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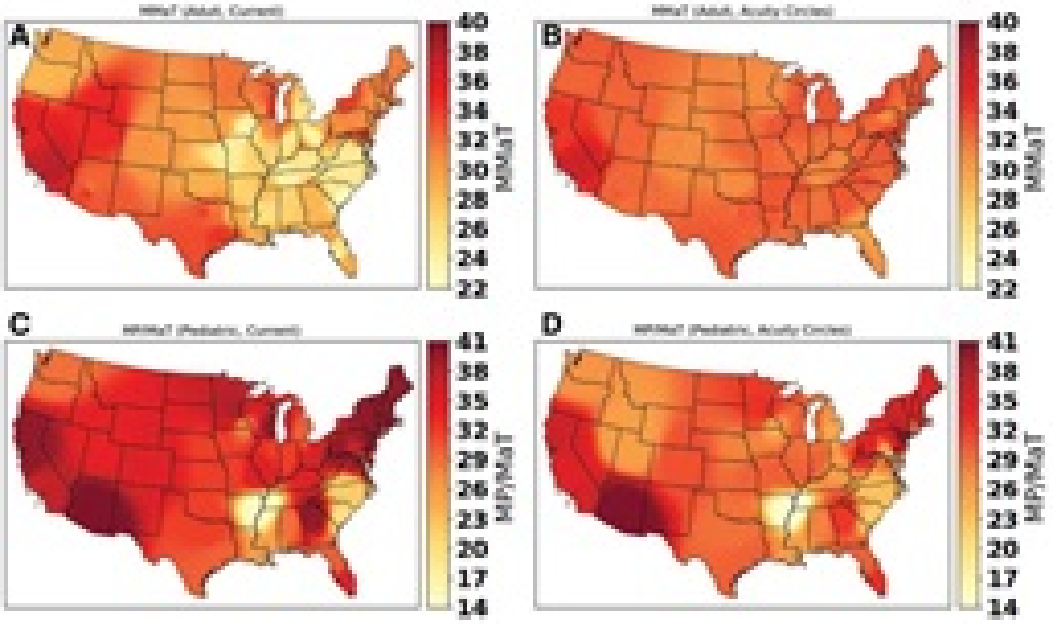

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CORONARY ARTERY INTIMAL THICKENING IN THE TRANSPLANTED HEART: An In Vivo Intracoronary Ultrasound Study of Immunologic and Metabolic Risk Factors

Rickenbacher Peter R.; Kemna, Mariska S.; Pinto, Fausto J.; Hunt, Sharon A.; Alderman, Edwin L.; Schroeder, John S.; Stinson, Edward B.; Popp, Richard L.; Chen, Ida; Reaven, Gerald; Valantine, Hannah A.

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Author Information

Divisions of Cardiovascular Medicine and Endocrinology, and Department of Cardiothoracic Surgery, Stanford University School of Medicine, Stanford, California 94305

[2]Division of Cardiovascular Medicine.

[3]Department of Cardiothoracic Surgery.

[5]Division of Endocrinology.

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[4]Dr. Popp is consultant to and holds stock options with CVIS Inc., manufacturer of the imaging system used.

[6]Address correspondence to: Hannah A. Valantine, MD, Division of Cardiovascular Medicine, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305-5246.

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Abstract

This study examined the hypothesis that immunologic factors are the major correlates of coronary artery intimal thickening and luminal stenosis. The study population included 116 adult heart transplant recipients with a mean age of 44.7±12.0 years (89 men and 27 women) undergoing annual coronary angiography and intracoronary ultrasound 3.4±2.7 (range, 1.0-14.6) years after transplantation. Mean intimal thickness was obtained from several distinct sites along the left anterior descending and/or left circumflex coronary artery by intracoronary ultrasound. Coronary artery stenosis defined by angiography was classified as mild (<30% luminal stenosis), moderate (≥30-70% luminal stenosis), or severe (>70% luminal stenosis or diffuse pruning of distal vessels). Prevalence of any transplant coronary artery disease (TxCAD) was 85% by intracoronary ultrasound and 15% by angiography. By multiple regression analysis, only average fasting plasma triglyceride level ($P<0.006$) and average weight($P<0.007$) were significantly correlated with severity of intimal thickening (R=0.54, $P<0.0001$). Donor age($P<0.006$) and average fasting plasma triglyceride level ($P<0.009$) were significantly correlated with stenosis by angiography.

Correlation of multiple immunologic and metabolic factors with intimal thickness by univariate analysis suggests a multifactorial etiology for TxCAD. Among the multiple univariate correlates of TxCAD, higher fasting plasma triglyceride levels and body weight are the only independent correlates of TxCAD. The absence of acute rejection as an independent predictor of intimal thickening suggests that mechanisms

beyond those mediating typical cellular rejection should be targeted for advancing our understanding of TxCAD.

