Prevention of Cardiac Allograft Vasculopathy in Heart Transplantation

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Abstract

Heart transplantation is a well established therapy for end-stage heart failure. Long-term results are limited by malignancy and cardiac allograft vasculopathy. The causes for cardiac allograft vasculopathy and the predictors for its onset and progression are multifactorial and determined by both immunological and non-immunological risk factors. The detection of cardiac allograft vasculopathy is difficult owing to the denervation of the allograft and the diffuse disease progression, especially in medium-sized and smaller arteries, in a diffuse and concentric fashion. The treatment options are limited and often do not provide long-term success so that retransplantation remains the only solution in some cases.

Therefore, the emphasis has to be placed on cardiac allograft vasculopathy prevention. A variety of strategies for endothelial protection exist, beginning with matching and virtual crossmatching prior to transplantation. Further approaches involve preservation solutions and additives as well as transportation modalities and the prevention of ischemic injury during reperfusion. After transplantation, the interplay of recipient nonimmune cardiovascular risk factors and the effects of cellular and antibody mediated rejection both injuring the allograft endothelium have to be considered, leading to an optimized medical prevention of cardiac allograft vasculopathy. Established medical approaches for cardiac allograft vasculopathy prevention involve statins, vasodilators, infection prophylaxis, proliferation signal inhibitors, mycophenolate mofetil, and endothelial protective agents.

This review will look at the pathophysiology of cardiac allograft vasculopathy, its diagnosis, and current and future concepts for cardiac allograft vasculopathy prevention. (Trends in Transplant. 2010;4:58-67)

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Introduction

Cardiac allograft vasculopathy (CAV) is a rapidly progressive form of coronary artery disease occurring in heart transplant recipients. It is a major therapeutic challenge, limiting long-term success after heart transplantation. According to the registry of the International Society for Heart and Lung Transplantation (ISHLT), CAV is diagnosed by coronary angiography in 8% of survivors within the first year, in 32% within five years, and in 43% within eight years after heart transplantation. In addition to malignancy, CAV and graft failure represent the leading causes of death in recipients beyond the first year after transplantation.

Early after heart transplantation, donor-transmitted focal, non-circumferential lesions are detectable, whilst late after transplantation, diffuse intimal thickening is predominant in the presence of focal atherosclerotic plaques. The diffuse and progressive nature is one of the factors limiting the success of interventional therapies for CAV. Intimal thickening might be caused by cell infiltration and the consecutive production of cytokines, growth factors, and matrix deposits. Due to the denervation of the donor heart, CAV presents as silent myocardial ischemia with consecutive congestive heart failure or sudden death. There is no effective treatment for CAV apart from retransplantation, which raises ethical questions in times of donor organ shortage. Therefore, emphasis has to be placed on the prevention of CAV by avoiding and treating risk factors. This article reviews established and future strategies for CAV prophylaxis.

Risk factors and pathogenesis

In order to develop preventive strategies against CAV, its multifactorial pathogenesis has to be considered. Referring to CAV as “chronic rejection” underlines the importance of alloimmunity, but does not consider alloantigen-independent factors adequately. Although the complex pathophysiologic mechanisms involved in CAV development are not completely understood, a large variety of immune and nonimmune risk factors have been identified. Nonimmune factors include hyperlipidemia, diabetes mellitus, hypertension, hyperhomocysteinemia, older donor age, and the explosive etiology of brain death. Most of these nonimmune risk factors are aggravated after heart transplantation by side effects of the immunosuppressive therapy. Pretransplant nonimmune risk factors are HLA donor/recipient mismatches, with a crucial role for HLA-donor/recipient mismatches and pre-sensitization of recipients with alloantibodies (predominantly anti-HLA alloantibodies and MICA antibodies). The occurrence of de novo donor-specific alloantibodies (HLA and non-HLA) have been recently linked to adverse outcome and CAV progression. Furthermore, the combination of antibodies and complement activation and deposition in humoral rejection promotes CAV.

