OPEN

Coronary Collateral Circulation: A New Predictor of Mortality in Heart Transplant Recipients With Allograft Vasculopathy

Giovanni Civieri, MD,[1] Giulia Masiero, MD,[1] Elena Osto, MD, PhD,[2,3] Antonio Gambino, MD,[4] Annalisa Angelini, MD,[5] Angela Fraiese, MD,[2] Marny Fedrigo, MD,[5] Giuseppe Toscano, MD,[2] Tomaso Bottio, MD,[2] Martina Perazzolo Marra, MD,[1] Sabino Iliceto, MD,[1] Gino Gerosa, MD,[2] and Francesco Tona, MD, PhD[1]

Background. Coronary collateral arteries (CCAs) are anastomotic channels between vessels; although beneficial in atherosclerosis, their role in heart transplantation (HT) recipients is underinvestigated. CCAs initially develop as microcirculation and cardiac allograft vasculopathy (CAV), promoting immune-dependent proliferative angiogenic response, and play a role in their development. In our hypothesis, ischemia induced by coronary microvascular dysfunction (CMD) triggers the development of CCAs, which are, in turn, less functional as affected by CAV themselves. **Methods.** One hundred twenty-one patients receiving HT at our institution were retrospectively evaluated and were included if transthoracic echocardiography with coronary flow velocity reserve (CFVR) assessment and coronary angiography were performed. CMD was defined as CFVR of ≤2.5. Patients with CAV were enrolled, and their angiograms were reviewed to evaluate the presence of CCAs. Cardiovascular mortality was assessed as the main clinical outcome. **Results.** Forty patients were found to have CCAs. Patients with CCAs have lower CFVR than those without CCAs (2.22 ± 0.72 versus 2.69 ± 0.92; $P = 0.003$), reflecting in different rates of CMD in the 2 groups (72.5% versus 37%; $P < 0.001$). CMD is associated with higher CAV grades ($P < 0.001$), which are also associated with CCAs ($P < 0.001$). Patients with poorly developed CCAs have lower CFVR ($P < 0.001$). At multivariable analysis, CMD ($P = 0.008$) and higher CAV grades ($P = 0.005$) are independent predictors of CCAs. During the median follow-up time of 10.2 (6.6-13.3) y, patients with CCAs have been found to have higher mortality than those without CCAs (57.5% versus 32.1%; $P = 0.007$). CCAs are associated with a lower probability of survival also in patients with CMD ($P < 0.001$) and are independent predictors of mortality ($P < 0.001$). **Conclusions.** Our results demonstrate an interplay between CAV, CMD, and CCAs. We confirm that CAV is associated with CMD, and we show, for the first time, that CMD is associated with CCAs. CCAs are pathophysiologically associated with more severe graft vasculopathy and independently predict mortality after HT.

(*Transplantation Direct* 2023;9: e1470; doi: 10.1097/TXD.0000000000001470.)

Coronary collateral arteries (CCAs) develop between adjacent epicardial coronary arteries in response to coronary stenosis to provide alternative sources of blood supply to a myocardial area jeopardized by ischemia.[1,2] In coronary atherosclerotic disease (CAD), CCAs are considered a beneficial adaptive response[2,3] that protects the myocardium from infarction during ischemia.[4] Instead, in heart transplantation (HT) recipients, the overall contribution of CCAs is less

Received 1 December 2022. Revision received 23 January 2023.

Accepted 25 January 2023.

[1] Cardiology Unit, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Padua, Italy.

[2] Department of Cardiology, Heart Center, University Hospital Zurich and University of Zürich, Zurich, Switzerland.

[3] Institute for Clinical Chemistry, University Hospital Zurich and University of Zürich, Zurich, Switzerland.

[4] Division of Cardiac Surgery, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Padua, Italy.

[5] Cardiac Pathology Unit, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Padua, Italy.

The authors declare no funding or conflicts of interest.

G.C. and F.T. generated figures and tables, and drafted the article. S.I., G.G., and F.T. contributed to the conception and design of the research as well as editing of the article. A.A., G.T., and M.F. contributed to the conception and design of the project. E.O., M.P.M., and G.M. contributed to the conception and design of the project, and editing of the article. A.G., A.F., and T.B. contributed to the conception and design of the research, generation of figures and tables, and drafting to editing of the article. All authors contributed to the article and approved the submitted version.

Correspondence: Francesco Tona, MD, PhD, Department of Cardiac, Thoracic and Vascular Sciences, University of Padua, Via Giustiniani, Padua 2 5128, Italy (francesco.tona@unipd.it).

Copyright © 2023 The Author(s). Transplantation Direct. Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001470

defined. The incidence of CCAs in HT is controversial, with studies reporting similar,[5] lower,[6] or higher[7,8] incidence compared with CAD patients.

Cardiac allograft vasculopathy (CAV) is the leading cause of long-term graft dysfunction after HT, accounting for the majority of patients' mortality at 5 to 10 y.[9,10] CAV develops in about 50% of patients 10 y after HT.[9] Histologically, CAV is a constellation of vascular changes characterized by intimal fibromuscular hyperplasia, atherosclerosis, and vasculitis.[11] Differently from atherosclerosis, it involves not only epicardial coronary arteries but also coronary microcirculation.[12,13] Coronary angiography with intravascular ultrasoundis a well-established method[14,15] to assess coronary intimal thickening only of epicardial vessels. Coronary flow velocity reserve (CFVR) evaluation by transthoracic echocardiography, on the other hand, can accurately detect CAV,[16,17] as well as coronary microvascular dysfunction (CMD) in HT patients.[18,19]

CCAs first develop as capillaries in a process called angiogenesis.[4] Therefore, coronary microcirculation is a major determinant of collateral circulation[5] and, although being early affected by CAV, there is no evidence, to our knowledge, about the relationship between CMD, CAV, and collateral circulation. We aimed to evaluate the interplay between CAV, CMD, and CCAs. Moreover, we aimed to investigate the prognostic implications of CCAs in HT recipients.

MATERIALS AND METHODS

Study Population

In this single-center retrospective study, patients receiving HT at the Padua University Hospital between November 1985 and November 2015 were included. Study patients underwent within 24 h transthoracic Doppler echocardiography to assess CFVR and coronary angiography to evaluate the presence of CAV and CCAs. Only patients with CAV were enrolled. Exclusion criteria included any of the following conditions: cerebral vascular disease, carotid artery bruit, peripheral bruit, or abnormal pulse. All participants had normal electrocardiogram at rest and during adenosine-induced hyperemia. The immunosuppression protocol has been previously described.[20,21] Ongoing medical therapy at the moment of coronary angiography was recorded. The study protocol was approved by the institutional ethical committee. All participants gave written informed consent.

Acute Rejection Scores

Acute graft rejection was monitored by periodical endomyocardial biopsies according to standardized protocols.[18] After the first year of HT, endomyocardial biopsies were performed only in the presence of clinical suspicion of acute rejection. On the basis of modification[20] of the International Society for Heart and Lung Transplantation (ISHLT) grading,[22] a rejection score (RS) was assigned for each patient. For each patient, the following scores were calculated: RS in the total follow-up (TRS); RS in the first y (RS 1st y), RS including only severe grades (≥3A) in the total follow-up (SevTRS); and first-y RS including only severe grades (1styrSevRS). All scores were subsequently normalized for the number of biopsies performed on each patient.

Echocardiography and CFVR Assessment

Transthoracic Doppler echocardiography (Vivid 7, GE Medical System, Inc., Horten, Norway) was performed. Left ventricular ejection fraction was measured according to American Society of Echocardiography criteria.[23] Coronary images were obtained in the distal part of the left anterior descending (LAD) artery with a 7-MHz transducer. After recordings of peak coronary diastolic flow velocity (DPV) at rest (DPV_r), adenosine was intravenously infused (140 μg/kg/min) for 3 min, obtaining hyperemic DPV (DPV_h). CFVR was the ratio of DPV_h and DPV_r. A CFVR of ≤2.5 was considered abnormal and diagnostic for CMD.[18,24] The population was dichotomized according to this cutoff point.

