.95 (0.88 – 1.01)		0.98 (0.93 – 1.03)	
Donor CMV serostatus (negative)	0.43 (0.014 – 1.40)			0.46 (0.14 – 1.66)		0.71 (0.26 – 2.06)	
CsA use (no)	2.16 (0.66 – 9.8)*	1.61 (0.5 - 7.9)		----		1.76 (0.64 – 4.62)	
Tacrolimus use (no)	0.57 (0.13 – 1.95)*		0.8 (0.2 - 2.82)	----		1.04 (0.33 – 3.95)	
CMV Seroconcordance (vs discordance)	0.45 (0.15 – 1.24)			1.14 (0.34 – 3.7)*	1.08 (0.32 – 3.6)	1.33 (0.52 – 3.4)	
QE Ever	**14.2 (4.2 – 57.4)***	**13.2 (3.87 – 54)**	**13 (3.8 – 52.8)**	1.88 (0.33 – 35.66)*	1.8 (0.3 – 34.3)	1.65 (0.41 – 11.2)*	1.9 (0.5 – 12.8)

*Variables included in the multivariate model

Figure 4. Time to first biopsy-proven (in days) QE (A), ACR (B), pAMR (C), and death (D) by CMV serostatus pairs

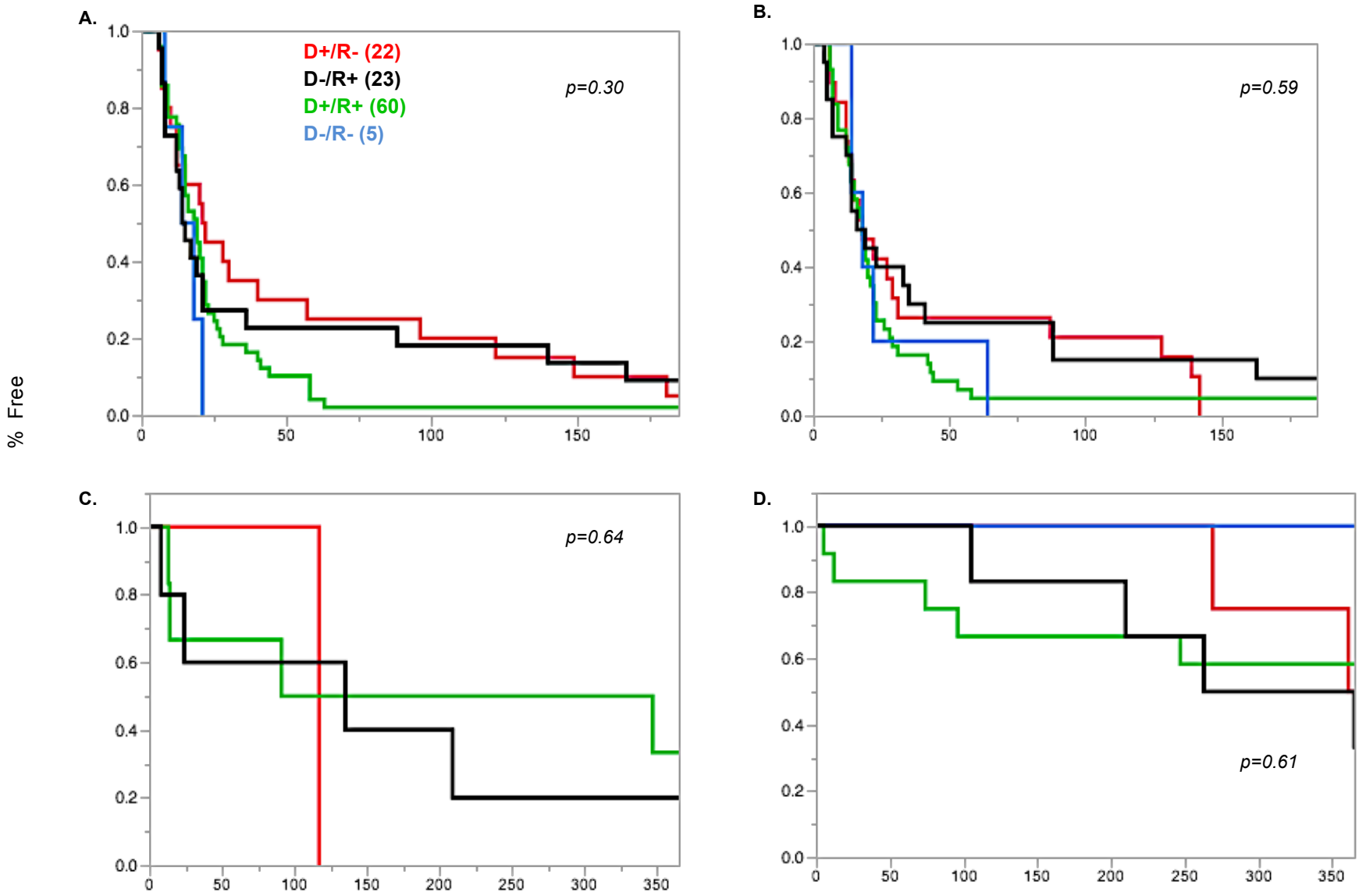


Figure 5. Time to first biopsy-proven QE (A), ACR (B), and pAMR (C) and time to death (D) by CsA use.