Current concepts suggest that after transplantation, direct allorecognition via the recognition of foreign major histocompatibility molecules on the surface of the allograft by recipient dendritic cells (DC) promotes acute rejection via T-cell responses. Indirect allorecognition via the internalization, processing, and presentation of host DC in the allograft endothelium is suspected to mediate CAV by activating T-cells. These invade the graft and contribute to an ongoing subendothelial inflammation and endothelial dysfunction via cytokine activation. Interferon-γ as an inductor of NO synthetase is a key cytokine, linking early endothelial dysfunction to later structural damage in CAV. In addition there is accumulating evidence for an impact of innate immune responses via toll-like receptor (TLR) signaling in the development
of CAV\textsuperscript{19}. After ischemia reperfusion injury, a subsequent allograft injury by reactive oxygen molecules results in the release of innate immune ligands, e.g. heat shock proteins, which can be detected by TLR-4 on mononuclear cells\textsuperscript{20}. Overexpression of TLR-4 can be linked to coronary endothelial dysfunction, and TLR ligation mediates the maturation of DC and might therefore contribute to subsequent adaptive immune responses\textsuperscript{21}. Considering these complex mechanisms, it might not be confusing that even human graft endothelial cells can directly activate host T-cells via direct presentation of HLA molecules and co-stimulators\textsuperscript{22}.

Finally, the role of viral infections, especially cytomegalovirus (CMV) and to a lesser amount even hepatitis B and C, in the development of CAV is increasingly recognized\textsuperscript{8,23}. Considering this large variety of risk factors, the concept of “chronic rejection” might be outdated and replaced by the concept “response to injury”\textsuperscript{17,24}. This concept takes into account that CAV is a result of cumulative endothelial injury caused by both alloimmune responses and nonimmune risk factors. There is even growing evidence for the interaction of these immunologic and nonimmune risk factors, e.g. the upregulation of HLA-DR and CD86 in immature DC by low-density lipoprotein (LDL) and oxidized LDL\textsuperscript{25} and sequestration of activated DC in the allograft by hyperlipidemia\textsuperscript{26}. These studies link the interaction between immune and nonimmune risk factors to CAV development\textsuperscript{27}.

**Diagnosis**

Early diagnosis of CAV is limited due to the lack of symptoms for ischemia in the denervated allograft and the insensitivity of coronary angiography, which underestimates the extent of CAV in most cases. Coronary angiography is still the standard for the diagnosis of CAV in many transplant centers. The angiographic detection of significant coronary artery stenoses conveys a poor prognosis. Cardiac allograft vasculopathy is diagnosed by coronary angiography in 30-50\% of heart transplant recipients after five years\textsuperscript{1,7}.

Intravascular ultrasound (IVUS) is the most sensitive examination for the diagnosis of CAV and has gained widespread acceptance\textsuperscript{28}. During the first year after heart transplantation, a rapid progression in intimal thickening is observed, followed by slower but steady progression over time. By IVUS criteria, CAV is usually defined as intimal thickness > 0.5 mm. Rapid progression of intimal thickness > 0.5 mm during the first year is a strong predictor for mortality and later angiographic changes\textsuperscript{29}. A major limitation of IVUS alone in CAV diagnosis is the fact that the complete coronary artery tree can not be reached.

Histologic examinations can also detect CAV in the form of stenotic microvasculopathy, which has recently been introduced as a new predictor for adverse outcome after heart transplantation\textsuperscript{30}.

There are encouraging results for noninvasive screening methods for CAV such as dobutamine stress echocardiography, single-photon emission CT (SPECT), and multidetector CT (MDCT), recently discussed in detail by Kass, et al.\textsuperscript{31}.

**Cardiac allograft vasculopathy prophylaxis by HLA matching and virtual crossmatching**

The best way to prevent CAV is prophylaxis. This should begin before transplantation in the allocation process. Since the number of HLA-DR mismatch is a predictor for long-term mortality after heart transplantation\textsuperscript{1}, an allocation taking into account HLA-DR matching might help to improve survival. The HLA-DR
locus is not as heterogeneous as HLA-A or HLA-B and in a population of heart transplant recipients the chance of getting one or two mismatches in a random allocation process is approximately 50%. The difference of one compared to two mismatches on the HLA-DR locus resulted in a significant increase in survival in the better-matched group in a long-term single-center analysis of 250 heart transplant recipients9.

The next target for CAV prophylaxis is the preservation solution.

Elevated panel reactive antibodies in the recipient are a risk factor for the development of antibody mediated rejection and CAV. Preformed anti-HLA antibodies acquired through transfusions or pregnancies become relevant in a high percentage of patients when the transplanted organ carries the specific HLA antigens. Solid-phase assays covered with single HLA molecules – such as single-antigen flow-beads – allow determining the presence of donor-specific HLA antibodies (HLA-DSA) “virtually” by comparison of the HLA-antibody specificities of the recipient with the HLA typing of the donor32. The use of more sensitive methods in pretransplant antibody detection, such as single-antigen flow-beads, offers the identification of minor amounts of antibodies, which might not be detected by the conventional crossmatch. A negative virtual crossmatch results in less rejection and favorable long-term results after solid organ transplantation and might therefore be an additional tool for CAV prophylaxis. On the other hand, some of the detected antibodies might not be clinically relevant33.