The evaluation of coronary flow involved the assessment of microvascular resistance. Coronary microvascular resistance (mm Hg·s/cm) was obtained from the mean blood pressure measured in the arm by a sphygmomanometer (mean pressure = [2 × diastolic + systolic]/3) divided by DPV,[25] both at rest and during hyperemia, assuming that the distal pressure in the microvascular bed can be neglected. In particular, we assessed coronary microvascular resistance in the basal (BMR, basal microvascular resistance) and in the hyperemic condition (HMR, hyperemic microvascular resistance). The arteriolar resistance index (ARI), defined as the difference between BMR and HMR, was calculated. ARI was considered a marker of vascular compliance and expressed the vessel capacity to dilate under maximal hyperemia.

Coronary Angiography

All HT patients at our institution undergo routine coronary angiography following a standardized protocol: (1) at baseline, (2) every y for 3 y, and (3) every 2 y thereafter. For every patient, an angiogram performed in a 24-h interval before or after CFVR assessment was chosen as a reference for the assessment of CAV and CCAs. Angiograms were reviewed by a cardiologist (G.M.) who was unaware of clinical findings and were compared, when available, with the first angiogram performed after HT.

CAV was defined and classified according to ISHLT criteria,[26] which take into account stenosis of left main coronary artery (LM), primary vessels, and secondary branch vessels, as well as graft dysfunction and evidence of restrictive physiology. CAV was defined as (1) mild (CAV_1): angiographic LM < 50%, or primary vessel with maximum lesion of <70%, or any branch stenosis <70% (including diffuse narrowing) without allograft dysfunction; (2) moderate (CAV_2): angiographic LM < 50%; a single primary vessel ≥70%, or isolated branch stenosis ≥70% in branches of 2 systems, without allograft dysfunction; and (3) severe (CAV_3): angiographic LM ≥50%, or ≥2 primary vessels ≥70% stenosis, or isolated branch stenosis ≥70% in all 3 systems, or ISHLT CAV_1 or CAV_2 with allograft dysfunction or evidence of significant restrictive physiology. CAV_2 and CAV_3 will also be referred to as "higher" CAV grades. Collateral coronary circulation was visually and morphologically evaluated according to Rentrop classification.[27,28] Grades of collateral circulation from the contralateral vessel were Rentrop 0 = none; Rentrop 1 = filling of side branches of the artery without visualization of the epicardial segment; Rentrop 2 = partial filling of the epicardial segment via collateral channels; and Rentrop 3 = complete filling of the epicardial segment via collateral channels. Coronary occlusion with balloon catheters was not routinely performed, and data about this procedure are therefore not included in our study.

Clinical Outcomes

Two independent investigators (A.F. and T.B.), specifically assigned to this task and blinded to CFVR and CCAs

assessment, carefully reviewed clinical outcomes. For this study, we considered cardiovascular mortality (sudden cardiac death and death during heart failure hospitalizations) as the main clinical outcome. Data about mortality were collected from the medical records and from the medical information system of our region.

Statistical Analysis

Continuous variables with no/mild skew were presented as mean ± standard deviation; skewed measures were represented as median with first and third quartiles. Discrete variables were summarized as frequencies and percentages. The distribution of the data was analyzed with a 1-sample Kolmogorov-Smirnov test. Categorical variables were compared by the χ^2 test or the Fisher exact test as appropriate. Continuous data were compared using the Mann-Whitney *U* test. Kaplan-Meier curves were constructed to estimate the cumulative event-free survival and compared by the log-rank test. Univariable and multivariable logistic regression analyses were performed to investigate the determinants of CCAs. Univariable Cox regression analysis was performed for all predictors of survival, and the variables with a *P* value of <0.10 were included in a multivariable Cox regression analysis to identify independent predictors of the endpoint: hazard ratio and 95% confidence intervals (CIs) were calculated. To evaluate the incremental value of CCAs on top of clinical and standard echocardiographic parameters, calculation of the overall C-statistic as proposed by Harrell et al[29] was performed as an analog of the area under the receiver operating characteristic curve for survival analysis. Furthermore, we assessed the impact of adding CCAs to a basic model using the continuous net reclassification improvement.

The intraobserver and interobserver reproducibilities of CFVR were evaluated by linear regression analysis and expressed as correlation coefficients (r), the standard error of estimates (SEE), and the intraclass correlation coefficient (ICC). These reproducibilities were assessed by repeating the CFVR evaluation twice, 1 h apart, by the same operator (G.F.) in all patients and by another operator (F.T.) in all patients. Reproducibility was considered satisfactory if the ICC was between 0.81 and 1.0.

All tests were 2-sided, and statistical significance was accepted if the null hypothesis could be rejected at a *P* value of <0.05. Data were analyzed with SPSS software version 28.0 (Chicago, SPSS, Inc., Chicago, IL).

RESULTS

Eight hundred forty-three patients who underwent HT at the Padua University Hospital between November 1985 and November 2015 were screened. For 191 of them (22.7%), coronary angiography and contemporary (within 24 h) CFVR assessment were available. Among these 191 patients, 121 (63.3%) presented any-grade CAV at coronary angiography and were included in the study. Fifty-six patients (46.3%) had mild CAV (CAV_1) and 65 patients (53.7%) had higher CAV (CAV_2 = 31 and CAV_3 = 34, respectively; (Figure 1).

Baseline Characteristics

Mean age at HT was 50.6 ± 1.2 y and HT recipients were 19 females (15.7%) and 102 males (84.3%). Considering the whole study population, the median time between HT and

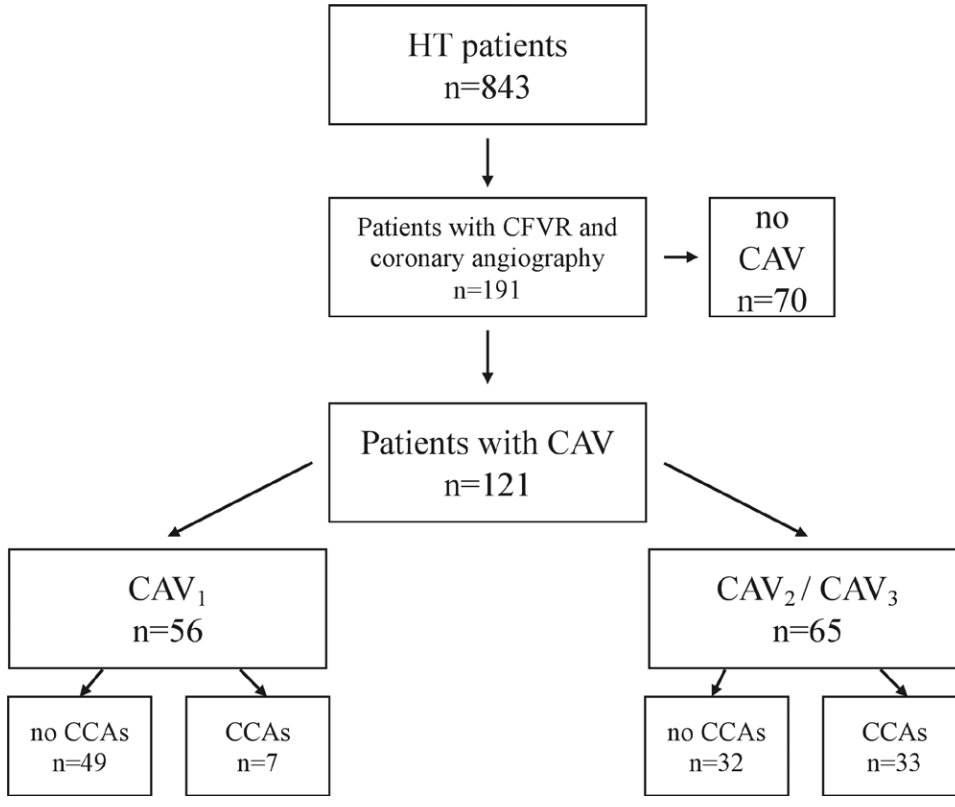

FIGURE 1. Study design. Among 843 screened patients, 191 underwent coronary angiography and transthoracic echocardiography with CFVR assessment within a 24-h period. One hundred twenty-one (63.3%) of them had CAV, which was mild (CAV_1) in 56 patients (46.3%) and more than mild (CAV_2 and CAV_3) in 65 patients (53.7%). The prevalence of CCAs was 12.5% CAV_1 and 50.8% in CAV_2 and CAV_3. CAV, cardiac allograft vasculopathy; CCA, coronary collateral artery; CFVR, coronary flow velocity reserve; HT, heart transplantation.