Accelerated transplant coronary artery disease(TxCAD*) has emerged as the major complication limiting long-term survival after heart transplantation (1, 2). Despite a number of analyses, the factors predisposing to or correlated with TxCAD remain controversial. A consensus exists that the disease is primarily immune-mediated because of its diffuse nature and its confinement to the vascular bed of the allograft. However, clinical studies that have attempted to clarify the relationship between acute cardiac allograft rejection and TxCAD have produced conflicting results. Whereas a positive correlation was found in some studies(3-7), we(8, 9) and others(10-12) have been unable to confirm a direct correlation of TxCAD with biopsy-diagnosed rejection incidence, duration, or severity. Other immunologic and nonimmunologic factors that have been associated with increased risk for TxCAD include HLA mismatch(3, 5, 11, 13), humoral rejection(14, 15), recipient and donor age(8, 9, 16), hyperlipidemia(8, 9, 17, 18), obesity(18), and cytomegalovirus infection(17, 19). In most of these studies, the diagnosis of TxCAD was based on coronary angiographic findings. Our recent experience with intracoronary ultrasound (ICUS) imaging in transplant patients indicates that qualitative coronary angiography grossly underestimates the severity of the disease (20, 21), thus potentially obscuring the analysis of clinical or laboratory correlates with TxCAD. ICUS has been shown to be a sensitive and reproducible method of measuring coronary artery lumen dimension (22) and intimal thickening following heart transplantation (23).

We have recently characterized the metabolic and lipoprotein abnormalities in heart transplant patients with reference to age-matched controls(24). This study demonstrated that a large proportion of patients were dyslipidemic, glucose intolerant, and insulin resistant. These observations provided our rationale to re-examine the relationship of immunologic versus metabolic factors with the development of TxCAD, using the more sensitive endpoint of intimal thickening. We hypothesized that immunologic factors would be the major predictors of TxCAD as assessed by ICUS. Therefore, the purpose of this study was to test this hypothesis by examining the correlation of

immunologic, metabolic, and donor-recipient demographics with both ICUS and angiographic evidence of TxCAD.

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METHODS

Study patients. Between January 1977 and February 1992, 546 heart transplantations were performed at our institution. The study population was drawn from the 265 recipients who were alive at the start of the present study, which began in June 1990. From 160 consecutive patients undergoing routine surveillance coronary angiography, 116 patients (27 women and 89 men) with a mean age of 44.7±12 years were enrolled who also consented to undergo ICUS study. Time since transplantation was 3.4±2.7 (range, 1.0-14.6) years. In a subset of these patients (n=70), ICUS was performed in 2 consecutive years. The mean age in the subset was 44±10 years and time since transplantation was 4.1±2 years.

All but 2 patients were managed with standard immunosuppressive regimens, including prophylactic antilymphocyte antibody therapy during the early postoperative period and maintenance with prednisone, azathioprine, and cyclosporine. The remaining 2 patients studied 13 and 14.6 years after transplantation did not receive cyclosporine. The cyclosporine dose was adjusted to maintain chronic trough serum levels between 100-200 ng/ml as measured by RIA, if permitted by normal renal function. The targeted prednisone dose was 0.2-0.5 mg/kg/day during the initial 3 months, and was tapered to 0.1-0.2 mg/kg/day by 1 year. Azathioprine was given in a dose of 2-4 mg/kg/day as tolerated by the white blood count. Surveillance endomyocardial biopsies were performed weekly during the initial month, biweekly during months 2-3, and monthly until the 6th month after transplantation. Thereafter, the frequency was decreased to 3-4 biopsies per year. The Billingham grading system for acute rejection(25) was used until 1990, and the grading system of the International Society for Heart and Lung Transplantation(26) was used thereafter. Biopsies were graded as: 1) no rejection; 2) mild rejection, indicating the presence of a lymphocytic infiltrate on 1 or more sections in the absence of myocyte necrosis (ISHLT grade IA or IB); 3) moderate rejection, indicating the presence of a cellular

infiltrate with evidence of myocyte necrosis ± evidence of interstitial edema (ISHLT grade II, IIIA, or IIIB); and 4) severe rejection, in the case of additional hemorrhagic changes (ISHLT grade IV). Rejection episodes were counted as one event from onset to resolution as defined histologically. Moderate or severe rejection episodes during the first 3 months after transplantation were treated with methylprednisolone 1.0 g intravenously for 3 consecutive days. Rejection occuring later in the course was treated with oral prednisone 100 mg/day for 3 days, with subsequent tapering to previous maintenance doses. Additional cytolytic therapy with rabbit antithymocyte globulin or OKT3 was reserved for refractory or severe rejection episodes.

The study protocol was approved by the Committee for the Protection of Human Subjects in Research at Stanford University Medical Center, and written informed consent was obtained from all subjects prior to inclusion in the study.

