Cardiac allograft vasculopathy: a review

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Cardiac allograft vasculopathy (CAV) is a major factor limiting long-term survival after cardiac transplantation. CAV is an accelerated form of coronary artery disease (CAD) that is characterized by concentric fibrous intimal hyperplasia along the length of coronary vessels. Both immunologic and non-immunologic risk factors contribute to the development of CAV by causing endothelial dysfunction and injury eventually leading to progressive intimal thickening. The diagnosis of CAV remains a challenge as angiography, the standard method for detecting focal plaques, lacks sensitivity in detecting CAV, and intravascular ultrasonography, a more sensitive method, lacks the ability to evaluate the entire coronary tree. The disease is difficult to treat and results in significant morbidity and mortality. Since treatment of CAV is limited and usually involves repeat transplantation, prevention or mitigation of immunologic and nonimmunologic risk factors is critically important. CAV prevention may involve therapy that provides protection against endothelial injury implemented just before transplantation, during storage and transplantation as well as after transplantation. This review addresses the frequency of occurrence, pathophysiology, diagnosis and treatment of CAV, highlighting areas of active research.
terial intima that develops in both the epicardial and intramyocardial arteries of the transplanted heart. The process is a concentric fibrous intimal hyperplasia that appears along the entire length of the affected arteries. Included in this form of arteriosclerosis are features of both atherosclerosis and arteritis. The atherosclerotic changes range from a diffuse incorporation of lipids to the development of classic focal plaques later in the disease process. With arteritis, there is thickening of the vessel due to infiltration by mononuclear inflammatory cells responding to alloimmune or infectious stimuli. Rarely, this arteritis may progress to destroy the internal elastic lamina and involve the media.

**Differences between cardiac allograft vasculopathy and coronary artery disease**

The pathological features of CAV differ significantly from those of CAD (Table 1). CAD is usually a focal, eccentric proliferation of the intima of proximal coronary vessels. There is usually sparing of the intramyocardial vessels. Fatty streaks are seen initially. Of importance in CAD is the deposition of calcium and disruption of the elastic lamina. Rarely, there are signs of inflammation, and veins are never involved.

CAV is typically characterized as a diffuse concentric proliferation of the intima. Intramyocardial vessels are usually involved, and the process can even involve the coronary veins. The initial lesions seen are smooth-muscle proliferation of the intima. There is rarely any calcium deposition, the internal elastic lamina is intact and inflammation is usually present.

**Pathological features**

CAV is mainly a disease of the intima. Changes in the intima can be seen as early as 6 months after transplantation. The lesion at this time is a mild intimal thickening. Mild fibrosis and increases in extracellular matrix proteins may be present. Early after transplantation, intimal thickening is limited to the proximal arteries. The lesions are characterized by hyperplastic fibrous thickening. Lesions then progress to a fibrofatty atheromatous plaque. Ultimately, the coronary vasculature progresses to a diffuse fibrous thickening of the intima, which can have superimposing atheromatous plaques.

The internal elastic lamina is almost always intact except for breaks that may be seen in more advanced stages of CAV. The media can be unaffected or almost completely replaced by fibrous tissue. As the intimal disease progresses in severity so does fibrosis of the media. The only vessels relatively unaffected are those with little or no muscular layer.

**Pathophysiologic characteristics**

The pathophysiologic features of CAV, although not completely understood, likely involve components of both immune-mediated and non-immune-mediated endothelial damage, and passenger “native vessel” atherosclerosis. There is substantial evidence that immunologic factors, including histocompatibility mismatch, acute rejection episodes and chronic inflammation, play a major role in CAV development. Nonimmunologic factors include cause of donor brain death, cytomegalovirus (CMV) infection, age, sex, obesity, dyslipidemia, hyperhomocysteinemia (HHcy), diabetes mellitus, hypertension, smoking and ischemia–reperfusion injury. In general, hyperlipidemia and insulin resistance are the most significant nonimmunologic factors, occurring in 50%–80% of the heart transplant population.

