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Non-Invasive Assessment of Coronary Microcirculation in Heart Transplantation

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Abstract

Heart transplantation (HT) is an accepted treatment for end-stage heart failure (HF). Heart transplantation significantly increases survival, exercise capacity, quality of life and return to work in selected patients with advanced heart failure compared with conventional treatment. The survival rates have improved with the use of new immunosuppressive drugs, with a median survival after transplantation of approximately 11 years. The shortage of donor hearts represents a major limitation in this field. In addition many are the consequences of the limited effectiveness and complications of immunosuppressive therapy (*i.e.* antibody-mediated rejection, infection, hypertension, renal failure, malignancy and coronary artery vasculopathy). In particular, chronic rejection may occur months to years after the transplantation and is referred to as cardiac allograft vasculopathy (CAV). CAV occurs in 32% of the patients after 5 years and ensuing allograft failure from CAV eventually accounts for 30% of recipient deaths after transplantation. Cardiac allograft vasculopathy, involving coronary macro- and microcirculation, is caused by complicated interplay between immunologic and non-immunologic factors resulting in repetitive endothelial injury and localized sustained inflammatory response. Early diagnosis of microvascular dysfunction is substantial. In this review we analyze signs and symptoms of CAV presentation and the different methodologies to achieve an early and precise diagnosis. We will discuss invasive and non-invasive diagnostic tools and their specific role in evaluating graft's function, morphology, the presence of coronary artery disease and possible microcirculation involvement.

Keywords

Heart Transplantation, Coronary Microcirculation, Imaging, Rejection, Echocardiography

1. Introduction

Heart transplantation (HT) is an accepted treatment for end-stage heart failure (HF). Although controlled trials have never been conducted, there is a consensus that transplantation significantly increases survival, exercise capacity, quality of life and return to work in selected patients with advanced heart failure compared with conventional treatment [1]. The survival rates have improved with the use of new immunosuppressive drugs, with a median survival after transplantation of approximately 11 years [2].

Heart transplantation is to be considered in end-stage HF patients with severe symptoms, a poor prognosis, and no remaining alternative treatment options. Patients must be motivated, well informed, emotionally stable and capable of complying with the intensive treatment required postoperatively. Many are the relative and absolute contraindications that must be analyzed case by case [3]. The shortage of donor hearts represents a major limitation in this field; in addition to this, the main challenges in transplantation are the consequences of the limited effectiveness and complications of immunosuppressive therapy (*i.e.* antibody-mediated rejection, infection, hypertension, renal failure, malignancy and coronary artery vasculopathy) [1].

Pre-transplant evaluation consists of several clinical, instrumental and laboratory evaluations; immune-compatibility testing should include ABO blood group typing. Although donor hearts are not selected on the basis of human leukocyte antigens (HLAs), screening for humoral sensitization is accomplished by means of panel-reactive antibody (PRA) testing to determine the presence of circulating anti-HLA antibodies [4]. Sensitization, although usually caused by pregnancy, blood transfusion, prior transplantation or placement of a ventricular assist device (VAD), occasionally occurs without an obvious sensitizing event, representing cross-reactivity between bacterial or viral epitopes and HLA antigens [4]. There are studies supporting the association of elevations in circulating antibodies (PRA > 10%) with an increase in mortality, rejection, and the development of cardiac allograft vasculopathy (CAV) in the post-transplant period, as well as longer waiting times and increased in-list risk of mortality [3].

After heart transplantation, patients may manifest unique clinical complications (associated with the immunosuppressive therapy and cardiac allograft rejection) as well as atypical clinical presentations for infections and systemic inflammatory response syndrome.

Early diagnosis and appropriate intervention for allograft-related and non-allograft-related syndromes with significant morbidity and mortality are the keys to long-term survival of patients after transplantation [2].

