Fall 9-2015

Association between recipient's preexisting antibodies and allograft vasculopathy and mortality in heart transplant patients

Hoang Tran
University of Massachusetts Medical School

Lorena Baccaglini
University of Nebraska Medical Center

Jane L. Meza
University of Nebraska Medical Center

Brian D. Lowes
University of Nebraska Medical Center

University of Nebraska Medical Center

SERVICE LEARNING
CAPSTONE EXPERIENCE PROPOSAL

Title:

Association between recipient's preexisting antibodies and allograft vasculopathy and mortality in heart transplant patients

STUDENT AND COMMITTEE INFORMATION

Name, Concentration:	Hoang Tran, M.D.
Chair, Credentials:	Lorena Baccaglini, D.D.S., M.S., Ph.D.
Faculty, Credentials:	Jane Meza, Ph.D.
Preceptor, Credentials:	Brian Lowes, M.D., Ph.D.

Table of Contents

CAPSTONE EXPERIENCE

Abstract

Objectives: To evaluate the role of preexisting Angiotensin II receptor type I antibodies (AT1RAb) and anti-HLA antibodies in predicting mortality and cardiac allograft vasculopathy (CAV) among heart transplant patients.

Methods: In this retrospective cohort study, we included 114 adults who received heart transplant from January 1st, 2007 to December 31, 2014 and were followed up at Nebraska Medicine. 48 hours pre-transplant sera sample were used to detect antibodies. A cutoff of 10UL/ml was used for AT1RAb positive and mean fluorescence intensity of 3,000 and 1,500 were used for anti HLA class I and class II, respectively. Patients were positive for composite antibodies if they were positive for anti-HLA, or AT1R antibodies. Survival analysis was conducted to compare the risk for mortality or CAV between antibody positive and negative groups.

Result: Participants who had positive composite antibodies had higher probability of having CAV (p=0.05). Participants who were negative for AT1RAb trended toward a lower risk of mortality or developing CAV compared to AT1RAb positive counterparts.

Conclusion: Positive status for any of anti-HLA or AT1RAb increased the risk of CAV. AT1RAb positivity is possibly linked with higher risk of death or developing CAV. Future study can focus on verifying these trends and the potential interaction effect between anti-HLA and AT1R antibodies.

INTRODUCTION

Heart transplantation is the treatment of choice for end-stage heart disease. Since the first human heart transplant in 1967, the number of heart transplantation has increased rapidly worldwide. According to the 2014 International Society for Heart and Lung Transplantation (ISHLT) official report, there were 104,027 heart transplants through June 30th, 2013 globally (Lund et al., 2014). In the United States, there were about 2,500 cases conducted in the year 2012 (The International Society of Heart and Lung Transplantation, 2014). Mortality rates in heart transplantation have been reducing for every ten years, with the mortality rate in the first year after transplantation is about 15% in the cohort of patients transplanted in 2006 to 2012 (Lund et al., 2014). There are several factors that are associated with a higher risk of death up to 1 year after transplant. These factors include recipients' pre-transplant severity of illness (measured by pre-operation hospitalization, mechanical ventilation, and temporary mechanical circulatory support), history of dialysis or blood transfusion, renal failure, and older age (Lund et al., 2014).

However, heart transplantation is not considered a curative therapy for heart disease because of the long-term complications or comorbidities. These complications include blood marrow suppression, opportunistic viral infection, malignancy, graft loss, graft dysfunction and even mortality (Griffin, Callahan, & Menon, 2012). Comorbidities, which contribute to graft failure and graft loss, are also common in heart transplant patients. The prevalence of chronic disease in heart transplant patients higher than that in general population, and increase with post-transplantation course. Specifically, prevalence of hypertension, hyperlipidemia,

cardiac allograft vasculopathy, and diabetes at 10 year post-transplantation are 97%, 93%, 59% and 39% respectively (The International Society of Heart and Lung Transplantation, 2014). Nonetheless, estimation of comorbidities at certain predetermined time point, such as ten year, does not account for dead patients and therefore underestimate the real prevalence.

Recipient selection

In general, patients are considered for heart transplantation when they have severe heart failure that does not response with medical or mechanical treatment. Peak exercise maximum oxygen consumption and percentage to predict maximum oxygen consumption are usually used to objective evaluate heart function. Common indications for heart transplantation are listed in Table 1 (Pham, Berry, & Hunt, 2011):

Table 1: Commonly Accepted Indications for Cardiac Transplantation

- Systolic heart failure with severe functional limitations or refractory symptoms despite maximal medical and device therapy
- LVEF usually <35%, but a low LVEF is not an adequate indication for transplantation
- NYHA functional class III-IV
- Maximal oxygen uptake (VO2max) of ≤12-14 cc/kg/min exercise testing
- Cardiogenic shock not expected to recover
- Acute myocardial infarction
- Acute myocarditis
- Ischemic heart disease with intractable angina not amenable to surgical or percutaneous revascularization and refractory to maximal medical therapy
- Intractable ventricular arrhythmias, uncontrolled with standard antiarrhythmic therapy, device therapy, or ablative therapy
- Severe symptomatic hypertrophic or restrictive cardiomyopathy
- Congenital heart disease in which severe, fixed pulmonary hypertension is not a complication
- Cardiac tumors with a low likelihood of metastasis

LVEF: Left ventricular ejection fraction. NYHA: New York Heart Association

Before being selected for transplantation, heart failure patients need to undergo a series of tests and examination to ensure that they will be an appropriate recipient. These measurements include clinical examination, family, social and medical history, standard serum and a 12-h urine collection laboratory tests.

Table 2: Contraindications for cardiac transplantation (Liao & Shumway, 2014)

Relative contraindication:

- Advanced age (>70)
- Active myocarditis
- Graft failure due to acute rejection

Temporary contraindications for cardiac transplantation

- Active infection
- Active peptic ulcer disease
- Diverticulitis
- Recent pulmonary/cerebral emboli
- Symptomatic cholelithiasis

Absolute contraindications for cardiac transplantation

- Positive prospective cross-match
- Irreversible pulmonary hypertension (pulmonary vascular resistance ≥ 5 Wood units)
- Malignancy
- Severe peripheral or cerebral vascular disease
- Irreversible renal dysfunction (Glomerular Filtration Rate ≤40 ml/min)
- Irreversible hepatic dysfunction
- Severe obstructive or restrictive lung disease
- Coexisting systemic disease
- Diabetes mellitus with end-organs disease
- Morbid obesity (≥30% or predicted ideal weight) or BMI ≥ 35
- Severe cachexia
- Ongoing tobacco use or drug addiction
- Ongoing alcohol abuse
- Noncompliance with medications
- Inability to fully understand the procedure and participate in follow-up care

Potential receivers are also ruled out of cancer with negative results from stool guaiac, mammography, prostate-specific antigen screening, Pap-smear. Tests of occult infections including hepatitis B and C, HIV, HTLV1, and HTLV2,

cytomegalovirus, Toxoplasma, Epstein–Barr virus, syphilis, and tuberculosis are also carried out before selection for transplantation is made. Pre-transplant data also include blood type, HLA–DR typing, and panel reactive antibody (PRA) screening (Liao & Shumway, 2014). Certain conditions that limit patients from being a recipient are listed in Table 2.

Donor selection

The donors are usually younger than 50 year old and do not have history of chest trauma or heart disease. Donor exclusion criteria include: malignancy with potential of metastasis to the heart, systemic sepsis or endocarditis, significant coronary artery disease, anatomical heart disease or poor ventricular function. While waiting for transplantation, the donor heart need to avoid prolong hypotension or hypoxemia. However, inotrope should be used as least as possible to reduce the oxygen demand of the donor heart.

There are several technique to preserve the donor heart, including hypothermia, cardioplegia, and preservation solutions. Cardioplegia is used to arrest the heart while preservation solution is used to keep the heart at 4 to 8 Celsius degree to reduce metabolism. However, all measures together, the donor heart is usually only kept for to 4-6 hour of cold ischemic time. Due to the shortage of donors and the increasing demand, the selection of donor heart is widening beyond some classic contraindications. These expended criteria include longer ischemic time, mild valvular abnormalities, mild coronary artery abnormalities or mild left ventricular dysfunction (Pham et al., 2011).