The endothelial damage involved in CAV can be categorized into either denuding or nondenuding injury. In nondenuding injury a rapid replacement of injured endothelial cells leads to endothelial dysfunction. Both immune-related and nonimmune-related factors contribute to nondenuding injury. In contrast, denuding injury is caused by ischemia–reperfusion injury during transplantation or during episodes of acute cellular rejection. This results in the loss of large stretches of endothelium along the vessel, which causes significant endothelial dysfunction. According to one hypothesis, it is the immune component or alloantigen-dependent mechanism of injury that acts principally to intensify initial nonimmune damage to the endothelial cells. Denuding injury allows for blood components and circulating cytokines to have direct contact with the subintimal layers. This can lead to significant proliferation of smooth-muscle cells. Therefore, CAV can be initiated or exacerbated by several processes that can lead to denuding or nondenuding injury. These include ischemia–reperfusion injury, immune activation, viral infection and injury from immunosuppressive drugs.

Hyperlipidemia is commonly seen in cardiac transplant patients for several reasons. Many of these patients are hyperlipidemic before transplantation. In addition, the immunosup-
pressive therapy given to patients, especially calcineurin inhibitors, may result in or exacerbate pre-existing dyslipidemia. Hypercholesterolemia, in a rabbit heterotopic cardiac transplant model, has been shown to be associated with CAV\textsuperscript{15,16} and transplanted coronary arteries were more affected by hypercholesterolemia than native coronary arteries. Hypercholesterolemia promotes fibrofatty proliferative changes to the intimal hyperplasia seen in most patients with CAV\textsuperscript{15}.

In solid-organ transplant recipients, HHcy is extremely common and occurs early with a rate as high as 80\%–90\%.\textsuperscript{17,29–31} HHcy can damage cells by several mechanisms, but primarily by affecting the endothelium.\textsuperscript{29–31} HHcy results in reduced endothelial nitric oxide production,\textsuperscript{32,33} impaired arterial response to vasodilators and increased expression of procoagulant factors.\textsuperscript{32,29–31} The neutrophil–endothelium interaction is promoted in the setting of HHcy, allowing for the presence of more neutrophils in the intima. All of these alterations in the endothelial wall are caused by alterations in the redox state induced by high homocysteine levels.\textsuperscript{30–32} Several investigators have demonstrated that HHcy is associated with the development of CAV.\textsuperscript{34,35}

Hypertension, smoking, diabetes mellitus and other risk factors for atherosclerosis are associated with CAV. Hypertension in transplant patients can be present preoperatively or postoperatively secondary to immunosuppressive medication, such as cyclosporine. Hypertension causes endothelial injury by promoting the formation of intimal hyperplasia, which eventually gives rise to atherosclerotic lesions.

Although the relative importance of the direct versus the indirect pathway of alloreactivity is still debated, one theory is that direct activation of recipient CD4+ T cells by donor allograft/nonself MHC class I molecules initiates graft rejection. CD8+ T cells can become activated by previously activated CD4+ T cells through the CD40L pathway and by nonself MHC class I molecules.

The activation of CD4+ and CD8+ T cells leads to further synthesis of cytokines, which perpetuate the ongoing cascade of events that lead to CAV. The most important cytokines in allograft rejection are interleukin-2 (IL-2), interferon-gamma (IFN-\(\gamma\)) and tumour necrosis factor-alpha (TNF-\(\alpha\)). IL-2 induces T-cell proliferation and differentiation, IFN-\(\gamma\) activates macrophages, and TNF-\(\alpha\) itself is cytotoxic to the transplanted heart. In addition, TNF-\(\alpha\) acts to increase MHC class I expression, while IFN-\(\gamma\) increases the expression of both MHC classes I and II molecules. Overall, these cytokines can lead to chronic graft rejection. IFN-\(\gamma\) and TNF-\(\alpha\) also induce the leukocyte vascular cell adhesion molecule-1, which promotes monocyte adhesion and entry through the endothelium, leading to CAV.