Graft rejection can be classified according to its acuity in hyperacute rejection, acute rejection and chronic rejection. It can be as well classified according to the mechanism of the rejection: cell-mediated rejection or antibody-mediated rejection. [2]

Hyperacute rejection is mediated by preexisting antibodies to allogenic anti-

gens and occurs within minutes to hours after the transplantation [5] while acute rejection can be categorized into cell-mediated and humoral-mediated rejection and occurs in the first week to several years after the transplantation. The inflammatory response of cell-mediated rejection consists mainly of T-cell lymphocytes [6] while humoral-mediated rejection consists of antibodies directed against the donor HLA [7].

Chronic rejection may occur months to years after the transplantation and can cause an irreversible graft dysfunction. In heart transplantation, chronic rejection is referred to as cardiac allograft vasculopathy (CAV); based on the ISHLT registry, CAV occurs in 32% of the patients after 5 years and ensuing allograft failure from CAV eventually accounts for 30% of recipient deaths after transplantation [2]. The occurrence of CAV has not decreased despite advancements in immunosuppressive therapies and better prevention of acute rejection [8]. Moreover, according to international registry data, more than 50% of long-term mortality due to graft failure is not attributed to CAV; a fraction of those deaths could be related to underestimated CAV, underlining the need for improving CAV diagnosis long-term after transplant [9].

Generally speaking, chronic rejection manifests similar pathological findings in different organs: obliterative vasculopathy, infiltration of leukocytes, luminal occlusion, and a marked fibrotic response [10].

Specifically, cardiac allograft vasculopathy is caused by complicated interplay between immunologic and non-immunologic factors resulting in repetitive endothelial injury and localized sustained inflammatory response (**Figure 1**) [11].

CAV results from both antigen-dependent and antigen-independent immune
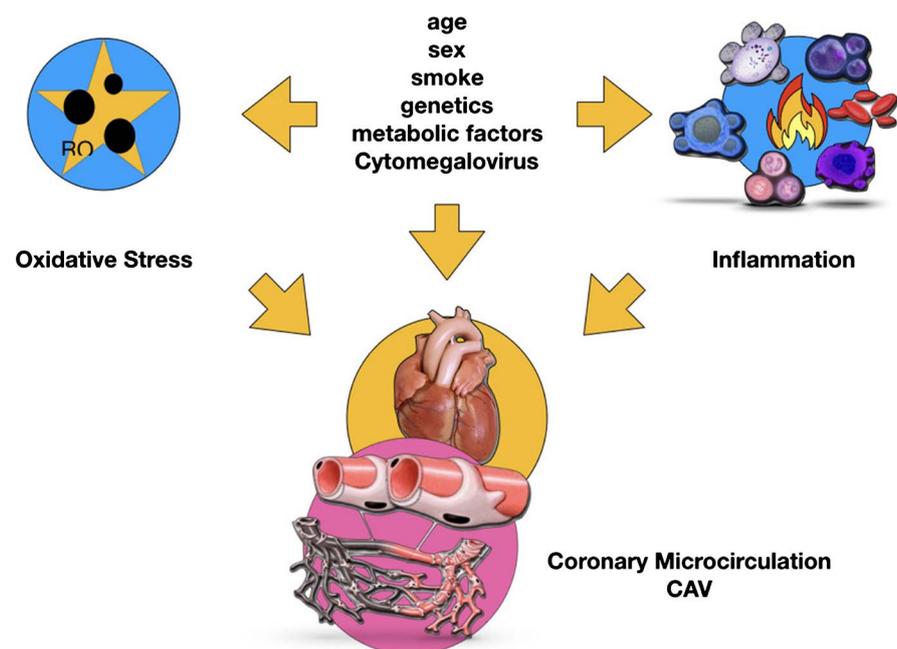
Figure 1. The development of CAV is a multifactorial and complex process initiated by heterogeneous factors that ultimately cause inflammation, oxidative stress and endothelial injury, the precursor to CAV and coronary microvascular dysfunction ([11]-[43]).

factors, and from autoimmune factors as well. Although numerous nonimmune entities are also implicated in the development of CAV, immune factors are the most important causes, given that CAV occurs within the arteries of the donor but not the recipient [12]. Nonimmunologic factors include cause of donor brain death, cytomegalovirus (CMV) infection, age, sex, obesity, dyslipidemia, hyperhomocysteinemia (HHcy), diabetes mellitus, hypertension, smoking and ischemia–reperfusion injury [2] [13].

