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Coronary Flow Reserve by Contrast-Enhanced Echocardiography: A New Noninvasive Diagnostic Tool for Cardiac Allograft Vasculopathy

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Noninvasive tests have proven unsatisfactory in cardiac allograft vasculopathy (CAV) diagnosis. We assessed coronary flow reserve (CFR) by contrast-enhanced transthoracic echocardiography (CE-TTE) in heart transplantation (HT). CFR was assessed in the left anterior descending coronary artery in 73 HT recipients (59 male, aged 50 $\pm$ 12 years at HT), at 8 $\pm$ 4.5 years post-HT. CFR measurements were taken blindly from coronary angiographies. CFR cut points were the standard value of $\leq$2 and those defined by receiver operating characteristics (ROC) curve analysis. CFR was lower in patients with CAV (2.3 $\pm$ 0.7 vs. 3.2 $\pm$ 0.5, $p < 0.0001$). The $\leq$2 cut point was 100% specific and 38% sensitive. The $\leq$2.7 cut point, optimal by ROC analysis, was 87% specific and 82% sensitive. Accuracy rose from 71% with the standard $\leq$2 cut point to 85% with the optimal cut point of $\leq$2.7. CFR by CE-TTE may offer promise as a novel, easily repeatable and accurate noninvasive tool in CAV detection. However, further longitudinal studies in larger patient cohorts are warranted before widespread adoption can be advocated.

Key words: Cardiac allograft vasculopathy, coronary flow reserve, echocardiography, heart transplantation

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Introduction

Cardiac allograft vasculopathy (CAV) is the main limiting factor of long-term survival in heart transplantation (HT), as well as a major cause of graft failure in the first year (1). CAV diagnosis is still based upon invasive methods, in particular selective coronary angiography, intravascular ultrasound (IVUS) (2) and coronary flow reserve (CFR) by intracoronary Doppler flow wire (3). Several noninvasive tests have proven unsatisfactory (4).

We have recently developed a new noninvasive technique based on contrast-enhanced transthoracic echocardiography (CE-TTE) for assessing CFR in the left anterior coronary descending artery (LAD) (5). CFR by CE-TTE correlates with angiographically detectable coronary artery lesion severity and intracoronary Doppler flow wire measurements in ischemic heart disease (6). The aim of this study was to assess the diagnostic potential of CFR by CE-TTE in CAV detection.

Methods

Study patients

Between January 2003 and March 2004, we studied 73 consecutive HT recipients (59 men, aged 50 $\pm$ 12 years, range 16–70, mean ischemia time 178 $\pm$ 46 min). Mean post-HT follow-up at study entry was 8 $\pm$ 4.5 years (range: 2 months to 17 years). Postoperatively, the majority (83%) of patients had received antilymphocyte and/or antithymocite globulin for 3–5 days. Our immunosuppression protocol consisted of standard cyclosporin A (CsA), azathioprine (Aza) and steroid therapy as previously detailed (7,8). The CsA daily dose was adjusted based on trough target levels (C_0), as well as the patient's renal function (assessed by blood creatinine): 150–400 ng/mL (first 3 months), 150–300 (4–12 months), 100–250 (>12 months). Oral prednisone (0.1 mg/kg/day) was associated to CsA and Aza for the first 6 months, than tapered off; low-dose steroids were maintained in the presence of repeated or persistent rejection or of CsA nephrotoxicity. However, standard immunosuppressive therapy was individualized during long-term follow-up. The institutional ethics committee approved the study, and all patients gave written, informed consent.

Angiography/diagnosis of CAV

Cardiac catheterization was performed within 24 h of CFR evaluation by CE-TTE. A cardiologist who was unaware of the clinical and echocardiographic findings reviewed angiograms. Data were analyzed using a qualitative grading system: grade I, normal angiogram; grade II, luminal irregularities, diameter reduction <30%; grade III, diameter reduction <50%; grade IV, diameter reduction $\geq$50% and/or diffuse narrowing of small vessels (9). CAV was defined as angiographic changes of grade II or greater, significant CAV as grade IV angiographic changes.