Matching recipients and donors

In order to anatomically matched heart size, the donors' and recipients' weight should also stay in the range from 80% to 120% of each other. However, body weight is not a sensitive tool to estimate the heart size. Chan et al. reported no correlation between body weights and heart size in adults who weigh from 40 to 99 kg (Chan et al., 1991). With the expansion of donor pool, older donors are becoming more common. In such cases, an older recipient with similar life expectancy is usually chosen for transplant (Esmore, 2005). Donor and recipient should have the same ABO blood group, or compatible blood group. Recipients are also tested for panel reactive antibody (PRA). Potential recipients in the transplant waiting list who have PRA positive more than 20% should be checked for their antibody status every two months. A PRA test positive more than 20% just before transplant requires actually donor-specific T cell cross-matches to see if donor specific antibodies are present in recipients. Positive cross-matches are also a contraindication to transplantation (Esmore, 2005).

Cardiac allograft vasculopathy

Cardiac allograft vasculopathy (CAV) is the most important chronic comorbidity in heart transplant patients. CAV is the leading single cause of re-transplantation in the US, accounting for 59% of total cases (Lund et al., 2014), and is associated with higher mortality rate at one year or five years after transplantation (Taylor et al., 2009). The mechanism of CAV is multifactorial, with the involvement of immune and non-immune factors (Griffin et al., 2012). These mechanisms include conventional risk factors for atherosclerosis, pre- and perioperative injury

to the graft vessels, innate immunity, cell-mediated rejection and antibody-mediated rejection (Pober, Jane-wit, Qin, & Tellides, 2014).

Table 3: ISHLT nomenclature for cardiac allograft vasculopathy (Mehra et al., 2010)

ISHLT CAV$_0$
• Not significant No detectable angiographic lesion
ISHLT CAV$_1$ (Mild)
• Angiographic left main (LM) < 50%, or primary vessel[†] with maximum lesion of <70%, or any branch[‡] stenosis <70% (include diffuse narrowing) without allograft dysfunction[§]
ISHLT CAV$_2$ (Moderate)
• Angiographic LM <50%; a single primary vessel ≥70% or isolated branch stenosis ≥70% in branches of 2 systems, without allograft dysfunction
ISHLT CAV$_3$ (Severe)
• Angiographic LM ≥50% or two or more primary vessels ≥70% stenosis, or isolated branch stenosis ≥70%, or ISHLT CAV1 or CAV2 with allograft dysfunction or evidence of significant restrictive physiology[$]

[†]A "primary vessel" denotes the proximal and middle 33% of the left anterior descending artery, left circumflex, the ramus and the dominant or co-dominant right coronary artery with the posterior descending and posterolateral branches.
[‡]A "secondary branch vessel" includes the distal 33% of primary vessels or any segment within a large septal perforator, diagonals or obtuse marginal branches or any portion of a non-dominant right coronary artery.
[§]Allograft dysfunction is defined as left ventricular ejection fraction ≤45% usually in the present of regional wall motion abnormalities.
[$]Restrictive cardiac allograft physiology is defined as symptomatic heart failure with echocardiographic E to A velocity ratio >2 (>1.5 in children), shortened isovolumetric relaxation time (<60 msec), shortened deceleration time (<150 msec), or restrictive hemodynamic value (right atrial pressure > 12 mmHg, pulmonary capillary wedge pressure >25 mmHg, cardiac index < 2l/min/m2)

The underlying pathology of CAV is a progressive, proliferative, diffuse, chronic inflammatory condition of allograft coronary arteries. The morphology of CAV is concentric intimal thickening with outward remodeling, which makes it difficult to diagnose by conventional angiography. Current antilymphocyte and immunosuppressant have not yet yielded promising results to prevent or treat CAV.

Therefore, identifying patients at risk and applying rigorous prevention is the current strategy to deal with CAV.

The risk of developing CAV is higher in patients of female gender, or with elevated pre-transplant panel-reactive antibodies (PRAs), de novo donor-specific antibodies (DSA) after transplantation, positive donor-specific crossmatch, prior sensitization to OKT3, cytomegalovirus (CMV) seropositivity, prior implantation of a ventricular assist device, and re-transplantation (J. Kobashigawa et al., 2011). Immunosuppressive agents such as cyclosporine or corticosteroid are also thought to contribute to endothelial cell injury, and ultimately to hyperplastic characteristic of CAV.

Mortality

The median survival time of heart transplant is 10 years. The mortality rate is highest in the first year, primarily due to graft failure and infection. For patients who survive the first year, the median survival time is 13 years. The improvement of survival in the first year after transplantation contributes greatly to the general improvement of survival of heart transplantation in the past decades, while long term survival has not changed much.

Factors associated with mortality post-transplantation vary by time. For the first year, mechanical circulatory bridging support, ischemic heart disease, renal failure and mechanical ventilation are among the most significant attributors to mortality. From one to ten years after transplantation, in addition to prior factors, history of previous stroke, female donor or recipient are also associated with higher mortality (David D. Yuh, Luca A. Vricella, Stephen C. Yang, John R. Doty, 2014).

Antibodies in heart transplantation

Human leukocyte antigen (HLA) antibodies are an important predictive factor for allograft failure (J. A. Kobashigawa, 2007; Nikaein, Alivizatos, Monahan, & Stone, 1995). The association between anti-HLA antibodies and cardiac allograft rejection was first described in the 1970s (Vasilescu et al., 2004). Since then, numerous studies have shown that the development of DSA to the HLA increased the risk of developing CAV in heart transplant patients (Raess et al., 2013; Reed et al., 1996; Suciu-Foca et al., 1991). The mechanism by which preexisting anti-HLA adversely affect transplant outcome has not been understood clearly. The presence of anti-HLA, although not directly specific against donor graft, is still associated with rejection post-transplant. Loupy et al. reported C1q-bound anti-HLA positivity associated with 11 times higher risk of graft lost in kidney transplantation compare to non C1q-bound anti-HLA (Loupy et al., 2013). Loupy also suggested that preformed C1q-bound anti-HLA had better predictive value for graft rejection than complement dependent cytotoxicity. However, these results were still controversial because half of patients who had been positive for C1q-bound antibodies became negative post-transplant, and posed a smaller risk of rejection post-transplantation (Baid-Agrawal, Lachmann, & Budde, 2014).

Recently, the role of non-donor specific antigen antibodies in graft rejection has been the subject of interest. These non-donor-specific antibodies include, but not limited to, antibodies against angiotensin II type 1 receptor, vimentin, endothelial cell antigens, cardiolipin, and cardiac myosin (Barz & Rummler, 2013;

Forman, Lin, Pascual, Denton, & Tolkoff-Rubin, 2004; Jurcevic et al., 2001; Kalache et al., 2011; Warraich et al., 2000).

Angiotensin II type 1 receptor (AT1R) is a transmembranous G-couple protein receptor, that mediates the majority of the physiologic effects of Angiotensin II, especially on blood pressure (Dzau, 2001). There are several pathways through which a human develops antibodies against AT1R. AT1RAb can develop similarly to HLA antibodies through blood transfusion, pregnancy, or prior transplant (Reinsmoen, 2013). AT1RAb activate AT1R and promote remodeling of allograft vasculature. AT1RAb was found to be associated with adverse outcomes in both heart and kidney transplantation (Reinsmoen, 2013). In heart transplantation, AT1RAb are linked to cell-mediated rejection (CMR), antibody-mediated rejection (AMR), and an early CAV onset at one year after transplant (Yamani et al., 2006). In kidney transplantation, AT1RAb are associated with AMR independently or synergistically with HLA class II DSA in pediatric patients (Kelsch et al., 2011; Reinsmoen, 2013). Reinsmoen et al. found an impact of AT1RAb and DSA positivity on the development of AMR and CMR at two years after transplant. However, the authors did not find a significant impact of AT1RAb and DSA positivity on the incidence of mortality or CAV (Reinsmoen et al., 2014). To our knowledge, the association between preexisting AT1RAb or anti-HLA antibodies with clinical outcomes, such as mortality or CAV has not been well studied in heart transplant patients.