Ardehali and associates\textsuperscript{36} used a murine CAV model with a compromised indirect alloreactivity pathway to show that this did not affect the extent of intimal thickening or lymphocyte and macrophage infiltration after heart transplantation when compared with wild type mice. They proposed 2 potential explanations: (1) an impaired indirect pathway is enough to cause severe CAV and the direct pathway does not play a major role in CAV; or (2) the direct pathway of alloreactivity can fully compensate for the impaired indirect pathway.\textsuperscript{36} Other studies, such as the one by Game and associates,\textsuperscript{37} have found a correlation between increased indirectly activated T cells and chronic rejection. As far as the importance of CD4+ versus CD8+ T cells, it appears that the CD4+ allorecognition pathway is required for CAV development, whereas the CD8+ pathway may act to increase the severity of CAV.\textsuperscript{38} In a study by Szeto and colleagues,\textsuperscript{39} hearts transplanted into CD8+ T-cell-depleted rats developed CAV, but there was no CAV in the CD4+ T-cell–depleted recipient.

Recent research has correlated ischemia–reperfusion injury with CAV. Determinants of ischemia–reperfusion injury are length of ischemic time and methods of allograft storage. Gohra and associates\textsuperscript{40} demonstrated in a rodent model of orthotopic aortic allograft transplantation that ischemia and reperfusion result in endothelial injury, leading to the development of transplant vasculopathy. They also found that endothelial cell loss occurred in both isografts and allografts due to ischemia and reperfusion.\textsuperscript{41} This initial loss of endothelial cells was replaced by 2 weeks; however, transplant vasculopathy developed within 60 days.\textsuperscript{42} Their study indicated that ischemia and reperfusion injury led to the development of transplant vasculopathy since isografts developed vasculopathy, although to a lesser extent than the allografts.

Several changes occur to the endothelium following hypoxia, including loss of the ability to release nitric oxide within minutes after reperfusion.\textsuperscript{43–46} This loss is related to the consumption of nitric oxide by superoxide radicals formed early during reperfusion.\textsuperscript{47} Ex-
Experimental evidence suggests that the oxygen free radicals are produced by neutrophils. In vitro exposure of coronary arteries to oxygen radicals produces endothelial dysfunction. In addition, ischemia–reperfusion causes the endothelial cells to become activated and express surface adhesion molecules. These molecules promote circulating leukocyte adhesion, which then causes endothelial damage by direct cytotoxicity. These leukocytes become activated and release cytokines, which enhance leukocyte and smooth-muscle cell proliferation and activation. Ischemia also promotes complement activation that causes not only cell lysis, but results in several other changes such as increased vessel permeability, leukocyte chemoattraction and smooth-muscle contraction. Complement 1q can increase platelet procoagulant activity, which can enhance CAV by the formation of thrombus but mostly by causing the release of several vasoactive substances and growth factors such as platelet-derived growth factor-beta, thromboxane A and prostacyclin. CMV has the ability to infect vascular endothelial cells and induce endothelial injury, which can lead to CAV. Weis and colleagues reported elevated asymmetric dimethylarginine (a nitric oxide synthase inhibitor) impairing vascular homeostasis in CMV-infected patients. These higher levels can lead to endothelial dysfunction and correlate with increased severity of CAV. CMV and herpes simplex viruses induce the host adaptive immune response, which leads to the release of cytokines, increased expression of adhesion molecules and activation of T-cell responses. Therefore, viral infection may result in CAV by impairing nitric oxide homeostasis, inducing proinflammatory cytokines, and enhancing T-cell-mediated alloreactivity.

The incidence of significant donor CAD remains low, at approximately 2%. Donor CAD can serve as a starting point for CAV and may accelerate the disease process. Donor CAD can be important in the prognosis of the transplant patient in that it can progress independently of the CAV process. However, Botas and associates found no significant difference in the rate of intimal thickening between patients with donor hearts having pre-existing coronary artery disease and those without. Thus, the impact of native vessel atherosclerosis on CAV remains controversial.

Finally, cause of donor heart death, more specifically explosive donor brain death (v. gradual brain death), causes an up-regulation of MHC classes I and II antigens, adhesion molecules and cytokine secretion, setting off an accelerated inflammatory response in the heart.

CAV is a complex disease with a multifactorial etiology, and several methods must be adopted to prevent its initiation and progression (Fig. 1).