Table 1: Comparison of recipient and donor features in patients with and without CAV

	Patients with CAV (n = 34)	Patients without CAV (n = 39)	p
Recipient age at HT, years	51 ± 11	49 ± 13	0.5
Follow-up, years	9.8 ± 4	6.4 ± 4.5	0.001
Recipient gender, M/F	32/2	27/12	0.007
ICD/no ICD pre-HT, n (%)	14 (41.2%)/20 (58.8%)	9 (23.1%)/30 (76.9%)	0.09
Body mass index (kg/m^2)	26 ± 4	25 ± 3	0.2
Donor age, years	37 ± 11	33 ± 12	0.2
Donor gender, M/F	26/8	18/21	0.008
Sex mismatch, n (%)	6 (17%)	9 (33%)	0.1
Hypertension after HT, n (%)	21 (62%)	26 (66%)	0.6
Diabetes after HT, n (%)	6 (17%)	5 (13%)	0.5
Serum cholesterol (mmol/L)	5.3 ± 0.9	5.5 ± 1	0.4
Serum triglycerides (mmol/L)	1.6 ± 0.6	1.8 ± 0.9	0.2
Angiographic EF (%)	71 ± 10	76 ± 8	0.01
Interventricular septum thickness (mm)	9.8 ± 0.8	10 ± 1	0.1
Posterior wall thickness (mm)	9.4 ± 0.7	9.7 ± 0.9	0.1
Echo end diastolic volume (mL/m^2)	56 ± 8	55 ± 8	0.8
Echo EF (%)	62 ± 6	63 ± 6	0.2
Haemoglobin (g/L)	13.7 ± 1.1	12.8 ± 1.4	0.006

CAV= cardiac allograft vasculopathy, EF = ejection fraction, F = female, HT = heart transplantation, ICD = ischemic cardiomyopathy, M = male, y = year.

Contrast-enhanced transthoracic Doppler echocardiography

Echocardiography was performed for CFR evaluation using CE-TTE before and after adenosine infusion, with an ultrasound system (Sequoia C256, Acuson, Mountain View, CA) connected to a broadband transducer with second harmonic capability (3V2c). All studies were continuously recorded on 0.5-in. (1.27 cm) S-VHS videotape. 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 (6). If the angle between color flow and the Doppler beam was >20°, angle correction was performed using the software package included in the software unit. Administration of the contrast agent (Levovist, Schering AG, Berlin, Germany) was performed both before and during adenosine intravenous administration (6). Cross-sectional area of the distal sample vessel remains constant at rest and during adenosine-mediated hyperemia, due to the endothelium-independent vasodilation caused by the drug (10).

Coronary flow velocity reserve assessment

All study patients were consecutive and had Doppler recordings of the LAD with adenosine infusion at a rate of 0.14 mg/kg/min for 5 min (6). 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 one experienced echocardiographer, blind to angiographic and clinical data. For each variable in the CFR calculation, the highest three cycles were averaged (6).

Statistical methods

Data were analyzed with SPSS software version 12.0 (Chicago, SPSS, Inc., Chicago, IL). Results are expressed as mean ± standard deviation. Student's *t*-test, chi-square test and analysis of variance (ANOVA) were used as appropriate. Sensitivity (Se), specificity (Sp), positive (PPV) and negative predictive values (NPV) were determined according to standard definitions. Angiographic 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. 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), by the Bland-Altman method and the intraclass correlation coefficient (ICC). Reproducibility is considered satisfactory if the ICC is between 0.81 and 1.0. Intraobserver reproducibility measurements were calculated in 25 randomly selected patients, interobserver reproducibility in the remaining 48 patients. A p value of <0.05 was considered to be significant.