Clinical data. Recipient pre- and posttransplant clinical characteristics recorded for each patient included age and gender, pretransplant cardiac diagnosis, blood pressure, weight, posttransplant treatment with diltiazem, and time since transplantation. Seropositivity for cytomegalovirus (CMV) was defined as a more than 4-fold rise of IgG anti-CMV antibody titer above baseline or preoperative values. CMV infection was defined as either a rise in CMV IgG or appearance of anti-CMV IgM in conjunction with a clinical syndrome consistent with CMV infection, or confirmed infection as evidenced by the presence of inclusion bodies on histologic examination, with positive CMV culture from the same tissue specimen. The donor factors examined were age, graft ischemic time, and CMV serology. An analysis of potential immunologic risk factors was made for each patient. These factors included number of HLA-A and -B mismatches between donor and recipient, number of rejection episodes tabulated as mild (ISHLT grade IA or IB) or moderate (ISHLT grade II, IIIA, or IIIB), and total number of rejection episodes. Maintenance immunosuppression was analyzed in terms of the average daily weight-adjusted doses of each immunosuppressive drug(cyclosporine, azathioprine, and prednisone) and the average cyclosporine level. Immunosuppression for treatment of acute rejection episodes was quantified as the total “pulsed” corticosteroid dose, computed as the sum of all prednisone-equivalent doses administered to each patient.

Potential metabolic risk factors examined were fasting serum lipoproteins measured prior to transplantation, average values obtained at annual intervals after transplantation, and average fasting serum glucose.

Two subset analyses were performed. First, the risk factor profile for early TxCAD was assessed in the subset of patients studied 1 year after transplantation (n=39). Second, the risk factors for progression of TxCAD were examined in a subset of patients (n=70) in whom ICUS was performed in 2 consecutive years; risk factors were compared in patients with disease progression versus no progression.

Coronary angiography and intracoronary ultrasound. Coronary anteriograms were performed using the percutaneous femoral approach and standard angiographic techniques. Multiple projections of both right and left coronary artery systems were obtained after sublingual nitroglycerin premedication. Coronary arteriograms were assessed visually by two independent, experienced angiographers blinded to clinical data. TxCAD was classified as mild (<30 luminal stenosis), moderate (≥30-70% luminal stenosis), or severe (>70% luminal stenosis or diffuse pruning of distal vessels).

ICUS imaging was accomplished with a 30-MHz ultrasound transducer and rotating mirror enclosed within an acoustic housing at the tip of either a 5-French or 4.3-French, 135-cm-long, flexible rapid exchange catheter (CVIS Inc., Sunnyvale, CA). The catheter characteristics have been reported in detail previously (20). After completion of the standard coronary angiography, 0.4 mg of sublingual nitroglycerin was given before the intracoronary imaging system was introduced through a high-flow 8-French guiding catheter over a 0.014-inch coronary guidewire from the ostium of the left main coronary artery to the midportion of the left anterior descending and/or left circumflex coronary arteries. Several segments (n=4-8), separated by at least 1 cm, were imaged sequentially with ultrasound and cine angiography in each patient. All ultrasound studies were recorded on 0.5-inch videotape for subsequent analysis.

Ultrasound gain settings were adjusted for optimal visualization of the vessel-lumen interface and images were digitized onto a 512×512×8-bit matrix in 34-frame sequences, obtained at 30 frames/sec by an image-

processing computer (Dextra Medical, Inc.) dedicated to echocardiographic analysis. The frame at end-diastole with the largest vessel diameter from the cardiac cycle immediately before the injection of contrast medium was selected for analysis. Both the luminal area (obtained by tracing the inner boundary of the vessel wall-lumen interface) and the total cross-sectional area of the vessel lumen (obtained by tracing the intima-media interface) were planimetered at each site. By subtracting the luminal area from the total cross-sectional area, an intimal area was obtained that allowed calculation of mean intimal thickness. The measurements from all sites were averaged for each patient.

Good reproducibility and minimal inter- and intraobserver variability for these parameters have been shown by our group (22). For the purpose of comparison, a modification of the Stanford classification(21) was used to categorize patients on the basis of intimal thickening: group 1 (n=79) included patients with a mean intimal thickness ≤0.30 mm and group 2 (n=37) comprised patients with a mean intimal thickness >0.30 mm. The rationale for the use of this dichotomous variable of intimal thickness above or below 0.3 mm to categorize patients was derived from prior work from this laboratory (27) and from pathological observations in 164 unselected subjects between the ages of 21 and 35 (28). The range of intimal thickness in the coronary arteries was 0.028-0.301 mm in this population.

In the subset of patients who had two consecutive annual ICUS examinations, disease progression was defined as a significant increase in the mean intimal thickness classification (21) based on development of one of the following criteria during the follow-up period: 1) moderate intimal thickening, defined as an intimal layer of 0.3-0.5 mm involving > 180° of the lumen circumference, or intimal layer >0.5 mm involving less than 180° vessel circumference; or 2) severe intimal thickening, defined as an intimal layer >0.5 mm involving more than 180° of the vessel circumference, or intimal layer >1.0 mm involving any area of the vessel circumference.

