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Coronary Flow Reserve by Transthoracic Echocardiography Predicts Epicardial Intimal Thickening in Cardiac Allograft Vasculopathy

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Cardiac allograft vasculopathy (CAV) is the leading cause of morbidity and mortality in heart transplantation (HT). We sought to investigate the role of coronary flow reserve (CFR) by contrast-enhanced transthoracic echocardiography (CE-TTE) in CAV diagnosis. CAV was defined as maximal intimal thickness (MIT) assessed by intravascular ultrasound (IVUS) ≥0.5 mm. CFR was assessed in the left anterior descending coronary artery in 22 HT recipients at 6 ± 4 years post-HT. CAV was diagnosed in 10 patients (group A), 12 had normal coronaries (group B). The mean MIT was 0.7 ± 0.1 mm (range 0.03–1.8). MIT was higher in group A (1.16 ± 0.3 mm vs. 0.34 ± 0.07 mm, $p < 0.0001$). CFR was 3.1 ± 0.8 in all patients and lower in group A (2.5 ± 0.6 vs. 3.7 ± 0.3, $p < 0.0001$). CFR was inversely related with MIT ($r = -0.774$, $p < 0.0001$). A cut point of ≤2.9, identified as optimal by receiver operating characteristics analysis was 100% specific and 80% sensitive (PPV = 100%, NPV = 89%, Accuracy = 91%). CFR assessment by CE-TTE is a novel noninvasive diagnostic tool in the detection of CAV defined as MIT ≥0.5 mm. CFR by CE-TTE may reduce the need for routine IVUS in HT.

Key words: Cardiac allograft vasculopathy, coronary flow reserve, diagnosis, follow-up studies, heart transplantation

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Introduction

Cardiac allograft vasculopathy (CAV) is the leading cause of mortality after heart transplantation (HT) (1). In CAV, both epicardial coronary vessels and the microvasculature may be affected (2). Histopathologically, CAV is characterized by discrete intracellular endothelial changes and diffuse concentric intimal thickening (3). Coronary angiography is the most common tool of screening for CAV; however, it is limited in detecting diffuse intimal thickening (4). Intravascular ultrasound (IVUS) is more sensitive, but it requires some degree of expertise to perform and interpret the images, it is time consuming and expensive, and it only interrogates the epicardial coronary system. Coronary flow reserve (CFR) measurements by intracoronary Doppler flow wire may provide functional assessment of the microvasculature in CAV, but it is an invasive procedure. We have recently applied a new noninvasive technique based on contrast-enhanced transthoracic echocardiography (CE-TTE) for assessing CFR in the left anterior coronary descending artery (LAD) in HT patients (5,6). CFR by CE-TTE has been shown to correlate with angiographically detectable coronary artery lesion severity as well as intracoronary Doppler flow wire measurements (7), and to stratify the risk of cardiac events in HT patients (6). We assessed the diagnostic potential of CFR by CE-TTE in CAV detection defined by IVUS and to test whether the extent of intimal thickening affects coronary flow velocity during adenosine infusion in HT recipients with normal coronary angiograms.

Methods

Study patients

We studied 22 consecutive HT recipients with normal coronary angiogram (20 male, aged 50 ± 7 years at HT, range 36–61, mean ischemia time 169 ± 37 min), at 6 ± 4 years post-HT. Our immunosuppression protocol consisted of Cyclosporin A, Azathioprine or Mycophenolate mofetil and steroids (triple therapy) as previously detailed (2,8). Twenty-four healthy control subjects, matched for age and gender, were recruited from local community. In the control subjects, the absence of cardiovascular diseases was evaluated by a clinical history and examination and, when available, echocardiography and coronary angiography. The study was approved by the institutional ethics committee, and all patients gave written, informed consent.

Echocardiography

An echocardiogram was obtained in all patients within 48 h of coronary angiography. From the parasternal long-axis view, M-mode measurements were performed to determine the end-diastolic thickness of the interventricular septum and the left ventricular posterior wall. Left ventricular hypertrophy was defined as a septal plus posterior wall thickness ≥24 mm (9).

Left ventricular ejection fraction was measured using Simpson's method. CFR was evaluated using CE-TTE before and after adenosine infusion. The method has been previously described in detail (7).

Contrast-enhanced transthoracic Doppler echocardiography

Echocardiography was performed for coronary flow evaluation using CE-TTE before and after adenosine infusion, with an ultrasound system (Sequoia C256, Acuson, Mountain View, CA) connected to a broad-band transducer with second harmonic capability (3V2c). Briefly, CFR was measured in the distal portion of the LAD, first obtaining a modified foreshortened two-chamber view or, if a distal LAD flow recording was not feasible, using a low parasternal short-axis view of the base of the heart (7). Administration of the contrast agent (Levovist, Schering AG, Berlin, Germany) was performed both before and during adenosine intravenous administration (5).