Results

Baseline, clinical and diagnostic features

The baseline features of recipients and donors in patients with and without CAV are shown in Table 1. Patients with CAV had longer follow-up, lower angiographic ejection fraction and higher hemoglobin levels. The presence of CAV was associated with recipient as well as donor male gender. The remaining features were similar in the two groups. Medications in the two groups are shown in Table 2. A higher proportion of patients with CAV were on aspirin and statins. Conversely, CAV was less common among patients treated with CsA, prednisone and Mycophenolate mofetil.

Echocardiographic measurements were similar in patients with and without CAV (Table 1). No regional wall motion abnormality was detected. Of the 73 angiograms 34 (46%) were classified as abnormal, of which 9 (26%) had grade II lesions, 4 (12%) grade III and 21 (62%) grade IV.

CFR evaluation

CE-TTE studies were always well tolerated. Figure 1 shows two representative examples. Overall, during adenosine infusion heart rate increased compared to baseline,

Table 2: Comparison of therapy in patients with and without CAV

	Patients with CAV (n = 34)	Patients without CAV (n = 39)	p
Statins, n (%)	23 (67%)	17 (43%)	0.03
Beta-blockers, n (%)	4 (12%)	3 (8%)	0.5
ACE-inhibitors, n (%)	16 (47%)	16 (41%)	0.6
Diuretics, n (%)	17 (50%)	21 (54%)	0.7
Diltiazem, n (%)	2 (6%)	5 (13%)	0.3
Verapamil, n (%)	2 (6%)	0 (0%)	0.1
Diidropiridine drugs, n (%)	8 (23%)	8 (20%)	0.7
Aspirin, n (%)	25 (73%)	18 (46%)	0.01
Ticlopidine, n (%)	4 (12%)	2 (5%)	0.3
Triple immunosuppressive therapy, n (%)	12 (35%)	7 (18%)	0.09
Double immunosuppressive therapy, n (%)	4 (12%)	9 (23%)	0.2
CsA + PDN, n (%)	7 (20%)	6 (15%)	0.5
CsA, n (%)	9 (26%)	6 (15%)	0.2
CsA + PDN + MMF, n (%)	1 (3%)	8 (20%)	0.02
CsA + MMF, n (%)	0 (0%)	2 (5%)	0.1
Tacrolimus, n (%)	1 (3%)	1 (2%)	0.9

CsA = Cyclosporin A, MMF = Mycophenolate mofetil, PDN = prednisone; other abbreviations as in Table 1.

Table 3: Hemodynamic parameters during CFR evaluation in patients with and without CAV

	Patients with CAV (n = 34)	Patients without CAV (n = 39)	p
Basal heart rate (beats/min)	82 ± 9	87 ± 12	0.08
Adenosine heart rate (beats/min)	89 ± 12	95 ± 12	0.02
Basal systolic blood pressure (mmHg)	134 ± 16	135 ± 20	0.8
Basal diastolic blood pressure (mmHg)	83 ± 12	87 ± 11	0.1
Adenosine systolic blood pressure (mmHg)	126 ± 22	123 ± 21	0.5
Adenosine diastolic blood pressure (mmHg)	78 ± 13	79 ± 14	0.7
Basal peak diastolic velocity (cm/s)	32 ± 13	26 ± 6	0.01
Adenosine peak diastolic velocity (cm/s)	71 ± 29	82 ± 17	0.04
Coronary flow reserve	2.3 ± 0.7	3.2 ± 0.5	<0.0001

Abbreviations as in Table 1.