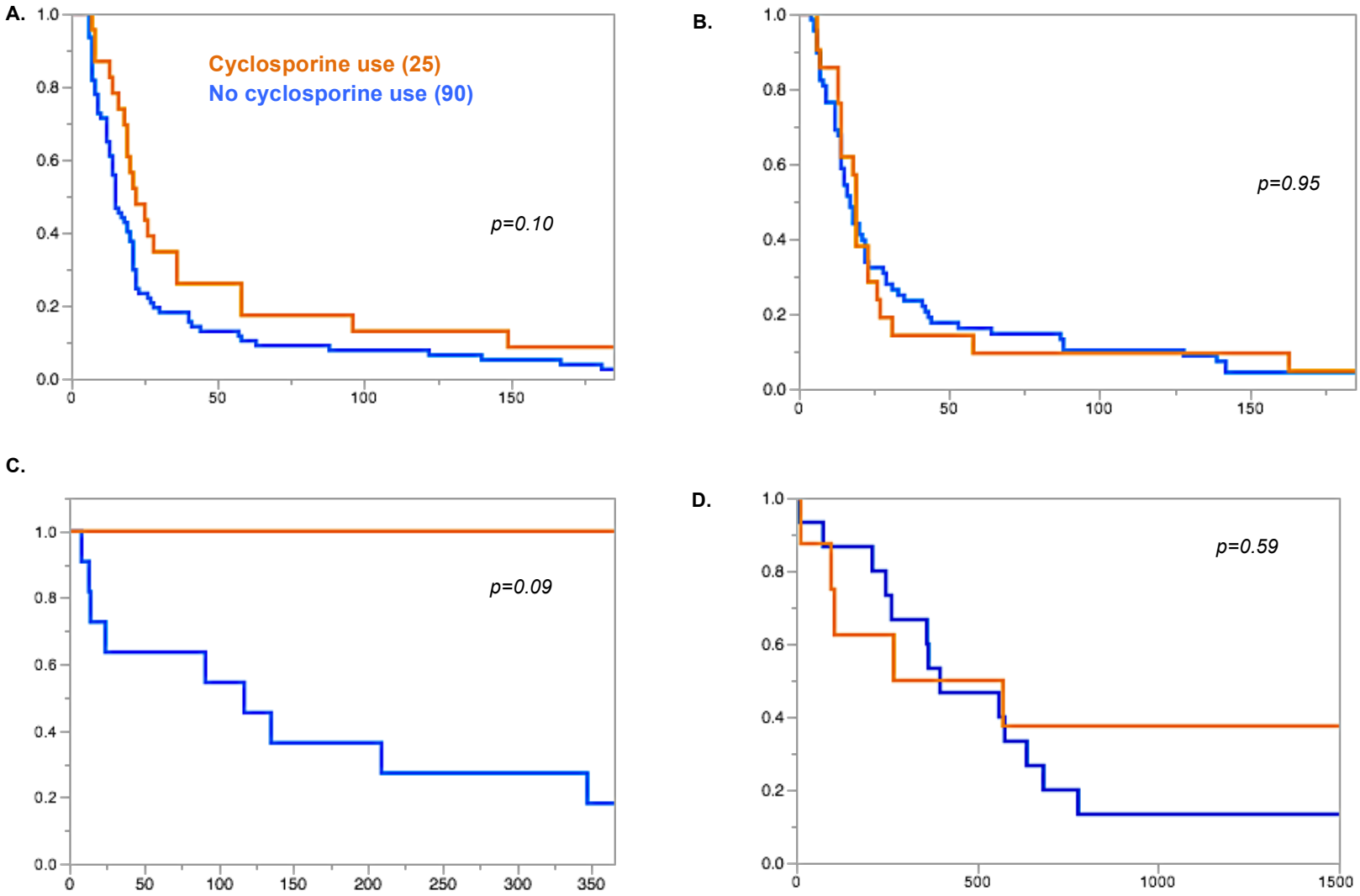


Figure 6. Time to event curves (in days) by CMV serodiscordant and seroconcordant groups; QE (A), ACR (B), pAMR (C), death (D).