Coronary flow velocity reserve assessment

All patients had Doppler recordings of the LAD with adenosine infusion at a rate of 0.14 mg/kg/min for 5 min (5). Cardiac drugs were not interrupted before testing, although all methylxantine-containing substances or medications were withheld 48 h before the study. CFR in the LAD was calculated, as the ratio of hyperemic to basal diastolic flow velocity, by an experienced echocardiographer, blind to angiographic and clinical data. For each variable in the CFR calculation, the highest three cycles were averaged (5).

IVUS/diagnosis of CAV

After anticoagulation with 5000–10 000 units of heparin and infusion of 200 μg intracoronary nitroglycerin, standard coronary angiography was performed in order to exclude LAD stenosis, which might contraindicate IVUS performance (10). IVUS images were obtained using a commercially available 3F IVUS catheter (Volcano Corporation, Rancho Cordova, CA, or Atlantis SR Pro 2, Boston Scientific, Natick, MA) placed under fluoroscopic guidance to the periphery of the LAD. Automatic pullback (1 mm/s motorized device) was performed and images were stored in a CD-ROM for subsequent analysis off line by an experienced observer, who was always blinded to the patients' characteristics and echocardiographic findings. External elastic membrane and lumen cross-sectional areas were identified and measured by manual planimetry. Following American College of Cardiology recommendations (11), we measured maximal intimal thickness (MIT); the average MIT was derived by averaging the MIT from the all sites examined. CAV was defined as MIT $\geq$0.5 mm (11). The area bounded by the external elastic membrane was considered the external vessel wall area and the difference between the external elastic membrane area and the lumen area was calculated to give the intimal (otherwise known as intima-media) area. An intimal index was calculated as intima area/(intima + lumen area). The luminal, vessel and plaque volumes (in cubic millimeters) of each segment were calculated as cross-sectional areas (lumen area, vessel area and plaque area) $\times$ segment length of 2 mm. Total plaque volume was obtained by adding up the measurements of all vascular segments. Since *de novo* graft atherosclerosis often has a diffuse distribution, unlike focal donor-related lesions, we averaged the measurements obtained from serial cross-sectional images taken every 2 mm of proximal 30 mm of LAD to minimize bias in the matching of individual sites in artery wall evaluation (12). To assess the reproducibility of IVUS measurements, we performed two subsequent motorized pullbacks of the IVUS catheter during the same IVUS examination. Mean values of total vessel and lumen areas were calculated on the basis of these two recordings, matching 28 coronary segments. The intraobserver error for vessel and lumen area analysis was 0.46 $\pm$ 0.61% and 1.95 $\pm$ 1.14%, respectively. The correlation coefficient between the two sets of measurements was 0.95 for vessel wall and 0.92 for lumen areas. The interobserver error for vessel and lumen analysis was 1.66 $\pm$ 1.25% and 3.01 $\pm$ 2.2%, respectively. The correlation coefficient between the measurements performed by two different observers was 0.99 for both vessel wall and lumen areas. These reproducibility assessments are in line with previous reports (13).

Fractional flow reserve (FFR) measurements

FFR, defined as the mean distal coronary pressure, measured with the pressure wire (Radi Medical Systems, Wilmington, MA), divided by the mean proximal coronary pressure, measured with the guiding catheter, at maximum hyperemia, was measured after administering 48 μg of intracoronary adenosine. The lower limit of the normal range for FFR was below 0.94 (14).

Statistical analysis

Results are expressed as mean $\pm$ standard deviation. CFR distribution was assessed by Shapiro–Wilk test, and it was not significantly different from normality ($p = 0.142$). Student's *t*-test and chi-square test were used as appropriate. Sensitivity, specificity, positive and negative predictive values were determined according to standard definitions. IVUS evidence of CAV was taken as the positive reference standard. Receiver operating characteristics (ROC) curve analysis was generated to test the predictive discrimination of patients with and without CAV. Pearson's test was used to correlate CFR and data derived from IVUS analysis (MIT, intimal index and plaque volume). Intraobserver and interobserver reproducibilities of CFR were evaluated by linear regression analysis and expressed as correlation of coefficients (r) and standard error of estimates (SEE), and by the intraclass correlation coefficient. Reproducibility is considered satisfactory if the intraclass correlation coefficient is between 0.81 and 1.0. Intraobserver and interobserver reproducibility measurements were calculated in all 22 patients. Probability levels of <0.05 were considered statistically significant. Data were analyzed with SPSS software version 13.0 (SPSS, Inc., Chicago, IL). The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Baseline clinical and diagnostic features

All patients had normal coronary angiograms. Of the 22 IVUS, 10 (45%) were classified as abnormal (MIT $\geq$0.5 mm) (group A), 12 (55%) had normal coronaries (MIT $<$0.5 mm) (group B). Time from HT was longer in group A (8 $\pm$ 3 vs. 6 $\pm$ 2 years, $p = 0.042$). Group A was associated with donor male gender (90% vs. 33%, $p = 0.02$). Recipient age at HT, male recipient gender, incidence of gender mismatch and donor age were similar in the two groups. Incidences of hypertension, diabetes and hypercholesterolemia after HT were comparable between the two groups. End diastolic dimensions, ejection fraction and mass were similar in the two groups. No regional wall motion abnormalities were detected. All patients were on aspirin and statins. No differences in immunosuppressive and cardiovascular therapies were observed (Table 1).