TABLE 1.

Clinical characteristics and microvascular coronary flow parameters of patients with and without CCAs

	No CCAs (n = 81)	CCAs (n = 40)	*P*
Clinical characteristics			
Age at HT, y	51.1 ± 13.7	49.5 ± 11.3	0.528
Donor age, y	34.8 ± 14.6	36.8 ± 15.3	0.485
Female recipient, n (%)	13 (16.0)	6 (15.0)	0.881
Female donor, n (%)	26 (32.1)	8 (20)	0.164
Sex mismatch, n (%)	19 (23.5)	6 (15.0)	0.280
Time from HT, y	18.3 (13.9–23.5)	18.3 (15.6–23.4)	0.781
Follow-up time, y	10.9 (8.4–13.4)	7.5 (4.6–12.8)	0.006
BMI at HT, kg/m^2	23.0 ± 3.1	23.2 ± 2.7	0.828
IHD pre-HT, n (%)	33 (40.7)	16 (40.0)	0.938
Hypercholesterolemia, n (%)	78 (96.3)	39 (97.5)	0.728
Diabetes, n (%)	12 (14.8)	6 (15.4)	0.935
Obesity, n (%)	15 (18.5)	10 (25.0)	0.407
CKD, n (%)	72 (90.0)	35 (89.7)	0.965
Ischemic time, min	175.7 ± 52.6	164.4 ± 46.5	0.253
LVEF, %	64.8 ± 6.8	65.5 ± 5.5	0.607
eGFR, mL/min/m^2	42.3 ± 17.1	41.5 ± 16.6	0.807
RS 1st y	1.2 (0.7–1.4)	1.5 (1.2–1.9)	0.069
TRS	1.2 (0.7–1.3)	1.4 (1.2–1.8)	0.042
1styrSevRS	0.7 (0.2–0.8)	1.1 (0.5–1.3)	0.121
SevTRS	0.6 (0.2–0.8)	0.8 (0.5–1.3)	0.094
CAV_2 and CAV_3, n (%)	32 (39.5)	33 (82.5%)	<0.001
Deaths, n (%)	26 (32.1)	23 (57.5)	0.007
Therapies			
Cyclosporine, n (%)	62 (76.5)	25 (62.5)	0.106
Tacrolimus, n (%)	1 (1.2)	0 (0)	0.480
Azathioprine, n (%)	16 (19.8)	3 (7.5)	0.081
Mycophenolate, n (%)	21 (25.9)	5 (12.5)	0.091
Prednisone, n (%)	28 (34.6)	15 (37.5)	0.751
Everolimus, n (%)	18 (22.2)	9 (22.5)	0.972
Statin, n (%)	26 (32.1)	11 (27.5)	0.606
ACEi/ARB, n (%)	30 (37)	7 (17.5)	0.028
β-blocker, n (%)	5 (6.2)	5 (12.5)	0.234
Spironolactone, n (%)	1 (1.2)	2 (5)	0.210
Ca antagonist, n (%)	13 (16)	5 (12.5)	0.606
Microvascular coronary flow parameters			
MAP_r, mm Hg	104.28 ± 14.36	102.50 ± 13.39	0.521
MAP_h, mm Hg	94.74 ± 15.50	95.67 ± 17.26	0.769
DPV_r, cm/s	26.80 ± 9.68	28.13 ± 8.39	0.462
DPV_h, cm/s	69.10 ± 25.95	66.75 ± 24.25	0.633
CFVR	2.69 ± 0.92	2.22 ± 0.72	0.003
BMR, mm Hg·s/cm	4. 28 ± 1.38	3.95 ± 1.24	0.203
HMR, mm Hg·s/cm	1.64 ± 0.85	1.66 ± 0.78	0.895
ARI, mm Hg·s/cm	2.69 ± 1.18	2.19 ± 1.08	0.036
CMD, n (%)	30 (37.0)	29 (72.5)	<0.001

Continuous variables with no/mild skew are presented as mean ± SD; skewed measures are represented as median with Q1–Q3. Discrete variables are summarized as frequencies and percentages.

1styrSevRS, severe rejection score within the first y; ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; ARI, arteriolar resistance index; BMI, body mass index; BMR, basal microvascular resistance; Ca antagonist, calcium antagonist; CAV, cardiac allograft vasculopathy; CCA, coronary collateral artery; CFVR, coronary flow velocity reserve; CI, confidence interval; CKD, chronic kidney disease; CMD, coronary microvascular dysfunction; DPV_h, hyperemic diastolic peak velocity; DPV_r, rest diastolic peak velocity; eGFR, estimated glomerular filtration rate; HMR, hyperemic microvascular resistance; HT, heart transplantation; IHD, ischemic heart disease; LVEEF, left ventricular ejection fraction; MAP_r, mean arterial pressure at rest; MAP_h, mean arterial pressure during hyperemia; OR, odds ratio; Q1–Q3, first and third quartiles; RS 1st y, rejection score in the first y; SD, standard deviation; SevTRS, severe total rejection score; TRS, total rejection score.

CFVR/CCAs assessment was 18.3 (14.2–23.3) y, whereas the median follow-up time thereafter was 10.2 (6.6–13.3) y. The clinical characteristics of the patients are reported in Table 1. Donor/recipient sex mismatch was reported in 25 cases (20.7%). CAV_2 and CAV_3 and mortality were significantly more frequent among patients with CCAs ($P < 0.001$ and $P = 0.007$, respectively). Therapy with ACE inhibitors (ACEi) or angiotensin receptor blockers (ARBs) was significantly more frequent among patients without CCAs ($P = 0.028$).

Microvascular Coronary Flow Parameters

All included patients underwent noninvasive evaluation of microvascular coronary flow parameters using transthoracic Doppler echocardiography. The main findings are reported in Table 1.

Patients with CCAs showed a significantly lower CFVR ($P = 0.003$), suggesting a lower capacity of these patients to increase coronary blood flow after hyperemic stimulation as confirmed by a lower ARI. As a consequence, higher rates of CMD (CFVR ≤2.5) were found among patients with CCAs ($P < 0.001$). A lower ARI was detected in patients with CCAs ($P = 0.036$). No significant differences were found regarding other microvascular coronary flow parameters.

Coronary Angiography

Different CAV grades were reported among included patients: 56 (46.3%) CAV_1, 31 (25.6%) CAV_2, and 34 (28.1%) CAV_3. CMD was significantly more frequent in patients with higher CAV grades (28.6% in CAV_1, 61.3% in CAV_2, and 70.6% in CAV_3; $P < 0.001$; Figure 2A).

Prevalence of CCAs was also significantly higher among patients with higher degrees of CAV (12.5% in CAV_1, 45.2% in CAV_2, and 55.9% in CAV_3; $P < 0.001$; Figure 2B).

As regards CFVR, it was significantly different in CAV subgroups ($P < 0.001$), and it was higher in CAV_1 than in CAV_2 ($P = 0.009$) and CAV_3 ($P = 0.003$). No difference was found between CAV_2 and CAV_3 ($P = 0.982$; Figure 3A).

Coronary collaterals were present in 40 of 121 patients (33.1%). According to the Rentrop score, these patients were divided into Rentrop 1 (21; 52.5%), Rentrop 2 (16; 40%), and Rentrop 3 (3; 7.5%).

CFVR was found to be significantly different between subgroups of patients with different Rentrop scores, being lower in patients with less developed CCAs (Rentrop 1: 1.88 ± 0.12; Rentrop 2: 2.44 ± 0.14; and Rentrop 3: 3.51 ± 0.45; Figure 3B).

Among subgroups of patients with different Rentrop scores, there was a higher prevalence of CMD in patients with Rentrop 1 (85.7%) and Rentrop 2 (68.8%) than in patients with Rentrop 3 (0%; $P = 0.007$).