Study specific aim

Investigate whether the presence of antibodies against HLA or AT1R increases the risk for cardiac allograft vasculopathy or mortality in heart transplant patients.

Study hypothesis

We hypothesized the presences of at least one type of anti-HLA antibodies or Angiotensin II type 1 receptor antibodies increase the risk for the development of cardiac allograft vasculopathy or death in heart transplant patients. We also hypothesized that AT1RAb, when being considered alone, is associated with a higher risk of mortality or CAV.

METHODS

Study Design and participants

This was a retrospective cohort study to investigate the association between antibody positivity and time to death or time to development of CAV. Eligible participants included patients aged 18 or above, who had heart transplantation from January 1st, 2007 to December 31, 2014 and received post-transplantation care at Nebraska Medicine. A total sample of 114 participants with at least one pre-transplantation antibody test record was used for analysis. The study protocol was approved by University of Nebraska Medical Center Institutional Review Board.

Outcomes

Our primary outcome was time to all-cause mortality and the secondary outcome was time to the first CAV diagnosis since transplantation. Time to death censored status calculated by time from transplantation to either the last visit or the last day of study (31st December 2014), whichever came first. Two researchers with formal cardiology training independently reviewed all available participants' coronary angiographs. In case there were discrepancies of diagnoses between two researchers, the coronary angiography results from medical record were used as a third judgment. CAV was identified by coronary angiograph and allograft function and physiology following the International Society for Heart and Lung Transplant (ISHLT) 2010 guidelines. In our study, patients were considered to have CAV if they felt into one of the categories from ISHLT CAV_1 to ISHLT CAV_3 and not to have CAV if they were ISHLT CAV_0. Time to CAV censored status was calculated by time from transplantation to either the last normal angiography or the last day of study (31st December 2014), whichever came earlier.

Exposures

The primary exposure was pre-transplant composite antibodies to donor, including preexisting anti-HLA antibodies class I and class II, and anti-AT1R antibodies (AT1RAb). Participants were considered positive for composite antibodies if they had antibodies against at least one type of HLA or AT1R. We also conducted exploratory analyses for AT1RAb alone as the secondary exposure. We detected anti-HLA antibodies using flow cytometric technology with LABScreen™ products. We incubated 48h-pretransplant sera with purified antigens-coated

microbeads and pre-optimized reagents. Any antibodies present in the patients' sera bound to the antigens on the beads and then were bound by anti-human IgG labeled with R-Phycoerythrin (PE). We detected AT1RAb using ELISA assay. AT1RAb in patient's serum was bounded by anti-human IgG labeled with peroxidase enzyme. Based on our laboratory protocol, AT1RAb was considered to be positive when plasma concentration greater than 10 U/ml. Mean fluorescence intensity >3,000 and >2,500 were used as a cut off for HLA class I positive and HLA class II positive, respectively.

Other measurements

Sociodemographic information was obtained through electronic medical records. We categorized age as younger than 55, or 55 and above following the median age of 55 in our sample. We classified race as two groups: white and other races. Smoking status was divided into have ever been a smoker or have never been a smoker. In our sample, only 3 patients were smoking at the time of transplant. Heart disease diagnosis was either coded to ischemic or non-ischemic heart disease.

Statistical analyses

Participants' baseline characteristics were described by univariate analyses. We used Chi-square test or Fisher's exact test where appropriate to compare baseline categorical variables. We used two independent samples T-test and Wilcoxon-signed rank test to compare continuous variables. We conducted Kaplan-Meier plot and a log-rank test to compare the time to event (death, CAV) by antibody strata. In addition to the analyses for primary exposure, exploratory analyses on a

sample of 67 patients who were tested for AT1RAb were also conducted. We used a 2-sided alpha of 0.05 as the cut-off value for statistical significance. All the analyses were performed in SAS 9.3 statistical software.

RESULTS

In our sample of 114 patients, 25 patients were positive for anti HLA (22%) and 89 were negative (78%). A subsample of 67 were tested for AT1RAb, among them 57 were positive (85%) and 10 were negative (15%).

Baseline characteristics

The median age at transplantation was 55 years, ranging from 20 to 69 years. The distribution of age at transplantation was not different by antibody status (Table 5). Males and whites were predominant, accounting for 80-90% of the sample. Current or past smoker tended to be more common among participants who were negative for all antibodies compared to the positive counterparts (68% vs. 54%, p=0.14). Similarly, hypertension tended to be more common among patients who were negative for all antibodies compared to those who were positive (86% vs. 73%, p=0.09). These results were also observed similarly for AT1RAb alone. The percentage of ischemic heart disease was seen equally (40%) between the positive and negative AT1R antibody group. However, when DSA and anti HLA were taken into consideration, ischemic heart disease was more commonly seen in the negativity group (62%) compared to the positive group (40%; p=0.03). Diabetes tended to be more common in the AT1RAb positive group than in the negative group (51% vs. 20%, p=0.09), whereas its distribution was not different (50%) between the

composite antibody positive and negative groups. More than 40% of participants who were positive for at least one type of antibodies had utilized LVAD before transplantation compared to just about 25% among participants who were negative for these antibodies (p=0.05).

Survival analyses

Time to death

The overall mean follow up time was 69 months, ranging from 1 to 94 months post-transplantation. Survival probability tended to be higher in antibody positive group around the first 3 years. The probability of survival at 36 months post-transplantation was 89% in the composite antibody negative group and 95% in the positive group (Figure 1-A). However, at the end of the study, survival probability of antibodies positive and negative groups were not different (p=0.29). Among participants with a valid AT1RAb test, the mean follow up time was 54 months, ranging from 1 to 57 months post-transplantation. Three years post-transplantation, 100% of participants who were negative for AT1RAb survived and compared with 94% of participants who were positive for AT1RAb (Figure 1-B). The difference was more prominent at the end of follow up. However, it did not reach the level of significant.

Time to cardiac allograft vasculopathy

The overall mean follow up time for CAV was 64 months, ranging from 3 to 84 months post-transplantation. The probability of CAV-free at 36 months post-transplantation was 88% in the composite antibody negative group, while 77% of positive group stayed free of CAV. The trend of developing CAV was not different in

the first 24 months after transplantation between the negative (22%) and positive (23%) groups (Figure 1-C). However, after 35 months, CAV-free time declined more rapidly in patients who had positive antibodies compared to those who were negative (p=0.05). History of ischemic heart disease showed a trend of increase CAV hazard, while hypertension associated with lower hazard. Both these trends did not meet significant level. History of LVAD use did not affect the risk of having CAV between composite antibody positive and negative groups. At 36 months post-transplantation, 100% of AT1RAb negative patients stayed CAV-free, compared to only 74% in the AT1RAb positive group were free of that condition (Figure 1-D). The decline trend was more obvious later in the follow up time in AT1RAb positive group, although this comparison did not reach the level of significant (p=0.23).

DISCUSSION

With the improvement of survival in the first year after transplantation, identifying risk factors that are associated with longer term complication such as CAV and long-term mortality is the new focus to further improve prognosis of heart transplantation. In the present study, we found an increased risk for developing CAV among participants who had pre-formed antibodies to at least HLA or AT1R.

