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Early invasive assessment of the coronary microcirculation predicts subsequent acute rejection after heart transplantation

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ABSTRACT

Background: Acute allograft rejection (AAR) plays an important role in patient and graft survival; therefore, more emphasis should be placed on its prediction. This study aimed to investigate baseline clinical and diagnostic variables associated with subsequent AAR during the first year post-transplant, especially focusing on early physiologic and anatomic measures.

Methods: This study enrolled 88 heart transplant patients who underwent fractional flow reserve (FFR), coronary flow reserve (CFR), the index of microcirculatory resistance (IMR) and intravascular ultrasound (IVUS) in the left anterior descending artery at baseline (within 8 weeks post-transplant). Cardiac index (CI), pulmonary capillary wedge pressure (PCWP), mean pulmonary artery pressure (mPAP), right atrial pressure and left ventricular ejection fraction were also evaluated. AAR was defined as acute cellular rejection of grade ≥2R and/or pathological antibody-mediated rejection of grade ≥pAMR2.

Results: During the first year post-transplant, 25.0% of patients experienced AAR. Patients with AAR during the first year showed higher rates of recipient obesity, lower rates of recipient-donor sex mismatch and rATG and tacrolimus uses, higher PCWP, mPAP and IMR, and lower CFR at baseline, compared with those without. In the multivariate analysis, only baseline IMR ≥ 16.0 was independently associated with AAR during the first year, demonstrating high negative predictive value (96.7%).

Conclusions: Invasively assessing microvascular resistance (baseline IMR ≥ 16.0) in the early post-transplant period was an independent determinant of subsequent acute allograft rejection during the first year post-transplant, suggesting that early assessment of IMR may enhance patient risk stratification and target medical therapies to improve patient outcome.

1. Introduction

Acute allograft rejection (AAR) plays an important role in the development of cardiac allograft vasculopathy (CAV, a major cause of long-term morbidity and mortality) and mortality after heart transplantation [1,2]; therefore, more emphasis should be placed on its prediction and early detection. Previous transplant studies, including previous work from our institution, have used invasive or noninvasive modalities, such as hemodynamic and coronary physiologic measurements, and intravascular ultrasound (IVUS), to evaluate CAV and to predict late graft failure [3–10]. Some of these studies have also shown the association between incidence and severity of AAR, and subsequent CAV progression [3,4,6,7]. However, it remains unknown whether the use of these modalities in the early post-transplant period can predict subsequent AAR and thereby identify high-risk patients who may benefit from closer follow-up and targeted medical therapies. AAR is an evolving process and may start immediately after heart transplantation [11]. Hence, we hypothesized that early evolution of rejection might manifest as impairment of invasive or noninvasive measures early after heart transplantation and changes in these measures could be used to predict or detect subsequent clinically apparent or biopsy proven AAR. Therefore, the aim of this study was to investigate baseline clinical and diagnostic variables associated with subsequent AAR during the first year post-transplant, focusing especially on early physiologic and anatomic measures.

Abbreviations: AAR, acute allograft rejection; ACR, acute cellular rejection; AMT, antibody-mediated rejection; CAV, cardiac allograft vasculopathy; CFR, coronary flow reserve; FFR, fractional flow reserve; IMR, the index of microcirculatory resistance; LAD, left anterior descending artery; LVEF, left ventricular ejection fraction; IVUS, intravascular ultrasound; mPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; ROC, receiver operating curve; rATG, rabbit antithymocyte globulin.

