Risk Stratification for Cardiac Allograft Vasculopathy in Heart Transplant Recipients

– Annual Intravascular Ultrasound Evaluation –

Takuma Sato, MD; Osamu Seguchi, MD, PhD; Hatsue Ishibashi-Ueda, MD, PhD; Masanobu Yanase, MD; Norihiro Okada, MD; Kensuke Kuroda, MD; Eriko Hisamatsu, MD; Haruki Sunami, MD; Takuya Watanabe, MD; Seiko Nakajima, MD; Kyoichi Wada, PhD; Hiroki Hata, MD, PhD; Tomoyuki Fujita, MD, PhD; Norihide Fukushima, MD, PhD; Junjiro Kobayashi, MD, PhD; Takeshi Nakatani, MD, PhD

Background: Cardiac allograft vasculopathy (CAV) limits long-term success after heart transplant. We assessed the post-transplant risk factors for CAV development.

Methods and Results: Patients who underwent heart transplant between May 1999 and December 2013 were included in this study. Patients (n=54) were divided into 2 groups according to the presence or absence of CAV progression after transplant. Coronary angiogram and intravascular ultrasound were conducted within 5–11 weeks after transplant, at 12 months, and annually thereafter. Scheduled endomyocardial biopsies were performed after transplant or whenever acute cellular rejection (ACR) or antibody-mediated rejection was suspected. Twenty-five of 54 patients (46.2%) had CAV progression. ACR ≥ International Society for Heart and Lung Transplantation grade 2 (ACR ≥2) and donor age >50 years were significantly associated with CAV development compared with ACR <2 and donor age ≤50 years. Patients with no history of ACR ≥2 and donor age ≤50 years had a significantly low risk of developing CAV compared with the other groups.

Conclusions: Donor age and history of ACR ≥2 are independent risk factors for CAV development. Identifying patients at risk of developing CAV is important for appropriate direction of resources and intensity of follow-up. (Circ J 2016; 80: 395–403)

Key Words: Acute cellular rejection; Cardiac allograft vasculopathy; Donor age; Heart transplantation; Intravascular ultrasound
IVUS, a more sensitive method than angiography to detect and quantify early transplant coronary disease.

Methods

Study Design
This was a single-center, retrospective, observational analysis to assess the influence and interdependence of immunologic and non-immunologic risk factors in the development of CAV after HTx. Consecutive patients who received HTx between May 1999 and December 2013 at the National Cerebral and Cardiovascular Center, Osaka, Japan, were included in this study. Patients were excluded when they did not attend at least 2 IVUS examinations (baseline examination and another) or did not have adequate coronary examination after transplantation. This study was approved by the local Ethics Committee and was in compliance with local laws and regulations.

Immunosuppression and Follow-up
After transplantation, all patients received a standard triple-drug combination immunosuppressive therapy consisting of calcium inhibitor (cyclosporine or tacrolimus) plus mycophenolate mofetil and prednisolone. Induction therapy with monoclonal or polyclonal antibodies, such as a murine monoclonal CD3 antibody or an interleukin-2 monoclonal antibody (basiliximab), was also used in patients with renal dysfunction in the perioperative period. Everolimus with reduced calcium inhibitor was used in patients with renal dysfunction and/or CAV since 2007.

Scheduled endomyocardial biopsies were performed at 1, 2, 3, 5, 7, 11 weeks, 4.5, 6, 9, 12 months, then every 6 months until 5 years, annually after 5 years after transplant or whenever clinically suspected. Histopathologic results were based on ISHLT standardized cardiac biopsy grading. Additionally, AMR was monitored by checking the panel reactive antibody test, presence of donor-specific antibody (DSA) and pathological findings on endomyocardial biopsy.