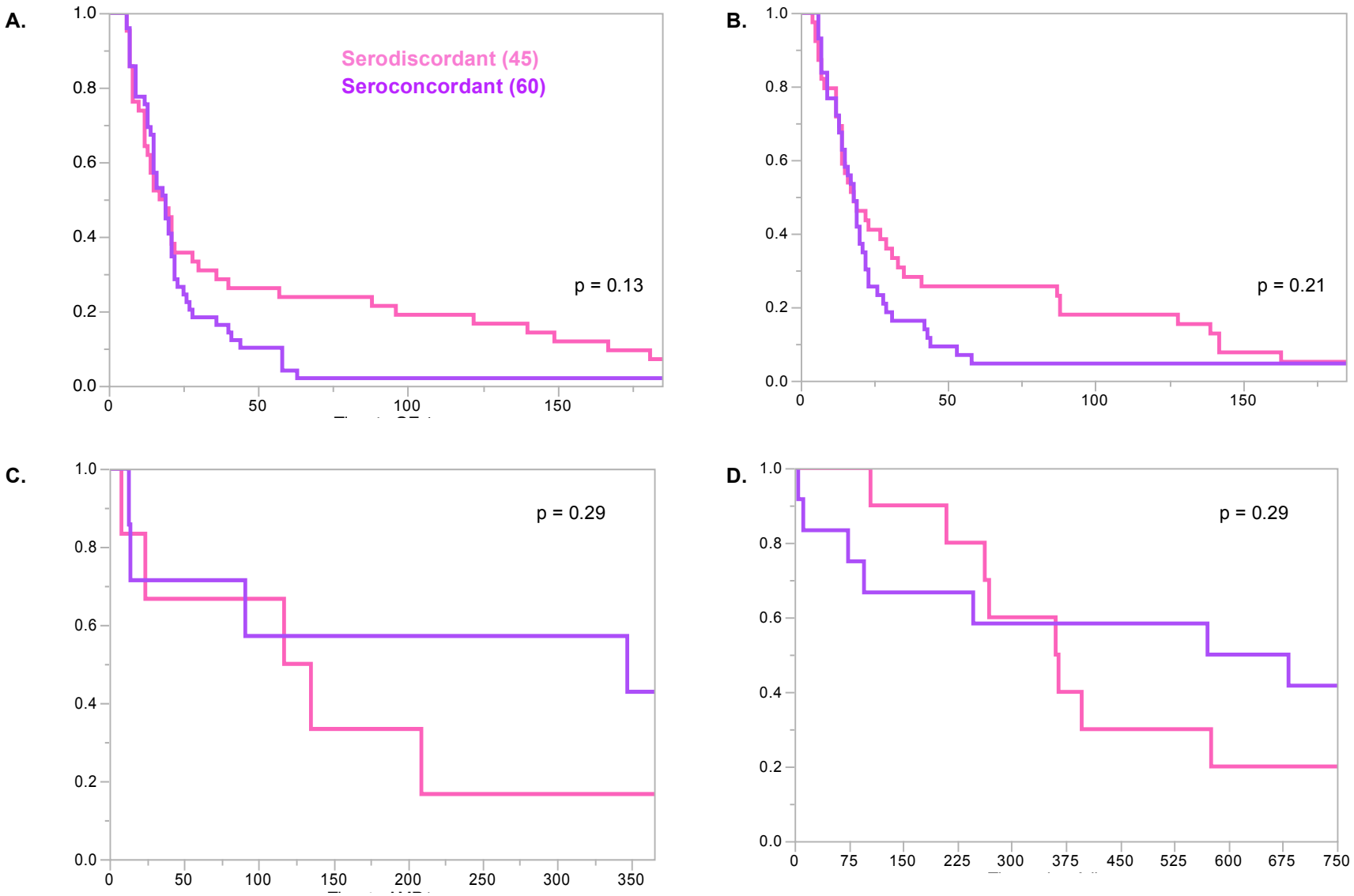


Table 7. Cox Proportional Hazards Regression Model: Time to first QE		
Parameter	**Univariate,** HR (95% CI)	**Multivariate,** HR (95% CI)
Recipient Age, per 10 years	0.94 (0.79 – 1.11)	0.87 (0.73 – 1.03)
Recipient gender (female)	1.19 (0.61 – 2.32)	1.85 (0.85 – 3.99)
Recipient CMV serostatus (negative)	1.3 (0.75 – 2.27)	0.87 (0.48 – 1.58)
MCSD Pump Type (pulsatile)	1.04 (0.59 – 1.85)	---
Duration of MCSD support	1.00 (0.99 – 1.00)	---
Donor gender (female)	1.10 (0.55 – 2.13)	0.67 (0.31 – 1.44)
Donor age, per 10 years	**0.70 (0.54 – 0.90)**	**0.61 (0.46 – 0.81)**
Donor CMV serostatus (negative)	0.75 (0.43 – 1.30)	0.69 (0.39 – 1.22)
CsA use (no)	0.99 (0.57 – 1.72)	0.77 (0.41 – 1.42)

Figure 7. Schematic illustrating theory of relationship between CMV seropairs and QE development >15 days post-OHT