Determinants of Collateral Circulation

We performed univariable logistic regression analysis to investigate the determinants of CCAs. Beyond CFVR ($P = 0.007$) and CMD ($P < 0.001$), also higher CAV ($P < 0.001$), ACEi/ARBs therapy ($P = 0.032$), and higher TRS ($P = 0.032$) were associated with CCAs. In the final multivariable regression model, CMD (odds ratio [OR] 23.3, $P = 0.008$), higher CAV grade (OR 3.48, $P = 0.005$), and TRS (OR 9.33, $P = 0.021$) were independently associated with CCAs (Table 2).

Long-Term Survival

Differences between survivors and nonsurvivors are shown in Table 3. The presence of CCAs ($P = 0.007$) and presence of higher CAV grade ($P < 0.001$), higher donor age ($P = 0.046$), higher 1styrSevRS ($P = 0.036$), and higher SevTRS

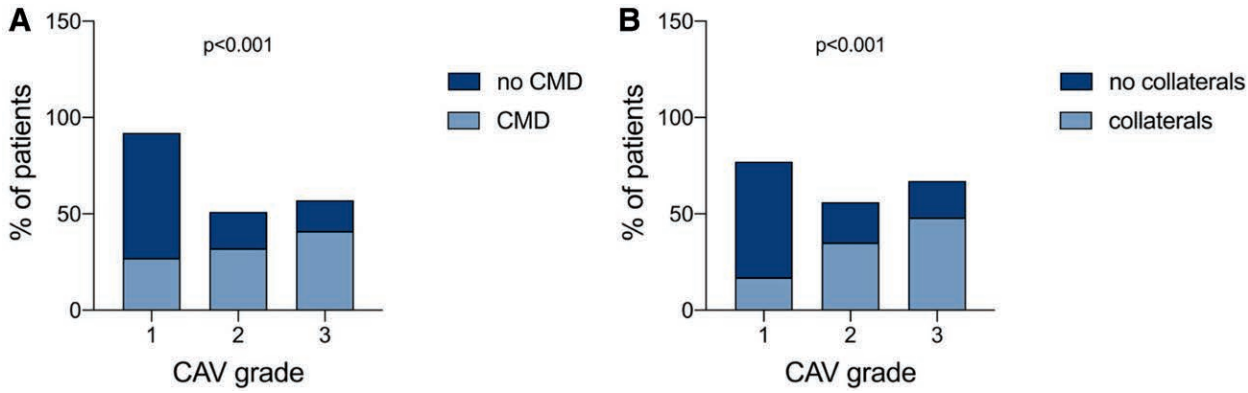

FIGURE 2. CMD and collateral arteries in different CAV grades. A, Patients with higher CAV grades (CAV_2 and CAV_3) have significantly higher rates of CMD ($P<0.001$). B, Patients with higher CAV grades (CAV_2 and CAV_3) have a significantly higher incidence of CCAs ($P<0.001$). CAV, cardiac allograft vasculopathy; CCA, coronary collateral artery; CMD, coronary microvascular dysfunction.

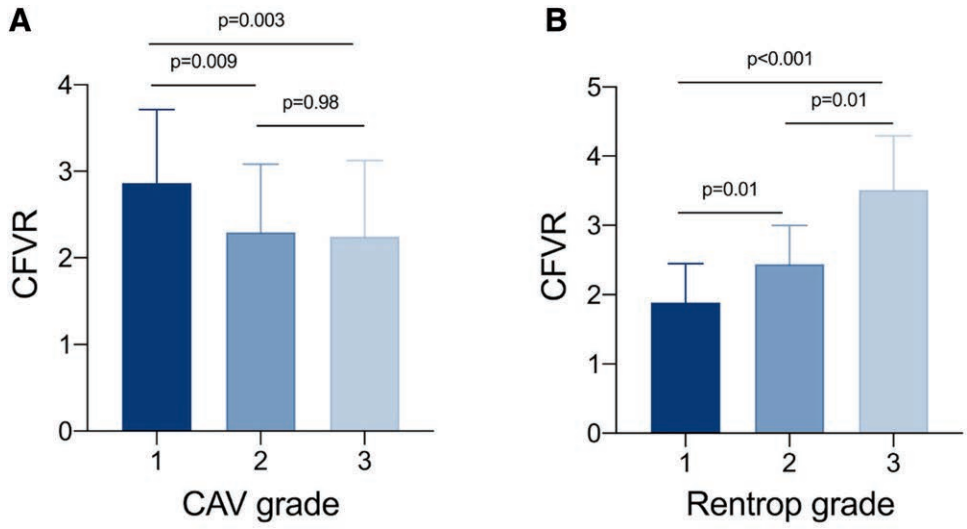

FIGURE 3. CFVR in different subgroups of patients. A, Patient with higher CAV grades (CAV_2 and CAV_3) have significantly lower CFVR ($P=0.009$ CAV_1 versus CAV_2 and $P=0.003$ CAV_1 versus CAV_3, respectively). B, Patients with higher Rentrop grades have higher CFVR ($P=0.01$ Rentrop 1 versus Rentrop 2, $P=0.01$ Rentrop 2 versus Rentrop 3 and $P<0.001$ Rentrop 1 versus Rentrop 3). CAV, cardiac allograft vasculopathy; CFVR, coronary flow velocity reserve.

($P=0.001$) were clinical characteristics associated with mortality. Obviously, nonsurvivors also presented shorter follow-up time ($P<0.001$). Therapies with cyclosporine ($P<0.001$), azathioprine ($P=0.004$), statin ($P=0.045$), and ACEi/ARBs ($P=0.045$) were more frequent among survivors. As regards microvascular coronary flow parameters, lower DPV_h ($P=0.027$), lower CFVR ($P<0.001$), and CMD ($P<0.001$) were significantly more frequent among nonsurvivors. Indexes of microvascular resistance (such as BMR, HMR, and ARI) were comparable in the 2 groups.

Figure 4 shows the Kaplan-Meier curves for different variables. We found that survival was lower in CAV_2 and CAV_3 patients ($P=0.003$; Figure 4A), in patients with CMD ($P=0.0003$; Figure 4B), and in patients with CCAs ($P=0.001$; Figure 4C).

Regarding CMD and CCAs, we further categorized the patient population into 4 different subgroups (no CMD/no CCAs, CMD/CCAs, no CMD/CCAs, and CMD/CCAs). As shown in Figure 5, there was a significantly different probability of survival among different subgroups ($P<0.0001$) and among patients with CMD, CCAs conferred a worse prognosis.

At multivariable Cox survival analysis (Table 4), CMD ($P=0.006$), CCAs ($P=0<0.001$), and therapy with cyclosporine ($P<0.001$) were found to be independent predictors of mortality.

Consequently, we evaluated the impact of strategies including CCAs on a prognostic model covering only the independent clinical predictors of mortality (referred to as model 1: CMD, donor age, SevTRS, cyclosporine, and azathioprine treatment). The inclusion of CCAs to model 1 permitted better prediction of survival in HT patients ($P=0.03$; Figure 6).

Intraobserver and Interobserver Reproducibilities of CFVR

The intraobserver reproducibility was high ($r=0.92$, SEE = 0.11); the mean difference was −0.004; the upper and lower limits of agreement between the measurements were +0.19 (95% CI, +0.11 to + 0.23) and −0.15 (95% CI, −0.21 to −0.10), respectively; and the ICC was 0.968. The interobserver reproducibility was also high ($r=0.89$, SEE = 0.10); the mean difference was −0.02, the upper and lower limits of agreement between the 2 measurements were +0.33 (95% CI,

TABLE 2.