Statistical analysis. All data are expressed as mean ± 1 SD for continuous variables and as percentages for discrete variables. For comparisons between groups, the unpaired Student's *t* test was used for continuous variables and the chi-square test was used for categorical variables. The

relationships between potential predisposing factors and ICUS parameters were explored by either chi-square tests for categorical variables or simple linear regression for continuous variables. Statistically significant parameters by simple linear regression analysis were subjected to multivariate analysis, using Cox's multiple regression model. The relationships between potential predictors for TxCAD and angiographic data were analyzed by chi-square test for discrete variables and with an unpaired*t* test for continuous variables, respectively. Since a number of variables were screened for a potential association with TxCAD and many of the potential predictors were closely related, a *P*-value of <0.01 was established for variables to be entered in the multivariate model, but with this exception: statistical significance was assigned to two-sided*P*-values <0.05. Commercially available statistics software(Statview 4.01, Abacus Concepts, Inc.) was used.

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RESULTS

Diagnosis of TxCAD by ICUS and angiography. Mean intimal thickening by ICUS for the whole group was 0.24 mm (range, 0-0.76 mm), and was present in 85% of patients. Intimal thickening was undetectable in 17 patients(15%). In contrast, by qualitative angiography, TxCAD was diagnosed in 18(15%) patients, but was absent in 98 (85%). Among the 18 patients with angiographic TxCAD, 5 (4%) were classified as having severe disease, 6 (5%) as having moderate disease, and 7 (6%) as having mild disease. Although the overall correlation of lumen dimension by angiography and ICUS was good(R=0.85, *P*<0.0001), 83 of the 98 patients with normal angiograms(85%) had evidence of intimal thickening by ICUS. Correlation of angiographic TxCAD with intimal thickness decreased with more severe intimal thickness: in the group with intimal thickness <0.3 mm, 74/79 (94%) of the angiograms were considered normal (R=0.95, *P*=0.0001), whereas in the group with intimal thickness of ≥0.3 mm, only 13/37 (35%) of angiograms showed evidence of TxCAD (R=0.34, *P*=0.01).

Predisposing factors for transplant coronary artery disease. Clinical characteristics before and after transplantation and immunologic and

metabolic parameters were compared in patients categorized according to the severity of intimal thickening by ICUS(Tables 1-4). Patients with intimal thickness >0.3 mm had a longer time since transplantation(P<0.005) and had higher average weight following transplantation(P<0.02) and lower prevalence of treatment with diltiazem(P<0.008) (Table 1). With respect to potential immunologic factors (Table 2), patients with intimal thickness >0.3 mm had higher total pulsed corticosteroid dose(P<0.009) and lower daily weight-adjusted cyclosporine(P<0.05) and azathioprine dose (P<0.02). Analysis of the potential metabolic factors (Table 3) shows that patients with intimal thickness >0.3 mm had higher average fasting serum triglycerides (P<0.002) and lower average fasting high density lipoprotein (HDL) cholesterol (P<0.03).

Several factors were positively correlated with intimal thickness by univariate regression analysis (Table 4): pretransplant low density lipoprotein cholesterol, time since transplantation, average weight after transplantation, number of moderate rejection episodes, total number of rejection episodes, total pulsed corticosteroid dose, average fasting triglyceride level, and average fasting glucose.

Inverse correlates of intimal thickness >0.3 mm (Table 4) included diltiazem treatment, average daily weight-adjusted doses of prednisone, cyclosporine and azathioprine, and average fasting HDL cholesterol. By multiple regression analysis with ICUS-evident disease as the dependent variable, average triglycerides (P<0.005) and mean weight after transplantation (P<0.02) remained the independent predictors for TxCAD (R=0.55, P<0.0001). Average weight was correlated with average fasting triglyceride level (R=0.26,P<0.007) and pulsed corticosteroid dose (R=0.37,P<0.006), but none of these three parameters was significantly correlated with time since transplantation (R=0.03, P<0.8; R=0.04, P<0.7; and R=0.03, P<0.5, respectively).

The relationship of potential risk factors with angiographic evidence of TxCAD is shown in Table 5. Patients with angiographic evidence of TxCAD had higher donor age, higher total pulsed corticosteroid dose, lower average daily maintenance cyclosporine dose, and higher fasting plasma triglyceride and glucose levels.

By univariate regression analysis, the only significant correlates of angiographic evidence of TxCAD were donor age (R=0.3, $P<0.006$) and average fasting plasma triglyceride level (R=0.34,$P<0.009$).

In the subgroup analysis of the 39 patients studied 1 year after transplantation, higher average triglyceride level (R=0.55,$P<0.003$) alone was found to be correlated with intimal thickening by univariate regression analysis. Multivariate analysis was not applied in this subgroup because triglyceride level alone qualified for entry into the model. Also, the same parameters found to be predictive for angiographic evidence of TxCAD in the total population correlated with the angiographic disease in this subgroup (donor age, $P<0.05$, and average fasting plasma triglyceride level, $P<0.006$).