Variables

Immunologic Factors
The immunosuppressive agents are controlled by pharmacokinetic parameters, including trough levels and the area under the plasma concentration curve. ACR surveillance was standardized and performed in all patients. All endomyocardial biopsy specimens were graded based on the standard biopsy grading scheme, according to the 1990 ISHLT classification. The 1990 ISHLT grading system is as follows: grade 0, no rejection; grade 1A, focal infiltrate without myocyte damage; grade 1B, diffuse infiltrate without myocyte damage; grade 2, 1 focus of infiltrate with associated myocyte damage; grade 3A, multifocal infiltrate with myocyte damage; grade 3B, diffuse infiltrate with myocyte damage and grade 4, diffuse, polymorphous infiltrate with extensive myocyte damage. ISHLT grade 3A or higher rejection is usually treated with steroid pulse therapy. If there is resistance to pulse therapy, cytolytic therapy consisting of monoclonal or polyclonal antibodies is instituted. A rejection episode ≥ISHLT grade 2 occurring any time during the follow-up period was analyzed as a risk factor for developing CAV in this study.

Diagnosis of AMR was made based on the 2013 ISHLT consensus, and described as follows: histological evaluation for endothelial activation, intravascular macrophages and capillary destruction. Additionally, immunofluorescence (C3d, C4d, HLA class I and II) and immunoperoxidase (C4d, CD68) were necessary for evaluation of AMR. Pathologic AMR (pAMR) grading categories were: pAMR 0, negative; pAMR 1, (++) immunohistologic AMR alone, (H+) histologic AMR alone; pAMR 2, pathologic AMR both (H+) and (I+); and pAMR 3, severe pathologic AMR. PAMR >1 occurring at any time during the follow-up period was analyzed as a risk factor for CAV development in this study. In addition, the presence of preformed and de novo DSA was confirmed both before transplant and in the follow-up period after HTx using Flow PRA or Luminex SA (Luminex LABSCREEN Single Antigen, One-Lambda, Canoga Park, CA, USA), and mean fluorescence intensity >500 was also defined as a risk factor.

Diagnosis of CAV Progression
Baseline evaluation coronary angiogram and IVUS were conducted each year at National Cerebral and Cardiovascular Center within 5–11 weeks after HTx, according to protocol. Maximum intimal thickness (MIT) was obtained by tracing the lumen vessel wall interface and the external border of the intimal layer. Severe intimal thickness was defined based on the scheme previously described by St. Goar et al. Approximately 3–5 matched cross-sections, predominantly in the left anterior descending coronary artery or right coronary artery, at baseline and upon annual follow-up thereafter, were studied. In addition, IVUS was obtained with an angiographic roadmap of where the initial IVUS examination was performed along the length of the vessel. Two IVUS systems were used during the study period. That used from May 1999 to December 2003 was a combination of a 3.5-F, 30-MHz short monorail imaging catheter (Sonicath, Boston Scientific, Boston, MA, USA) and an imaging console (SONOS100, Hewlett-Packard, Andover, MA, USA). The other used from January 2004 to December 2013 was the 40-MHz mechanical IVUS (ViewIT, Terumo, Tokyo, Japan). A slow, steady pull-back was performed from the mid-distal portion of the study vessel, manually in the former and automatic at 1 mm/s in the latter system. The IVUS cross-sections were matched using identifiable landmarks in the images, such as bifurcations or external landmarks such as coronary veins or pericardium. Diagnosis of CAV was based on a minimum of 0.5-mm progression in MIT from baseline on IVUS. Because there is no way to distinguish whether the intimal thickening observed at baseline IVUS is donor transmitted disease or reaction of vascular endothelial injury in hyperacute state after HTx, the absolute value of MIT progression after HTx was the only parameter taken into account in this study.
Risk Stratification for CAV

Results

Subjects
Overall, 55 patients received HTx during the study period. We excluded 1 patient who did not undergo IVUS after HTx. Thus, 54 HTx recipients were included. CAV progression was detected in 25 of the 54 patients (46.2%; Figure 1).

Clinical Characteristics
Table 1 lists the main baseline characteristics of the cohort. Baseline characteristics of patients with CAV progression and non-CAV progression groups did not differ significantly. Median follow-up was 6.6±4.0 years, with an overall mortality of 3.7% (n=2). Cardiac events developed in 5.5% of patients (n=3) during follow-up.

Risk Factor Analysis for CAV Progression
On univariate analysis several factors were significantly associated with the development of CAV. These included immunologic factors such as ACR≥ISHLT grade 2 (ACR ≥2) and de novo DSA, and non-immunologic factors such as donor age and dyslipidemia.