In 2004, Caforio *et al.* found that risk factors for CAV onset were older donor, male donor, high rejection scores (RS) for severe grades and high cyclosporine at 3 months after transplantation. Risk factors for CAV severity and diffusion were higher donor weight, high prednisone dosage at 1 year and coronary disease pre-HT. High RS was an independent predictor for CAV onset, not severity/diffusion. This suggested an immune basis for CAV onset and nonimmune modulation for progression. High RS for severe grades were supposed to provide a predictor for patients at risk.

More recently, risk factors for development of CAV were proved to include also ischemic cardiomyopathy prior to transplant and re-transplant [14], while statins and mTOR inhibitors resulted preventative [15].

CAV is indeed a progressive and worsening condition characterized by diffuse, concentric thickening of the epicardial and intramyocardial coronary arteries; the obstructive process can progress to near-complete occlusion of the coronary arteries causing micro- and macroinfarctions [16]. The remodeling process can affect the epicardial coronary arteries primarily together with or without the intramyocardial arteries involvement, or the intramyocardial coronary microvasculature as primary and sole involvement. Predominant allograft microvascular dysfunction is detectable in around 15% of patients after HT [16]. Early graft vascular lesions seem to interest mostly small coronary arteries, supporting the hypothesis that microvasculopathy is an immune-mediated phenomenon, similar to epicardial CAV, but which could precede the onset of epicardial CAV [17].

Histologically, repetitive endothelial injury and a localized sustained inflammatory response are followed by intimal hyperplasia and proliferation of vascular smooth muscle cells. Intramyocardial microvasculature shows not only concentric intimal thickening but the presence of plump endothelial cells [18]. Morphologic evaluation of microcirculation can be performed on endomyocardial biopsies to quantify the microvessels remodeling; the main indices utilized for the quantification of microvasculopathy are the microvascular density and arterioles or small arteries percent stenosis [16].

Early diagnosis of microvascular dysfunction in heart transplantation patients is substantial; as a matter of fact, in 2015 Tona *et al.* proved that microvascular dysfunction is independently associated with the onset of epicardial CAV, and associated with a higher risk of death, regardless of CAV onset [19].

So, how to perform an accurate and prompt diagnosis of microvascular and

epicardial CAV?

Cardiac denervation at the time of heart transplantation usually prevents patients from experiencing angina, which is an important warning sign for heart disease. Because of this lack of typical symptoms, transplant patients with CAV usually present with silent myocardial infarction, loss of allograft function or sudden death [13]. Symptoms can be atypical, such as exertional dyspnea, gastrointestinal symptoms, or may have a severe initial presentation such as heart failure or even fatal arrhythmias [8] (**Table 1**).

In order to provide early diagnosis of chronic rejection, post-transplant patients undergo screening with coronary angiography starting at the first year post transplant and annually or bi-annually thereafter. Current guidelines indicate angiography, coupled with the assessment of graft function, as the imaging procedure of choice for CAV diagnosis and classification and to predict long-term prognosis.

2. Evaluation of Graft's Function and Morphology as Indirect Signs of CAV

Echocardiography is the first line imaging modality to assess graft function and morphology; in the immediate post-operative period, echocardiography enables identification of surgical complications and early allograft dysfunction, while in long-term follow-up, serial echocardiographic studies are useful to detect acute graft rejection, CAV and to monitor pulmonary artery systolic pressure [20].

Echocardiography can highlight new wall motion abnormalities that could be associated with the presence of CAV; late reduction of left ventricle ejection fraction (LVEF) is however often associated with progression of CAV with a subsequent poor prognosis [21].

Classical diastolic parameters can be altered in HT patients even without a

Table 1. Signs and symptoms of CAV ([13]-[45]).