Comparison between HT recipients and control subjects

CFR in HT recipients was comparable to control subjects (3.1 $\pm$ 0.8 vs. 3.4 $\pm$ 0.7, $p = 0.3$). CFR in HT patients with MIT $\geq$0.5 mm was lower than in controls (2.5 $\pm$ 0.6 vs. 3.4 $\pm$ 0.7, $p = 0.001$). CFR in HT patients without

Table 1: Recipient and donor characteristics

	Group A (MIT ≥0.5 mm) (n = 10)	Group B (MIT <0.5 mm) (n = 12)	p
Age at HT, years	48 ± 6	51 ± 7	0.6
Male gender, n (%)	9 (90)	11 (92)	0.8
Ischemic time, min	170 ± 37	183 ± 30	0.5
Time from HT, years	8 ± 3	6 ± 2	0.04
Hypertension, n (%)	8 (80)	8 (66)	0.2
Diabetes, n (%)	2 (20)	2 (16)	0.7
Hypercholesterolemia, n (%)	4 (40)	3 (25)	0.6
Donor age, years	35 ± 12	37 ± 10	0.5
Donor male gender, n (%)	9 (90)	4 (33)	0.02
Gender mismatch, n (%)	3 (30)	3 (25)	0.5
End-diastolic diameter, mm	46 ± 6	48 ± 5	0.9
End-systolic diameter, mm	25 ± 3	27 ± 2	0.7
LVEF (%)	68 ± 5	66 ± 3	0.7
Interventricular septum thickness, mm	12 ± 0.5	12 ± 0.3	0.8
Posterior wall thickness, mm	11 ± 0.4	11 ± 0.3	0.9
IHD pre-HT, n (%)	4 (50)	3 (25)	0.6
Total numbers of rejections	3.1 ± 2.5	3 ± 2	0.5
Cyclosporine, n (%)	10 (100)	12 (100)	1
Mycophenolate mofetil, n (%)	5 (50)	5 (42)	0.6
Azathioprine, n (%)	5 (50)	7 (58)	0.8
Prednisone, n (%)	10 (100)	12 (100)	1
ACE-inhibitors, n (%)	4 (40)	4 (33)	0.5
Beta-blokers, n (%)	1 (10)	3 (16)	0.4
Calcium antagonists, n (%)	2 (20)	3 (16)	0.7

Unless specified otherwise, the values are means ± SD.
HT = heart transplantation; IHD = ischemic heart disease; LVEF = left ventricular ejection fraction.

MIT ≥0.5 mm was comparable to controls (3.7 ± 0.3 vs. 3.4 ± 0.7, p = 0.2). The prevalence of CFR ≤2.5 was higher in HT patients compared to controls (27.3% vs. 4.3%, p = 0.04) and CFR is significantly lower in HT patients with CFR ≤2.5 compared with the remaining patients' population (2 ± 0.4 vs. 3.5 ± 0.4, p < 0.0001).

IVUS and FFR analysis

IVUS was performed successfully in all patients. The mean MIT was 0.7 ± 0.1 mm (range 0.03–1.8 mm) and intimal index was 0.13 ± 0.11 (range 0.05–0.55). MIT was higher in group A (1.16 ± 0.3 mm vs. 0.34 ± 0.07 mm, p < 0.0001). FFR was successfully measured in all patients. The mean FFR was 0.90 ± 0.05. In 64% of cases, the FFR was less than the normal threshold of 0.94. In only one patient (4.5%), FFR was ≤0.80, the upper boundary of the gray zone of the ischemic threshold, and in none the FFR was ≤0.75 (15). FFR was inversely related to MIT (r = −0.399, p = 0.054).

Noninvasive CFR evaluation

CE-TTE studies were always well tolerated. Overall, during adenosine infusion heart rate increased compared to baseline (90 ± 13 beats/min vs. 83 ± 14 beats/min, p < 0.0001), systolic blood pressure decreased (127 ± 18 mmHg vs. 135 ± 22 mmHg, p = 0.04), as well as diastolic blood pressure (77 ± 13 mmHg vs. 82 ± 15 mmHg, p = 0.03), whereas peak diastolic velocity in the LAD increased (82 ± 27 cm/s vs. 26 ± 7 cm/s, p < 0.0001). CFR was 3.1 ± 0.8 in the whole patient group. Adenosine peak diastolic velocity and CFR were lower in group A (68 ± 28 cm/s vs. 100 ± 13 cm/s, p = 0.01 and 2.5 ± 0.6 vs. 3.7 ± 0.3, p < 0.0001, respectively) (Figure 1). Figure 2 shows two representative examples.