For the multivariate analysis, ACR ≥2 (P=0.067; OR, 3.43) and donor age (P=0.0004; OR, 33.15) were significantly associated with CAV progression (Table 2).

ACR and CAV Progression
Patients with a history of ACR ≥2 were significantly associated with risk of CAV progression. Figure 2A shows the relation-
Table 1. Patient Background

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total n=54</th>
<th>CAV (-) n=29</th>
<th>CAV (+) n=25</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.5±11.7</td>
<td>33.2±12.4</td>
<td>38.1±10.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Male</td>
<td>43</td>
<td>25</td>
<td>18</td>
<td>0.31</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.4±3.8</td>
<td>21.6±4.2</td>
<td>21.2±3.2</td>
<td>0.74</td>
</tr>
<tr>
<td>Time after HTx (days)</td>
<td>2,415±1,482</td>
<td>2,120±1,418</td>
<td>2,757±1,510</td>
<td>0.11</td>
</tr>
<tr>
<td>Etiology (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCM</td>
<td>41</td>
<td>24</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>dHCM</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>ICM</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>LVAD</td>
<td>49</td>
<td>27</td>
<td>22</td>
<td>0.65</td>
</tr>
<tr>
<td>Age at LVAD implantation (years)</td>
<td>34.1±12.3</td>
<td>31.4±12.6</td>
<td>37.0±11.7</td>
<td>0.15</td>
</tr>
<tr>
<td>LVAD support period (days)</td>
<td>901±354</td>
<td>908±376</td>
<td>894±348</td>
<td>0.88</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>41.0±12.2</td>
<td>38.6±12.6</td>
<td>43.7±11.6</td>
<td>0.13</td>
</tr>
<tr>
<td>Ischemic time (min)</td>
<td>200.9±32.8</td>
<td>196.7±37.1</td>
<td>205.5±27.1</td>
<td>0.33</td>
</tr>
<tr>
<td>Episode of CPA</td>
<td>22</td>
<td>14</td>
<td>8</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Data given as mean±SD or n (%). BMI, body mass index; CAV, cardiac allograft vasculopathy; CPA, cardio pulmonary arrest; DCM, idiopathic dilated cardiomyopathy; dHCM, dilated phase hypertrophic cardiomyopathy; HTx, heart transplantation; ICM, ischemic cardiomyopathy; LVAD, left ventricular assist device.

Table 2. Risk Factors for CAV

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR ISHLT ≥ grade 2</td>
<td>0.0186</td>
<td>0.0067</td>
<td>3.43</td>
<td>1.28–9.70</td>
</tr>
<tr>
<td>DSA Preformed</td>
<td>0.2952</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSA De novo</td>
<td>0.13</td>
<td>0.254</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pAMR ≥ grade 2</td>
<td>0.7185</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor age</td>
<td>0.0151</td>
<td>0.0004</td>
<td>33.15</td>
<td>4.36–297.4</td>
</tr>
<tr>
<td>Recipient age</td>
<td>0.3636</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic time</td>
<td>0.3802</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity (BMI ≥25)</td>
<td>0.3985</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>0.2882</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.0208</td>
<td>0.0622</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV infection</td>
<td>0.9507</td>
<td></td>
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</tbody>
</table>

ACR, acute cellular rejection; CMV, cytomegalovirus; DM, diabetes mellitus; DSA, donor specific antibody; ISHLT, International Society for Heart and Lung Transplantation; pAMR, pathologic antibody-mediated rejection.

Donor Age and CAV Progression

Advanced donor age was associated with a 33.15-fold higher risk of CAV. We divided patients into 2 groups: >50 years of age or ≤50 years of age, according to the criteria for marginal donor.23 As shown in Figure 4A, the patients with a donor age >50 (n=16) had a significant association with CAV development (P=0.0462), compared with the donors <50 years of age (n=38). The time to develop CAV after HTx was not statistically different between the 2 groups (donor age >50 years, 1.8±0.5 years; donor age ≤50 years, 2.6±2.2 years; P=0.69; Figure 4B). Mean follow-up in these 2 groups was not statistically different (donor age >50 years, 6.1±3.6 years; donor age ≤50 years, 8.6±4.2 years, P=0.15).