CAV CLINICAL PRESENTATION
SIGNS
• Signs of heart failure
• Ankle oedema
• Pulmonary crackles
• Pulmonary oedema
• Hepatomegaly and ascites
• Instrumental evidence of allograft dysfunction
• Instrumental evidence of silent myocardial infarction
SYMPTOMS
• Non-specific fatigue
• Exertional dyspnoea
• Worsening effort intolerance
• Rarely angina

concomitant diastolic dysfunction; as a matter of facts, Tona *et al.* evaluated the role of myocardial performance index (MPI, meaning the sum of isovolumetric contraction time and isovolumetric relaxation time divided by ejection time) as a marker of long-term allograft dysfunction in 154 patients; they found a progressive increase in MPI during long-term follow-up in HT patients with preserved LV systolic function and MPI resulted higher in patients with multiple rejection episodes but no correlation was found with the occurrence of CAV [22].

So, the presence of alterations in LV systolic and diastolic function has an important role in the assessment of prognosis in HT patients, but it is not an accurate marker of graft rejection or CAV.

Worsening of longitudinal strain has been associated with acute cellular rejection [23]. Moreover, segmental longitudinal strain was found to be reduced in LV segments which showed inducible wall motion abnormalities during stress test and strain values could predict CAV [24].

Global longitudinal strain can be considered as a suitable parameter to diagnose subclinical allograft dysfunction, regardless of etiology, by comparing the changes occurring during serial evaluations [20].

Stress echocardiography has been reported to increase the specificity in detecting CAV. Dobutamine has been the most frequently used pharmacological stressor and a sensitivity between 70% and 80% to detect significant CAV at coronary angiography has been reported [20].

Three-dimensional echocardiography (3DE) could have an important role in assessing HT patients since it has been reported to be more accurate and reproducible than two-dimensional echocardiography in quantitating heart chambers volumes and mass. Moreover, during stress echocardiography, 3DE may improve the assessment of regional wall motion, possibly improving the accuracy of acute GR and CAV screening [20].

Cardiovascular magnetic resonance (CMR) imaging provides accurate and reproducible assessment of cardiac structure and function, independent of image plane and BMI, and in addition allows characterization of myocardial tissue [25]. CMR allows evaluation of kinetics alterations, left ventricle and right ventricle mass, volumes, coronary imaging and the in vivo tissue characterization; it also allows identification of areas of delayed hyperenhancement on post-contrast sequences identifying silent myocardial infarction in transplant recipients and distinguishing areas of fibrosis not-related to CAD [26]. In fact, LGE detects 'silent' infarcts in up to approximately a quarter of patients who have only mild vasculopathy angiographically; the majority of infarcts are found in mid and apical segments and is distributed across coronary territories [25].

3. Evaluation of Epicardial Coronary Lesions and Microvascular Dysfunction

In relation to coronary anatomy and physiology, as previously mentioned, angiography is the first invasive tool to be considered; the Stanford classification

system is used to describe the morphology of coronary lesions from a discrete atherosclerosis to concentric arterial obliteration [20]. However, typical CAV features (such as its histological features and microvascular impairment) reduce the diagnostic sensitivity of coronary angiography [27]; coronary angiography can indeed underdiagnose the prevalence and extent of CAV due to the vascular remodeling which in an early stage does not necessarily reduce the luminal diameter [20].

Intravascular ultrasound (IVUS) has emerged as the gold standard for early detection of CAV thanks to high-resolution images of the cross-section of the vessel [20]. It allows the accurate quantitative assessment of lumen size, intimal thickening, vessel wall morphology, and composition [28] and therefore allows detection of angiographically silent early CAV. Combined imaging analysis of progression of angiographic lesions and IVUS-detected maximal intimal thickness (MIT), with a cutoff value of ≥0.35 mm, between 1 and 5 years post-HT allows discriminating patients at high, intermediate, and low risk for adverse long-term cardiovascular outcomes [9]. IVUS should also be performed when there is discrepancy between non-invasive imaging tests and coronary angiography concerning the presence of CAV [20].

