DNA Microarray in Cardiac Allograft Vasculopathy

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A. Study Purpose and Rationale

Cardiac allograft vasculopathy (CAV) remains the most significant impediment to successful long-term engraftment of cardiac allografts.[1] It is the leading cause of death one year after heart transplantation and contributes significantly to morbidity and long-term costs of heart transplantation.[1] This process is characterized by a diffuse neointimal thickening that develops throughout donor vessels, including smaller, more distal intramyocardial vessels as well as larger coronary arteries. The neointimal thickening results in the progressive narrowing of the vascular lumen. By 5 years after transplant, over 40% of heart transplant recipients will have developed CAV, including 7% of all recipients who will die or be re-transplanted due to CAV-related event.[1] A 70% stenosis of one of the major coronary arteries lowers 2 year survival to 60%. Survival drops to 20% in 6 months when all three coronary arteries are involved.[2]

Risk factors for CAV development include older donor and recipient age, ischemia-reperfusion injury, major histocompatibility mismatch, hyperlipidemia, insulin resistance, CMV infection, recurrent or persistent allograft rejection.[3] Studies suggest a complex interplay between immune, inflammatory and vascular mediated mechanisms underlying the development of CAV. Immunostaining techniques have shown that the neointima of these lesions is composed of inflammatory cells.[4] CAV is characterized by early recipient derived macrophage, T-cell and B-cell infiltration of the interstitial and perivascular areas.[5] The alloimmune response appears to be primarily caused by T-lymphocytes. Damaged endothelial and inflammatory cells produce cytokines including IL-1beta, IL-6, and TNF-alpha and growth factors that lead to smooth muscle cell proliferation, extracellular matrix synthesis, lipid accumulation and late intimal fibrosis. Endothelial cell activation as determined by MMP-2 and -9, ET-1, and ICAM-1 are also correlated with the development of CAV.[3] Furthermore, studies of diltiazem, statins and rapamycin indicate potential regulatory points involved in CAV.[3]

Recently, Horwitz et al demonstrated in a proof of principle study that DNA microarray profiles correlate with biopsy-proven acute cardiac allograft rejection.[6] The complex interplay between risk factors and mechanisms for CAV may provide a further area in cardiac transplant that expression profiling may have utility.

B. Study Design and Statistical Analysis

This will be a nested case-control study of endomyocardial tissue expression within the cohort of cardiac transplant patients. Consecutive cardiac transplant recipients performed and followed at Columbia University Medical Center (CUMC) will be enrolled at the time of transplantation. As the current standard of care, patients will have regularly scheduled follow-ups (weekly for the first month, then every 3 months for the first year, and then yearly afterwards) when transvenous endomyocardial biopsies are performed for surveillance of acute rejection. An additional tissue sample will be taken and frozen. Once cases and controls are determined, the samples will be prepared for microarray analysis. Case patients will be the anticipated 7% seen in prior studies by 5 years post transplant who either die or are re-transplanted due to CAV as determined on angiographic examination. Given that CUMC performs approximately eighty cardiac transplants per year, the conservatively anticipated recruitment time necessary to ascertain 6 cases will be 4 years. Control patients (n=6) will be selected from those without CAV on angiography at the end of the study and have been in the study the longest. Routine surveillance angiography will be done as the standard of care annually post transplant.

Statistical analysis includes cluster analysis of the microarray data.[7] Clusters will be construed with average linkage clustering and Pearson correlation coefficients as a measure of similarity with

Cluster software. This study will focus on genes involved in the inflammatory (IL-1, IL-6, TNF-alpha), endothelial (ET-1, ICAM-1, MMP-2, MMP-9), and rapamycin pathways that are believed involved in the development of CAV. With 6 subjects in each group, an effect size of 2.1 of the standard deviation should be detected with 90% power, testing at p=0.05.

C. Study Procedure

Standard of care will be followed as in all patients after receiving a cardiac transplant. This includes annual coronary angiography for routine surveillance for development of CAV and routine surveillance with right heart catheterization and endomyocardial biopsies weekly for the first month, then every 3 months afterwards and as needed as determined the primary cardiologist. The only difference will be the ascertainment of an additional biopsy sample at baseline and every 6 months afterwards until completion of the study. Given that CUMC performs approximately eighty cardiac transplants per year, the conservatively anticipated recruitment time necessary to ascertain 6 cases will be 4 years.

D. Study Drugs

Not applicable.

E. Medical Device

Not applicable.

F. Study Questionnaires

Not applicable.

G. Study Subjects

Inclusion criteria for this study will be cardiac transplant recipients followed at Columbia University Medical Center with the transplant occurring from the onset of this study. Exclusion criteria will be age$\leq$18. It is anticipated that women, racial and ethnic groups, and non-English speaking populations will reflect the transplant population at CUMC. Vulnerable patients will not be eligible because these patients are not transplant candidates.

H. Recruitment of Subjects

All potential subjects will be identified prior to transplantation. Subjects will be informed of this study once listed for transplant. When a subject arrives for transplantation, s/he will be approached by either the transplant surgeon or cardiologist to ascertain that s/he is willing to discuss the study with the research team.

I. Confidentiality of Study Data

A unique study code will be assigned each subject. Tissue samples and data will only be labeled using the unique study code. All data and samples will be stored in a secure location, accessible only to the investigators.

J. Potential Conflict of Interest

None.

K. Location of Study

The study will be done in clinical care areas at CUMC including, but not limited to, the Cardiology facilities at CUMC.

L. Potential Risks

Patients will only be subjected to the risk from an additional endomyocardial biopsy sample that will be taken at the time of their scheduled follow-up as standard of care. This risk is minimal given that multiple samples are taken frequently from these patients as standard of care for rejection surveillance.

M. Potential Benefits

Patients may not benefit as a result of their participation in the study because no additional treatments are being performed. There is the potential benefit to society from the gain in knowledge about the mechanisms of CAV.

N. Alternative Therapies

Patients may choose to not participate in the study and will continue to receive the standard of care post-transplant.

O. Compensation to Subjects

No compensation will be provided.

P. Costs to Subjects

The subject will not incur any additional costs as a result of participating in the study.

Q. Minors as Research Subjects

Not applicable.

R. Radiation or Radioactive Substances

Not applicable.

S. References

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