Subset analysis of intimal thickness progression over a 1-year interval(Table 6) revealed that patients with disease progression(n=27) had a higher incidence of total and moderate rejection episodes($P<0.03$ and 0.015, respectively), higher pulsed corticosteroid dose ($P<0.01$), and higher fasting serum triglyceride levels($P<0.006$). By multiple regression analysis, only total pulsed corticosteroid dose and fasting plasma triglyceride level were found to be independently correlated with TxCAD (R=0.5, $P<0.0001$).

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DISCUSSION

Identification of factors predisposing to intimal proliferation may provide an important contribution to the understanding of the pathophysiology of TxCAD and to development of preventive and therapeutic strategies. Since angiographic-pathologic correlation studies (29) and our recent experience with ICUS in transplant patients(20, 21) have demonstrated that ICUS is more sensitive than qualitative coronary angiography for detection of TxCAD, we examined the correlation of potential risk factors with ICUS measurements of intimal thickness and with angiographic findings. We hypothesized that coronary artery intimal thickening occurs as a direct consequence of the alloimmune response, and therefore acute rejection should be an independent risk factor for this disease. Three important observations refuting our hypothesis regarding the role of acute rejection as

an independent risk factor for TxCAD emerge from this study. First, average fasting plasma triglyceride level and average weight after transplantation, but not rejection incidence or severity, were shown to be independently correlated with intimal thickening by ICUS measured at a single time point. Second, using the less sensitive endpoint of angiography, only fasting plasma triglyceride level was independently correlated with TxCAD, although donor age emerged as a nonindependent correlate. Third, with respect to the endpoint of intimal thickness progression, average fasting plasma triglyceride and total pulsed corticosteroid dose were independent correlates, while rejection incidence correlated only in the univariate analysis.

Several lines of evidence suggest a major role for the alloimmune response in the development of TxCAD. First and most convincing, the disease is limited to the vascular bed of the allograft, which is diffusely involved. Second, this proliferative vascular lesion is not unique to cardiac allografts but has also been described in kidney, liver, and lung allografts(30). Third, experimental data in animal models point to a correlation of rejection, immunosuppression, and TxCAD(31, 32). However, clinical studies examining the correlation of TxCAD with cellular rejection have produced conflicting results(3-12), and the addition of cyclosporine to the immunosuppressive regimen has not decreased the incidence of TxCAD, despite a reduction in the incidence of acute rejection episodes (5, 33). These clinical observations, which question the role of acute rejection in the pathophysiology of TxCAD, are consistent with the results of the current study, which failed to document acute rejection as an independent correlate of intimal thickening or coronary artery stenosis.

Several possible explanations for the conflicting results of clinical studies addressing the relationship of acute rejection to TxCAD(3-5, 11, 13, 34, 35) can be postulated. All the studies cited have been retrospective, and inclusion criteria, diagnosis of rejection, and immunosuppressive protocols varied widely. The diagnosis of TxCAD was based on visual assessment of coronary angiograms in most studies. However, the absence of an independent correlation of rejection with TxCAD in the present study can be explained only partly by the described limitations, and suggests that factors other than the alloimmune response might confer an independent risk for

TXCAD. In particular, we observed that hypertriglyceridemia and obesity were independently correlated with both coronary artery intimal thickening and stenosis in long-term patients. Furthermore, even in the subset of patients studied 1 year after transplantation, fasting plasma triglyceride levels were identified to be independently correlated with intimal thickening. These observations suggest an important role for the metabolic consequences of high dose corticosteroids, independent from the acute rejection process per se. In support of this explanation is the observation that hypertriglyceridemia was independently correlated with all three endpoints of TxCAD, namely coronary artery intimal thickness measured at a single time point, progression of intimal thickness, and the presence of coronary stenosis. In further support of this explanation is the observation that although progression of intimal thickness significantly correlated with both acute rejection incidence and pulsed corticosteroid dose, only the latter proved to be independently correlated. In addition to hypertriglyceridemia, high dose corticosteroids used for the treatment of acute rejection result in severe metabolic derangement, including decreased HDL cholesterol, which was also found to be significantly correlated with intimal thickening. These observations are, therefore, consistent with the classical injury-response model of atherosclerosis. In the case of TxCAD, the initiating injury is the alloimmune response, and the proliferative response leading to intimal thickening is driven by dyslipidemia, as seen in animal models of accelerated atherosclerosis. Indeed, the inverse relationship of intimal thickening with the use of a calcium channel blocker in the current study also parallels observations in animal models that use dyslipidemia as a means of accelerating the atherosclerotic process. Thus, our results suggest a pathophysiologic mechanism in which dyslipidemia accelerates the development of intimal proliferation in a fashion similar to native atherosclerosis. Further studies are required to test this mechanism, since if a causal role for dyslipidemia in the pathophysiology of TxCAD can be established, it would imply great potential for currently available and inexpensive preventive and therapeutic options. There is only one other study evaluating predisposing factors for TxCAD with ICUS. Anderson et al. (36) surveyed 40 heart transplant patients with ICUS in order to study the functional significance of intimal thickening. They identified a pretransplant history of hypercholesterolemia and years after transplantation as predictors

of increased intimal index. Other lipids, weight, and immunosuppressive therapy were not evaluated by these authors.