**Diagnosis**

Cardiac denervation at the time of heart transplantation usually prevents transplant patients from experiencing angina, which is an important warning sign for heart disease. Only 10%–30% of heart transplant recipients regain any innervation to the heart. Because of this lack of early clinical symptoms, transplant patients with CAV typically present late with silent myocardial infarction, loss of

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**FIG. 1. Pathophysiology of cardiac allograft vasculopathy (CAV).** CHF = congestive heart failure, CHOL = cholesterol, CNl = calcineurin inhibitors, DM = diabetes mellitus, HHcy = hyperhomocysteinemia, HTN = hypertension, MI = myocardial infarction.
allograft function or sudden death.5

Another difficulty faced by clinicians in diagnosing CAV is coronary remodelling and the diffuse nature of the disease. Angiography measures luminal diameter and compares the narrowing at plaques to normal reference diameters and previous angiograms in order to understand the severity and rate of disease progression. CAV, however, shows no initial decrease in luminal diameter due to vascular remodelling.63 Only when the process is more advanced does the lumen narrow and angiographic detection become possible. Since CAV involves the entire coronary arterial tree, angiography may convey the impression of less-than-actual vessel narrowing at plaque sites. Thus, angiography, although it is a good screening tool for CAD, often underestimates CAV, and in some patients with evenly distributed disease throughout the coronary tree, CAV can be missed altogether.64

Despite the poor sensitivity of angiography, it is still widely used as a screening test for vascular disease. Johnson and associates6 developed a classification system based on the varying morphologies in CAV to aid in its diagnosis using angiography. Briefly, type A lesions appear as discrete proximal tubular stenoses, type B as diffuse concentric middle or distal stenoses, with type B1 having an abrupt narrowing and type B2 having a smooth concentric tapering. Finally, a type C angiographic appearance indicates irregular vessels with distal lesions and loss of small branches. Diagnosis of CAV requires type B or C lesions and comparison with previous and recent angiograms to note disease progression.16

A more sensitive tool is intravascular ultrasonography (IVUS). IVUS is useful for detecting the extent of intimal thickening by imaging vessel wall structure rather than simply luminal diameter. IVUS has an axial resolution of 50–80 µm.63 Unfortunately, it is physically restricted to the larger epicardial arteries, and thus cannot be used to screen for CAV throughout the entire heart. One year after transplantation, IVUS detects CAV in 80% of patients whereas angiography detects disease in only 10%–20% of patients.65,66

With IVUS, normal coronary intimal thickness ranges between 0.10 and 0.30 mm. Hence, CAV is considered present when intimal thickness exceeds 0.3 mm or when the sum of the intimal and medial thickness exceeds 0.5 mm. At greater than 0.6-mm intimal thickening, patients are 10 times more likely to experience a cardiac event.67

Since angiography and IVUS are invasive tests, they pose increased risks for the patient.68 Dobutamine stress echocardiography is currently the most sensitive noninvasive test for cardiac disease; it measures wall motion and can detect CAV with a sensitivity and specificity of 79% and 83% respectively.69 Possible future modalities include both pulse-wave tissue Doppler imaging and electron-beam CT. Since both modalities are noninvasive they may replace angiography as screening tools, allowing IVUS to be used in high-risk patients or those with equivocal or positive test results.

Treatment and prevention

Treatment of established CAV in humans remains limited. Encouraging research, however, has been done in small animals. For example, treatment with anti-CD154 in a rat cardiac allograft rejection model prevents acute rejection and drastically slows the development of CAV.70 In this study, early treatment was required to inhibit CAV.70

In clinical heart transplantation the focus remains on prevention of CAV via attenuation of adverse non-immunologic and immunologic reactions. Before transplantation, preventing endothelial injury at brain death, reducing cold ischemic time and subsequent tissue damage, and improving myocardial preservation during storage and transportation of the graft all aid in post-transplant cardiac function and longevity. In a study on prolonged cold storage, Kevelaitis and associates71 demonstrated that longer cold ischemic times produced greater endothelial dysfunction in cardiac allografts and that the composition of the storage medium affected the extent of allograft tissue damage. Our group has shown that profound hypothermic storage results in depressed myocardial metabolic and functional recovery72 and that shed donor blood perfusion can permit cardiac allograft storage at tepid temperatures, resulting in improved myocardial performance.72,73 We have also shown that the addition of insulin to the blood perfusate during storage results in improved functional and metabolic recovery during heart transplantation.74 Fedak and associates75 demonstrated that bosentan, an endothelin-1 antagonist, added to shed blood perfusion improves both the functional recovery of the myocardium and endothelium. Several other groups have demonstrated that endothelin antagonism reduces CAV.76,77