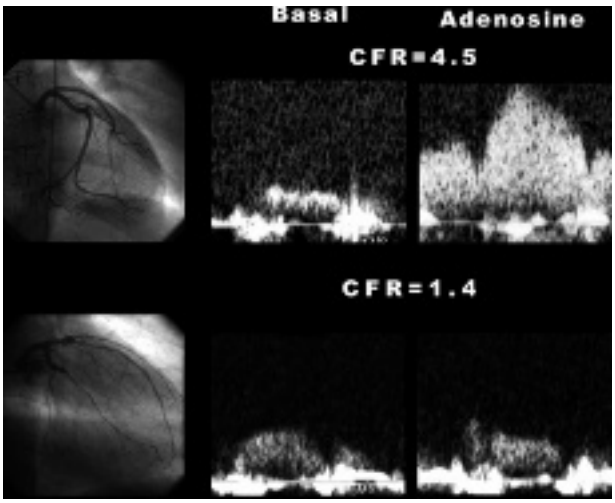

Figure 1: (A) Absence of CAV at nine-year follow-up angiography in a HT recipient (upper left panel). Coronary flow velocity assessed by CE-TTE on the same day of angiography increased from baseline (upper middle panel) to postadenosine administration (upper right panel), with a calculated CFR of 4.5. (B) Diffuse CAV at 10-year follow-up angiography in a heart transplantation recipient (lower left panel). Coronary flow velocity assessed by CE-TTE on the same day of angiography increased from baseline (lower middle panel) to postadenosine administration (lower right panel), with a calculated CFR of 1.4.

(92 ± 13 beats/min vs. 85 ± 11 beats/min, $p < 0.0001$), systolic blood pressure decreased (124 ± 21 mmHg vs. 135 ± 18 mmHg, $p < 0.0001$), as well as diastolic blood pressure (79 ± 13 mmHg vs. 85 ± 12 mmHg, $p < 0.0001$), whereas peak diastolic velocity in the LAD increased (77 ± 23 cm/s vs. 28 ± 10 cm/s, $p < 0.0001$). CFR was 2.79 ± 0.77 in the whole patient group. Patients with CAV had lower adenosine heart rate, higher basal peak diastolic velocity, lower adenosine peak diastolic velocity and lower CFR than those without (Table 3 and Figure 2, top). CFR was negatively associated with higher CAV grades (3.2 ± 0.5 in grade I, 2.7 ± 0.7 in grade II, 2.3 ± 0.3 in grade III and 2 ± 0.6 in grade IV, $p < 0.0001$) (Figure 2, bottom). Severe (≤2) CFR impairment was found in 13 out of 34 (38%) patients with CAV, but in none of those without ($p < 0.0001$).

Diagnostic power of CFR evaluation by CE-TTE

ROC analysis for separation of the presence or absence of CAV is shown in Figure 3. The area under the ROC curve (AUC) of 0.861 has a SE of 0.048, yielding a 95% confidence interval of 0.768 to 0.954 ($p < 0.0001$). A severe CFR impairment (≤2) was 100% specific and 38% sensitive for detecting CAV, with PPV and NPV of 100% and 65%, respectively ($p < 0.0001$). A cut point of ≤2.7, identified as optimal by ROC analysis, was 87% specific and 82% sensitive (PPV = 85%, NPV = 85%) ($p < 0.0001$). Accuracy rose from 71% with the standard ≤2 cut point to 85% with the optimal cut point of ≤2.7.