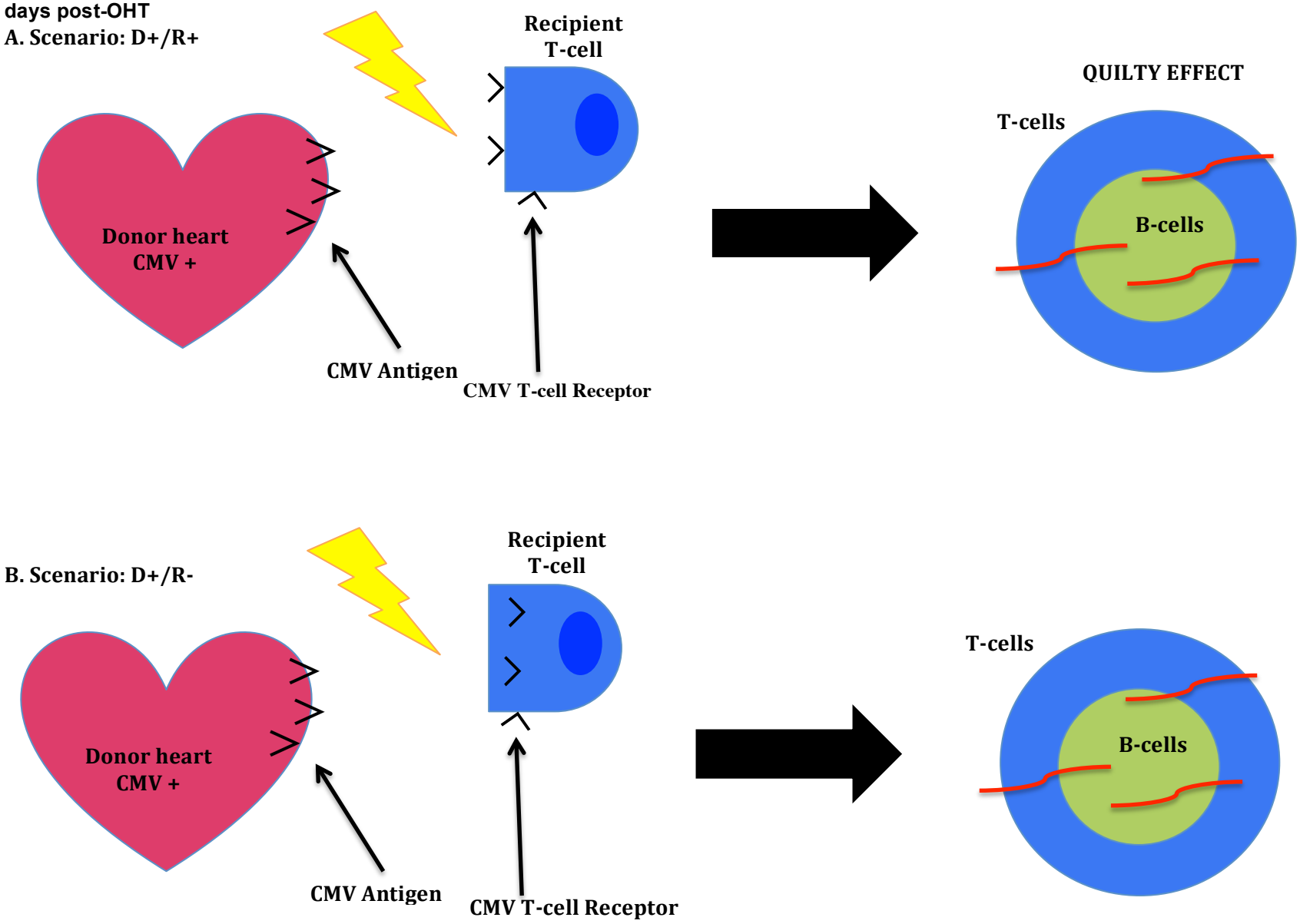