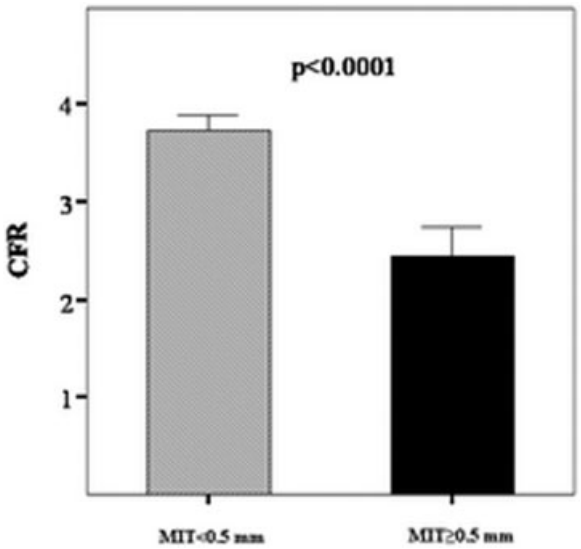

Figure 1: Coronary flow velocity reserve by contrast-enhanced transthoracic echocardiography in patients with and without maximal intimal thickness ≥0.5 mm. Error bars express standard deviations.

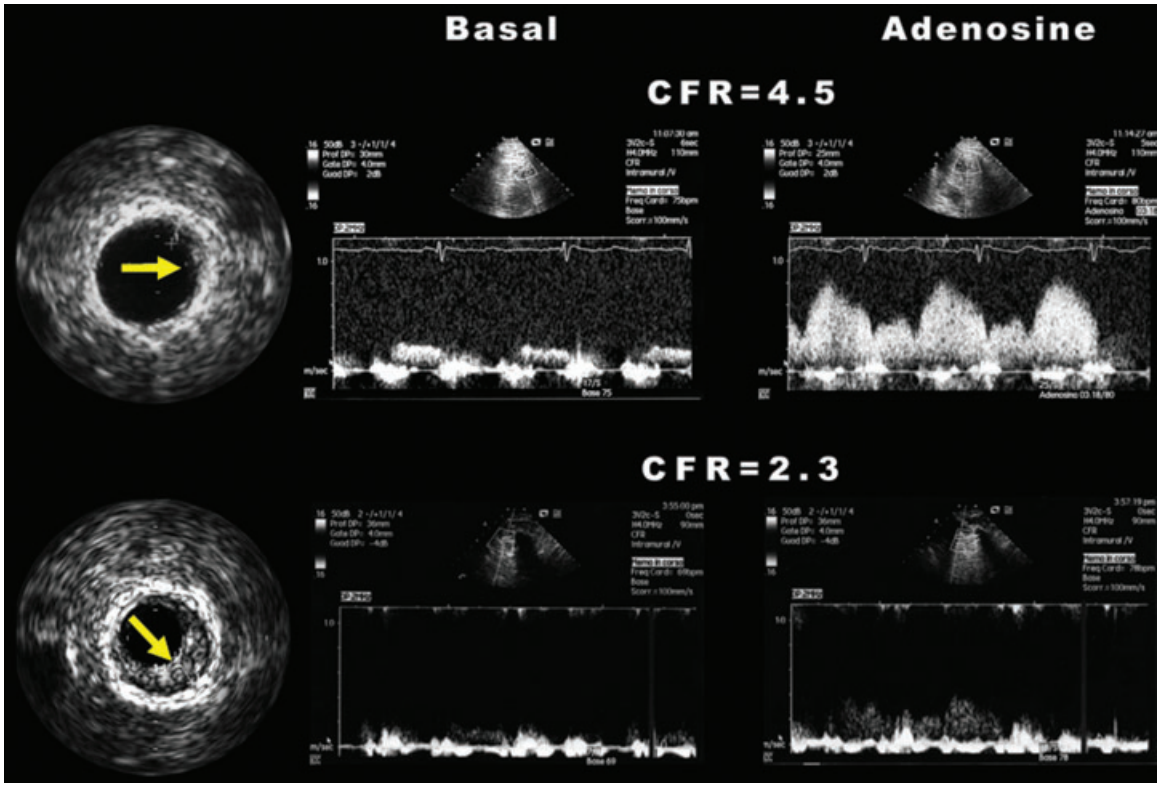

Figure 2: (A) Maximal intimal thickness (MIT) <0.5 mm (upper left panel). Coronary flow velocity assessed by contrast-enhanced transthoracic echocardiography (CE-TTE) on the same day of intravascular ultrasound (IVUS) increased from baseline (upper middle panel) to postadenosine administration (upper right panel), with a calculated coronary flow reserve (CFR) of 4.5. (B) MIT >0.5 mm (lower left panel). Coronary flow velocity assessed by CE-TTE on the same day of IVUS increased from baseline (lower middle panel) to postadenosine administration (lower right panel), with a calculated CFR of 2.3.

