

Early growth response-1 (Egr-1) as a predictor of TxCAD in cardiac transplant patients

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A. Study Purpose and Rationale

Cardiac allograft vasculopathy (CAV), also known as transplant coronary artery disease (TxCAD), is the major late complication after cardiac transplantation [1,2]. Angiographic evidence of TxCAD was present in 42% of over 3800 cardiac transplant patients 5 years post-transplant in one study [1]. Unlike conventional coronary artery disease, which is characterized by focal atherosclerotic plaques, TxCAD leads to diffuse concentric lesions that begin in the distal arterioles and eventually span the donor coronary arterial tree. A number of immunologic and nonimmunologic factors have been implicated in the pathogenesis of TxCAD. Nonimmunologic factors include donor hypertension, donor age and sex, recipient sex and race, dyslipidemia, and insulin resistance. Immunologic factors include both cellular and humoral immunity, cytokines, nitric oxide synthesis, and possibly CMV infection. These factors interact to cause endothelial cell damage, inflammatory cell recruitment and cytokine secretion, upregulation of cell adhesion molecules, smooth muscle cell proliferation and ultimately intimal thickening and vessel stenosis.

Early growth response factor-1 (Egr-1) is 82-kd 533 amino acid zinc finger proinflammatory signal transduction factor [3]. It is upregulated in response to vascular injury, hypoxia, cytokines, and growth factors and induces expression of vascular genes including FGF, TGF- β , TNF- α and ICAM-1 [4]. Egr-1 is also induced in the smooth muscle cells of human atheromas as well as in atherosclerotic lesions of animal models [5]. Many of the molecules induced by egr-1 such as PDGF and ICAM-1 are also upregulated in TxCAD [6,7]. In several animal cardiac allograft and xenograft models egr-1 expression is induced in animals that develop TxCAD. Furthermore, these animal models suggest that egr-1 gene expression precedes the morphologic vascular changes associated with TxCAD [8-10]. In addition, egr-1 null donor allografts do not develop TxCAD nor are the downstream target genes ICAM-1, VCAM-1 and PDGF upregulated to levels observed in wild-type control animals [10].

a. Hypothesis

Among cardiac transplant patients, those that develop TxCAD express egr-1 at significantly higher levels than those that do not develop disease. Furthermore, this upregulation will precede the development of TxCAD by at least 1 year so that this gene might be used as an early marker of patients predisposed to TxCAD.

B. Study Design and Statistical Analysis

a. Study Design

This is a retrospective case-control study designed to identify egr-1 upregulation as a predictor of TxCAD in cardiac transplant recipients. All heart transplant patients at CPMC's Heart Institute who received transplants between 1995 and 1998 will be eligible for the study. During that period, 329 orthotopic cardiac transplants were performed at CPMC. These patients were scheduled to receive endocardial biopsies at 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 12 weeks, 26 weeks and annually post-transplant. They were also scheduled to receive annual angiograms. Because acute rejection is a complication that usually occurs within the first year and TxCAD is seldom observed prior to one year, all cardiac transplant patients that survived one year post-transplant will be included. With an approximate 90% survival rate at 1 year, close to 300 patients would be included in the study. A portion of the first endocardial biopsy at 1 week as well as annual biopsies will be evaluated by RT-PCR for egr-1 expression levels which will be measured densitometrically and expressed as a ratio to expression of β -

actin, a constitutively expressed gene. Any biopsy with signs of acute rejection will be omitted from the analysis. Immunostaining with egr-1, PDGF, and ICAM-1 will be performed on a portion of the biopsies to look for an in-situ difference and cellular expression patterns of patients who develop TxCAD and those who do not. Angiograms performed annually will be reviewed by two independent blinded angiographers to assess for the presence of CAD.

b. Statistical Analysis

Because approximately half (42%) of cardiac transplant patients develop TxCAD by five years, patients will be followed for 5 years after their transplant. Levels of egr-1 will be determined by mRNA expression in endocardial biopsies measured by densitometric quantitation expressed as a ratio to β -actin mRNA levels in the same animals. The presence or absence of TxCAD will be determined from annual angiographic data by two blinded angiographers. All of the data assessing levels of egr-1 expression after cardiac transplantation are based on animal models. Extrapolating from these models, a ratio of approximately 3.75 of egr-1/ β -actin expression is observed in mice that develop TxCAD whereas a ratio of approximately 2 is observed in animals that do not develop TxCAD. Based on these numbers and the incidence of disease in humans and underestimating the study population at 200, this study would be able to detect a difference of 0.2 to achieve a power of 80% and $p < 0.05$ using the unpaired t-test. Based on the hypothesis, this observed difference in egr-1 expression would be detectable at least 1 year prior to the onset of TxCAD.

C. Study Procedures

As stated above, egr-1 levels will be determined by mRNA expression from endocardial biopsies. These biopsies were performed one week after transplant and annually for five years post-transplant. TxCAD will be determined review of angiograms performed annually after transplantation. All of these procedures were part of the routine clinical management of cardiac transplant patients.

D. Study Drugs

N/a

E. Medical Device

N/a

F. Study Questionnaires

N/a

G. Study Subjects

Approximately 300 cardiac transplant patients from CPMC's Heart Institute from 1995-1998 will be included in the study. As stated above, patients with acute rejection leading to premature death within 1 year of transplant will be excluded from the study. All minors will also be excluded from the study. Patients lost to follow-up will be included if they are followed for at least one year. Patients who did not survive the 5-year study period will be included up until their death.

H. Recruitment of Subjects

N/a

I. Confidentiality of Study Data

All study data will be coded for each patient. Copies will be made of all angiograms and these too will be coded.

J. Potential Conflict of Interest

N/a

K. Location of the Study

All biopsies and angiograms were performed in the catheterization laboratory at CPMC as is per routine.

L. Potential Risks

The risks of the study were the standard procedural risks of catheterization and biopsy including bleeding, infection, and arrhythmias. As this is a retrospective study no additional risks will be incurred by the study population.

M. Potential Benefit

This study is of no benefit to the participants.

N. Alternative Therapies

N/a

O. Compensation to Subjects

None

P. Costs to Subjects

None

Q. Minors as Research Subjects

Minors who underwent cardiac transplantaton will be excluded from the study.

R. Radiation or Radioactive Substances

N/a

S. References

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