Cardiac allograft vasculopathy prophylaxis during donation, preservation, and implantation

The high potassium content of the University of Wisconsin organ preservation solution (UW) has lead to the hypothesis that UW solution might account for late occurrence of CAV. The literature search revealed controversial results. In a consecutive cohort of 195 heart transplants comparing Stanford solution to UW solution, significantly more patients developed CAV after a two-year follow-up34. A similar study in 159 patients found no difference in CAV development between UW and Stanford solution35. On the other hand, a small prospective trial divided 48 patients into three groups (UW = 17, Celsior = 16, HTK = 15) and found significantly less CAV in the one-year IVUS examination in the Celsior group36. Considering these data, UW might not be the solution of choice in terms of CAV prophylaxis, but conclusive long-term data are needed.

Not only the choice of the preservation solution but also some additives to these solutions are under investigation, targeting myocardial and endothelial protection. Traditional preservation techniques focus on immediate cardioplegia, without particularly considering vascular demands. Recently, the endothelial surface layer, composed of the endothelial glycocalyx and plasma proteins, was discovered to play a major role in vascular barrier function, edema formation, and leukocyte-to-endothelial interaction. Addition of albumin to HTK in a guinea pig heart transplant model significantly decreased edema formation and increased right heart cardiac output. Glycocalyx shedding was significantly reduced when the hearts were stored under albumin protection. For the prevention of acute and chronic graft failure, the glycocalyx might represent a new target37.

According to the ISHLT registry, donor age and ischemia time are further risk factors for CAV development.

Since donor organ shortage increased, donor ages increased over time1 and this might not change in the near future.
Ischemia time has to be kept short, which could be reached by regionalization of organ donation and allocation. A negative example in terms of ischemic times was introduced in the year 2000 by the German transplantation law, which de-regionalized the organ allocation to a nationwide system. At least in our center, average ischemia time increased and higher transportation costs were caused. The effect on CAV has to be seen. A promising approach to cut short ischemic time is the start of oxygenated 10 °C cold blood cardioplegia via a coronary sinus catheter immediately during the implantation described by Beyersdorf, et al. Long-term results, especially in terms of vasculopathy, are not available.

A promising new technique for the minimization of ischemia time and ischemia reperfusion injury seems to be the organ care system developed by TransMedics. The donor hearts can be transported to the recipient in a warm and beating condition with two minor cardioplegic episodes when the hearts are connected to and disconnected from the system. The first study is ongoing and late results on vasculopathy have to be awaited.

Cardiac allograft vasculopathy prevention by pharmacotherapy

Statins

The use of statins is well established after heart transplantation since hyperlipidemia represents one major risk factor for the progression of CAV and is aggravated by immunosuppressive therapy. Statin treatment has proven to prevent early CAV development and finally lead to improved survival after heart transplantation in long-term investigations. Undoubtedly, the routine use of statins is recommended after heart transplantation, but a word of caution has to said since the drug-drug interactions between HMG-coenzyme A inhibitors and calcineurin inhibitors (CNI) might result in a higher degree of myositis and rhabdomyolysis. Elevated creatine kinase levels as a sign for increased muscle toxicity under lipid-lowering therapy in heart transplant recipients can be ameliorated by the use of fluvastatin, which is not primarily metabolized by the P450-isoenzyme CYP3A4.

There are some reports that aggressive lipid-lowering by HELP-LDL-apheresis decelerates CAV progression after heart transplantation, but the therapy is costly and time consuming compared to the approach with oral medication so that it is nowadays reserved for severe refractory cases of hyperlipidemia.

Cytomegalovirus prophylaxis

Grattan, et al. reported that CMV infection was associated with a 28% rate of obstructive CAV at five years compared to 10% in noninfected patients. Furthermore, CMV prophylaxis with ganciclovir for 28 days reduced the prevalence of angiographically detected CAV in a placebo-controlled trial. A lack of intensive CMV prophylaxis results in a more intense luminal narrowing early after heart transplantation. Mechanisms involved are the dysregulation of the NO pathway by CMV and the acceleration of CAV by the recruitment of inflammatory chemokines and cytokines. These recruit a multitude of host cellular infiltrates. In summary, CMV infection promotes mononuclear adhesion and transmigration into the graft endothelium where it induces a procoagulant state and affects angiogenesis, smooth muscle cell migration, and vessel remodeling. Further evidence for a central role of CMV in CAV development was reported by Tu, et al. who reported that T-cell immunity to subclinical CMV infection reduces cardiac allograft disease.
Vasodilators

Arterial hypertension is a common side effect of CNI and develops in the majority of heart transplant recipients. Calcium channel blockers, such as diltiazem, might decelerate CAV progression and improve vasomotor function51.

