Infectious Burden and Accelerated Atherosclerosis in the Renal Transplant Population

A. Study Purpose and Rationale

Cardiovascular complications remain the leading cause of morbidity and mortality for patients undergoing renal transplantation. In addition to classic cardiovascular risk factors, pretransplantation dialysis, immunosuppressive regimen choice, and allograft losses may contribute to post-transplantation atherosclerotic events. As advances in the development of immunosuppressive agents have brought steroid-free or steroid-sparing regimens into the forefront of routine maintenance for renal transplantation patients, adverse events related to long-term immunosuppression, particularly cardiovascular disease, infection, and malignancy remain significant problems for renal transplant patients [1]. The incidence of atherosclerotic events is significantly higher in renal transplant recipients [2]. Immunosuppressive agents have predisposed to higher rates of hypertension, renal graft dysfunction, hyperlipidemia, and hyperglycemia in the transplant population, contributing to a high rate of cardiovascular events in this population.

The mechanism of accelerated atherosclerosis in the transplant population remains unclear. Framingham Risk Score (FRS) is an insufficient predictor of cardiovascular events in kidney transplant recipients [3]. Additionally, novel risk factors such as CRP, uric acid, and urine albumin-to-creatinine ratio did not improve the predictive value of FRS in this population [3]. A recent model proposed by the Predicting Outcomes in Renal Transplantation (PORT) investigators has developed a model that better predicts CHD using transplant-specific risk factors such as pretransplant diabetes, new onset post-transplant diabetes, delayed graft function, estimated glomerular filtration rate, age, sex, race, and duration of pre-transplant hemodialysis[4]. This accounts for much of the variation of CHD in renal transplant patients but does not for all of it. Notably missing from much of this data is complete seroprevalence data for chronic infections that have been associated with atherosclerotic events in immunocompetent patients.

Seroepidemiologic studies in patients with coronary artery disease and stroke have provided evidence of risk in patients demonstrating prior infection with common pathogens such as *Chlamydia pneumonia*, *Mycoplasma pneumoniae, Haemophilus influenzae, Helicobacter pylori,* and herpesviruses, including cytomegalovirus (CMV), Epstein-Barr Virus (EBV), and herpes simplex viruses 1 and 2 (HSV1/HSV2) [5 6]. Studies in immunocompetent individuals have demonstrated a positive correlation between high infectious burden with multiple agents and the development of both atherosclerotic disease and stroke [7 8]. The risk posed by infectious burden may be through inflammatory responses rather than through direct infection as demonstrated by correlation of seropositivity with CRP levels [7].

Additionally, in a population-based cross sectional study in Iran, CRP demonstrated a relationship with ECG-defined CAD only in a dependent fashion with positive IgG titers for C. pneumonia, H. pylori, CMV, and HSV1 [9]. Pathogen burden and CRP levels interacted significantly, demonstrating that carriers of 4-5 pathogens with elevated levels of CRP had higher odds of coronary artery disease compared with those who carried fewer pathogens [10]. There exists a growing body of indirect evidence that chronic infections are indeed related to the development of atherosclerosis.

Among infectious complications of transplant, morbidity from CMV remains significant. CMV causes more clinically relevant disease in immunosuppressed individuals, but can readily infect immunocompetent individuals. There is a high burden of CMV infection and disease in the renal transplant population. In the absence of any preventative therapy, CMV infection occurs in 30-75% of renal transplant recipients, and CMV disease occurs between 8 and 80% dependent upon type of transplantation, donor/recipient CMV serostatus, and immunosuppression.

Although directly implicated in retinitis, pneumonia, hepatitis, pancreatitis, colitis, meningoencephalitis, and rarely myocarditis, a causal relationship between CMV and atherosclerotic disease is not clearly delineated. But data do exist that suggest an association between CMV infection and the development of atherosclerotic events. Since the advent of improved treatment options for CMV as well as alternative immunosuppressive choices, CMV can now present more insidiously, in the absence of fever or systemic symptoms. CMV has been implicated not only in cardiac transplant patients with allograft vasculopathy [11] but also in restenosis of native coronary arteries [12]. In a European retrospective study of patients with end stage renal disease, 408 patients followed from 2002-2006 seropositive for CMV demonstrated a significant increased risk for accelerated atherosclerosis [13]. Additionally, a Turkish study of renal transplant patients identified a significant association of CMV infection with atherosclerotic events [14]. A retrospective analysis of 1859 adult renal transplant recipients at the University of Minnesota demonstrated an increased risk of cardiac complications in patients with CMV disease [15]. We hypothesize that patients who are CMV seronegative and remain seronegative will have a lower rate of major adverse cardiovascular events. We also hypothesize that patients who are IgA seropositive for *Chlamydia pneumoniae* may also demonstrate increased risk along with patients seropositive for IgG specific for CMV. We suspect that the comorbid infectious burden is high in these common pathogens, and that coinfection may contribute additive risk of cardiovascular disease in the renal transplant population.