Preexisting anti AT1R antibodies were commonly present among heart transplant patients in our study. The prevalence of preexisting AT1RAb was reported from 17% to 47% among kidney transplant patients (Giral et al., 2013; Taniguchi et al., 2013). Urban et al. reported a lower prevalence of AT1RAb (38%) among heart transplant patients, however, the authors used higher cutoff (17U/ml) compared to our study (10U/ml) (Urban, Gazdic, Slavcev, & Netuka, 2015). Among baseline

characteristics, history of LVAD use and ischemic heart disease were significantly associated with positivity of composite antibodies. Similar association was also noted between AT1RAb and history of LVAD use in our study and in another study in Germen with similar percentage of AT1RAb positive in LVAD group (Sandy von Salisch et al., 2013). The association between history of LVAD use and anti-HLA antibodies was reported before (Kaczorowski, Datta, Kamoun, Dries, & Woo, 2013). The mechanism by which LVAD patients became sensitized to alloantigen was not clear. It is proposed that the exposure of blood products via transfusion in LVAD implantation procedure could induce allosensitization (McKenna Jr., Eastlund, Segall, Noreen, & Park, 2002). However, Drakos et al. and Itescu et al. in independent studies found avoiding blood transfusion or applying leukocyte-filtered cellular blood product did not reduce the risk of allosensitization (Drakos et al., 2007; Itescu, Ankersmit, Kocher, & Schuster, 2000). In our study, when considering the hazard of developing CAV, patients with history of LVAD use did not have higher hazard than patients without LVAD use. This finding was consistent with previous studies which found no risk of LVAD use on clinical outcome such as mortality or CAV post-transplantation (Baran et al., 2005), even though it was associated with positive HLA antibodies and allograft rejection (John et al., 2003).

Our study's results suggested that positive preexisting composite antibody participants had higher hazard of developing CAV compared to negative counterparts. Pre-transplant anti-HLA antibodies was not associated with CAV in a study by Gandhi (Gandhi et al., 2010). However, the sample size in that stud was relatively small (19 patients). Eschborn in a study with 92 patients found a higher prevalence of CAV among preexisting HLA antibody patients compared to negative

counterparts, but the authors failed to establish a statistical difference (Eschborn et al., 2013). The mechanism by which HLA antibodies acquire their effect on vasculopathy is mainly via transplant rejection. Circulating antibodies attack donor antigen on the graft, activate complement system, cause C4d deposition on endothelial cells and start inflammation processes. In our study, the difference of CAV risk was not evident in the first 24 months after transplantation between then composite antibody positive and negative groups. This finding was consistent with the knowledge that CAV usually develops later in the course of transplantation, in the relation with both cell mediated and antibody mediated chronic rejection.

The increase risk of developing CAV also noted when only AT1RAb positivity was taken into account in our study. AT1RAb was found to be associated with early onset of microvasculopathy in heart transplant before (Hiemann et al., 2012). The transplant procedure itself or the ischemic condition post-transplant promotes the expression of AT1R on endothelial cells and the development of AT1RAb (Reinsmoen, 2013). Since Angiotensin II also functions as moderator for cell growth, apoptosis, fibrosis and inflammation (Ruiz-Ortega et al., 2001), upregulating AT1RAb is thought to be associated with endothelial activation, leading to smooth muscular cell proliferation and microvascular disease (Reinsmoen, 2013). In our study, all patients without antibody against AT1R were free of CAV in the follow up time while the patients with AT1RAb accumulated CAV along with time. Although the follow up time and the number of participants in the AT1RAb negative group were relatively shorter and smaller than those in the positive group (Figure 1-D), we expect to see the trend to continue when more patients are enrolled and followed for a longer time in the future.

In our study, participants who were positive for either donor specific antigen or AT1R did not showed higher mortality hazard compared to negative counterparts. Although post-transplant anti HLA antibodies were found to increase the risk of mortality in heart transplant, preexisting antibodies against HLA were not clearly associated with mortality in these patients (Ho et al., 2011). Tambur et al. found that majority of pre-existing anti HLA antibodies do not act directly against donor antigen found by lymphocyte cross match (Tambur et al., 2000). Thus, these antibodies would not trigger vigorous immune activity against the allograft, especially under the immunosuppressive therapy post-transplantation. When AT1RAb was considered alone, there was a minor trend of worse survival outcome in positive participants after 24 months. Due to no event in the AT1RAb negative group and short follow up period, mortality hazard ratio was not calculated for AT1RAb status. This finding calls for further investigation on the role of preexisting AT1RAb on mortality in heart transplant patients.

Our study has some limitations. First, our sample was relatively small, which reduced our ability to detect small differences or conduct subgroup analyses. The selection of participants based on availability of test results could have introduced selection bias. On the other hand, our study was among the first study to look at the predictive value of AT1RAb on CAV and survival in heart transplantation. The assessment of CAV was done by two independent researchers, which would reduce observer bias. Future studies would assess the relationship between antibodies and CAV or mortality over a longer period of time, or evaluate the potential interaction between AT1RAb and donor specific antibodies on clinical outcomes in heart transplant patients.

Importance of the Capstone project

This study allowed us to better understand the involvement of donor specific antibodies and non-donor specific antibodies, more specifically, anti-HLA antibodies and AT1RAb in the development of cardiac allograft vasculopathy as well as survival. This knowledge can help cardiologists identify the best donor-receiver match for transplant as well as transplanted patients who are at higher risk for these conditions. Further, these knowledge can be translated into a more appropriate prevention or treatment strategy for high risk heart transplant recipients.

For the public health, the study's finding can contribute to better outcomes of heart transplantation, including lower re-transplant rates, mortality and dysfunction in heart transplantation. Further, these better outcomes will result in more patients receiving heart transplant, less medical cost and more effective and efficient heart transplantation programs.

Table 5: Pre-transplantation characteristics of participants by positivity to pre-transplant antibodies (n=114)

Characteristics	Composite antibodies‡			Anti AT1R antibodies			
	Positive (n=70)	Negative (n=44)	*p-value*	Not tested (n=47)	Positive (n=57)	Negative (n=10)	*p-value*
	n (%)	n (%)		n (%)	n (%)	n (%)	
Age			*0.95*				*0.99**
<55	33 (47)	21 (48)		20 (43)	29 (51)	5 (50)	
≥55	37 (53)	13 (52)		27 (57)	28 (49)	5 (50)	
Gender			*0.67*				*0.99**
Male	55 (79)	36 (82)		37 (79)	46 (81)	8 (80)	
Females	15 (21)	8 (18)		10 (21)	11 (19)	2 (20)	
Race			*0.99**				*0.99**
White	64 (91)	40 (91)		44 (94)	51 (89)	9 (90)	
Others	6 (9)	4 (9)		3 (6)	6 (11)	1 (10)	
Smoking status†			*0.14*				*0.49**
Never smoker	31 (46)	13 (32)		15 (36)	26 (46)	3 (30)	
Ever smoker	36 (54)	28 (68)		37 (64)	30 (54)	7 (70)	
Heart disease			*0.05*				*0.99**
Ischemic	18 (40)	26 (59)		27 (57)	23 (40)	4 (40)	
Non-ischemic	42 (60)	18 (41)		20 (43)	34 (60)	6 (60)	
Diabetes			*0.99*				*0.09**
Yes	35 (50)	22 (50)		26 (55)	29 (51)	2 (20)	
No	35 (50)	22 (50)		21 (45)	28 (49)	8 (80)	
Hypertension			*0.09*				*0.43**
Yes	51 (73)	38 (86)		39 (83)	41 (72)	9 (90)	
No	19 (27)	6 (14)		8 (17)	16 (28)	1 (10)	
Hyperlipidemia			*0.43*				*0.73**
Yes	46 (66)	32 (73)		35 (74)	37 (65)	6 (60)	
No	24 (34)	12 (27)		12 (26)	20 (35)	4 (40)	
LVAD			*0.05*				*0.17**
Yes	30 (43)	11 (25)		11 (23)	28 (49)	2 (20)	
No	40 (57)	33 (75)		36 (77)	29 (51)	8 (80)	

AT1R: Angiotensin II type 1 receptor, LVAD: Left ventricular assist device.
All the p-values are from Chi-Square test comparing the positive to the negative groups, otherwise stated.

*p-values from Fisher's exact test comparing the positive to negative group.

†Sum less than total due to missing values.

‡Composite antibodies positive if positive for at least one of anti-HLA class I and II or AT1R antibodies.