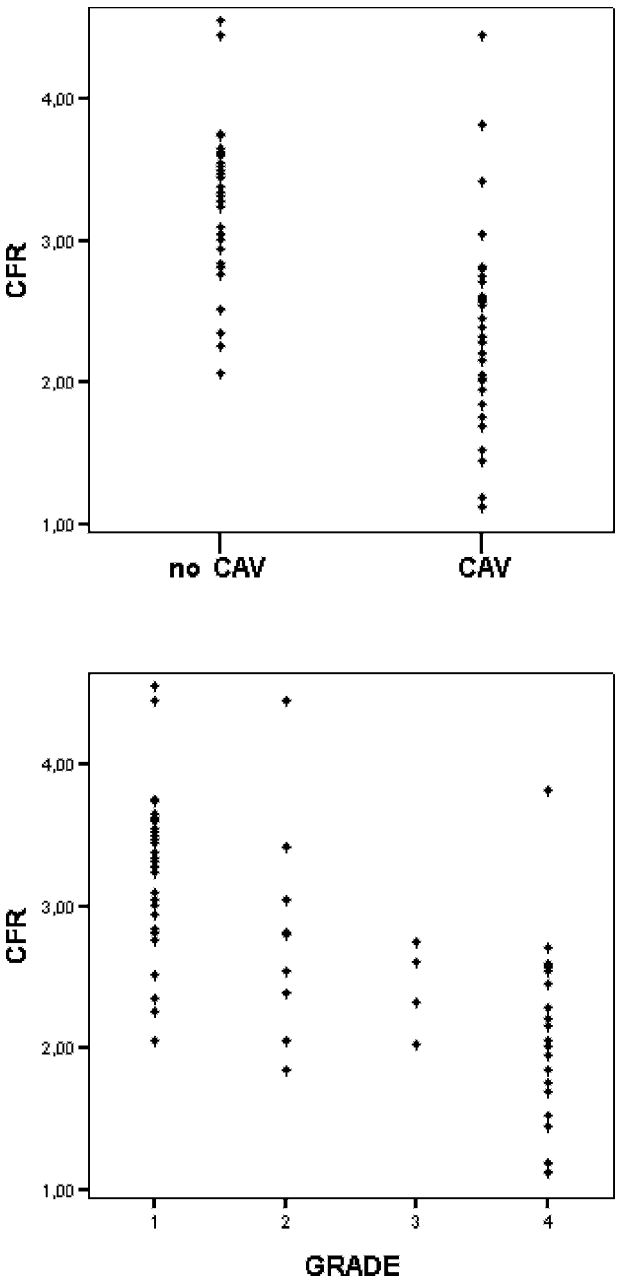

Figure 2: (Top) distribution of CFR results in patients with and without CAV by ANOVA ($p < 0.0001$). (Bottom) distribution of CFR results according to CAV grades by ANOVA ($p < 0.0001$).

By ROC analysis for separation of the presence or absence of significant CAV (defined as any lesion ≥50%), the AUC of 0.881 has a SE of 0.047, yielding a 95% confidence interval of 0.790–0.972 ($p < 0.0001$). The CFR value of ≤2 was 96% specific and 47% sensitive, with PPV and NPV of 85% and 80%, respectively ($p < 0.0001$); the CFR value of ≤2.7 was confirmed as the optimal cut point, with 78% Sp, 96% Se, PPV 67% and NPV 97% ($p < 0.0001$). Accuracy

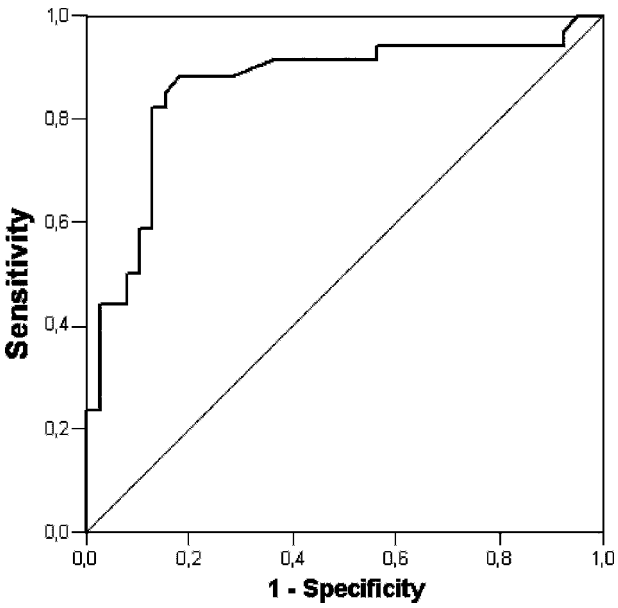

Figure 3: ROC analysis for separation of the presence or absence of CAV. True-positive rate (Se) on the ordinate is plotted against false-positive rate (1—Sp) on the abscissa. The AUC of 0.861 has a SE of 0.048, yielding a 95% confidence interval of 0.768 to 0.954, indicating that this area is significantly different from the area of 0.500 under the diagonal identity line ($p < 0.0001$).

increased from 81% with the standard ≤2 cut point to 83% with the optimal cut point of ≤2.7.