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2. Methods

2.1. Study population

This retrospective study included patients from two consecutive prospective trials performed to evaluate the role of cytomegalovirus or an angiotensin converting enzyme inhibitor in CAV development (NCT01078363) at Stanford University Medical Center and the Palo Alto Veterans Affairs Health Care System between 2002 and 2015. Patients were included if they were >18 years old, clinically stable with preserved renal function (serum creatinine < 2.0 mg/dL), provided informed and written consent, and underwent pre-scheduled baseline (within 8 weeks post-transplant) coronary angiogram with measurements of fractional flow reserve (FFR), coronary flow reserve (CFR), the index of microcirculatory resistance (IMR) and IVUS. Patients were excluded if they were undergoing more than one solid organ transplant or repeat cardiac transplantation. All patients received standard immunosuppressive therapy, including induction therapy (daclizumab, anti-interleukin-2 monoclonal antibody, OKT3 or rabbit antithymocyte globulin (rATG)), corticosteroids, an antiproliferative agent (sirolimus or mycophenolate mofetil) and a calcineurin inhibitor (cyclosporine or tacrolimus); dose adjustments were made as indicated by adverse effects and/or acute rejection. At our centers, by one month, heart transplant recipients were usually on a stable medical regimen, including the immunosuppressant regimen. The steroid dosage was gradually weaned during the first year post-transplant. Patients were monitored for AAR by routine surveillance endomyocardial biopsies at scheduled intervals post-transplant: weekly during the first month, biweekly until the third month, monthly until the sixth month, and then at 8, 10 and 12 months. AlloMap was also performed as a screening of rejection instead of biopsy in patients who were ≥6 months post-transplant, at a very low risk for rejection, and on low dose steroid. If the AlloMap score was >33, then the patients were invited back for a biopsy within a week. According to the International Society for Heart and Lung Transplantation (ISHLT) grading scale, AAR was defined as acute cellular rejection (ACR) of grade ≥2R/3A and/or biopsy-proven (pathologic) antibody-mediated rejection (AMR) of grade ≥pAMR2 during the first year post-transplant [8,12]. Cardiac index, pulmonary capillary wedge pressure (PCWP), mean pulmonary arterial pressure (mPAP), right atrial pressure (RAP), and left ventricular ejection fraction (LVEF) were also measured as invasive and noninvasive hemodynamic indexes. Patients were followed beyond one year post-transplant and clinical outcomes (all-cause death or re-transplantation) were evaluated. The study protocols were approved by the Stanford Institutional Review Board and informed consent was obtained from all patients.

2.2. Coronary physiology measurements

FFR, CFR and IMR were measured in the left anterior descending (LAD) artery as previously described [8]. After performing coronary angiography with a 6-French guiding catheter, intracoronary nitroglycerin and intravenous heparin (60 U/kg) were administered. Following calibration, a 0.014 inch pressure-temperature sensor guidewire (PressureWire™Certus™, Abbott Vascular) was advanced to the distal two-thirds of the LAD artery. With commercially available software (Radi Analyzer®; Abbott Vascular, Santa Clara, CA), the transit time of room-temperature saline injected down a coronary artery was determined with a thermodilution technique. During administration of intravenous adenosine (140 μg/kg/min) to induce maximal hyperemia, 3 intracoronary injections of 3 mL of room-temperature saline were performed and hyperemic mean transit time was calculated. Simultaneous measurements of mean proximal and distal coronary pressures were also acquired during maximal hyperemia. FFR was calculated as the mean distal coronary pressure divided by the mean proximal pressure at hyperemia. CFR was calculated as resting mean transit time divided by hyperemic mean transit time. IMR was calculated as the distal coronary pressure at hyperemia multiplied by the hyperemic mean transit time.

2.3. IVUS measurements

IVUS was performed using a 40-MHz mechanical system (Galaxy with Atlantis SR Pro or OptiCross with iLab. Boston Scientific Corporation). Images were analyzed with a validated system (echoPlaque. Indec Systems, Santa Clara, CA) at a core laboratory blinded to clinical and angiographic information. Vessel, lumen and intimal areas were traced at 1-mm intervals throughout the first 50 mm of the LAD artery with automated interpolated measurements of the remaining frames [5]. Volumes calculated using Simpson's method were measured as volume index (volume/analyzed length, mm^3/mm) and then standardized by recipient body surface area ($0.007184 \times \text{body weight (kg)}^{0.425} \times \text{height (cm)}^{0.725}$, m^2). Donor-transmitted atherosclerosis was defined as maximum intimal thickness (MIT) at baseline ≥0.5 mm [5]. Paradoxical vessel remodeling of the proximal LAD segment was also evaluated [5].