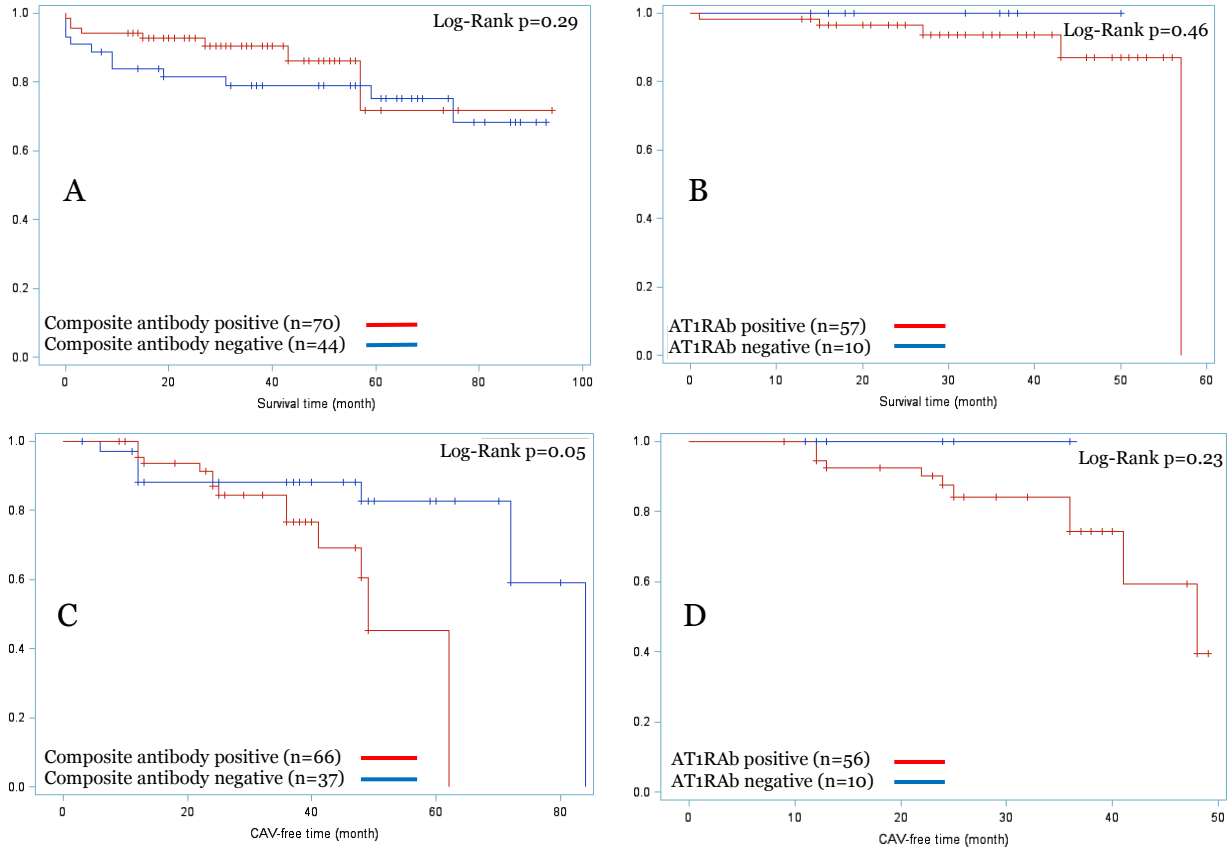

Figure 1: Post-transplantation survival time by composite antibody status (DSA class I, II, anti HLA class I, II and AT1RAB) in (A) or by AT1RAb in (B) and cardiac allograft vasculopathy (CAV) free time by composite antibody status in (C) or by AT1RAb in (D).

REFERENCES

Baid-Agrawal, S., Lachmann, N., & Budde, K. (2014). Complement-binding anti-HLA antibodies and kidney transplantation. *The New England Journal of Medicine, 370*(1), 84. doi:10.1056/NEJMc1313506#SA2 [doi]

Baran, D. A., Gass, A. L., Galin, I. D., Zucker, M. J., Arroyo, L. H., Goldstein, D. J., . . . Lansman, S. L. (2005). Lack of sensitization and equivalent post-transplant outcomes with the novacor left ventricular assist device. *The Journal of Heart and Lung Transplantation, 24*(11), 1886-1890. doi:http://dx.doi.org.library1.unmc.edu:2048/10.1016/j.healun.2005.03.010

Barz, D., & Rummler, S. (2013). Detection of antibodies in eluates of immunoadsorption causing humoural rejection in patients after solid organ transplantation. *Atherosclerosis.Supplements, 14*(1), 191-197. doi:10.1016/j.atherosclerosissup.2012.10.031 [doi]

Chan, B. B., Fleischer, K. J., Bergin, J. D., Peyton, V. C., Flanagan, T. L., Kern, J. A., . . . Kron, I. L. (1991). Weight is not an accurate criterion for adult cardiac transplant size matching. *The Annals of Thoracic Surgery, 52*(6), 1230-1236.

David D. Yuh, Luca A. Vricella, Stephen C. Yang, John R. Doty. (2014). *Johns hopkins textbook of cardiothoracic surgery* (Second ed.). New York, NY: McGraw-Hill.

Drakos, S. G., Stringham, J. C., Long, J. W., Gilbert, E. M., Fuller, T. C., Campbell, B. K., . . . Lindblom, J. M. (2007). Prevalence and risks of allosensitization in

HeartMate left ventricular assist device recipients: The impact of leukofiltered cellular blood product transfusions. *The Journal of Thoracic and Cardiovascular Surgery, 133*(6), 1612-1619.

Dzau, V. J. (2001). Theodore cooper lecture: Tissue angiotensin and pathobiology of vascular disease: A unifying hypothesis. *Hypertension, 37*(4), 1047-1052.

Eschborn, J. M., Kemper, D., Schönemann, C., Knosalla, C., Hetzer, R., & Hiemann, N. E. (2013). Effects of anti-HLA antibodies present before heart transplantation on survival, acute cellular rejection and coronary allograft vasculopathy: A single center experience. *The Journal of Heart and Lung Transplantation, 32*(4, Supplement), S261. doi:http://dx.doi.org/10.1016/j.healun.2013.01.679

Esmore, D. (2005). Cardiac transplantation, the columbia university medical center/new York–Presbyterian hospital manual. *ANZ Journal of Surgery, 75*(5), 369-369. doi:10.1111/j.1445-2197.2005.03363.x

Forman, J. P., Lin, J., Pascual, M., Denton, M. D., & Tolkoff-Rubin, N. (2004). Significance of anticardiolipin antibodies on short and long term allograft survival and function following kidney transplantation. *American Journal of Transplantation, 4*(11), 1786-1791.

Gandhi, M. J., De Goey, S. R., Periera, N., Kushwaha, S., Edwards, B., Park, S., . . . Daly, R. C. (2010). 483: Significance of pre transplant donor specific HLA antibodies and post transplant cardiac allograft vasculopathy. *The Journal of*

Heart and Lung Transplantation, 29(2, Supplement), S158. doi:http://dx.doi.org/10.1016/j.healun.2009.11.499

Giral, M., Foucher, Y., Dufay, A., Van Huyen, J. P. D., Renaudin, K., Moreau, A., . . . Dragun, D. (2013). Pretransplant sensitization against angiotensin II type 1 receptor is a risk factor for acute rejection and graft loss. *American Journal of Transplantation, 13*(10), 2567-2576. doi:10.1111/ajt.12397

Griffin, B. P., Callahan, T., & Menon, V. (2012). *Manual of cardiovascular medicine* (4th ed.) Lippincott Williams & Wilkins.

Hiemann, N. E., Meyer, R., Wellnhofer, E., Schoenemann, C., Heidecke, H., Lachmann, N., . . . Dragun, D. (2012). Non-HLA antibodies targeting vascular receptors enhance alloimmune response and microvasculopathy after heart transplantation. *Transplantation, 94*(9), 919-924. doi:10.1097/TP.0b013e3182692ad2 [doi]

Ho, E. K., Vlad, G., Vasilescu, E. R., de la Torre, L., Colovai, A. I., Burke, E., . . . Mancini, D. (2011). Pre-and posttransplantation allosensitization in heart allograft recipients: Major impact of de novo alloantibody production on allograft survival. *Human Immunology, 72*(1), 5-10.