Severe (<2) CFR impairment was found in 3 out of 10 (30%) patients with MIT ≥0.5 mm, but in none of those with MIT <0.5 mm ($p = 0.04$).

Diagnostic power of CFR by CE-TTE in the identification of MIT ≥0.5 mm

ROC analysis for separation of the presence or absence of MIT ≥0.5 mm was performed. The area under the ROC curve (AUC) of 0.903 has an SE of 0.022, yielding a 95% confidence interval of 0.941 to 1 ($p < 0.0001$) (Figure 3). A cut point of ≤2.9, identified as optimal by ROC analysis, was 100% specific and 80% sensitive (positive predictive value = 100%, negative predictive value = 89%) (OR = 8, $p = 0.007$). Accuracy was 91%.

Intra- and interobserver reproducibility of CFR by CE-TTE

Intraobserver and interobserver reproducibilities of CFR measurements were assessed by repeating CFR evaluation twice, 1 h apart, by the same operator (FT) in all patients and by another operator (EO) in all patients as well. The intraobserver reproducibility was high ($r = 0.95$, SEE = 0.11); intraclass correlation coefficient was 0.976. The interobserver reproducibility was also high ($r = 0.94$, SEE = 0.12); intraclass correlation coefficient was 0.968.

Relation between CFR and risk factors for coronary allograft vasculopathy

No relation between CFR and number or severity of previous rejections was observed ($r = -0.386$, $p = 0.3$ and $r = -0.350$, $p = 0.4$, respectively). Moreover, there was no correlation between CFR and cytomegalovirus status or cytomegalovirus infection after HT. CFR was 3 ± 0.8 in donor CMV−/recipient CMV− patients, 3.1 ± 0.8 in donor CMV−/recipient CMV+ patients, 2.9 ± 0.7 in donor CMV+/recipient CMV− patients and 3 ± 0.7 in donor CMV+/recipient CMV+ patients ($p = 0.6$). CFR was comparable between patients with and without documented CMV infection (2.8 ± 0.8 vs. 3 ± 0.7, $p = 0.2$). CFR was unrelated to pre-HT risk factors for coronary artery disease, donor/recipient gender (male donor CFR 3 ± 0.8 vs. female donor CFR 2.9 ± 0.7, $p = 0.5$; male recipient CFR 3 ± 0.8 vs. female recipient CFR 2.9 ± 0.8, $p = 0.4$), gender mismatch (mismatch + 2.8 ± 0.6 vs. mismatch − 3 ± 0.8, $p = 0.2$), and donor/recipient age at HT ($r = 0.601$, $p = 0.1$ and $r = 0.012$, $p = 0.9$, respectively). The time from HT to CE-TTE and IVUS was unrelated to the degree of intimal

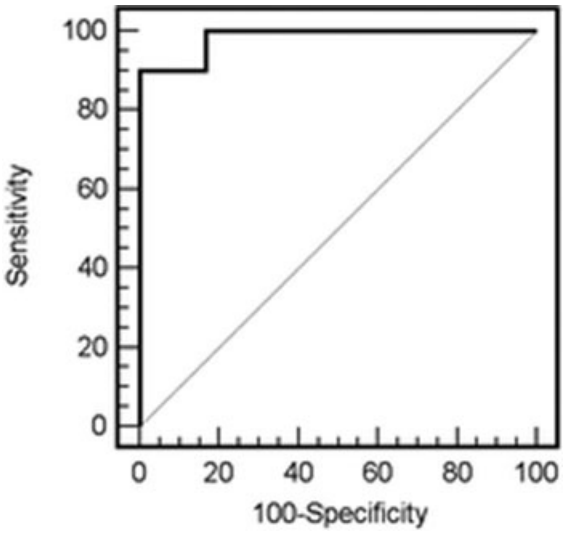

Figure 3: Receiver operating characteristics analysis for separation of the presence or absence of cardiac allograft vasculopathy. True-positive rate (sensitivity) in the ordinate is plotted against false-positive rate (100-specificity) on the abscissa. The area under the ROC curve of 0.903 has an SE of 0.022, yielding a 95% confidence interval of 0.941 to 1, indicating that this area is significantly different from the area of 0.500 under the diagonal identity line ($p < 0.0001$).