Intra- and Interobserver Reproducibility

Intraobserver reproducibility was high ($r = 0.98$, SEE = 0.12); the mean difference was −0.02 and the upper and lower limits of agreement between the measurements were +0.14 (95% CI, +0.08 to +0.2) and −0.19 (95% CI, −0.26 to −0.13), respectively (Figure 4); ICC was 0.986. Interobserver reproducibility was also high ($r = 0.97$, SEE = 0.16); the mean difference was 0 (no bias was found) and the upper and lower limits of agreement between the two measurements were +0.33 (95% CI, +0.24 to +0.41) and −0.33 (95% CI, −0.41 to −0.24), respectively (Figure 5); ICC was 0.975.

Discussion

This study demonstrates, for the first time, that CFR by CE-TTE in the LAD is a feasible and accurate noninvasive tool for CAV detection, as previously shown and validated against Doppler flow wire measurements in coronary artery disease (5,6). Noninvasive tests are insensitive in CAV diagnosis (4). The role of stress ECG is limited, because of the diminished chronotropic response to physical exercise (4). In the setting of significant CAV, it has been suggested that a strategy, which reserves angiography for patients with echocardiographic resting wall motion

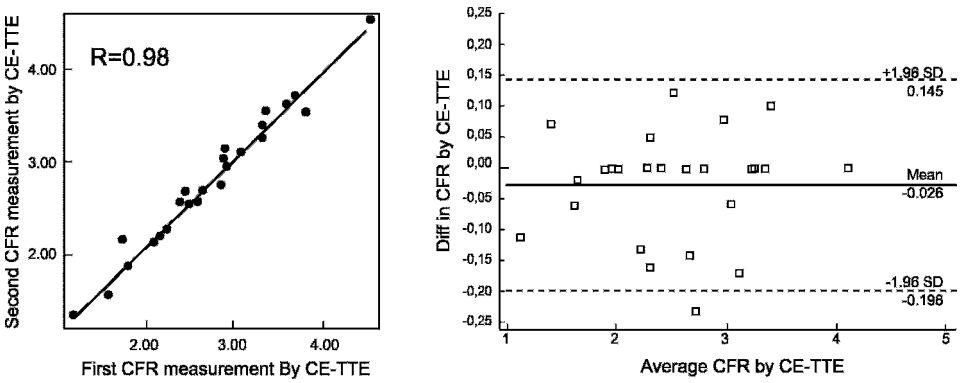

Figure 4: Scattergram (left panel) showing the relation by regression analysis between CFR obtained by the same operator by CE-TTE. Plot of the difference (right panel) between the CFR measurements against their mean is shown (Bland-Altman method). Dotted lines represent boundaries of means ± 2 SD.

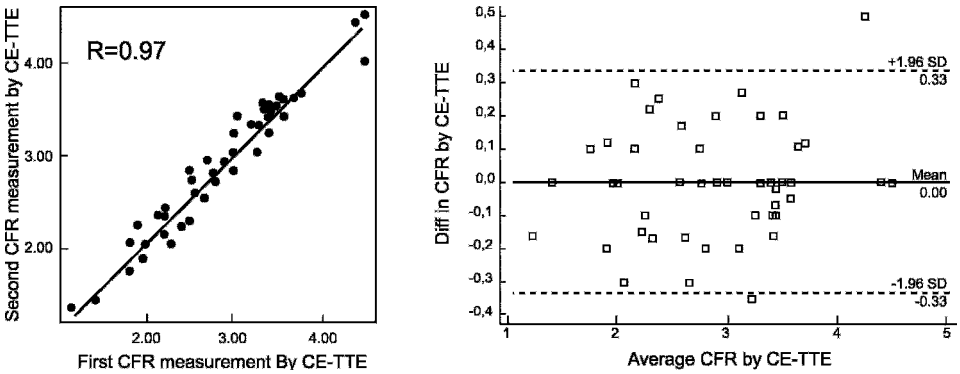

Figure 5: Scattergram (left panel) showing the relation by regression analysis between CFR obtained by two different operators. Plot of the difference (right panel) between the CFR measurements against their mean is shown (Bland-Altman method). Dotted lines represent boundaries of means ± 2 SD.