Hosenpud, J. D., Everett, J. P., Morris, T. E., Mauck, K. A., Shipley, G. D., & Wagner, C. R. (1995). Cardiac allograft vasculopathy. association with cell-mediated but not humoral alloimmunity to donor-specific vascular endothelium. *Circulation, 92*(2), 205-211.

Itescu, S., Ankersmit, J., Kocher, A. A., & Schuster, M. D. (2000). Immunobiology of left ventricular assist devices. *Progress in Cardiovascular Diseases, 43*(1), 67-80. doi:http://dx.doi.org/10.1053/pcad.2000.7191

John, R., Lietz, K., Schuster, M., Naka, Y., Rao, V., Mancini, D. M., . . . Edwards, N. M. (2003). Immunologic sensitization in recipients of left ventricular assist devices. *The Journal of Thoracic and Cardiovascular Surgery, 125*(3), 578-591.

Jurcevic, S., Ainsworth, M. E., Pomerance, A., Smith, J. D., Robinson, D. R., Dunn, M. J., . . . Rose, M. L. (2001). Antivimentin antibodies are an independent predictor of transplant-associated coronary artery disease after cardiac Transplantation1. *Transplantation, 71*(7), 886-892.

Kaczorowski, D. J., Datta, J., Kamoun, M., Dries, D. L., & Woo, Y. J. (2013). Profound hyperacute cardiac allograft rejection rescue with biventricular mechanical circulatory support and plasmapheresis, intravenous immunoglobulin, and rituximab therapy. *Journal of Cardiothoracic Surgery, 8*(1)

Kalache, S., Dinavahi, R., Pinney, S., Mehrotra, A., Cunningham, M. W., & Heeger, P. S. (2011). Anticardiac myosin immunity and chronic allograft vasculopathy in heart transplant recipients. *Journal of Immunology (Baltimore, Md.: 1950), 187*(2), 1023-1030. doi:10.4049/jimmunol.1004195 [doi]

Kelsch, R., Everding, A. S., Kuwertz-Broking, E., Brand, E., Spriewald, B. M., Sibrowski, W., . . . Dragun, D. (2011). Accelerated kidney transplant rejection and hypertensive encephalopathy in a pediatric patient associated with antibodies against angiotensin type 1 receptor and HLA class II. *Transplantation, 92*(10), e57-9. doi:10.1097/TP.0b013e318234b337 [doi]

Kobashigawa, J. A. (2007). Contemporary concepts in noncellular rejection. *Heart Failure Clinics, 3*(1), 11-15.

Kobashigawa, J., Crespo-Leiro, M. G., Ensminger, S. M., Reichenspurner, H., Angelini, A., Berry, G., . . . Kfoury, A. G. (2011). Report from a consensus conference on antibody-mediated rejection in heart transplantation. *The Journal of Heart and Lung Transplantation, 30*(3), 252-269.

Liao, K. K., & Shumway, S. J. (2014). Cardiac transplantation. In D. D. Yuh, L. A. Vricella, S. C. Yang & J. R. Doty (Eds.), *Johns hopkins textbook of cardiothoracic surgery* (). New York, NY: McGraw-Hill Education.

Loupy, A., Lefaucheur, C., Vernerey, D., Prugger, C., van Huyen, J. D., Mooney, N., . . . Desgrandchamps, F. (2013). Complement-binding anti-HLA antibodies and kidney-allograft survival. *New England Journal of Medicine, 369*(13), 1215-1226.

Lund, L. H., Edwards, L. B., Kucheryavaya, A. Y., Benden, C., Christie, J. D., Dipchand, A. I., . . . Meiser, B. (2014). The registry of the international society for heart and lung transplantation: Thirty-first official adult heart transplant

report—2014; focus theme: Retransplantation. *The Journal of Heart and Lung Transplantation, 33*(10), 996-1008.

Maldonado, G., & Greenland, S. (1993). Simulation study of confounder-selection strategies. *American Journal of Epidemiology, 138*(11), 923-936.

McKenna Jr., D. H., Eastlund, T., Segall, M., Noreen, H. J., & Park, S. (2002). HLA alloimmunization in patients requiring ventricular assist device support. *The Journal of Heart and Lung Transplantation, 21*(11), 1218-1224. doi:http://dx.doi.org/10.1016/S1053-2498(02)00448-5

Mehra, M. R., Crespo-Leiro, M. G., Dipchand, A., Ensminger, S. M., Hiemann, N. E., Kobashigawa, J. A., . . . Uber, P. A. (2010). International society for heart and lung transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy—2010. *The Journal of Heart and Lung Transplantation, 29*(7), 717-727.

Nikaein, A., Alivizatos, P. A., Monahan, K., & Stone, M. J. (1995). The role of anti-class II HLA antibodies in heart transplantation. *Transplantation, 59*(3), 439-442.

Pham, M. X., Berry, G. J., & Hunt, S. A. (2011). Chapter 29. cardiac transplantation. In V. Fuster, R. A. Walsh & R. A. Harrington (Eds.), *Hurst's the heart, 13e* (). New York, NY: The McGraw-Hill Companies.

Pober, J. S., Jane-wit, D., Qin, L., & Tellides, G. (2014). Interacting mechanisms in the pathogenesis of cardiac allograft vasculopathy. *Arteriosclerosis,*

Thrombosis, and Vascular Biology, 34(8), 1609-1614. doi:10.1161/ATVBAHA.114.302818 [doi]

Raess, M., Frohlich, G., Roos, M., Rusi, B., Wilhelm, M. J., Noll, G., . . . Enseleit, F. (2013). Donor-specific anti-HLA antibodies detected by luminex: Predictive for short-term but not long-term survival after heart transplantation. *Transplant International : Official Journal of the European Society for Organ Transplantation, 26*(11), 1097-1107. doi:10.1111/tri.12170 [doi]

Reed, E. F., Hong, B., Ho, E., Harris, P. E., Weinberger, J., & Suciu-Foca, N. (1996). Monitoring of soluble HLA alloantigens and anti-HLA antibodies identifies heart allograft recipients at risk of transplant-associated coronary artery disease. *Transplantation, 61*(4), 566-572.

Reinsmoen, N. L. (2013). Role of angiotensin II type 1 receptor-activating antibodies in solid organ transplantation. *Human Immunology, 74*(11), 1474-1477.

Reinsmoen, N. L., Lai, C. H., Mirocha, J., Cao, K., Ong, G., Naim, M., . . . Kobashigawa, J. (2014). Increased negative impact of donor HLA-specific together with non-HLA-specific antibodies on graft outcome. *Transplantation, 97*(5), 595-601. doi:10.1097/01.TP.0000436927.08026.a8 [doi]

Ruiz-Ortega, M., Lorenzo, O., Ruperez, M., Esteban, V., Suzuki, Y., Mezzano, S., . . . Egido, J. (2001). Role of the renin-angiotensin system in vascular diseases: Expanding the field. *Hypertension, 38*(6), 1382-1387.

Sandy von Salisch, Maja-Theresa Dieterlen, Jens Garbade, Sara Klein, Stefan Dhein, Friedrich W. Mohr, . . . Markus J. Barten. (2013). Identification of non-HLA antibodies in ventricular assist device recipients. *Open Access Journal CARDIOMETRY,* (3), 82-99.

Suciu-Foca, N., Reed, E., Marboe, C., Harris, P., Yu, P. X., Sun, Y. K., . . . King, D. W. (1991). The role of anti-HLA antibodies in heart transplantation. *Transplantation, 51*(3), 716-724.

Tambur, A. R., Bray, R. A., Takemoto, S. K., Mancini, M., Costanzo, M. R., Kobashigawa, J. A., . . . Gebel, H. M. (2000). Flow cytometric detection of HLA-specific antibodies as a predictor of heart allograft rejection. *Transplantation, 70*(7), 1055-1059.