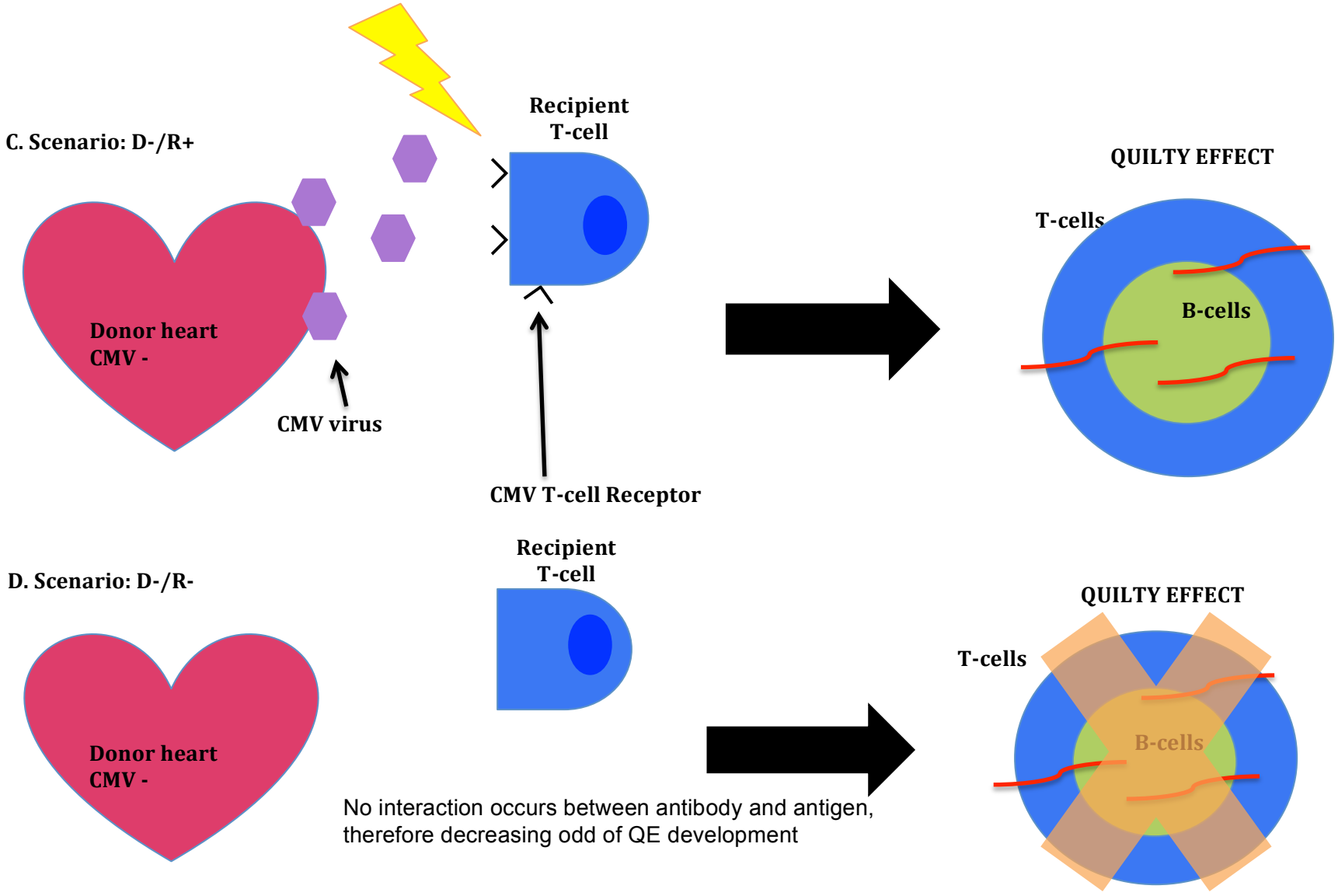