abnormalities and/or stress perfusion defects may be safe and cost-effective, leading to 85% accuracy (11). Others reported a limited sensitivity (57%) for resting echocardiography (9); in keeping with this, we did not detect resting wall motion abnormalities in patients with significant CAV. Anyhow, our CFR results in significant CAV indicate that CE-TTE, using the optimal ROC defined cut point of 2.7, is comparable to the combination of resting echocardiography and stress scintigraphy (11), with 83% accuracy.

CFR in HT: CE-TTE versus other methods

Although to the best of our knowledge the present study is the first on CFR by CE-TTE, CFR has been previously studied in HT patients by other methods (3,12–16).

Some studies, mainly using Doppler flow wire and hyperemic response to the nonendothelial dependent vasodilator stimulation, have shown that, in keeping with our observations, coronary vasodilatory capacity is preserved in HT recipients without angiographic CAV (12,17) and is impaired in patients with minor epicardial lesions (3). CFR reduction in CAV may reflect a loss in the number of resistance vessels and/or functional impairment of the allograft coronary microvasculature (18). A proportion of our patients (12%) had CFR < 2.5 without CAV; this may relate to functional impairment and/or the fact that standard angiography is relatively insensitive compared with IVUS. Using CFR in conjunction with fractional flow reserve might discriminate these mechanisms in individual patients (3), but this was beyond our study aim.

Conversely, others found that CFR by Doppler flow wire was preserved in HT patients with mild-to-moderate diffuse CAV and was impaired in the absence of CAV (15). The discrepancy may relate to: small sample size and/or very few patients with CAV (13,15), selection bias due to technical difficulty in performing the invasive measurements in severe CAV (14), various definitions of CAV by angiography (13,14) and/or by IVUS (14,15). In addition, noninvasive studies, which failed to show a correlation between the reduced CFR and CAV, used positron emission tomography (15) or MRPI (13), therefore they are not comparable with CE-TTE.

Study limitations

At present, CE-TTE-derived CFR evaluation can be achieved in the LAD, the circumflex and right coronary artery territories; good correlations with invasive intracoronary Doppler flow wire measurements have been shown both in LAD (10) and right coronary artery (19). However, the feasibility of CE-TTE-derived CFR is higher in the LAD (80–98%) than in the right coronary (50–87%) or in the circumflex (43–72%) artery (10). In addition CE-TTE-derived CFR measurements relate to the microcirculatory function, thus the choice of the sample vessel does not affect the results (10). Thus, in this noninvasive study we, as other groups who used invasive Doppler flow wire measurements in HT patients (3), sampled the LAD. In keeping with this, CFR measurements were successfully achieved in the absence of contrast agents in 80% of our patients.

Acute rejection (AR), accordingly to some but not all studies, may affect CFR (17). In the present study, on stable long-term HT patients with preserved ejection fraction, no endomyocardial biopsies were taken. The possibility that our CFR findings are related to undetected AR seems unlikely. AR frequency is low after the first year, and in none of our patients AR was clinically suspected or diagnosed in the following months.

Last but not least, CFR by CE-TTE in the LAD has already been validated against Doppler flow wire measurements (6), thus it was felt ethically unacceptable to perform an additional invasive procedure (20).

Conclusions

CFR by CE-TTE may offer promise as a relatively simple, readily available, objective, noninvasive diagnostic tool for the detection of early and severe CAV. However, further longitudinal studies in larger patient cohorts are warranted before widespread adoption can be advocated.

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