Taniguchi, M., Rebellato, L. M., Cai, J., Hopfield, J., Briley, K. P., Haisch, C. E., . . . Terasaki, P. I. (2013). Higher risk of kidney graft failure in the presence of anti-angiotensin II type-1 receptor antibodies. *American Journal of Transplantation, 13*(10), 2577-2589. doi:10.1111/ajt.12395

Taylor, D. O., Stehlik, J., Edwards, L. B., Aurora, P., Christie, J. D., Dobbels, F., . . . Hertz, M. I. (2009). Registry of the international society for heart and lung transplantation: Twenty-sixth official adult heart transplant report-2009. *The*

Journal of Heart and Lung Transplantation : The Official Publication of the International Society for Heart Transplantation, 28(10), 1007-1022. doi:10.1016/j.healun.2009.08.014 [doi]

The International Society of Heart and Lung Transplantation. (2014). Overall heart transplantation statistics. Retrieved from https://www.ishlt.org/registries/slides.asp?slides=heartLungRegistry

University of Nebraska Medical Center. (2014). Division of cardiology. Retrieved from http://www.unmc.edu/intmed/divisions/cardiology/index.html

Urban, M., Gazdic, T., Slavcev, T., & Netuka, I. (2015). The impact of anti angiotensin II type 1 receptor antibodies in human leucocyte antigen antibodies negative recipients on post-heart transplantation outcome. *Journal of Heart and Lung Transplantation, 4*(34), S286.

Vasilescu, E. R., Ho, E. K., de la Torre, L., Itescu, S., Marboe, C., Cortesini, R., . . . Mancini, D. (2004). Anti-HLA antibodies in heart transplantation. *Transplant Immunology, 12*(2), 177-183. doi:10.1016/j.trim.2003.08.002 [doi]

Walter, S., & Tiemeier, H. (2009). Variable selection: Current practice in epidemiological studies. *European Journal of Epidemiology, 24*(12), 733-736.

Warraich, R. S., Pomerance, A., Stanley, A., Banner, N. R., Dunn, M. J., & Yacoub, M. H. (2000). Cardiac myosin autoantibodies and acute rejection after heart transplantation in patients with dilated cardiomyopathy. *Transplantation, 69*(8), 1609-1617.

Yamani, M. H., Cook, D. J., Rodriguez, E. R., Thomas, D. M., Gupta, S., Alster, J., . . . Starling, R. C. (2006). Increased expression of angiotensin II type 1 receptor (AGTR1) in heart transplant recipients with recurrent rejection. *The Journal of Heart and Lung Transplantation : The Official Publication of the International Society for Heart Transplantation, 25*(11), 1283-1289. doi:S1053-2498(06)00650-4 [pii]

SERVICE LEARNING REFLECTION

Organization

DIVISION OF CARDIOLOGY, DEPARTMENT OF INTERNAL MEDICINE, UNMC

The Division of Cardiology has 23 physicians. Its physicians are subspecialized in cardiac electrophysiology, interventional cardiology, diagnostic cardiovascular imaging, heart failure and heart transplantation (University of Nebraska Medical Center, 2014).

ORGANIZATION FUNCTION

The division of Cardiology functions as both a treatment center and an academic center. The Division provides patient care services to all adults with various type of cardiovascular disease, ranging from ischemic heart disease, valvular heart disease, arrhythmia, heart failure, and heart transplantation care. The cardiologists work closely with thoracic surgeons to provide the exceptional, comprehensive medical and surgical cardiovascular care.

In addition to patient care services, the Division of Cardiology providing support for medical student, resident, and cardiology fellow training. The Division also offers clinical and research oriented conferences. The Division is also actively involved in research, ranging from bench research to clinical and community based research (University of Nebraska Medical Center, 2014).

ORGANIZATION AIMS

- Working with surgical partners to become a destination for patients to receive exceptional, comprehensive medical and surgical cardiovascular care.

- Be nationally recognized for our research programs.
- Be a leader in innovative educational programs for students, house officers and clinicians across the region and provide
- Attract and retain top clinicians, educators and scientists.

Service performed

The Service learning activities was conducted in the 2015 spring semester, from January 2015 to May 2015. The goal of the service that student provides to the site was to create a dataset that contains heart transplant patients which would be used for future research activities.

A part of the Service Learning activities, the student reviewed coronary angiographs of heart transplant patients. The results then transferred into electronic records with corresponding anatomical lesions. For each patient, the coronary angiograph results included the latest test, if those patients did not have any coronary artery lesion on the film, or the earliest angiograph that showed abnormal anatomy if they had coronary artery lesions.

Student also reviewed echocardiograph and cardiac catheterization results from the medical record system (EPIC) for patients who have lesions on coronary angiography. The results were recoded into electronic form which contains left ventricular ejection fraction, right atrial pressure, pulmonary arterial pressure, diastolic function, and cardiac index. The results of coronary angiography, echocardiography and cardiac catheterization then graded for diagnosing of cardiac allograft vasculopathy following the International Society of Heart and Lung Transplantation guidelines.

In the second part of the Service learning, the student created an online database using REDCap web application. The REDCap database contained heart transplant patients currently in the research projects of the Cardiology Division. The variables were organized by variable domains, which included pre-transplantation variables, post-transplantation variables and donor-related variables. The database is able to be added more records in the future when the projects go on. Access and right of modification to the database can be granted by Dr. Brian Lowes. The REDCap database will be maintained and used for research activities of the Division in the future.

Learning experience

When I came to UNMC, Vietnam started heart transplant program with the first four patients. Sadly, after 6 months, all patients died because of acute rejection, a catastrophic complication that has been well prevented and treated in the US for the last ten years. The service learning and capstone project provides me a wonderful opportunity to pursuit my interest and apply my knowledge and skills. The first thing I learned from this project was current literature on heart transplantation, which provided me a more comprehensive view of transplantation process, from selecting the patients, selecting donor, screening and matching donor-recipient, post-transplant care and especially the role of antibodies in heart transplantation. From literature review, I also identified potential risk factors for mortality or CAV in heart transplant recipients. This knowledge was applied to identify potential confounders or interactions in our analysis plan. Secondly, this was my first time to conduct a longitudinal study. I have learned the vital role of

time measurement for both exposures and outcomes, and how an obscure measurement could largely distort the relationship. More important, I learn how to apply epidemiology to test a scientific hypothesis, and ultimately to improve quality of patient care and common health at large.

The second big gain was my experience of using REDCap. REDCap is a secure web application, developed by Vanderbilt University, for building and managing online surveys and databases. The application offers many options for designing questionnaire, checking data quality, export data dictionary or copy the format and structure of the project to use in a similar study. There is also a REDCap shared library of validated instruments and forms that can be easily downloaded and used by researchers partnered with REDCap. While working on service learning, I had an opportunity to explore and use most of the relevant options in REDCap.

The biggest challenge to me was how to coordinate and collaborate between a busy research team. The challenge can be as simple as how to set up a meeting with the committee members or with members in the team to more complicated as how to exploit the expertise of each member of the project in the most valuable way. For these challenge the best way for me was to discuss with my advisor and other students to collect their experience, and plan everything in advance.

Administrative Resources

Offices supplies such as paper, pen, copying and printing are self-supported by students and committee members. Student used SAS 9.3 statistical software for all the analysis. SAS 9.3 is provided free of charge to all College of Public Health students through secure clustered computers in the computer lab. Rooms for

committee meetings are located in College of Public Health, which is provided by the college for Service Learning and Capstone Projects. No travel will be needed to accomplish the course. Other costs will be covered by the student.

Service Learning/ Capstone Experience Goals and Objectives

Goal 1: Completing service learning in spring semester

Objective 1: Creating a REDCap database for data entry.

Objective 2: Read coronary angiographs of heart transplant patients

Objective 3: Enter data into the REDCap project

Goal 2: Completing capstone experience in summer semester

Objective 1: Analyze data for study questions

Objective 2: Write and review the Capstone Experience report and manuscript

Objective 3: Prepare and defend the capstone experience and submit paper

TIMELINE FOR SERVICE LEARNING AND CAPSTONE EXPERIENCE

The timeline for Service Learning and Capstone Experience is depicted bellow:

Table 4: Timeline for Service Learning and Capstone Project

	Spring Semester	Summer Semester
Service Learning		
Objective 1	X	
Objective 2	X	
Objective 3	X	
Capstone Experience		
Objective 1		X
Objective 2		X
Objective 3		X

ETHICS ISSUES REGARDING RESEARCH

PROTECTION OF PERSONAL IDENTIFICATION

Since the data that we used include critical personal identification of the patients, there was a chance that such information might accidently be disclosed and/or obtained by a third party without the authorization of participants. To avoid any unauthorized access, all the data were stored in encrypted computer inside locked cabins or rooms. All personal identification was available only to the investigators. The access of personal identification to collaborators in the future can be safely granted by Dr. Lowes via REDCap project. All the statistics analysis was processed at secure computer lab at College of Public Health, UNMC.