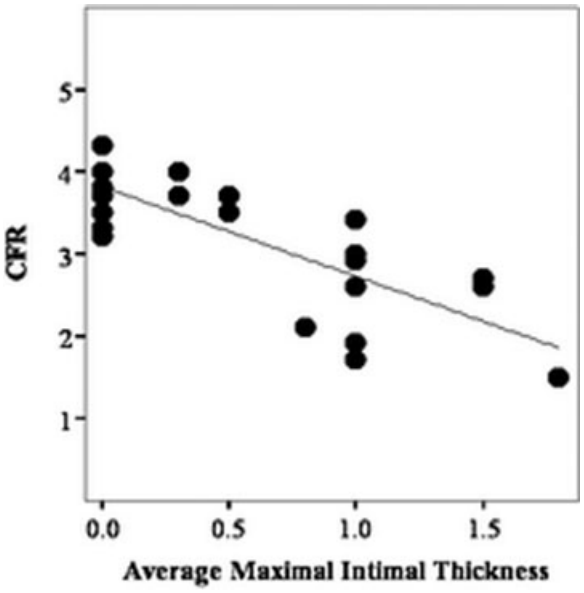

Figure 4: Coronary flow reserve (CFR) by contrast-enhanced transthoracic echocardiography (y-axis) as a function of average maximal intimal thickness (x-axis) in the territory of the left anterior coronary descending artery. Increases in intimal thickness were associated with decreases in CFR ($r = -0.796$; $p < 0.0001$).

thickening, resting and hyperemic coronary flow velocity or CFR and with FFR.

Relation between CFR by CE-TTE and MIT

CFR was inversely related to MIT ($r = -0.796$; $p < 0.0001$) (Figure 4), to intimal index ($r = -0.454$; $p = 0.01$) (Figure 5) and to plaque volume ($r = -0.775$; $p < 0.0001$) (Figure 6).

To determine whether the intimal hyperplasia of epicardial arteries contributed to the CFR reduction, we separately analyzed patients with normal ($\geq$0.94) FFR. In these patients, CFR by CE-TTE was correlated with MIT ($r = -0.814$; $p = 0.01$). The correlation between CFR and MIT was no more present in patients with FFR $<$0.94 ($p = 0.151$). MIT is minor and CFR is higher in patients with a FFR $\geq$0.94 (0.26 ± 0.1 vs. 0.81 ± 0.1, $p = 0.01$ and 3.7 ± 0.3 vs. 2.7 ± 0.7, $p = 0.002$, respectively).

Relation between CFR by CE-TTE and FFR

CFR by CE-TTE correlated weakly with FFR ($r = 0.436$, $p = 0.048$). In 14% of cases, CFR by CE-TTE was $\leq$2. CFR and FFR were normal in 8 (36%) patients. FFR was abnormal and CFR normal in 11 (50%), and FFR and CFR were both abnormal in 3 (14%) patients. In one patient, FFR was almost normal (0.93) and CFR was severely reduced (1.9), suggesting predominant microcirculatory dysfunction (Figure 7); in this patient MIT was 1 mm.

Discussion

This study demonstrates, for the first time, that CFR by CE-TTE in the LAD is a feasible and accurate noninvasive tool for the detection of MIT $\geq$0.5 mm identified by IVUS, the gold standard for the diagnosis of CAV in HT (11).

Many authors studied the relation between CFR and angiographic CAV. Some studies, mainly using invasive Doppler flow wire and endothelial-independent vasodilatation, showed that coronary vasodilatatory capacity is preserved in HT patients without angiographic CAV (16,17) and is impaired in patients with mild CAV (5). Our group demonstrated that CFR by CE-TTE may offer promise as a simple, readily available, objective, noninvasive diagnostic tool for the detection of early and severe CAV (5), and that a lower CFR is the main independent predictor of poor outcome in long-term clinically stable HT patients (6). However, to the best of our knowledge, this study is the first on CFR by CE-TTE and CAV in epicardial arteries defined by IVUS.

Echocardiography for the diagnosis of CAV defined by IVUS

Dobutamine for the assessment of wall motion and left ventricular size and function is the most frequently used technique. Spes et al. analyzed the diagnostic value of dobutamine stress echocardiography for noninvasive assessment of CAV (18). They found that resting 2D

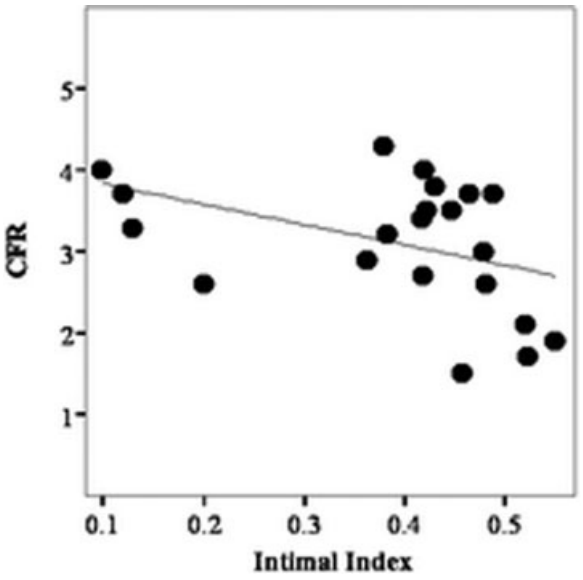

Figure 5: Coronary flow reserve (CFR) by contrast-enhanced transthoracic echocardiography (y-axis) as a function of intimal index (x-axis) in the territory of the left anterior coronary descending artery. Increases in intimal index were associated with decreases in CFR ($r = -0.454$; $p = 0.01$).