2.4. Statistical analysis

Statistical calculations were performed with JMP® 10 (SAS Institute Inc., Cary, NC). Data are expressed as frequencies and percentages for categorical variables, and as mean ± SD for continuous variables. Categorical comparisons were performed using a chi-square test or Fisher's exact test. Continuous values were compared by using the unpaired or paired Student *t*-test, Wilcoxon rank sum test, Mann-Whitney *U* test, or one-way analyses of variance (ANOVA), as appropriate. When there were significant differences in baseline diagnostic variables between patients with and without subsequent AAR, receiver operating characteristics (ROC) curve analysis was performed to identify the optimal cutoff values to predict subsequent AAR. Associated sensitivity, specificity, positive predictive value, negative predictive value, area under the curve (AUC) and overall accuracy were also calculated to identify the diagnostic efficacy of each measure. Logistic regression analysis was performed to find the relationship between baseline variables and subsequent AAR during the first year post-transplant and variables with a p value of ≤0.05 on univariate analysis were entered into the multivariate analysis. Survival analysis was performed by applying the Kaplan-Meier method and the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were analyzed with Cox proportional hazards regression models. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Clinical characteristics

A total of 88 patients met inclusion and exclusion criteria and underwent both physiology and IVUS examinations at baseline. Baseline clinical characteristics are presented in Table 1. Mean age was 54 ± 10 years, and 73.9% of patients were men. 28.4% of patients received heart transplantation as a result of ischemic cardiomyopathy. Recipient-donor CMV IgG mismatch was present in 40.9% of patients.

During the first year post-transplant, AAR occurred in 22 (25.0%) patients between the time of baseline invasive and noninvasive measurements and one year post-transplant: 20 patients had ACR (including 4 ACR of grade 3R/3B); 1 had biopsy-proven AMR; and 1 had both ACR and AMR. About two third of the first episode of ACR (65.0%) occurred within 6 months (median: 4 months) post-transplant; 85.7% of patients with ACR had single episode of ACR within one year.

3.2. AAR, CAV progression and mortality

After one year post-transplant, patients were followed for up to 12 years (median 4.1 years). During this period, 16 patients (18.2%) died (12 were cardiac deaths) and 1 (1.1%) underwent re-transplantation. Kaplan-Meier analysis demonstrated a significantly lower event-free survival rate in patients with AAR during the first year post-transplant, compared with those without (Fig. 1). A similar result was also observed when the analysis was limited to patients with ACR during the first year post-transplant ($p = 0.0001$ for Log-rank), whereas the grade (grade 3R/3B vs. grade 2R/3A), time of the onset (within 6 months vs. after 6 months post-transplant) and frequency (single vs. multiple) of ACR during the first year was not associated with subsequent mortality or re-transplantation ($p = 0.58$, 0.90, and 0.90 respectively).

Among the study cohorts, serial coronary physiologic measurements at baseline and at one year post-transplant were performed in 83 patients (94.3%), while serial IVUS measurements were performed in 80 (90.0%). Overall, FFR, IMR and vessel and lumen volumes decreased, while CFR and intimal parameters (intimal volume and average and maximum intimal thickness) increased from baseline to one year post-transplant (Table 1). Patients with AAR during the first year post-transplant showed greater intimal growth, a reduction in FFR, and higher rates of paradoxical vessel remodeling of the proximal LAD segment at one year compared with those without, whereas changes in vessel and lumen volumes and CFR were not significantly different between the groups. There were also a greater reduction in IMR at one year in patients with AAR during the first year than those without, this may be attributable to higher baseline IMR in these patients (Table 1). IMR at one year tended to remain higher in patients with AAR compared with those without.

3.3. Baseline variables associated with subsequent AAR

Patients with AAR showed significantly higher rates of recipient obesity, lower rates of recipient-donor sex mismatch and rATG and tacrolimus uses, higher PCWP, mPAP and IMR, and lower CFR at baseline, compared with those without (Table 2). There was also a trend towards higher RAP in patients with subsequent AAR than

Table 1
Baseline variable and CAV progression: patients with versus without AAR.