In the present study, the two strongest correlates of TxCAD, fasting plasma triglyceride level and posttransplant weight were significantly correlated with each other, but neither was related to time since transplantation. Dyslipidemia following heart transplantation has been well documented(37). Several epidemiologic studies have evaluated the relationship between hyperlipidemia and TxCAD(3, 5, 8, 16, 38-40). In native coronary atherosclerosis, hypertriglyceridemia has not generally been recognized as a major risk factor, although some reports suggest that it may be an important predictor of mortality from native coronary artery disease (41). The results of the current study, suggesting a strong relationship of TxCAD with triglycerides but not total cholesterol, are consistent with our previous experience correlating lipid levels with angiographic TxCAD (8). Contrary results have been reported by other groups (5, 16). The important question of whether hypertriglyceridemia promotes TxCAD or is only a marker of immunosuppressive therapy remains unanswered. Experimental evidence indicates the ability of hypercholesterolemia to induce a phenotypic change in vascular smooth muscle cell, which results in proliferation and intercellular matrix production, typical of early atherosclerosis (42). Obesity, the second independent predictor of intimal proliferation in the present study, has previously been reported as a risk factor for TxCAD (43), and may simply reflect a side effect of high dose corticosteroids. However, Winters et al.(18), in a study of failed cardiac allografts, found body mass index to be the single most predictive risk factor for TxCAD, emphasizing the need for further studies to determine mechanisms that underlie these epidemiologic observations.

Another possible explanation for the discordance between acute rejection and TxCAD in the current study and prior studies may be related to the nature of the alloimmune response that is characterized. The standard histologic grading does not identify the phenotype of infiltrating cells, nor does it characterize their cytokine content, both of which could have important implications for vascular injury and intimal proliferation(44). Thus, alternative pathways of immunologic injury, distinct from acute cellular rejection, may contribute to the development of TxCAD. For example,

vascular rejection, characterized by endothelial activation and accumulation of immunoglobulins and complement in the microvasculature, has been recognized using immunofluorescence techniques(45). In this form of rejection, postulated to be mediated by humoral immunological mechanisms(14, 46, 47), a correlation with angiographic evidence of TxCAD has been observed by several authors(14, 15). Indeed, an important role for an immunologic mechanism in the pathophysiology of TxCAD is supported by our observation of an inverse correlation of intimal thickness with the maintenance doses of all three immunosuppressive drugs. Whether suboptimal dosing of immunosuppressive drugs relates to inadequate suppression of alloimmune responses, or to an inflammatory process independent of alloimmunity, requires further testing.

Consistent with other reports(3, 5, 8, 16, 18), we could not identify a relationship between other "conventional" coronary artery disease risk factors and TxCAD. None of the other parameters analyzed identified patients with a high probability of TxCAD with the exception of donor age, which was significantly correlated with angiographic but not ICUS-measured TxCAD. Although this is in accordance with earlier large angiographic series from Stanford (8) and Papworth(16), no correlation of donor age with intimal thickness assessed by ICUS was evident in this study. Given the paucity of other clearly defined posttransplant risk factors for TxCAD, this observation emphasizes the need for further investigating the contribution of donor factors in predisposing to the disease.

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STUDY LIMITATIONS

Several limitations apply to this study. First, the analysis of the clinical data was retrospective in nature, and does not address the issue of mortality. This very important issue cannot be appropriately addressed in a retrospective study and is therefore the subject of a prospective analysis. The potential for selection bias is inherent in this design, since only survivors were analyzed. However, this bias is addressed by the subgroup analysis of year 1 patients, which only excluded patients dying during the first year, when the incidence of TxCAD is low (<10%).

Second, the range of time since transplantation in the population analyzed was wide. However, the subgroup analysis of patients studied 1 year after transplantation identified average fasting plasma triglyceride level as the most powerful predictive variable, in accordance with the analysis in the total population. Furthermore, time since transplantation was not an independent predisposing factor for TxCAD in the multivariate analysis. Whether different factors may influence the course of TxCAD early and late after transplantation is not known and cannot be answered by this study.

Third, the large number of variables evaluated in this study mandates a careful interpretation of positive results. It was for this reason that multivariate analysis was performed, and only those parameters showing a high correlation with TxCAD ($P<0.01$) by univariate analysis were entered into the multivariate model. Attempts were also made to address this issue by studying disease progression during a 1-year interval.

Fourth, we did not compare ICUS with quantitative coronary angiography. This was not the purpose of this study, and we have shown previously that this method correlates closely with ICUS measurements of coronary artery lumen dimension (22). Qualitative angiography was used in the present study to allow comparison with previous reports evaluating predisposing factors for TxCAD. Quantitative coronary angiography is not routinely performed in this patient population in most centers.