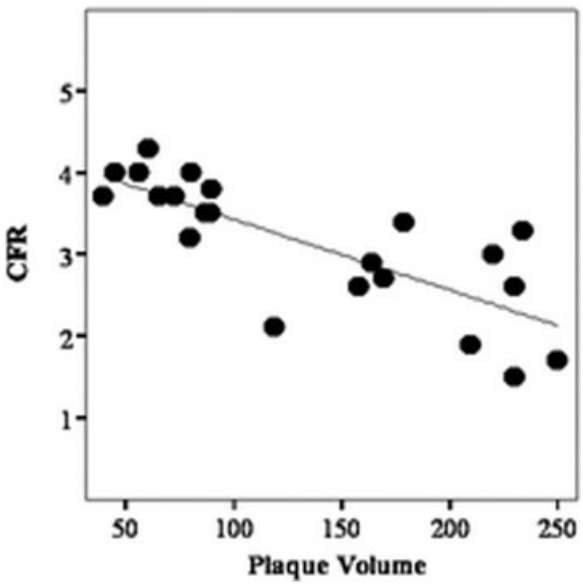

Figure 6: Coronary flow reserve (CFR) by contrast-enhanced transthoracic echocardiography (y-axis) as a function of plaque volume (x-axis) in the territory of the left anterior coronary descending artery. Increases in plaque volume were associated with decreases in CFR ($r = -0.775$; $p < 0.0001$).

echocardiography detects CAV defined by IVUS with a sensitivity of 57% and a specificity of 88%. Dobutamine stress echocardiography increased the sensitivity to 72%. Moreover, M-mode analysis increased the sensitivity of 2D rest and stress analysis to 85% but reduced the specificity to 82% (18). In other studies, the sensitivity of stress echocardiography ranged between 15% and 79%, and the specificity ranged between 83% and 85%. In some, but not all studies, the accuracy was comparable to CFR by CE-TTE. However, dobutamine stress echocardiography depends on image quality and cannot be used in all patients. Furthermore, dobutamine echocardiography requires more experience than CFR by CE-TTE (6,18). CFR by CE-TTE is also more accurate for predicting cardiac events (6) and for verifying the functional significance of CAV.

Tissue Doppler imaging is another growing practice in the nontransplant population. The limited data in HT showed that there are significant changes for both systolic and diastolic parameters. Compared with patients without CAV, even those with CAV defined by IVUS showed significant differences for all parameters (19). In particular, sensitivity for systolic and diastolic parameters ranged between 83% and 93%, and the specificity between 92% and 96% (19). However, this methodology has some limitations. All parameters should be corrected to the heart rate and this is time consuming. Moreover, the reproducibility of measurements can be very high for clinical use.

Influence of intimal thickening on CFR

The relation between CFR and epicardial intimal thickening has been previously studied by other methods (20,21).

CFR was not reduced in our whole patient's population and in HT patients without MIT $\geq$0.5 mm. According to these results, previous studies described a normal endothelium-independent flow response in HT recipients using IVUS, intracoronary Doppler flow wire or PET (20,21).

In our study, CFR was reduced in patients with CAV defined by IVUS, and average MIT and CFR were significantly correlated. By using histology or Doppler flow wire, previous studies investigating whether microvasculopathy occurs in concordance with intimal hyperplasia in epicardial arteries yielded conflicting results (20–22). However, these studies enrolled patients with either angiographic stenosis or with abnormal epicardial coronary physiology (14,23). The inverse correlation between MIT and CFR is evident in our patients with functionally normal epicardial coronary arteries (FFR $\geq$0.94). This correlation may indicate that, in the early stage of CAV, both epicardial arteries and microvasculature are concordantly involved. To the best of our knowledge, this is the first study showing a relation between MIT and CFR by CE-TTE and identifying a CFR cutoff for the diagnosis of CAV defined by IVUS. However, CFR interrogates the entire coronary circulation (25% epicardial function and 75% microvascular system). Therefore, the concordance between CFR and MIT could be because there was little microvascular dysfunction, as evidenced