Figure 7. Schematic illustrating theory of relationship between CMV seropairs and QE development >15 days post-OHT



CHAPTER 2: STATISTICAL APPENDIX

Repeated QE measurements: A limitation of the statistical methods proposed in the manuscript, is that it does not take into account the recurrent nature of QE. Given that it is not entirely known if the recurrent nature of QE is dependent on prior QE events or if each QE finding is an independent phenomenon, two statistical methods were utilized to further investigate the repeated occurrence of QE: Anderson-Gill counting process and Prentice-Williams-Peterson counting process. These analyses were performed using Statistical Analysis System (SAS) Version 9.3 ®.

The total number of QE events in this cohort was 565, with a mean of 5.9 events per patient. Figure 8 illustrates the number of patients for a given number of QE events. For example, ten patients had one QE event, and one patient had 24 QE events.

Anderson-Gill Counting Process (AG-CP) Model for recurrent QE: This model assumes that the recurrent QE events are independent of each other, as well as a common baseline hazard function for all events.

The AG-CP model revealed that the recipient age and post-OHT cyclosporine use were statistically significant in the univariate and multivariate models. In the univariate model, for every 10-year increase in recipient age, the hazard of QE will decrease by 11.6%. Additionally, cyclosporine use increases the hazard of QE by 87.6%. The multivariate model included recipient age, recipient gender, recipient CMV serostatus, donor gender, donor age, donor CMV serostatus, and cyclosporine use. After adjusting for other covariates, every 10-year increase in

recipient age, the hazard in QE will decrease by 14.6%; where as, after adjusting for all other covariates, the hazard of QE will increase by 108%. These results have been summarized in Table 8.

Prentice-Williams-Peterson Counting Process (PWP-CP) Model for recurrent QE events: This is a conditional model, where QE events are dependent upon each other. Each recurrent event is assumed to have its own baseline hazard function.

The PWP-CP model revealed similar results as the AG-CP model (Table 9). In the univariate model, for every 10-year increase in age, there was a 5.6% decrease in the hazard of QE (though this was not statistically significant, with a p-value of 0.06). Additionally, cyclosporine use was, again, statistically significant, with an increase in the hazard of QE by 51.5% (p-value <0.0001). Lastly, in the univariate model, when the donor was older than the recipient, there was a 27.2% increase in the hazard of QE development, which was statistically significant (p-value = 0.02).

Conclusion: Interestingly, when QE is evaluated as a repeated measure, not only does CsA remain significant, but recipient age also became an important predictor. Given that donor age was relevant in the analyses presented earlier (assessing QE as a single event), and since recipient age becomes an important predictor in the repeated measurement analyses, perhaps immunosenescence phenomenon is at play. These findings, however, are similar to that of prior studies: older patients have a lower incidence of QE when compared to pediatric transplant

recipients (2, 7). As mentioned previously, a study comparing an MCSD population to those who proceed directly to OHT would be beneficial. Additionally, if these popualations were stratified between younger recipients/donors and older recipients/donor, a further trend may be identified. This type of study is quite feasible at our institution, as we frequently transplant those >65 years old, and often >70 years of age.

Figure 8. Frequency of QE per patient.