Intracoronary imaging with optical coherence tomography (OCT) adds additional spatial resolution that may provide further diagnostic benefit, although it is a currently a research rather than a clinical tool [29].

Coronary flow reserve (CFR), meaning the ratio between resting and maximal possible flow in coronary arteries, is an important functional parameter commonly used to investigate the pathophysiology of coronary circulation. CFR is dependent on the combined effects of epicardial coronary flow and coronary microvascular function. Therefore, impaired CFR may reflect the presence of coronary microvascular dysfunction in the absence of obstructive coronary artery narrowing.

The evaluation of endothelial function and CFR have been investigated invasively by intracoronary Doppler flow wire (IDFW) in many studies, but it is not suitable to detect early onset of endothelial alterations or to be repeated many times during follow-up; it may provide a valuable functional assessment of the microvasculature in CAV, but it is an invasive and expensive procedure [16].

In 2006, Tona *et al.* demonstrated for the first time that CFR evaluated by contrast-enhanced transthoracic echocardiography (CE-TTE) in the left descendent coronary artery (LAD) was a feasible and accurate noninvasive tool for CAV detection [30]; this method was previously shown and validated against Doppler flow wire measurements in coronary artery disease [31]. CFR by CE-TTE has indeed been shown to correlate with angiographically detectable coronary artery lesion severity as well as intracoronary Doppler flow wire measurements [31], and to stratify the risk of cardiac events in HT patients [32].

Moreover, CFR assessment by CE-TTE could detect CAV defined as MIT ≥ 0.5 mm. Microvascular dysfunction, as assessed by CFR, was indeed proved to

be correlated 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 [33].

In 2015, Tona *et al.* demonstrated that coronary microvascular dysfunction assessed after the first year post-HT was likely to become a future independent predictor of new onset epicardial CAV [19]. CFR evaluated by transthoracic Doppler echocardiography (TDE) provided indeed prognostic information on clinically stable HT recipients: a CFR ≤ 2.5 was independently associated with a higher probability of new onset CAV and a higher probability of death, regardless of CAV onset [19].

Non-invasive detection of impaired CFR in HT recipients has been studied also with cardiac magnetic resonance; this examination though is not available for all transplantation recipients due to frequent renal impairment that limits the utilization of gadolinium contrast-based agents; moreover, the high cardiac rate, due to denervation of the heart, may limit the quality of images. In general population with suspected coronary artery disease, the clinical role of stress CMR has been validated in a multicenter study (MR-IMPACT [34]) in 241 patients demonstrating that perfusion CMR is either equivalent or superior to perfusion single photon emission computed tomography (SPECT) for the detection of coronary artery lesions.

In transplant recipients without demonstrable CAV on IVUS, resting myocardial blood flow (MBF) evaluated by CMR is elevated and, consequently, transplant recipients have decreased myocardial perfusion reserve (MPR). In one study, an MPR of >2.3 post-transplant was found to exclude angiographic CAV [35] [36]. In these patients, stress perfusion CMR with adenosine was tested in different studies, demonstrating reduced myocardial blood flow in patients with CAV compared with the normal population, allowing stratification of vascular disease severity. The application of stress-CMR to assess reduced CFR in transplant patients has been used and described by Muehling and co-workers [35]; they proved that non-invasive determination of myocardial perfusion reserve with magnetic resonance perfusion imaging (MRPI) allows exclusion of transplant arteriopathy and closely correlates with the invasive data on coronary flow reserve. Furthermore, they could identify patients with allograft vasculopathy using only the Endo/Epi ratio when LV hypertrophy and/or prior rejection were excluded. In addition, they thought that MRPI in combination with magnetic resonance cine imaging for cardiac function might be a good method for routine surveillance of patients after cardiac transplantation.