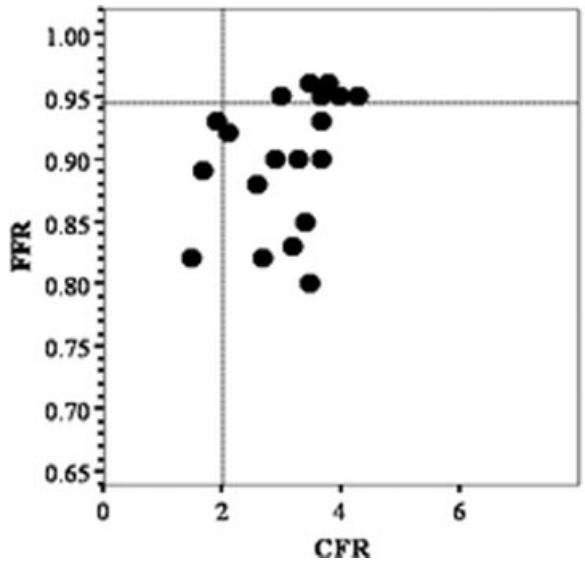

Figure 7: Scatterplot of fractional flow reserve (FFR) and coronary flow reserve (CFR) by contrast-enhanced transthoracic echocardiography values in each patient. Dashed lines represent FFR and CFR normal cutoff values.

by the fact that there were no patients with normal FFR and abnormal CFR (<2). The only way to distinguish between epicardial and microvascular dysfunction would be to have a simultaneous, invasive and independent assessment of the epicardial artery by measuring FFR and of the microvasculature by calculating the index of microvascular resistance with a single coronary pressure wire, as suggested in the PITA II study (24). However, this independent assessment is, by now, noninvasively impossible.

The combination of FFR and CFR by CE-TTE may provide information about the functional status of the coronary system that would not be available with either each measurement alone or coupled with IVUS. In particular, the combination of FFR and CFR by CE-TTE may identify patients with prevalent microvasculopathy. This finding is clinically relevant because the presence of microvascular dysfunction has been correlated with future development of epicardial allograft vasculopathy and cardiac events (6,25). Fearon et al., evaluating a new method for simultaneously measuring FFR and CFR with a single wire, achieved similar results (14). In this very elegant study, Fearon et al. showed that the assessment of FFR and CFR simultaneously help us to distinguish between abnormal epicardial and microvascular physiology and revealed that a different proportion of patients have predominant microvascular dysfunction. They conclude that the ability to detect and distinguish changes in epicardial and microvascular function in HT patients may aid in identifying modifiable factors that lead to transplant arteriopathy. We think that PITA study is a milestone on this topic. However, these authors applied, even if very valid, an invasive method to study the coronary circulation. We aimed to identify a noninvasive method that allows us to reduce the number of coronary angiography and IVUS, and at the same time allows us to have information about the function of the coronary circulation. While remaining the undisputed superiority of invasive methods, every cardiac transplant group feels the need for a noninvasive method that can be applied to large populations of patients and at a lower cost compared to costs of invasive procedures and with low risk to the patient. We do not believe that the noninvasive CFR measurement may replace the invasive one, which allows us to have information also on FFR, but certainly it can be very useful in the clinical follow-up of HT patients.

Study Limitations

The sample size is relatively small, even if similar to previous studies (23–26), and not powered to state with certainty that the groups were comparable. Nevertheless, our study represents the largest CE-TTE and IVUS study after HT reported to date.

A recent study suggests that progression of maximal intimal thickening $\geq$ 0.5 mm in the first year after HT appears to be a reliable marker for subsequent outcome (26). In this study, without serial examinations, the progression of intimal thickness and CFR cannot be estimated. Therefore, a larger study, with serial measurements would provide a better insight into the correlation between macro- and microvasculopathy. Currently, we are following these patients for cardiac events and by repeating IVUS and CFR by CE-TTE.

A limitation of this study is that the 1 mm/s pull back of the IVUS catheter does not allow as accurate a determination of intimal thickening as 0.5 mm/s.

CFR was not quantified invasively. Therefore, we do not have a comparison of an invasive method of calculating CFR with the noninvasive method. However, CFR by CE-TTE in the LAD has already been validated against Doppler flow wire measurements by our group, thus the validation was beyond the aim of our study (7,27).

The individual therapeutic protocol might have affected CFR. However, no differences in CFR were detected between patients who were taking calcium antagonists or ACE inhibitors and in patients with different immunosuppressive regimen.

Acute rejection may affect CFR. In this study, on stable long-term HT recipients with preserved ejection fraction, no biopsies were taken. The possibility that CFR impairments are related to undetected acute rejection is unlikely. Acute rejection prevalence is low after the first year, and in none of our patients acute rejection was clinically suspected or diagnosed in the following months.

Conclusions

CFR assessment by CE-TTE is a novel noninvasive diagnostic tool in the detection of CAV defined as MIT $\geq$0.5 mm.

The microvascular dysfunction, as assessed by CFR, correlates with intimal hyperplasia measured by IVUS in patients with physiologically normal epicardial coronary arteries, suggesting the possible concordant involvement of both macro- and microvascular system in early CAV.

CFR by CE-TTE, coupled with IVUS, may help to detect and distinguish epicardial disease and microvascular dysfunction, emerging as a new noninvasive, useful tool to monitor the course of CAV. Thus, our data provide a rationale for including CFR by CE-TTE in future clinical trials aimed at assessing short-term or long-term pharmacological interventions for CAV prevention or stabilization.

Conflict of Interest

None declared.

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