Baseline variables	All ($n = 88$)	AAR during the first year post-transplant		
		Present ($n = 22$)	Absent ($n = 66$)	p value
Recipient profile				
Age, years	54 ± 10	53 ± 9	54 ± 11	0.38
Male	73.9%	86.4%	69.7%	0.11
Recipient-donor sex mismatch	23.9%	9.1%	28.8%	0.04
Body mass index, kg/m^2	26.5 ± 5.0	28.2 ± 4.0	26.0 ± 5.1	0.01
Body mass index ≥ 25 kg/m^2	58.0%	81.8%	50.0%	0.007
CMV IgG positive	63.6%	63.6%	63.6%	1.00
Recipient-donor CMV IgG mismatch	40.9%	36.4%	42.4%	0.62
Diabetes mellitus	17.0%	22.7%	15.2%	0.42
Hypertension	44.3%	40.9%	45.5%	0.71
Hyperlipidemia	25.3%	27.3%	24.6%	0.81
Ischemic cardiomyopathy	28.4%	36.4%	25.8%	0.35
Immunosuppressive regimen*				
rATG	51.1%	27.3%	59.1%	0.009
Tacrolimus	64.8%	45.5%	71.2%	0.03
Sirolimus	9.1%	18.2%	6.1%	0.11
Mycophenolate mofetil	81.2%	68.2%	86.4%	0.07
Statins at 1 year	93.2%	86.4%	95.5%	0.17
ACEI at 1 year	53.5%	59.1%	51.6%	0.54
Antidiabetic medications at 1 year	34.4%	36.4%	33.9%	0.83
Donor profile				
Age, years	32 ± 12	34 ± 12	32 ± 12	0.27
Male	75.0%	77.3%	74.2%	0.77
Cold ischemic time, min	228 ± 43	233 ± 43	227 ± 44	0.78
Echocardiography at baseline				
LVEF, %	61.4 ± 7.8	60.9 ± 10.2	61.5 ± 6.8	0.43
Right heart catheterization at baseline				
CI, L/min per m^2	3.4 ± 2.3	4.2 ± 4.9	3.1 ± 0.7	0.66
PCWP, mm Hg	14.5 ± 5.8	17.6 ± 4.7	13.7 ± 5.9	0.02
mPAP, mm Hg	23.0 ± 6.9	26.5 ± 7.1	22.0 ± 6.6	0.02
RAP, mm Hg	7.0 ± 4.2	8.5 ± 3.5	6.6 ± 4.3	0.053
Coronary physiologic indexes at baseline				
FFR	0.89 ± 0.05	0.90 ± 0.04	0.89 ± 0.06	0.28
Hyperemic mean transit time, s	0.35 ± 0.18	0.42 ± 0.20	0.32 ± 0.17	0.048
CFR	3.41 ± 1.65	2.72 ± 0.99	3.64 ± 1.76	0.04
IMR	23.3 ± 14.3	28.7 ± 13.9	21.5 ± 14.0	0.004
IVUS indexes at baseline				
Vessel volume, mm^3/mm per m^2	8.3 ± 1.7	8.6 ± 1.1	8.2 ± 1.8	0.34
Lumen volume, mm^3/mm per m^2	6.9 ± 1.5	7.3 ± 1.0	6.7 ± 1.6	0.15
Intimal volume, mm^3/mm per m^2	1.4 ± 0.7	1.3 ± 0.4	1.5 ± 0.7	0.996
Percent intimal volume, %	17.3 ± 7.0	15.7 ± 5.0	17.8 ± 7.5	0.60
Average intimal thickness, mm per m^2	0.11 ± 0.05	0.10 ± 0.03	0.11 ± 0.05	0.61
MIT, mm per m^2	0.41 ± 0.24	0.43 ± 0.23	0.40 ± 0.24	0.37
Maximum plaque burden, %	28.0 ± 11.9	26.8 ± 10.5	28.4 ± 12.4	0.81
Donor atherosclerosis (MIT 0.5 mm)	63.5%	75.0%	60.0%	0.21
CAV progression during the first year				
Changes in coronary physiologic indexes				
Number of patient analyzed	83	19	64	
Changes in FFR	−0.02 ± 0.06	−0.04 ± 0.07	−0.01 ± 0.06	0.06
Changes in CFR	0.83 ± 3.13	0.94 ± 1.37	0.80 ± 3.50	0.27
Changes in IMR	−4.9 ± 18.4	−10.6 ± 13.2	−3.2 ± 19.4	0.03
Increases in IMR during the first year	30.1%	15.8%	34.4%	0.10
IMR at one year post-transplant	17.9 ± 13.3	19.5 ± 8.5	17.4 ± 14.5	0.08
Changes in IVUS indexes				
Number of patient analyzed	80	18	62	
Changes in vessel volume, %	−7.0 ± 9.3	−6.7 ± 12.4	−7.1 ± 8.3	0.88
Changes in lumen volume, %	−12.2 ± 12.3	−14.5 ± 18.0	−11.5 ± 10.2	0.37
Changes in intimal volume, %	21.6 ± 38.2	41.1 ± 47.6	16.0 ± 33.4	0.03
Changes in average intimal thickness, %	27.3 ± 40.2	52.4 ± 52.6	20.2 ± 33.2	0.01
Changes in MIT, %	24.0 ± 42.5	37.2 ± 46.6	20.2 ± 40.9	0.13
Increases in MIT ≥ 0.5 mm within 1 year	10%	16.7%	8.1%	0.31
Paradoxical remodeling of the proximal LAD segments	41.3%	83.3%	29.0%	<0.0001