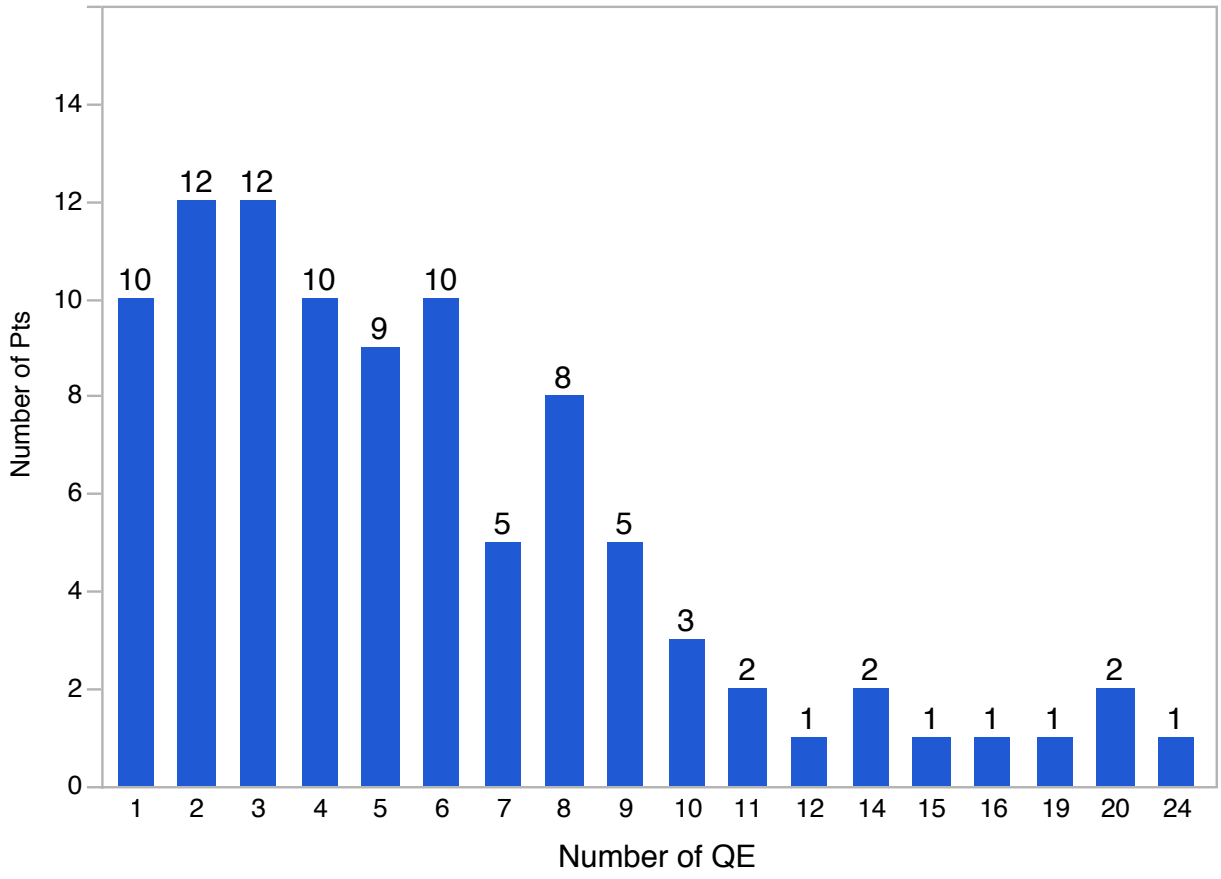


Table 8. AG-CP Model for recurrent QE, % change in hazard (p-value)				
	Univariate		Multivariate	
Parameter	**% Change in hazard**	**p-value**	**% Change in hazard**	**p-value**
Recipient Age, per 10 years	**11.6% decrease**	**0.03**	**14.6% decrease**	**0.001**
Recipient gender (female)	8.5% increase	NS	8.6% decrease	NS
Recipient CMV serostatus (negative)	7.5% increase	NS	8.83% increase	NS
MCSD Pump Type (pulsatile)	9.4% decrease	NS	---	---
Duration of MCSD support	0.05% decrease	NS	---	---
Donor gender (female)	24.2% increase	NS	5.76% increase	NS
Donor age, per 10 years	9.15% decrease	NS	6.7% decrease	NS
Donor CMV serostatus (negative)	5.1% decrease	NS	4.8% decrease	NS
CsA use (no)	**87.6% increase**	**0.0002**	**108% increase**	**<0.0001**
Donor age > Recipient age	25.6% increase	NS	---	---

Table 9. PWP-CP Model for recurrent QE, % change in hazard (p-value)				
	Univariate		Multivariate	
Parameter	**% Change in hazard**	**p-value**	**% Change in hazard**	**p-value**
Recipient Age, per 10 years	**5.6% decrease**	**0.06**	**7.7% decrease**	**0.004**
Recipient gender (female)	4.7% increase	NS	13.7% decrease	NS
Recipient CMV serostatus (negative)	3.7% increase	NS	8.5% increase	NS
MCSD Pump Type (pulsatile)	13.5% decrease	NS	---	---
Duration of MCSD support	0.05% decrease	NS	---	---
Donor gender (female)	27.8% increase	NS	20.8% increase	NS
Donor age, per 10 years	2.3% increase	NS	4.5% increase	NS
Donor CMV serostatus (negative)	8.7% increase	NS	14.4% increase	NS
CsA use (no)	**51.5% increase**	**<0.0001**	**65.6% increase**	**<0.0001**
Donor age > recipient age	**27.7% increase**	**0.02**	---	---

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