Univariable and multivariable logistic regression analyses of the determinants of CCAs

	Univariable			Multivariable		
Covariates	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
CFVR[a]	0.51	0.32-0.84	0.007	0.62	0.37-1.03	0.071
CMD[a]	4.48	1.95-10.25	<0.001	23.3	2.27-26.3	0.008
Ischemic time	0.99	0.99-1.00	0.252			
Donor age	1.0	0.97-1.05	0.482			
CAV_2 and CAV_3	3.98	1.72-9.18	<0.001	3.48	1.44-8.38	0.005
Age at HT	0.91	0.96-1.02	0.525			
IHD	0.97	0.44-2.09	0.938			
Female donor	1.89	0.76-4.77	0.167			
Female recipient	1.09	0.33-3.65	0.881			
Sex mismatch	2.05	0.56-7.53	0.284			
RS 1st y	1.77	0.97-3.22	0.059			
TRS	4.31	1.85-18.73	0.032	9.33	1.25-16.2	0.021
1^{st}yrSevRS	1.68	1.15-3.63	0.222			
SevTRS	1.40	0.58-3.39	0.444			
Hypercholesterolemia	1.50	0.15-14.80	0.729			
Obesity	1.46	0.59-3.64	0.409			
Metabolic syndrome	1.26	0.58-2.75	0.549			
LVEF	1.01	0.95-1.08	0.604			
CKD	0.97	0.27-3.44	0.965			
Diabetes	1.04	0.36-3.03	0.935			
Cyclosporine	1.95	0.86-4.44	0.109			
Tacrolimus	0.84	0.41-1.12	0.723			
Azathioprine	0.32	0.09-1.26	0.093			
Mycophenolate	0.40	0.14-1.17	0.098			
Prednisone	1.13	0.51-2.49	0.751			
Everolimus	1.01	0.41-2.52	0.972			
Statin	1.24	0.54-2.87	0.606			
ACEi/ARB	0.36	0.14-0.91	0.032	0.32	0.03-3.52	0.332
β-blocker	2.17	0.59-7.98	0.243			
Spironolactone	4.21	0.37-4.7	0.247			
Ca antagonist	0.74	0.24-2.26	0.607			

[a]These 2 covariates were included separately 1 at a time in the multivariable model.

1^{st}yrSevRS, severe rejection score within the first y; ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; Ca antagonist, calcium antagonist; CAV, cardiac allograft vasculopathy; CCA, coronary collateral artery; CFVR, coronary flow velocity reserve; CI, confidence interval; CKD, chronic kidney disease; CMD, coronary microvascular dysfunction; HT, heart transplantation; IHD, ischemic heart disease; LVEEF, left ventricular ejection fraction; OR, odds ratio; RS 1st y, rejection score in the first y; SevRS, severe rejection score; SevTRS, severe total rejection score.

+0.25 to +0.45) and −0.37 (95% CI, −0.45 to −0.25), respectively; and the ICC was 0.955.

DISCUSSION

In this study, CAV and CMD were associated with CCAs. Moreover, CCAs were found to be independent predictors of cardiovascular mortality, reflecting a higher severity of CAV disease. Our results have a conceivable background in the pathophysiology of CAV and CMD.

The role of CCAs is well established in CAD, where collaterals have a beneficial effect on the reduction of mortality and major adverse cardiovascular events.[2-4,30] In HT, the picture is less clear and underinvestigated. It was first found that coronary involvement in HT patients is represented by a progressive proliferative disease that occurs without collateral vessels development,[6] remarking the difference with CAD. Subsequent studies reported different results: CCAs were found in most HT patients with CAV, suggesting that they represent an angiogenic response to microvascular ischemia, similar to what also happens in CAD.[8] Moreover, functional testing using collateral flow index showed that HT

TABLE 3.

Baseline characteristics and coronary flow parameters of patients, divided into survivors and nonsurvivors

	Survivors (n = 72)	Nonsurvivors (n = 49)	*P*
Baseline characteristics			
Age at HT, y	50.9 ± 13.0	50. 0 ± 12.9	0.716
Donor age, y	33.2 ± 15.3	38.7 ± 13.6	0.046
Female recipient, n (%)	14 (19.4)	5 (10.2)	0.170
Female donor, n (%)	19 (26.4)	15 (30.6)	0.612
Sex mismatch, n (%)	13 (18.1)	12 (24.5)	0.391
Time from HT, y	16.3 (13.7-25.3)	20.1 (15.8-22.2)	0.530
Follow-up time, y	12.1 (8.5-13.9)	7.9 (4.8-11.4)	<0.001
BMI at HT, kg/m^2	23.1 ± 3.2	23.0 ± 2.6	0.914
IHD pre-HT, n (%)	26 (36.1)	23 (46.9)	0.234
Hypercholesterolemia, n (%)	68 (94.4)	49 (100)	0.093
Diabetes, n (%)	9 (12.7)	9 (18.4)	0.391
Obesity, n (%)	17 (23.6)	8 (16.3)	0.331
CKD, n (%)	63 (90.0)	44 (89.8)	0.971
Ischemic time, min	175.0 ± 53.8	167.4 ± 46.1	0.420
LVEF at HT, %	64.5 ± 7.1	65.9 ± 5.2	0.271
eGFR at HT, $mL/min/m^2$	41.6 ± 18.1	42.6 ± 15.1	0.751
RS 1st y	1.1 ± 0.6	1.3 ± 0.7	0.221
TRS	1.1 ± 0.5	1.4 ± 0.6	0.116
1^{st}yrSevRS	0.5 ± 0.5	0.7 ± 0.5	0.036
SevTRS	0.4 ± 0.4	0.8 ± 0.4	0.001
CAV_2 and CAV_3, n (%)	29 (40.3)	36 (73.5)	<0.001
CCAs, n (%)	17 (23.6)	23 (46.9)	0.007
Therapies			
Cyclosporine, n (%)	66 (91.8)	21 (42.9)	<0.001
Tacrolimus, n (%)	1 (1.4)	0 (0)	0.407
Azathioprine, n (%)	17 (23.6)	2 (4.1)	0.004
Mycophenolate, n (%)	17 (23.6)	9 (18.4)	0.491
Prednisone, n (%)	27 (37.5)	16 (32.7)	0.585
Everolimus, n (%)	20 (27.8)	7 (14.3)	0.080
Statin, n (%)	27 (37.5)	10 (20.4)	0.045
ACEi/ARB, n (%)	27 (37.5)	10 (20.4)	0.045
β-blocker, n (%)	6 (8.3)	4 (8.2)	0.973
Spironolactone, n (%)	2 (2.8)	1 (2)	0.798
Ca antagonist, n (%)	13 (18.1)	5 (10.2)	0.234
Coronary flow parameters			
DPV_r, cm/s	27.5 ± 9.3	26.8 ± 9.2	0.695
DPV_h, cm/s	72.5 ± 27.4	62.1 ± 20.7	0.027
CFVR	2.8 ± 0.9	2.2 ± 0.8	<0.001
BMR, mm Hg·s/cm	4.1 ± 1.3	4.2 ± 1.4	0.799
HMR, mm Hg·s/cm	1.6 ± 0.8	1.7 ± 0.8	0.352
ARI, mm Hg·s/cm	2.6 ± 1.1	2.5 ± 1.3	0.568
CMD, n (%)	25 (34.7)	34 (69.4)	<0.001

Continuous variables with no/mild skew are presented as mean ± SD; skewed measures are represented as median with Q1–Q3. Discrete variables are summarized as frequencies and percentages.

1^{st}yrSevRS, severe rejection score within the first year; ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; ARI, arteriolar resistance index; BMI, body mass index; BMR, basal microvascular resistance; Ca antagonist, calcium antagonist; CAV, cardiac allograft vasculopathy; CCA, coronary collateral artery; CFVR, coronary flow velocity reserve; CI, confidence interval; CKD, chronic kidney disease; CMD, coronary microvascular dysfunction; DPV_h, hyperemic diastolic peak velocity; DPV_r, rest diastolic peak velocity; eGFR, estimated glomerular filtration rate; HMR, hyperemic microvascular resistance; HT, heart transplantation; IHD, ischemic heart disease; LVEEF, left ventricular ejection fraction; MAP_r, mean arterial pressure at rest; MAP_h, mean arterial pressure during hyperemia; Q1–Q3, first and third quartiles; RS 1st y, rejection score in the first y; SD, standard deviation; SevTRS, severe total rejection score; TRS, total rejection score.