Values are mean ± SD or %. p values for patients with versus without AAR. Categorical comparisons were performed using chi-square test. Continuous values were compared using unpaired Student t-test or Wilcoxon rank-sum test. *Primarily used during the first year post-transplant. AAR = acute allograft rejection, ACEI = angiotensin converting enzyme inhibitor, CAV = cardiac allograft vasculopathy, CI = cardiac index, CFR = coronary flow reserve, CMV IgG = Cytomegalovirus immunoglobulin G, FFR = fractional flow reserve, IMR = the index of microcirculatory resistance, LAD = left ventricular descending artery, LVEF = left ventricular ejection fraction, MIT = maximum intimal thickness, mPAP = mean pulmonary artery pressure, PCWP = pulmonary capillary wedge pressure, RAP = right atrial pressure, rATG = rabbit antithymocyte globulin.

those without ($p = 0.053$). The ROC curve analyses identified the optimal cutoff values of baseline PCWP, mPAP, CFR, and IMR as 15.0 mm Hg, 27.0 mm Hg, 3.90, and 16.0 for predicting subsequent AAR during the first year post-transplant (AUC: 0.72, 0.68, 0.64 and 0.71, respectively) (Fig. 2). In the multivariate analysis including all variables with a p value of ≤0.05 on univariate analysis, baseline

IMR ≥ 16.0 and CFR ≤ 3.90 were or tended to be associated with subsequent AAR during the first year, demonstrating high negative predictive value (NPV) with similar diagnostic accuracy between IMR and CFR ($p = 0.51$ for DeLong method) (Table 2). Indeed, 36.2% of patients with baseline IMR ≥ 16.0 subsequently suffered AAR during the first year post-transplant, whereas it occurred only in 3.3% of patients with very low baseline IMR (<16.0) (Fig. 2). Although the present study was underpowered to detect statistical significance, similar trends were also found when using continuous values of baseline IMR ($p = 0.07$) and CFR ($p = 0.13$) in the regression analyses (Supplementary Table).

Baseline IMR was also associated with subsequent ACR during the first year post-transplant ($p = 0.0003$). Of note, all ACR of grade 3R/3B or multiple ACR were observed in patients with baseline IMR ≥ 16.

The combined assessment of baseline IMR with CFR, PCWP or mPAP appeared to enhance the diagnostic efficiency of IMR. Among patients with baseline IMR ≥ 16.0, subsequent AAR during the first year was more frequently observed in patients with low baseline CRF versus high baseline CFR (42.9% vs. 0%, $p = 0.003$), as well as in patients with high baseline PCWP versus low baseline PCWP (50.0% vs. 12.5%, $p = 0.004$) and in patients with high baseline mPAP versus low baseline mPAP (58.8% vs. 19.4%, $p = 0.006$).

3.4. Microvascular function and late mortality

Survival rate did not differ significantly between patients with high and low baseline IMR ($p = 0.55$) or CFR ($p = 0.45$) (both for Log-rank). In contrast, a significantly lower survival rate was or tended to be seen in patients with versus without increased IMR ($p = 0.04$) or decreased CFR ($p = 0.28$) during the first year post-transplant. Notably, the association between increased IMR and late mortality was more prominent in patients with higher versus lower baseline IMR (Supplementary Fig. 2).

4. Discussion

The main finding of this study is that invasively measuring IMR soon after heart transplantation is an independent predictor among baseline clinical and diagnostic variables for subsequent AAR during the first year post-transplant, demonstrating high NPV. To the best of our knowledge, this is the first study to suggest the potential clinical utility of early invasive assessment of the coronary microcirculation for predicting subsequent AAR during the first year post-transplant.