B. Study Design and Statistical Analysis

This will be a prospective observational cohort study of renal transplant recipients followed longitudinally for a 5-year period at transplant centers in New York State after transplant for the development of atherosclerotic events. Interval periods of follow-up will be 1, 3, and 5 years after transplant at which point patients will be assessed for interval seroconversion for the organisms of interest. At enrollment in the study, data will be obtained about the extent of atherosclerosis at time of transplant based on coronary angiography, carotid duplex sonography, and evaluation of ankle-brachial indices.

Data will be collected about infectious serologic status (seropositive or seronegative, or evidence of seroconversion) of the transplant donors and recipients, as well as information about classic cardiovascular risk factors (age, sex, smoking status, diabetes, lipid levels), novel risk factors (CRP, urine microalbumin, and previous major adverse cardiovascular events (previous MI, angina, revascularization procedures, intermittent claudication, stroke, or TIA). Possible confounders include administration of antiviral medications and antibiotics, use of lipid-lowering medications, episodes of acute rejection, and administration of steroids. Use of anti-thymocyte preparations such as OKT3 also have been shown to worsen CMV disease and will be documented. The study will be limited to patients with intact graft function at 1 year post-transplant.

Based on retrospective data from the PORT study, the cumulative incidence of CHD in a 19,578 renal transplant patients was 3.1%, 5.2%, and 7.6% at 1, 3, and 5 years post transplant respectively [4]. Adjustments for age, sex, cardiovascular risk factors, urine microalbumin, CRP, pre-transplant diabetes, new onset post transplant diabetes, acute graft rejection, will be performed. Based on testing and dividing the transplant recipients in two groups of patients exposed to Chlamydia pneumoniae and/or cytomegalovirus to compare then with unexposed patients, we would expect to see an increased effect of pathogen burden on incidence of CHD in our enrolled patients. We will measure serologic positivity at time of enrollment/transplant, and at 1,3, and 5 years post transplant.

Power Calculation:

To examine an effect size in difference between the two groups' CHD incidence rate at 5 years, a power calculation at goal of 80% was performed assuming that there are 4-fold as many patients positive for one or both markers of interest versus patients without serologic evidence of exposure to CMV or Cp. In examining a difference, we expect at least a two-fold increase in CHD in exposed patients versus unexposed. Assuming a baseline 7.6% CHD incidence in low group as reference with HR of 1) and CHD incidence of 15.2% in patients exposed to either or both agents, the power calculation peformed shows that the number of patients needed to adequately power the

study would be 197 patients in the double-seronegative group versus 788 patients in the single-or-double-seropositive group.

N = 8((p1q1 + p2q2)/ (p2-p1)^2) + 2/(p2-p1) + 2
Group2/Group1 ratio: 4

CMV+/Cp-IgA+	CMV+/Cp-IgA-	CMV-/Cp-IgA+	CMV-/Cp-IgA-
(0.5)x(0.6)=0.30	(0.5)x(0.6)= 0.30	(0.5)x(0.6)= 0.30	(0.5)x(0.4)=0.20

Group 1 = double seronegative = 0.20, Group 2 = single or double seropositive = 0.8.

Cox proportional hazard modeling will be performed to judge the association of the two groups with the development of CHD. Factors adjusted for will be those described in the PORT study described above. Both unadjusted and adjusted hazard ratios will be calculated.

Exclusion criteria: To specifically examine the role of infection or inflammation caused by chronic infection, we will aim to exclude patients with known autoimmune disease (SLE, rheumatoid arthritis). Additionally, we will exclude patients who are HIV+ from the analysis.

C. Study Procedures

There are no procedures to be performed in this study.

D. Study Drugs or Devices

Not applicable – no additional study drugs or devices will be included in the analysis. Patients will be restricted in the study to using FDA approved.

E. Study Questionnaires

A short questionnaire will be given to the patient at the time of enrollment, asking that they or their physician inform the study coordinators of any usage of antibiotics or antiviral agents during the course of the study.

F. Study Subjects

Study subjects will include adult renal transplant patients (>16 years old at time of transplant) transplanted at transplant centers in New York State. Patients excluded will be those with known diagnosed and treated autoimmune conditions (SLE, systemic sclerosis, rheumatoid arthritis, and antiphospholipid antibody syndrome). This will also exclude patients whose

primary indication for transplantation was the result of an autoimmune disease. Patients with known HIV disease will also be excluded from the analysis. Patients who secondarily develop autoimmune disease or HIV disease will be excluded from the study.

G. Recruitment
   Patients will be recruited for the study at the time of transplantation.
H. Confidentiality of Study Data
   Patients will be de-identified for the analysis, and their personal information kept in encrypted computer databases maintained at the transplant center.
I. Potential Risks
   There are likely no potential risks from participating in the study other than the loss of possible confidentiality.
J. Potential Benefits
   There are no potential benefits to participating in the study
K. Alternatives
   Not applicable

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