Immediately after transplantation, patients are placed on calcineurin immunosuppressive drugs (cyclosporine or tacrolimus), most commonly cyclosporine. Unfortunately, cyclosporine in high doses and for a long time can cause side effects such as renal failure4 and hypertension. Simonson and colleagues78 demonstrated in a Lewis to Fischer rat heart transplant model that the combination of low-dose cyclosporine with an endothelin-converting enzyme inhibitor resulted in long-term survival of the graft equal to that of high-dose cyclosporine alone. As an alternative to using cyclosporine, other immunosuppressive drugs such as mycophenolate mofetil, rapamycin or leflunomide, may inhibit CAV by limiting smooth-muscle cell proliferation.4 The newer immunosuppressive agent everolimus has recently been demon-
strated to reduce the frequency and severity of CAV\textsuperscript{99} in humans. Eisen and associates\textsuperscript{99} demonstrated that, in patients on cyclosporine and corticosteroids, everolimus reduces intimal thickness and index compared with azathioprine.

Hyperlipidemia is known to be a risk factor for both CAD and CAV. Unfortunately, immunosuppressive therapy with corticosteroids, cyclosporine, rapamycin and to a lesser extent tacrolimus and everolimus results in hyperlipidemia.\textsuperscript{89} To treat hyperlipidemia in post-transplant patients, lipid-lowering drugs are prescribed since lifestyle changes are usually not enough to lower lipid profiles to desired levels. HMG-CoA reductase inhibitors, or statins, are the most popular and are very effective at lowering total cholesterol, low-density lipoprotein (LDL) and very low-density lipoprotein, and increasing high-density lipoprotein (HDL).

Recently, it has been documented that statins have pleiotropic effects in heart transplant recipients. Unfortunately, these studies demonstrated to reduce the frequency and severity of CAV\textsuperscript{99} in humans. Eisen and associates\textsuperscript{99} demonstrated that, in patients on cyclosporine and corticosteroids, everolimus reduces intimal thickness and index compared with azathioprine.

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treatments such as coronary angioplasty, coronary stenting, and coronary bypass offer only palliative solutions. The only true solution to severe CAV is repeat heart transplantation. Even so, CAV is likely to recur, and there are significant moral and ethical issues that complicate repeat transplantation.5,9

Summary and conclusions

CAV is the major limiting factor for long-term survival after heart transplantation. It affects up to 75% of patients 3 years after transplantation. The risk factors for CAV can be divided into two categories (immunologic and nonimmunologic). Immunologic factors include the severity and frequency of acute rejection, and chronic rejection. Nonimmune factors include the classic risk factors for CAD, ischemia–reperfusion injury during organ retrieval and transplantation, CMV infection and endothelial injury from immunosuppressive drug therapy. Current areas of research focus on determining the etiology of CAV and the development of treatment strategies to prevent or limit its extent. These include endothelial protection during organ retrieval, limiting the use of calcineurin inhibitors and aggressive management of CAD risk factors. Another challenge in the management of CAV is its diagnosis. Early diagnosis of CAV will lead to earlier treatment and better outcomes. Angiography—the standard diagnostic modality for CAD—lacks sensitivity, and IVUS (the most sensitive method) lacks the ability to assess the entire coronary tree. New diagnostic tools are required for the more accurate and earlier diagnosis of CAV. The successful long-term survival of the cardiac transplant patient rests in our ability to understand, detect, treat, and prevent CAV.

CAV is a multifactorial disease that remains the major limitation to long-term survival after heart transplantation. Methods of diagnosis have improved significantly with the use of IVUS in addition to angiography. Since treatment of CAV is limited and usually involves repeat transplantation, prevention of immunologic and non-immunologic risk factors is of critical importance. CAV is conceptually very similar to post-transplant disorders in other organs (e.g., bronchiolitis obliterans with organizing pneumonia, biliary cirrhosis). Therefore, novel therapeutic strategies to prevent or attenuate the development of CAV may have clinical relevance to transplant recipients of other solid organs.

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