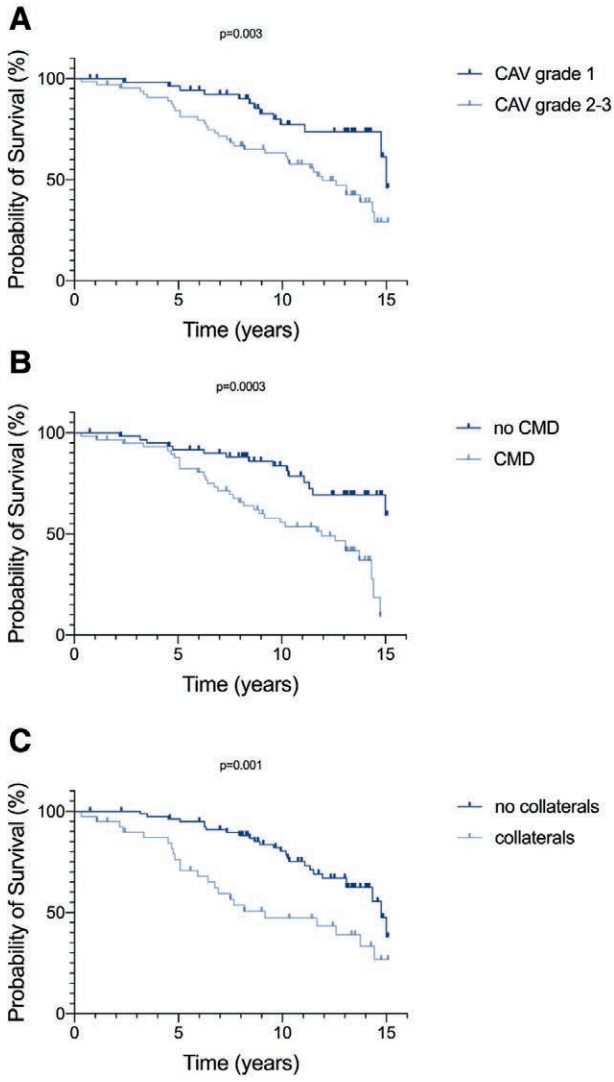

FIGURE 4. Probability of survival according to macro- and microvascular flow parameters. Kaplan-Meier curves show (A) lower survival in patients with CAV_2 and CAV_3 compared with CAV_1 ($P=0.003$); (B) lower survival in patients with CMD compared with those without ($P=0.0003$); and (C) lower survival in patients with CCAs compared with those without ($P=0.001$). CAV, cardiac allograft vasculopathy; CCA, coronary collateral artery; CMD, coronary microvascular dysfunction.

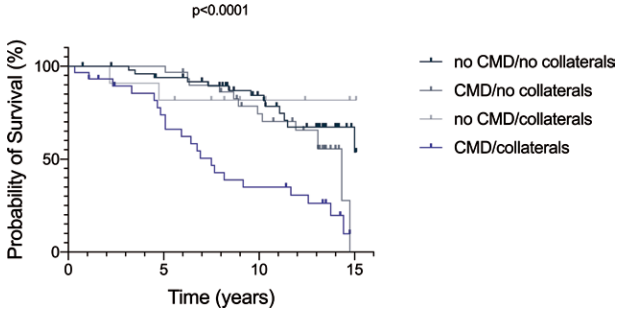

FIGURE 5. Probability of survival in different subgroups of patients. Kaplan-Meier curves show a significantly different probability ($P<0.001$) of survival among different subgroups (no CMD/no CCAs, CMD/CCAs, no CMD/CCAs, CMD/CCAs). Patients with CMD and CCAs show the lowest probability of survival. Interestingly, also among patients with CMD, those with CCAs have a lower probability of survival. CCA, coronary collateral artery; CMD, coronary microvascular dysfunction.

TABLE 4.

Univariable and multivariable Cox regression analyses of the determinants of survival

	Univariable			Multivariable		
Covariates	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
CMD	3.01	1.60-5.65	<0.001	2.51	1.31-4.34	0.006
Ischemic time	0.99	0.99-1.00	0.569			
Donor age	1.02	1.01-1.04	0.037	1.01	0.99-1.04	0.176
CCAs	2.44	1.39-4.30	0.002	2.71	1.52-4.83	<0.001
CAV grade 2–3[a]	2.48	1.31-4.68	0.005			
Age at HT	0.99	0.97-1.02	0.730			
IHD	1.73	0.97-3.01	0.061			
Female donor	0.93	0.51-1.71	0.817			
Male recipient	1.45	0.57-3.70	0.087			
Sex mismatch	1.25	0.65-2.39	0.505			
RS 1st y	1.12	0.73-1.74	0.597			
TRS	1.77	0.75-4-20	0.195			
SevRS 1st y	1.34	0.86-2.10	0.201			
SevTRS	2.58	1.13-5.86	0.024	1.71	0.98-2.97	0.057
Hypercholesterolemia	2.13	0.02-2.42	0.394			
Obesity	0.80	0.38-1-72	0.574			
Metabolic syndrome	1.10	0.62-1.96	0.735			
LVEF	1.02	0.97-1.06	0.429			
CKD	1.00	0.40-2.53	0.995			
Diabetes	1.30	0.62-2.70	0.484			
Cyclosporine	0.19	0.11-0.35	<0.001	0.22	0.12-0.40	<0.001
Tacrolimus	0.04	0.01-0.09	0.915			
Azathioprine	0.19	0.04-0.80	0.023	0.33	0.07-1.44	0.142
Mycophenolate	0.62	0.30-1.31	0.216			
Prednisone	0.81	0.44-1.47	0.494			
Everolimus	0.47	0.21-1.06	0.072			
Statin	0.51	0.25-1.03	0.064			
ACEi/ARB	0.49	0.24-0.99	0.048			
β-blocker	0.74	0.26-2.10	0.580			
Spironolactone	1.01	0.14-7.40	0.986			
Ca antagonist	0.49	0.19-1.26	0.143			

CCA, coronary collateral arteries.

[a]This covariate was not included in the multivariable model for its collinearity with CCAs.

1stYrSevRS, severe rejection score within the first y; ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; Ca antagonist, calcium antagonist; CAV, cardiac allograft vasculopathy; CCA, coronary collateral artery; CFVR, coronary flow velocity reserve; CI, confidence interval; CKD, chronic kidney disease; CMD, coronary microvascular dysfunction; HT, heart transplantation; IHD, ischemic heart disease; LVEEF, left ventricular ejection fraction; OR, odds ratio; RS 1st y, rejection score in the first y; SevRS, severe rejection score; SevTRS, severe total rejection score.

patients present with the same degree of functional collateral flow compared with CAD patients.[5] To our knowledge, only 1 study analyzed the prognostic implications of CCAs in HT patients: among HT patients with moderate-to-severe CAV, patients with CCAs had better outcomes compared with those without CCAs.[7]

CAV, Coronary Microcirculation, and CCAs

Differently from CAD, which predominantly affects epicardial coronary arteries, CAV also extensively affects coronary microcirculation.[31] Furthermore, as already shown, the presence of CMD can predict the occurrence of CAV, suggesting that microvascular involvement may precede epicardial vasculopathy.[12,17,32,33] Consistent with these observations, we found that patients with higher CAV grades also presented more severe microvascular impairment with low CFVR values and higher rates of CMD (Figures 2A and 3A). This is not surprising because microcirculation affected by CAV is dysfunctional

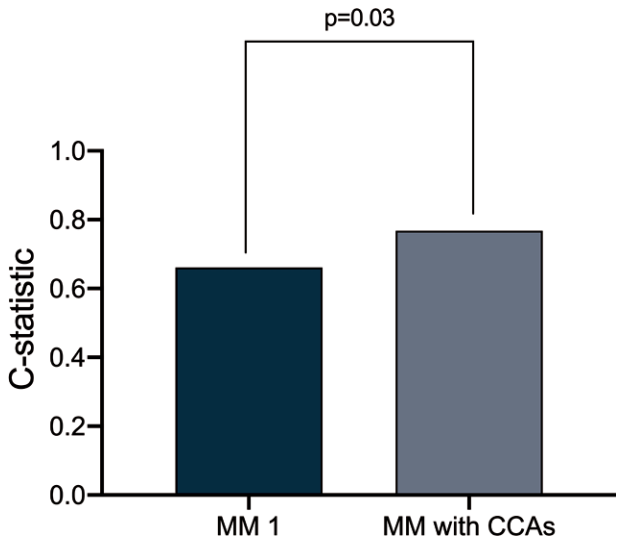

FIGURE 6. Performance of 2 survival prediction models among HT patients. CMD, donor age, SevTRS, cyclosporine treatment, and azathioprine treatment are independent predictors of survival (referred to as model 1). We evaluated the incremental prognostic value of CCAs to model 1 and found that the inclusion of CCAs permitted better prediction of survival among HT patients ($P = 0.03$). CCA, coronary collateral artery; CMD, coronary microvascular dysfunction; MM, multivariable model; HT, heart transplantation; SevTRS, severe total rejection score.

and loses its ability to increase blood flow when the myocardial demand is higher. From the data in our possession, we observed that CFVR is comparable in CAV_2 and CAV_3 patients (Figure 3A); this could suggest that microvascular impairment becomes overt when CAV is more than mild and that, thereafter, there is no graded relationship between CFVR and CAV grades, but further studies with higher numerosity will be required to confirm this hypothesis.