Fifth, this study does not compare the relative predictive value of risk factors versus noninvasive testing for myocardial ischemia. However, in general, functional testing for diagnosis of TxCAD, such as treadmill, electrocardiogram, or thallium tests, has been found to have a poor sensitivity and specificity (48). Newer modalities, such as dobutamine stress testing, suggest improved sensitivity and specificity when the endpoint for TxCAD is intimal thickening as measured by ICUS(49).

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CONCLUSION

The correlation of multiple immunologic and metabolic factors with intimal thickness by univariate analysis suggests a multifactorial etiology for TxCAD. Among these factors, it appears that higher average fasting plasma triglyceride levels and higher average body weight are independent predictors of TxCAD. The independent correlation of intimal thickness progression with total pulsed corticosteroid dose and hypertriglyceridemia suggests that the proliferative process of TxCAD is accelerated by metabolic consequences of high dose corticosteroid therapy.

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	Mean intimal thickness		*P*
	≤0.3 mm (n=79)	>0.3 mm (n=37)	
Pretransplant characteristics			
Recipient age (yr)	45.4±12.2	43.2±11.6	<0.4
Donor age (yr)	26.0±9.2	25.0±8.2	<0.6
Female gender (%)	21 (27)	6 (16)	<0.4
PreTx CAD (%)	43 (54)	18 (49)	<0.8
Donor ischemia (min)	146±79	130±61	<0.3
Donor CMV positive (%)	31 (42)	14 (42)	<1
Recipient CMV positive (%)	43 (57)	17 (47)	<0.5
Syst BP preTx (mmHg)	110±16	111±16	<0.9
Diast BP preTx (mmHg)	66±10	68±10	<0.3
Fasting triglycerides (mg/dl)	113±95	112±59	<1
Fasting total cholesterol (mg/dl)	166±53	172±52	<0.6
Fasting HDL-c (mg/dl)	36±15	34±12	<0.6
Fasting LDL-c (mg/dl)	108±39	120±44	<0.2
Posttransplant characteristics			
Time after Tx (yr)	2.9±2.4	4.5±3.1	**<0.005**
Syst BP postTx (mmHg)	132±15	130±14	<0.5
Diast BP postTx (mmHg)	84±10	86±9	<0.3
CMV infection	19 (24)	9 (24)	<1
Average weight (kg)	76.9±12.6	83.5±14.9	**0.02**
Diltiazem treatment (%)	36 (46)	7 (19)	**<0.008**

[a] Data are expressed as mean ± SD. All boldface values indicate items with a *P*-value <0.05. Abbreviations: CAD = coronary artery disease; HDL-c = high density lipoprotein cholesterol; LDL-c = low density lipoprotein cholesterol.

	Mean intimal thickness		*P*
	≤0.3 mm (n=79)	>0.3 mm (n=37)	
HLA mismatches (n)	3.2±0.9	2.8±0.9	<0.06
Moderate rejection episodes (n)	2.7±2.6	3.4±2.4	<0.2
Mild rejection episodes (n)	2.7±2.8	2.7±2.5	<1
Total rejection episodes (n)	5.4±4.6	6.1±4.0	<0.5
Total pulsed steroid dose (mg)	720±230	2785±950	**<0.009**
Avg daily PRED dose (mg/kg/day)	0.23±0.14	0.29±0.17	<0.06
Avg daily CsA dose (mg/kg/day)	5.1±2.4	4.3±1.9	**<0.05**
Avg CsA level (ng/ml)	136±43	131±33	<0.6
Avg daily AZA dose (mg/kg/day)	1.8±0.6	1.5±0.8	**<0.02**

[a] Data are expressed as mean ± SD. All boldface values indicate items with a *P*-value <0.05. Abbreviations: AZA, azathioprine; CsA, cyclosporine; PRED, prednisone.

	Mean intimal thickness		*P*
	≤0.3 mm (n=79)	>0.3 mm (n=37)	
Avg fasting triglycerides	158±90	235±160	**<0.002**
Avg fasting total cholesterol	222±42	227±47	<0.6
Avg fasting HDL-c	47±14	41±13	**<0.03**
Avg fasting LDL-c	140±36	135±38	<0.5
Avg fasting glucose	120±31	127±48	<0.5

[a] Data are expressed as mean ± SD mg/ml. All boldface values indicate items with a *P*-value <0.05. Abbreviations: HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol.