Angiotensin converting enzyme (ACE) inhibitors might improve the impaired allograft endothelial function and has been associated with plaque regression52 and improved survival in larger cohorts of patients53.

Interestingly, there might even be a synergistic beneficial effect on CAV in the concomitant use of calcium channel blockers and ACE inhibitors regardless of their antihypertensive capacities54.

Larger prospective trials do not exist, but nevertheless, antihypertensive therapy with ACE inhibitors and calcium channel blockers is well established in CAV prevention.

Endothelial protection

The NO pathway might be an interesting target for CAV prevention since its improvement results in reduced adhesion molecule expression and less leukocyte infiltration55. Oral administration of L-arginine, a precursor of NO synthase, has proven to reverse endothelial dysfunction after heart transplantation and is subject of ongoing research56.

Other strategies for endothelial protection involve the use of antioxidants such as vitamins C and E and flavonoids.

In a prospective, randomized, placebo-controlled trial, 40 cardiac transplant recipients received 500 mg vitamin C and 400 IU vitamin E twice daily or placebo for one year. The IVUS examinations revealed an average intimal index increased by 8% in the placebo group, with no changes in the vitamin group. Early administration of vitamin E and C might be a simple means in CAV prevention strategies57.

Riboflavin has proven to be efficacious in the reduction of oxidant injury, rejection, and CAV in animal models of heart transplantation, but is not yet established in the clinical setting58.

Antiplatelet therapy has not yet proven to be of any benefit in CAV prevention. Neither has the improvement of elevated homocysteine levels by administration of folic acid and vitamin B₆ resulted in a reduced disease progression of CAV.

Immunosuppressants – proliferation signal inhibitors

The immunosuppressive agents sirolimus and everolimus act through inhibition of the mammalian target of rapamycin (mTOR) and are therefore referred to as mTOR inhibitors. The blockade of mTOR leads to the desired immunosuppressive mechanism, the inhibition of cellular proliferation, and the two substances are nowadays referred to as proliferation signal inhibitors. Two landmark papers demonstrated the superiority of either sirolimus or everolimus in comparison with azathioprine in the prevention of CAV in large cohorts in IVUS-controlled, prospective, randomized trials59,60. Sirolimus and everolimus also resulted in a lower incidence of CMV infections in these studies, which might even have synergistic effects with anti-proliferation in CAV prevention. The addition of sirolimus to the immunosuppressive therapy also has proven to decelerate the disease progression when severe CAV occurs under a CNI-based regimen61. In minor studies, the combination...
of low-dose tacrolimus and low-dose sirolimus\(^6\) as well as the use of a \textit{de novo} CNI-free immunosuppression with sirolimus and mycophenolate mofetil (MMF) resulted in remarkably low rates of CAV\(^6\). The antiproliferative properties of the two compounds are undoubted, but nevertheless, a survival benefit has not been documented up to now. Furthermore, larger studies that evaluate the superiority of everolimus or sirolimus in comparison to the current "gold standard" MMF have not been published so far.

**Immunosuppressants – mycophenolate mofetil**

Mycophenolate mofetil with its active metabolite mycophenolic acid is an inhibitor of inosine monophosphate dehydrogenase, a key enzyme for purine synthesis. Being rather selective for B- and T-cells, it suppresses lymphocyte proliferation and the production of immunoglobulins with a low side effect profile apart from gastrointestinal toxicity. Its ability to reduce anti-endothelial vimentin antibodies has been linked to a lower incidence of CAV controlled by IVUS\(^6\). In a large multicenter trial comparing MMF and azathioprine, there was a trend towards less intimal thickening in IVUS examinations after three years in the MMF group\(^6\). A reanalysis of the IVUS data considering site-to-site comparisons revealed significantly more patients reaching the primary endpoint of this study in the azathioprine group presenting with intimal thickening \(\geq 0.3\) mm. Coronary lumen area and vessel area were also in favor of the MMF-treated patients\(^6\).