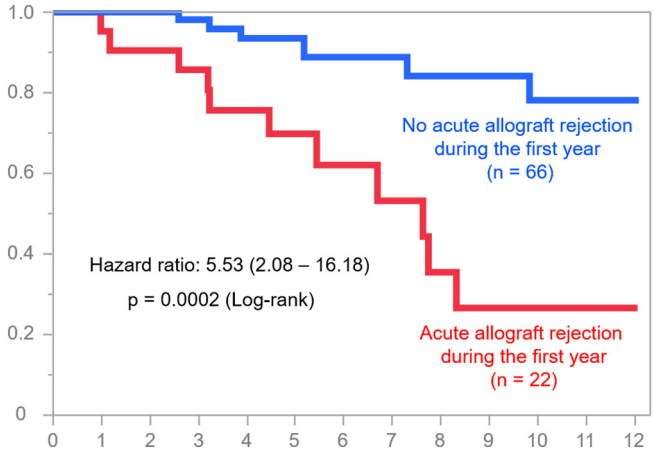

Fig. 1. AAR and subsequent mortality. Kaplan-Meier analysis over a median follow-up period of 4.1 years demonstrated a significantly lower event-free survival rate seen in patients with AAR during the first year post-transplant compared with those without. Abbreviations as in Table 1.

4.1. Acute allograft rejection

AAR is a major limitation to patient and graft survival and remains an important concern after heart transplantation [1]. Multiple studies have also repeatedly shown the association between AAR early post-transplant and subsequent CAV progression [3,4,7,10,13,14]. Although modern immunosuppressive regimens have substantially reduced the incidence of acute rejection, about one quarter of heart transplant recipients have still at least one rejection episode within the first year post-transplant [1,2]. The present study, as well as previous work from our group [5,8,15], confirmed these previous findings and demonstrated that AAR during the first year post-transplant is a consistent determinant of subsequent CAV progression (both in epicardial vessel and in microcirculation) and mortality in heart transplant recipients. As AAR is a modifiable risk, its prediction and early detection may allow earlier institution of more aggressive immunosuppressant therapies which could improve patient and graft survival.

4.2. Early invasive assessment of the coronary microcirculation

Theoretically, the rejection process could start immediately after heart transplantation and involve not only the vasculature (large vessels and microcirculation) but also the myocardium; moreover, one can hypothesize that impairments of invasive and noninvasive measures (such as coronary physiologic and IVUS measurements, right heart catheterization, and echocardiography, etc.) could also begin even in the early post-transplant period (within 8 weeks post-transplant) in patients who are subject to AAR. The present study supported the hypothesis and demonstrated higher IMR, PCWP, mPAP and RAP, and lower CFR at baseline in patients with subsequent AAR during the first year post-transplant compared with those without. Typically, these baseline measures have been performed serially in previous studies to evaluate the development of CAV and subsequent graft failure, however, the present study identified that assessment of these baseline measures at a single time point can be also useful for patient risk stratification (especially, as a potential surrogate marker for subsequent AAR during the first year post-transplant).

Among baseline diagnostic variables, baseline IMR ≥ 16.0 and CFR < 3.90 were or tended to be associated with subsequent biopsy-proven AAR during the first year post-transplant, indicating that invasive assessment of the coronary microcirculation is a more sensitive method to detect early changes associated with subsequent AAR, compared with the assessment of the epicardial vessel or right and left heart pressures. One plausible explanation for this observation may be discordant progression of AAR in the transplanted organ. Primarily, allograft rejection is identified as an evolving injury that results from repeated alloimmune attack on the transplanted graft [11]. Although this process occurs diffusely within the graft, the vasculature (especially, microvasculature) appears to be preferentially targeted by the alloimmune insult [11], leading to earlier impairment of the microcirculation compared with the larger vessels (epicardial vessels). Indeed, previous studies support this explanation showing that microvascular dysfunction often occurs in the absence of epicardial disease [13,15,16]. Others have shown that episodes of rejection are associated with microvascular dysfunction before the development of significant epicardial CAV [7]. Interstitial edema may also play an important role in the interaction between early changes in AAR and microvascular dysfunction [12]. However, the exact mechanisms of this observation need to be elucidated in further studies, including histopathological studies.