Clinical Outcome

During the follow-up period (mean follow-up, 6.6±4.0 years), the composite endpoint of cardiac events (PCI, n=2; CABG, n=1) occurred in 3 patients, and death (sepsis, n=1; post-transplantation lymphoproliferative disorder, n=1) occurred in 2 patients. All deaths were not related to cardiac events.

Cumulative cardiac event-free survival in patients with and without CAV progression is shown in Figure 5A. Two patients with CAV progression underwent coronary revascularization via PCI and CABG. Each patient had a history of refractory ACR or had been treated for pAMR2. One patient in the non-CAV progression group underwent PCI 1 month after HTx.
because of donor-transmitted disease. There was no significant difference between the 2 groups in overall mortality after HTx (Figure 5B). Cumulative cardiac event-free survival related to donor age is shown in Figure 5C. All cardiac events occurred in the group of donors aged ≤50 years, and there was no statistically significant difference between the groups (Wilcoxon P=0.36). With regard to overall mortality, there was also no significant difference (Wilcoxon P=0.42; Figure 5D).

Risk Evaluation
Based on the multivariate analysis of the Cox proportional hazards model, we divided all patients into 4 groups: history of ACR ≥2 and donor age >50; no history of ACR ≥2 and donor age >50; history of ACR ≥2 and donor age ≤50; and no history of ACR ≥2 and donor age ≤50, to further assess the risk stratification for developing CAV in the transplanted patients. The risk of developing CAV was significantly low in the patients without a history of ACR ≥2 and donor age ≤50 com-

Figure 2. Correlation between acute cellular rejection (ACR) and cardiac allograft vasculopathy (CAV). (A) Relationship between ACR grade and prevalence of CAV. (B) CAV timing. (C) ACR occurrence frequency and prevalence of CAV.

Figure 3. Cumulative incidence of cardiac allograft vasculopathy (CAV) in the whole group (n=54). HTx, heart transplantation.
Figure 4. Correlation between donor age and cardiac allograft vasculopathy (CAV). (A) Donor age (older or younger than 50 years of age) and prevalence of CAV. (B) Donor age and time to post-HTx diagnosis of CAV.

Figure 5. (A) Cumulative cardiac event-free survival and (B) cumulative post-heart transplantation (HTx) survival according to presence of cardiac allograft vasculopathy (CAV; n=54). (C) Cumulative cardiac event-free survival and (D) cumulative patient survival vs. donor age (n=54).
pared with that of the other groups (Figure 6). There was no difference in CAV development, however, between the other groups.

**Discussion**

To our knowledge, this is the first observational study of annual IVUS after HTx to assess the influence and interdependence of immunologic and non-immunologic risk factors. Several observational studies have used coronary angiography, which, although invasive, is the main modality to detect CAV, but these studies lack the sensitivity of IVUS to detect early arterial lesions. It was previously demonstrated that severe intimal proliferation on IVUS is predictive of cardiac events, even in the absence of angiographic abnormalities.

Because previous studies traditionally focused on the first year after HTx and performed only a single or a few follow-up angiography or IVUS examinations to diagnose CAV, the onset of intimal thickening progression and the strength of the association with the clinical condition may be underestimated. The present study has delineated a more precise timing of CAV development, and investigated the strength of the association with each clinical risk factor by examining annual IVUS results at every follow-up visit.

In this study, we defined CAV progression as >0.5-mm MIT progression on IVUS at any time after HTx to observe the effect and correlation of long-term clinical conditions. The progression of intimal thickening ≥0.5 mm in the first year after HTx is a reliable marker for adverse cardiac events, and late MIT increase on IVUS was also recently reported as an additional sensitive marker for cardiovascular events and long-term prognosis. Thus, continuous monitoring and the earliest possible intervention to reduce factors that will exacerbate CAV are important for improving late prognosis.

**Donor Age and CAV**

In the present study shows donor age was an independent risk factor associated with the development of CAV. A recent United Network for Organ Sharing (UNOS) report also identified more advanced donor age as an independent risk factor for development of CAV regardless of recipient age, similar to results from previous studies.

The reasons for the influence of donor age on the development of intimal hyperplasia remain unknown, but allografts from older donors may be more susceptible to endothelial damage resulting from immunologic or non-immunologic interaction via an age-related decline in endogenous cardioprotective mechanisms.