Since CAV is often a late phenomenon after heart transplantation and most studies are designed for one to three years, Kaczmarek, et al. reviewed their single-center data for all patients receiving a CNI-based regimen with either MMF or azathioprine. They confirmed by multivariate analysis that MMF treatment was associated with a significantly lower incidence of CAV after a mean follow-up of eight years after heart transplantation\(^6\). It has to be concluded that MMF has a preventive effect in CAV development, but it might be less intense than the antiproliferative capacities of proliferation signal inhibitors such as everolimus or sirolimus.

**Immunosuppressants – calcineurin inhibitors**

The calcineurin inhibitors, cyclosporine (CsA) and tacrolimus (TAC), effectively prevent acute rejection, but do not prevent the development of CAV\(^6\).

Several trials revealed that TAC-based immunosuppression provides superior prevention of acute rejection compared with CsA-based therapy\(^6\). Survival and angiographically detectable CAV were similar between microemulsion CsA- and TAC-treated groups after a five-year follow-up in a study by Kobashigwa, et al.\(^7\). A higher degree of intimal proliferation was detected by IVUS after one year in patients treated with CsA and MMF compared to TAC and MMF was reported in a study by Meiser, et al.\(^6\). Microvascular endothelial function reveals a higher degree of impairment in CsA-treated patients than in TAC-treated patients, which might be associated with enhanced endothelin-1 concentration and reduced vascular remodeling\(^7\). According to these findings, the choice of CNI for an immunosuppressive regimen in heart transplantation should consider the associated relative cardiovascular risks since there is not enough evidence for the long-term superiority of either of the CNI.

**Immunosuppressants – induction therapy**

There is some evidence that the use of a more intense immunosuppression early after
transplantation prevents or delays CAV in the long-term follow-up. The use of induction therapy is controversial since it does not lead to an improved long-term outcome according to large registries\(^1\). A recently published minor clinical trial reports a long-term advantage of antithymocyte globulin (ATG) in prevention of CAV. In contrast, OKT3 failed to show such benefit. Induction therapy with either ATG or OKT3 did not exhibit a significant beneficial effect on long-term patient survival\(^74\). In another trial, ATG was superior in prevention of CAV compared to an interleukin-2 receptor antagonist\(^75\). Based on these limited data, a general recommendation on the use of induction agents cannot be given, but there might be a protective effect for CAV development after ATG induction.

**Photopheresis**

A prospective randomized study in 23 cardiac transplant recipients receiving prophylactic photopheresis in addition to a conventional CsA-based immunosuppression revealed a significant reduction in panel reactive antibodies, and coronary artery intimal thickness was significantly reduced in the photopheresis group at two years (0.28 vs. 0.46 mm; \(p < 0.02\)). In this small pilot study, photopheresis was well-tolerated and was capable of decreasing the severity CAV\(^76\). The method is, on the other hand, costly and time-consuming and a beneficial effect has to be documented in larger studies.

**New immunosuppressants**

The CD-20 antibody rituximab has shown to be effective in the treatment of antibody mediated rejection, and its widespread use in desensitization protocols might have an impact on CAV development by a reduction in antibody mediated endothelial injury\(^77\). A study designed to elucidate these effects has not been published so far.

Co-stimulation blockade with belatacept proved to be non-inferior to CsA in a prospective randomized trial and, interestingly, preserved renal function better with less chronic allograft nephropathy, which might be comparable to CAV\(^78\). Results after clinical heart transplantation are to be awaited, but anyway, the superior side effect profile of co-stimulation blockade might as well be suitable to reduce CAV by reducing cardiovascular risk factors.

Janus kinase 3 (JAK3) is crucial for signal transduction downstream of various cytokine receptors in immune cells. The effect of JAK3 inhibition on graft survival in an animal model of heart transplantation was in part comparable to tacrolimus or sirolimus\(^79\). The low cardiovascular side-effect profile and high specificity of JAK inhibitors might determine their role in future immunosuppressive strategies.

**Conclusions**

Considering that the treatment of CAV is difficult and ineffective, its prevention and prophylaxis is of major importance.

Prevention of CAV begins with the allocation process, organ preservation, and transportation. Early effective immunosuppressive strategies and aggressive monitoring and therapy of cardiovascular risk factors are further tools for delaying CAV onset.

New promising immunosuppressants still have to prove their potentially beneficial effects. No matter which preventive strategies one applies, it has to be started at the time of transplantation as the determinants for late CAV development are triggered at this early stage.
References


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