Microvascular function can be measured using CFR or IMR. Although CFR did not meet significance in the multivariate analysis, its performance characteristics appeared to be comparable to IMR with similar NPV. Integrating assessments of IMR and CFR may also enhance the diagnostic efficiency of stand-alone assessment of IMR. This may reflect the absence of significant epicardial disease on the early study which would otherwise affect FFR and therefore CFR, and suggest the practical

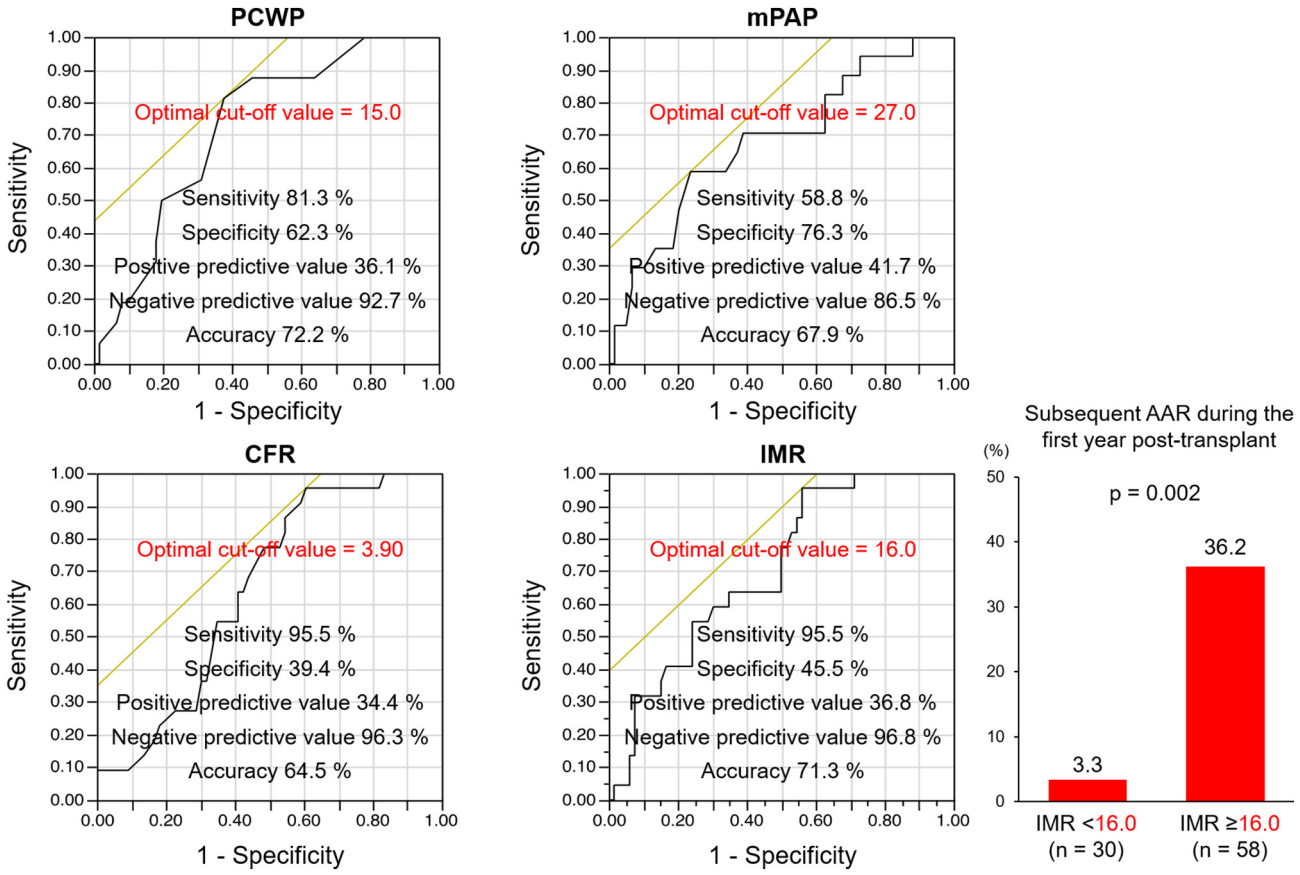

Fig. 2. ROC curve analysis and baseline IMR for predicting of subsequent AAR. Receiver Operating Characteristics (ROC) curve analysis identified the optimal cutoff values of baseline PCWP, mPAP, CFR, and IMR as 15.0 mm Hg, 27.0 mm Hg, 3.90, and 16.0 respectively for predicting subsequent AAR during the first year (Left). 36.2% of patients with baseline IMR ≥16.0 subsequently suffered AAR during the first year post-transplant, whereas it occurred only in 3.3% of patients with very low baseline IMR (<16.0) (Right). Abbreviations as in Table 1.

significance because CFR can also be measured by non-invasive modalities such as PET or echocardiography.