Even in the absence of epicardial obstructions, CMD can cause myocardial ischemia,[34-36] involving the mechanism of ischemia-induced angiogenesis.[1,4,37] We hypothesized that ischemia caused by CAV-induced CMD may trigger the formation of CCAs through angiogenesis, and this hypothesis was confirmed by our results because we showed that CFVR values are significantly lower among patients with CCAs (Table 1). In our hypothesis, CMD was caused by CAV and, indeed, patients with higher CAV grades more often presented CCAs (Figure 2B).

To further investigate the determinants of CCAs, we performed multivariable analysis that showed how CMD and CAV_2/CAV_3 are independent predictors of the presence of collaterals (Table 2). Also, TRS was an independent predictor of CCAs, and this suggests that severity of allograft disease, both in terms of vascular involvement and immune activation, may lead to the development of CCAs. Interestingly, medical therapy did not show significant influence on the development of CCAs.

Conflicting evidence are reported about the functionality of CCAs in HT patients. We found that the majority of patients with CCAs presented with Rentrop 1 and Rentrop 2 (52.5% and 40%, respectively), whereas only 7.5% had Rentrop 3 CCAs, suggesting a certain incapacity of patients with CAV to form fully functional CCAs. This might be the result of the immunological activation against the heart and its vessels. Moreover, we compared CFVR in different Rentrop classes and found a significant association between lower Rentrop grade and lower CFVR (Figure 3B). This was reflected by a significantly higher prevalence of CMD in lower Rentrop classes. These results may seem contradictory to the "angiogenetic hypothesis" of CCAs because low CFVR (and thus more ischemia) should trigger the development of more efficient CCAs. Anyway, we must not forget that CAV first affects microcirculation and that CCAs first develop as microcirculation: CCAs themselves could therefore be affected by CAV, and this could hamper their complete development. Again, immune derangement damages CCAs and impairs their protective function. This hypothesis is in line with the results of studies finding a >2-fold difference in microvascular density between CAV patients and controls.[7]

Our study is the first to noninvasively measure CFVR to find a possible correlation between microvascular function and CCAs. One study already assessed microvascular density in a similar subgroup of patients, finding increased microvascular density in CAV patients with CCAs.[7] Because microvascular evaluating techniques were substantially different, we believe that the results are not comparable with ours. Indeed, although we used transthoracic echocardiography to measure a functional index that mainly regards arterioles, they used endomyocardial biopsies to assess a structural parameter (such as microvascular density) that mainly regards capillaries.

Prognostic Implications

Prognostic implications of CAV and CMD in HT patients have been previously extensively described.[18,38] Also, in our cohort, patients with higher CAV and with CMD had a significantly lower probability of survival (Figure 4A and B), and CMD was an independent predictor of mortality at multivariable analysis (Table 4). As regards CCAs, we assessed the association between CCAs and cardiovascular mortality and found that patients with CCAs had a significantly higher mortality rate (Figure 4C); moreover, at multivariable Cox regression analysis, the presence of CCAs was the strongest predictor of survival (Table 4). Also, in patients with CMD, CCAs were a negative prognostic factor (Figure 5) and the inclusion of CCAs in a model with CMD (and other clinical predictors of mortality) allowed better prediction of survival (Figure 6). From a clinical perspective, this is especially relevant given the easy availability of CCAs assessment, which requires only routine coronary angiograms and not focused adenosine echocardiography such as CFVR. However, these results may seem counterintuitive when compared with the vast amount of data collected in CAD patients, showing how CCAs have a clear prognostic benefit.[2-4] Anyway, although atherosclerosis affects epicardial coronary arteries, in CAV, we must also take into account microvascular involvement that affects CCAs themselves, as reflected by the higher rates of lower Rentrop grades. Interestingly, also in CAD, in which CCAs have a clear overall beneficial effect, the presence of barely developed CCAs was reported to be a prognostic indicator of adverse cardiovascular outcome,[39] and this is what we believe also happens in HT with CAV: CAV causes the development of CCAs through CMD-induced ischemia (as in CAD), but these CCAs are poorly developed as they are affected by CAV itself, which is the result of a profound immune derangement because of the immunogenicity of HT. Therefore, CCAs are indirectly associated with the extent of CAV and with the overall severity of disease.

Only 1 study has already assessed the prognostic implications of CCAs in HT, reporting a favorable effect on survival.[7]

CCAs were found in 34 of 59 patients (57.6%), and the prevalence was comparable with our results. Regarding the timing of diagnosis, patients with CAV who formed coronary collateral vasculature were diagnosed later than those without CCAs (7.9 ± 3.6 *versus* 4.8 ± 3.1 y; $P = 0.001$). Because coronary angiography was performed on the basis of a strategy driven by a combination of factors (routine screening but also high-risk stress testing or heart failure symptoms), the authors concluded that collateral formation may protect patients from the development of symptoms, thus leading to a later time of diagnosis of CAV. However, as the authors also stated, an earlier diagnosis of CAV may reflect a more aggressive disease. Indeed, those patients could not have had enough time to form CCAs. Vice versa, patients with CAV and CCAs may have had time to form collaterals as a consequence of milder disease. Given these premises, it is questionable whether the prognostic benefit of CCAs is driven by an effective protective role or it is only a reflection of a milder form of disease. In our study, we did not analyze the coronary angiography of CAV diagnosis but a routine angiography performed within 24 h from CFVR assessment, in patients in whom CAV could be previously known or not. Indeed, in our study, there was no difference between patients with and without CCAs as regards the time of assessment after HT (Table 1), and the prognostic value of CCAs could not be influenced by this bias.

Study Limitations

Some important limitations must be taken into account. Firstly, all patients included in the study have CAV. Our study is, therefore, not adequate to demonstrate that the presence of CCAs gives a benefit compared with the evaluation of CAV alone but shows, among patients with CAV, a negative prognostic impact of CCAs. Secondly, the relatively small sample size makes this study hypothesis-generating, and further studies will be needed to confirm our results. Moreover, our population mainly consists of male patients, and thus results cannot be with certainty extrapolated to women. Thirdly, there is a lack of gold-standard invasive data that would provide evidence of which patients definitively had CMD and CCAs. Obviously, because we collected data from routine coronary angiography, these data were not available and we relied on noninvasive studies. However, the close relationship between invasive and noninvasive measurements of CFVR has already been described.[40] Fourthly, a reduction of CFVR could be caused by both a significant epicardial coronary stenosis and CMD; therefore, the measurement of distal LAD flow may result in misleading conclusions in case there is a severe stenosis in the proximal LAD, and this is an intrinsic limitation of CFVR assessment by echocardiography. However, in our study, only 3 of 121 patients (2.5%) had impaired CFVR and concomitant CAV_3 involving LAD: our results are therefore not invalidated, given the small percentage of patients in this "gray zone." Finally, we do not have histological data to validate our hypothesis on the relationship between CAV and CCAs development.

As regards medical therapy, we collected these data at the moment of CFVR assessment, and they do not necessarily reflect the medical therapy of the patient for the entire follow-up. Cumulative doses, which might be more accurate for investigating the impact of medical therapy,[20] were not available. Moreover, cyclosporine was the most frequently used calcineurin inhibitor, and this may not reflect the actual therapeutic preferences of most centers.

CONCLUSION

In HT patients with CAV, the presence of noninvasively assessed CMD and the severity of CAV correlate with CCAs at coronary angiography. We found that, on top of other well known risk factors, the presence of CCAs has a negative prognostic impact on cardiovascular mortality. Assessment of CCAs may, therefore, contribute to better risk stratification of HT recipients and should be routinely adopted.