	R	*P*
PreTx characteristics		
Donor age (yr)	−0.04	<0.8
Gender	—	<0.2
PreTx CAD	—	<1
Donor ischemia (min)	−0.11	<0.3
Donor CMV positive	—	<0.9
Recipient CMV positive	—	<0.9
Syst BP preTx (mmHg)	0.17	<0.2
Diast BP preTx (mmHg)	0.11	<0.4
Fasting triglycerides (mg/dl)	0.11	<0.3
Fasting total cholesterol (mg/dl)	0.18	<0.08
Fasting HDL-c (mg/dl)	−0.13	<0.3
Fasting LDL-c (mg/dl)	0.25	**<0.02**
PostTx characteristics		
Time after Tx (yr)	0.30	**0.001**
Syst BP postTx (mmHg)	0.001	<1
Diast BP postTx (mmHg)	0.17	<0.07
CMV infection	—	<1
Avg weight (kg)	0.34	**0.0002**
Diltiazem treatment	−0.20	**<0.03**
Immunologic parameters postTx		
Moderate rejection episodes (n)	0.29	**<0.002**
Mild rejection episodes (n)	0.07	<0.5
Total rejection episodes (n)	0.21	**<0.03**
HLA mismatches (n)	−0.07	<0.6
Total pulsed steroid dose (g)	0.30	**0.001**
Daily prednisone dose (mg/kg/day)	−0.25	**<0.008**
Avg daily CsA dose (mg/kg/day)	−0.22	**<0.02**
Avg CsA level (ng/ml)	−0.09	<0.4
Avg daily AZA dose (mg/kg/day)	−0.18	**<0.05**
Metabolic parameters postTx		
Avg triglycerides (mg/dl)	0.32	**0.0007**
Avg total cholesterol (mg/dl)	0.08	<0.5
Avg HDL-c (mg/dl)	−0.23	**<0.02**
Avg LDL-c (mg/dl)	−0.04	<0.7
Avg fasting glucose (mg/dl)	0.21	**<0.04**

[a] Univariate linear regression analysis for continuous variables and chi-square test for discrete variables. All boldface values indicate items with a *P*-value <0.05. Abbreviations: CAD, coronary artery disease; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol.

	TxCAD		P
	Present (n=18)	Absent (n=98)	
PreTx characteristics			
Recipient age (yr)	45.6±12.0	44.5±12.1	<0.8
Donor age (yr)	30.9±10.7	24.7±8.2	**<0.006**
Gender (% male)	14 (78)	75 (77)	<1
PreTx CAD (%)	9 (50)	52 (53)	<1
Donor ischemia (min)	123±71	144±74	<0.3
Donor CMV positive (%)	8 (50)	37 (41)	<0.7
Recipient CMV positive (%)	8 (47)	52 (55)	<0.8
Syst BP preTx (mmHg)	117±19	109±15	<0.08
Diast BP preTx (mmHg)	70±9	65±11	<0.2
Fasting triglycerides (mg/dl)	129±59	110±89	<0.5
Fasting total cholesterol (mg/dl)	190±59	164±51	<0.1
Fasting HDL-c (mg/dl)	33±8	36±15	<0.5
Fasting LDL-c (mg/dl)	128±53	109±38	<0.1
PostTx characteristics			
Time after Tx (yr)	3.9±3.3	3.3±2.6	<0.5
Syst BP postTx (mmHg)	135±16	131±14	<0.2
Diast BP postTx (mmHg)	86±7	85±11	<0.6
CMV infection	4 (22)	24 (25)	<1
Avg weight (kg)	82±15	78±13	<0.3
Diltiazem treatment	7 (39)	36 (37)	<0.9
Mild rejection episodes (n)	2.5±1.9	2.7±2.8	<0.8
Total rejection episodes (n)	6.3±3.6	5.5±4.6	<0.5
HLA mismatches (n)	3.0±0.8	3.1±1.0	<0.8
Total pulsed steroid dose (g)	2270±202	821±230	**0.04**
Avg daily PRED dose (mg/kg/day)	0.24±0.14	0.28±0.17	<0.5
Avg daily CsA dose (mg/kg/day)	4.2±2.3	5.0±2.2	**<0.03**
Avg CsA level (ng/ml)	136±39	134±41	<0.9
Avg daily AZA dose (mg/kg/day)	1.7±0.9	1.7±0.7	<1
Metabolic parameters postTx			
Avg fasting triglycerides (mg/dl)	254±168	170±107	**0.009**
Avg total cholesterol (mg/dl)	233±52	222±42	<0.4
Avg HDL-c (mg/dl)	41±13	46±14	<0.2
Avg LDL-c (mg/dl)	137±39	139±36	<0.9
Avg fasting glucose (mg/dl)	131±68	121±29	**<0.03**

[a] Unpaired *t*-test for continuous variables and chi-square test for discrete variables. All boldface values indicate items with a *P*-value <0.05. Abbreviations: AZA, azathioprine; CsA, cyclosporine; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; PRED, prednisone.

	Progression (n=27)	No progression (n=43)	*P*
Total rejections (n)	3.6±3.9	1.4±1.9	0.03
Moderate rejections (n)	1.5±1.8	0.6±1.1	0.015
Pulsed steroids (mg)	2566±598	839±235	0.01
Fasting serum triglyceride (mg/dl)	233±97	135±61	0.006
Donor age (yr)	29±9	23±7	0.02
Time since Tx	1.6±1.2	2.5±2.7	0.08

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Footnotes

Abbreviations: CMV, cytomegalovirus; HDL, high density lipoprotein; ICUS, intracoronary ultrasound; TxCAD, transplant coronary artery disease.

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