According to the International Heart and Lung Transplantation Registry in 2013, the mean age of heart donors was 34 years. The mean donor age in this study was 41 years (range, 16–68 years), which is older compared with other countries. Through frequent precise IVUS evaluation, our analysis further indicates that allografts from older donors (>50 years) tend to develop CAV more easily after HTx than those from younger donors. Although CAV remains the main cause of cardiac-related death after HTx, the actual post-transplant survival curve and cardiac events did not differ in acute or long-term survival in the present study. This might be due to the use of annual IVUS, which enables implementation of an immediate intervention at the earliest sign of CAV development, before progressing to an angiographic change that would require intervention medication or immediate tailoring of immunosuppressive regimens to minimize progression. Conversion to everolimus with a reduced-exposure calcineurin inhibitor, recently became a widely-used treatment because of its potential to decrease the severity and incidence of CAV.

Everolimus was introduced in 12 of 25 CAV patients (48%) from 2007 in this study, earlier than in other countries. As a result of increased demand for cardiac transplantation and a small donor pool, older donors are being used with increasing frequency despite several concerns that have been described. Careful selection of recipients and closer monitoring of CAV and early therapeutic intervention including optimization of immunosuppressive regimens are still warranted in these patients.
ACR and CAV
In the present study a history of ACR ≥2 was an independent risk factor associated with the development of CAV. ACR has the highest incidence in the first postoperative year, and the effect of heightened immune response on CAV is cumulative and extends beyond the first year. Rahmani et al reported that the immunological response is the principal initiating stimulus and it results in endothelial injury and dysfunction and altered endothelial permeability, with consequent myo-intimal hyperplasia and extracellular matrix synthesis. Jimenez et al additionally reported that, after examining IVUS results after HTx, even a minimal endomyocardial rejection contributed insidiously to the development of CAV. This experimental and clinical evidence shows that ACR and CAV are closely related to the immune process, which is consistent with the present results. In addition, our study showed the importance of the association between ACR frequency and CAV development, in that the more often ACR ≥2 occurs, the higher the tendency to develop CAV. CAV also tends to occur after an episode of more severe grade of ACR, as shown on annual IVUS with pathological evidence of cellular rejection, and the mean period between the first major ACR and detection of CAV was 61±597 days.

Clinical Implications
We studied additional risk stratification for developing CAV based on statistical results. As shown in Figure 6, the group of patients without a history of ACR ≥2 and donor age ≤50 had a significantly lower risk of developing CAV than the other groups. The effect remains controversial, however, because of the present small sample size. Further research is required on important clinical implications of patient follow-up. Avoiding ACR is important in reducing the incidence of CAV, and these data suggest that more careful follow-up and intensive intervention for the patients with a frequent history of ACR ≥2 or a heart from an older donor are required. Moreover, the present results suggest the possibility of avoiding invasive and frequent examination in the patients with well-controlled cellular rejection, a younger donor heart and those who have no other clinical arteriosclerosis factors.

Conclusions
Donor age and history of ACR ≥2 were independent risk factors associated with the development of CAV. The post-transplant survival curve and cardiac events, however, did not differ between acute or long-term survival, which might be a result of the proactive diagnosis of CAV as early as possible using annual IVUS with prompt therapeutic intervention to minimize further development. Identification of patients at risk of developing CAV may have relevant implications in appropriately guiding resources and intensive follow-up. Patients without these risks, however, may be able to avoid frequent invasive testing and the associated increased costs and complication risks.

Study Limitations
Our study had several limitations. First, it was retrospective and conducted at a single center and, consequently, included a relatively small number of patients. Second, baseline donor vascular conditions (whether the patient had donor-transmitted lesions at baseline) were not examined separately. The absolute value of MIT progression was the only parameter taken into account. Third, 2-D or 3-D IVUS analysis would provide much more quantitative information, although it was difficult to obtain quantitative data due to the retrospective observational nature of the study. Fourth, we were unable to draw any firm conclusions about the influence of different immunosuppressive therapies on outcomes, because the regimens targeted trough levels, and the timing of dose changes was different for each patient.

References
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