SAFETY OF SUBJECTS

This was a retrospective study. All the information was available from the electronic health record system or in other hard copy forms. No procedure or medication was applied to patients for purpose of this study. Post-transplantation care was given to patients regardless of participation status. Other safety procedures were followed at the primary clinical trial and were approved by the UNMC IRB. Therefore, this study is considered to be minimal risk.

CONFLICTS OF INTEREST

The student and other researchers declare that they have no conflict of interest. The student, Hoang Tran, received a fellowship from the Vietnam Education Foundation to support his study at UNMC. The fellowship has no known benefits from this study.

APPLICATION OF PUBLIC HEALTH COMPETENCIES

Competency Activity/Application	Reflection of Competency Strength/ Professional Growth	Committee Assessment
CORE COMPETENCIES		
Competency 1B: Applied descriptive and inferential methodologies according to the type of study design **Activity/Application:** Applied univariate analysis with Chi-Square and Fisher's Exact tests to describe the baseline characteristics. Applied Kaplan Meier curve and Log-Rank test to compare time to events. Applied Cox proportional regression to estimate hazard ratios for interest predictors	This was my first time to apply survival analysis outside class. There were several factors that need to be considered when I conducted survival analyses which were not relevant to logistic regression analyses that I have done before. These factors such as time of exposure, or variability of exposures (DSA, AT1RAb) along the follow up time could affect the accuracy of the measurement and there for bias the results. These factors should always be taken account in the plan of data collection and efforts to identify these potential bias should be made in order to have accurate results.	☐ Not Competent ☐ Somewhat Competent ☐ Competent ☐ Highly Competent ☐ Uncertain
Competency 1C: Interpret results of statistical analyses in public health studies. **Activity/Application**: Interpreted results from analysis, presented data in a variety of formats: table, plots, narrative.	Interpreting the results in my project was challenging. Because of the unexpected missing data on CAV outcome, I have less power to detect small differences. For example, although the Kaplan Meier curves showed different patterns between the negative and positive exposure groups, the p-value was still non-significant. Therefore, recognizing the trends or evaluating the absolute estimates, and confident intervals was important in interpreting the results rather than just focusing on p-value.	☐ Not Competent ☐ Somewhat Competent ☐ Competent ☐ Highly Competent ☐ Uncertain
Competency 3B: Identify key sources of data for epidemiological purposes. **Activity/Application:** Identified potential source epidemiological data for Capstone Experience.	I was able to identify several sources of epidemiological data such as Maine's Behavior Risk Factor Survey (BRFSS), Veteran Affair Hospital Rheumatoid Arthritis Registry (VARA), and California Health Interview Survey. In my project, the data mainly came from NMC electronic medical records (EPIC). EPIC can be a very good source of research data with lots of information such as history, laboratory tests, comorbidities, procedures and treatments. However, some of the information was stored in an unextractable forms such as pdf or scanned pictures, which requires researchers to manually mine the data.	☐ Not Competent ☐ Somewhat Competent ☐ Competent ☐ Highly Competent ☐ Uncertain
Competency 7A: Identify and apply fundamental research skills in public health.	From the Capstone experience paper, I was able to write a scientific manuscript which could be submitted for publication in a peer-review journal. The challenge that I faced to	☐ Not Competent

Activity/Application: Applied research skill to write a scientific manuscript.	when I transformed the paper into the manuscript was the succinct format of the manuscript. Unlike the paper which was relatively flexible in format, the manuscript needed to be very concise, with around 3,000 words to covey the study findings and discussion, in addition to a throughout and detailed method description.	☐ Somewhat Competent ☐ Competent ☐ Highly Competent ☐ Uncertain
Competency 10A: Applied ethical principles to the collection, maintenance, use, and dissemination of public health information. **Activity/Application:** Secured patients' confidentiality and safety throughout the study	Although my study was a retrospective cohort study and no procedures, drugs or experiments were given to the patients, we dealt with lots of personal information which also need to be secured. I always adhered to patients' information protection rule in my study. All the data were stored or transferred via HIPPA-compliant, secured server provided by UNMC information services. Data were not exposed to anyone who did not have the right to access determined by UNMC IRB.	☐ Not Competent ☐ Somewhat Competent ☐ Competent ☐ Highly Competent ☐ Uncertain
CONCENTRATION COMPETENCIES		
Competency 1A: Conceptualize epidemiologic research questions and hypotheses. **Activity/Application:** Created study objectives and hypotheses	From literature review, I was able to identify the potential association between AT1RAb and CAV or mortality following heart transplant and propose the testable hypotheses through which AT1RAb affect heart transplant patients. I also identify the knowledge gap and proposed the study objective to fill in the gap.	☐ Not Competent ☐ Somewhat Competent ☐ Competent ☐ Highly Competent ☐ Uncertain
Competency 1C: Review and critique published epidemiologic studies. **Activity/Application:** Reviewed and critiqued published epidemiologic studies. Compared previous studies' findings with the present study's findings.	There were a bundle of literature related to heart transplant and post-transplant complications or mortality. I was able to review the current literature to describe the donor-recipient selection process, which essential to understand the role of antibodies in rejection post-transplant. I also compared the findings in my study with previous studies, and discussed potential explanation for any differences among the findings.	☐ Not Competent ☐ Somewhat Competent ☐ Competent ☐ Highly Competent ☐ Uncertain
Competency 3A: Choose a study design appropriate for a particular epidemiologic question **Activity/Application:** Chose retrospective cohort study as the design for my Capstone project.	Choosing the proper study design to test the study hypotheses is crucial. With the current data, I was be able to conduct a case-control study or a retrospective cohort study. The case-control study would be easier for me to conduct since I was familial with logistic regression. Since the study outcomes (CAV, death) would not occur at once, the case-control study would not be able to reflect time to event and therefore would fail to test the hypotheses. On the other hand, a	☐ Not Competent ☐ Somewhat Competent ☐ Competent ☐ Highly Competent ☐ Uncertain

	retrospective cohort study would enable to measure time from transplant until the occurrence of outcomes, which will provide more information in the results, even with potential explanation of the underlying mechanism.	
Competency 4c: Identify potential sources and effects of bias in epidemiologic studies. **Activity/Application:** Identified potential bias in the present study and minimized the bias by analytical method.	The present study employed time to event analyses. Besides the potential confounders that can affect the association (which partially adjust in multivariate analyses), time measurement of exposures or outcomes can also bias the results. For example, if the preexisting antibody status change, measure it at one point in time would potentially resulted in different result if it was measured in another time point. Therefore, the classification of patients to either exposed or non-exposed group could be changed.	☐ Not Competent ☐ Somewhat Competent ☐ Competent ☐ Highly Competent ☐ Uncertain

ACKNOWLEDGEMENT

I would like to express the deepest appreciation to my committee chair as well as my advisor, Dr. Lorena Baccaglini for her support, guidance, motivation extended to me throughout this project.

I sincere thank would go to Dr. Brian Lowes, for giving me a chance to work in the field that I am interested in, and for his insightful input, his patience and encouragement toward me in the time I worked in this project.

I also would like to thank Dr. Jane Meza for her analytical and statistical insights.

In addition, I would like to thank Dr. Catherine Gebhart, Dr. Eugenia Raichlin, Dr. Adam Burdorf, and Mohamed Awaji for their supports in this project.

Finally, I would like to thank to my family, my friends, and the Vietnam Education Foundation for all of their support toward my study time as well as the time I carried out this project.