4.3. Clinical implication

The present study suggests several potential clinical implications. First, baseline IMR measurement identifies patients at higher risk for rejection and potentially late mortality, who may benefit from closer follow-up with more gradual tapering of immunosuppressants. Conversely, its high NPV of subsequent AAR may allow for earlier steroid withdrawal. Second, baseline IMR measurement might guide transplant medical therapy to prevent subsequent AAR and ultimately improve patient and graft survival. In the present study, significantly lower rates of rATG and tacrolimus use were seen in patients with AAR during the first year. Previous studies have observed that tacrolimus-based immunosuppression provides superior prevention of AAR compared with cyclosporine-based therapy [16–18]. Several transplant studies, including a randomized trial, have demonstrated a lower incidence and severity of AAR using rATG versus interleukin-2 receptor antagonist induction [19]. Other studies have shown that administration of everolimus was associated with a lower incidence of AAR [16]. However, as retrospective analyses are susceptible to confounding factors, further studies are warranted to determine the effects of specific medical therapies on AAR and to investigate the potential clinical utilities of baseline IMR measurement in these clinical settings.

Table 2
Factors associated with subsequent AAR.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Model 1						
Recipient-donor sex mismatch	0.25	0.04–0.96	0.04	0.71	0.08–4.49	0.72
rATG use	0.26	0.08–0.72	0.009	0.78	0.15–4.06	0.78
Tacrolimus use	0.34	0.12–0.91	0.03	0.95	0.18–4.94	0.95
PCWP ≥ 15.0 mm Hg	7.16	2.05–33.71	0.002	2.63	0.51–15.94	0.25
mPAP ≥ 27.0 mm Hg	4.59	1.50–14.92	0.008	2.25	0.55–9.60	0.26
CFR ≤ 3.90	13.65	2.60–251.95	0.0006	5.55	0.84–109.46	0.08
Model 2						
Recipient-donor sex mismatch	0.25	0.04–0.96	0.04	1.05	0.11–7.67	0.97
rATG use	0.26	0.08–0.72	0.009	0.97	0.18–5.69	0.97
Tacrolimus use	0.34	0.12–0.91	0.03	0.75	0.13–4.31	0.74
PCWP ≥ 15.0 mm Hg	7.16	2.05–33.71	0.002	4.31	0.82–27.84	0.09
mPAP ≥ 27.0 mm Hg	4.59	1.50–14.92	0.008	2.71	0.62–12.48	0.18
IMR ≥ 16.0	16.46	3.15–303.55	0.0002	12.70	2.10–247.41	0.003

All variables with a *p* value of ≤0.05 on univariate analysis were included in the multivariate analysis. OR = odds ratio, CI = confidence interval. Other abbreviations as in Table 1.

4.4. Limitations

Several limitations should be noted. First, endomyocardial biopsy was used as the primary endpoint of this study; however, it may not be definitive in detecting AAR. Second, coronary physiologic and IVUS measurements were performed only in the LAD. Therefore, it remains unknown whether 3-vessel analyses could offer additional insights to our findings. Third, although this study used right heart catheterization and LVEF to measure hemodynamic function, LV diastolic function by echocardiography was not assessed. Fourth, it remains unknown about

how much microvascular dysfunction was pre-existent to heart transplantation and whether donor-derived microvascular dysfunction could expose the recipient to AAR. Also, whether hyperemic response to adenosine in the peculiar setting could provide additional insights to our results remains unclear. Fifth, this study represents a retrospective analysis with relatively small sample size of a selected patient population with preserved renal function to allow angiography/IVUS. Diverse immunosuppressive agents were also used in this population. Finally, our findings, including optimal cut-off values of IMR and CFR for predicting subsequent AAR, need to be confirmed in prospective studies with pre-defined endpoints.

5. Conclusions

Invasively assessing microvascular resistance (baseline IMR ≥ 16.0) in the early post-transplant period was independently associated with subsequent AAR during the first year post-transplant. Our findings suggest that early invasive assessment of the coronary microcirculation may enhance patient risk stratification and targeted medical therapy to improve clinical outcomes.

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