ACKNOWLEDGMENTS

This study would also not have been possible without the tireless support of the catheter laboratory staff of the Padua University Hospital. The authors are also grateful to the patients who participated.

REFERENCES

1. Seiler C. The human coronary collateral circulation. *Eur J Clin Invest.* 2010;40:465–476.
2. Meier P, Hemingway H, Lansky AJ, et al. The impact of the coronary collateral circulation on mortality: a meta-analysis. *Eur Heart J.* 2012;33:614–621.
3. Meier P, Gloekler S, Zbinden R, et al. Beneficial effect of recruitable collaterals: a 10-year follow-up study in patients with stable coronary artery disease undergoing quantitative collateral measurements. *Circulation.* 2007;116:975–983.
4. Koerselman J, van der Graaf Y, de Jaegere PPT, et al. Coronary collaterals: an important and underexposed aspect of coronary artery disease. *Circulation.* 2003;107:2507–2511.
5. Rutz T, Gloekler S, de Marchi SF, et al. Coronary collateral function in the transplanted heart: propensity score matching with coronary artery disease. *Heart.* 2011;97:557–563.
6. Gao SZ, Alderman EL, Schroeder JS, et al. Accelerated coronary vascular disease in the heart transplant patient: coronary arteriographic findings. *J Am Coll Cardiol.* 1988;12:334–340.
7. Lavine KJ, Sintek M, Novak E, et al. Coronary collaterals predict improved survival and allograft function in patients with coronary allograft vasculopathy. *Circ Heart Fail.* 2013;6:773–784.
8. Bajaj S, Shah A, Crandall C, et al. Coronary collateral circulation in the transplanted heart. *Circulation.* 1993;88(5 Pt 2):II263–II269.
9. Khush KK, Hsich E, Potena L, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: thirty-eighth adult heart transplantation report—2021; focus on recipient characteristics. *J Heart Lung Transplant.* 2021;40:1035–1049.
10. Pober JS, Chih S, Kobashigawa J, Madsen JC, Tellides G. Cardiac allograft vasculopathy: current review and future research directions. *Cardiovasc Res*. 2021;117:2624–2638.
11. Lu W, Palatnik K, Fishbein GA, et al. Diverse morphologic manifestations of cardiac allograft vasculopathy: a pathologic study of 64 allograft hearts. *J Heart Lung Transplant.* 2011;30:1044–1050.
12. Tona F, Osto E, Famoso G, et al. Coronary Microvascular dysfunction correlates with the new onset of cardiac allograft vasculopathy in heart transplant patients with normal coronary angiography: microvascular dysfunction correlates with CAV. *Am J Transplant.* 2015;15:1400–1406.
13. Labarrere CA, Jaeger BR, Kassab GS. Cardiac allograft vasculopathy: microvascular arteriolar capillaries ('capioles') and survival. *Front Biosci (Elite Ed).* 2017;9:110–128.
14. Rickenbacher PR, Pinto FJ, Lewis NP, et al. Prognostic importance of intimal thickness as measured by intracoronary ultrasound after cardiac transplantation. *Circulation.* 1995;92:3445–3452.
15. Kobashigawa JA, Tobis JM, Starling RC, et al. Multicenter intravascular ultrasound validation study among heart transplant recipients. *J Am Coll Cardiol.* 2005;45:1532–1537.

16. Tona F, Caforio ALP, Montisci R, et al. Coronary flow reserve by contrast-enhanced echocardiography: a new noninvasive diagnostic tool for cardiac allograft vasculopathy. *Am J Transplant.* 2006;6:998–1003.
17. Tona F, Osto E, Tarantini G, et al. Coronary flow reserve by transthoracic echocardiography predicts epicardial intimal thickening in cardiac allograft vasculopathy. *Am J Transplant.* 2010;10:1668–1676.
18. Tona F, Caforio ALP, Montisci R, et al. Coronary flow velocity pattern and coronary flow reserve by contrast-enhanced transthoracic echocardiography predict long-term outcome in heart transplantation. *Circulation.* 2006;114(1 Suppl):I49–I55.
19. Osto E, Tona F, Angelini A, et al. Determinants of coronary flow reserve in heart transplantation: a study performed with contrast-enhanced echocardiography. *J Heart Lung Transplant.* 2009;28:453–460.
20. Caforio ALP, Tona F, Fortina AB, et al. Immune and nonimmune predictors of cardiac allograft vasculopathy onset and severity: multivariate risk factor analysis and role of immunosuppression. *Am J Transplant.* 2004;4:962–970.
21. Caforio AL, Fortina AB, Piaserico S, et al. Skin cancer in heart transplant recipients: risk factor analysis and relevance of immunosuppressive therapy. *Circulation.* 2000;102(19 Suppl 3):III222–III227.
22. Billingham ME, Cary NR, Hammond ME, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: heart rejection study group. The International Society for Heart Transplantation. *J Heart Transplant.* 1990;9:587–593.
23. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* 2015;28:1–39.e14.
24. Rubinshtein R, Yang EH, Rihal CS, et al. Coronary microcirculatory vasodilator function in relation to risk factors among patients without obstructive coronary disease and low to intermediate Framingham score. *Eur Heart J.* 2010;31:936–942.
25. Sezer M, Kocaaga M, Aslanger E, et al. Bimodal pattern of coronary microvascular involvement in diabetes mellitus. *J Am Heart Assoc.* 2016;5:e003995.
26. Mehra MR, Crespo-Leiro MG, Dipchand A, et al. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy—2010. *J Heart Lung Transplant.* 2010;29:717–727.
27. Rentrop KP, Cohen M, Blanke H, et al. Changes in collateral channel filling immediately after controlled coronary artery occlusion by an angioplasty balloon in human subjects. *J Am Coll Cardiol.* 1985;5:587–592.
28. Piek JJ, van Liebergen RA, Koch KT, et al. Clinical, angiographic and hemodynamic predictors of recruitable collateral flow assessed during balloon angioplasty coronary occlusion. *J Am Coll Cardiol.* 1997;29:275–282.
29. Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med.* 1996;15:361–387.
30. Seiler C, Stoller M, Pitt B, et al. The human coronary collateral circulation: development and clinical importance. *Eur Heart J.* 2013;34:2674–2682.
31. Rahmani M, Cruz RP, Granville DJ, et al. Allograft vasculopathy versus atherosclerosis. *Circ Res.* 2006;99:801–815.
32. Vecchiati A, Tellatin S, Angelini A, et al. Coronary microvasculopathy in heart transplantation: consequences and therapeutic implications. *World J Transplant*. 2014;4:93.
33. Hollenberg SM, Klein LW, Parrillo JE, et al. Coronary endothelial dysfunction after heart transplantation predicts allograft vasculopathy and cardiac death. *Circulation.* 2001;104:3091–3096.
34. Camici PG, Crea F. Coronary microvascular dysfunction. *N Engl J Med.* 2007;356:830–840.
35. Suppogu N, Wei J, Quesada O, et al. Angina relates to coronary flow in women with ischemia and no obstructive coronary artery disease. *Int J Cardiol.* 2021;333:35–39.
36. Sasayama S, Fujita M. Recent insights into coronary collateral circulation. *Circulation.* 1992;85:1197–1204.
37. Arras M, Ito WD, Scholz D, et al. Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. *J Clin Invest.* 1998;101:40–50.
38. Tremblay-Gravel M, Racine N, de Denus S, et al. Changes in outcomes of cardiac allograft vasculopathy over 30 years following heart transplantation. *JACC Heart Fail.* 2017;5:891–901.
39. Koerselman J, de Jaegere PPT, Verhaar MC, et al; SMART Study Group. Prognostic significance of coronary collaterals in patients with coronary heart disease having percutaneous transluminal coronary angioplasty. *Am J Cardiol.* 2005;96:390–394.
40. Caiati C, Montaldo C, Zedda N, et al. Validation of a new noninvasive method (contrast-enhanced transthoracic second harmonic echo Doppler) for the evaluation of coronary flow reserve: comparison with intracoronary Doppler flow wire. *J Am Coll Cardiol.* 1999;34:1193–1200.