On the basis of the previously reported correlation between Doppler velocities and CMR phase contrast measurement, Kennedy *et al.* compared in 17 transplants recipients the CFR, measured by ratio between rest flow in the coronary sinus and during hyperemia (obtained by dipyridamole infusion), with angiography findings [37]. In this study, during hyperemia, a significant difference was seen between the control group and the "severe disease" CAV group.

In 2009, in a large population study [38], besides the quantification of myocardial perfusion reserve, the estimation in HT patients of the mean diastolic strain rate using Strain-Encoding MR resulted a useful parameter for the detection of chronic allograft vasculopathy. In combination with the clinical evaluation, perfusion reserve and diastolic strain represented early markers of this disease and therefore they were considered as an effective tool for the routine surveillance of HTx-recipients, reducing the number of patients in need of invasive testing.

In 2014, Miller CA *et al.* [39] studied 48 transplant recipients with invasive fractional flow reserve (FFR), microvascular resistance and IVUS to assess performance of CMR first-pass perfusion to detect CAV. They proved that, in a comprehensive assessment of cardiac structure and function in the medium to long term after transplantation, CMR-based MPR was independently predictive of both epicardial and microvascular components of CAV. Furthermore, the diagnostic performance of CMR-MPR was significantly higher than that of coronary angiography, the current clinical screening technique.

Computer tomographic (CT) coronary angiography can be employed to exclude relevant CAV. This imaging modality offers the possibility of evaluating the coronary lumen, as well as the wall thickness and intimal hyperplasia, with a potential for early CAV detection. [20] CT coronary calcium score may have utility in the evaluation of post-transplant coronary calcification that is associated with the presence of CAV as defined by current ISHLT guidelines, yet the absence of coronary calcification does not exclude CAV [29]. Dynamic myocardial perfusion imaging during pharmacological vasodilation allows quantification of myocardial perfusion and may provide a non-invasive alternative to Positron Emission Tomography (PET) imaging for the detection of microvascular disease in the future [20].

About the field of nuclear cardiac imaging, in 2003 Yen-Wen Wu *et al.* [40] studied 47 patients at a mean of 34 months after transplantation who received dobutamine thallium-201 (^{201}TI) singol photon emission computed tomography (SPECT), echocardiography and coronary angiography within one months of each other. Dobutamin ^{201}TI SPECT was found to be a useful method for detecting patients with significant CAV and assessing prognosis; the sensitivity, specificity, positive predictive value and negative predictive value of SPECT for the detection of significant angiographic CAV were 89%, 71%, 42% and 96%, respectively [40]. Large reversible perfusion defect was a significant predictor of cardiac death (p = 0.002) [40].

On the other hand, Thompson *et al.* [41] more recently showed that adenosine stress/rest technetium-99m tetrofosmin-gated SPECT was not a sensitive test for detection of CAV in heart transplant recipients; diastolic dysfunction, assessed by SPECT, was not shown to be associated with development of CAV. As a predominantly qualitative technique, perfusion SPECT does not detect CAV until flow-limiting disease of epicardial coronary arteries is present [29].

Cardiac position emission tomography, on the other hand, has been used extensively to study graft perfusion after cardiac transplantation. As mentioned before for CMR perfusion evaluation, normal graft perfusion differs from native heart perfusion, and is increased at rest by approximately 40%; resting MBF decreases however with time after transplant, and may eventually return near normal levels [29].

Flow quantification is suited to evaluate CAV because it can determine global MBF, which detects balanced or diffuse epicardial and microvascular coronary disease. Cardiac PET is the clinical gold standard for non-invasive quantification of MBF and myocardial flow reserve (MFR). It has also been proved the prognostic value of reduced stress MBF and MFR on PET to predict adverse events after heart transplantation [42].

In 2018, Chih *et al.* evaluated forty patients that underwent coronary angiography, rubidium 82 (Rb-82) PET, multivessel intravascular ultrasound (IVUS), and intracoronary hemodynamics. CAV was defined as International Society of Heart and Lung Transplantation CAV1-3 on angiography and maximal intimal thickness ≥ 0.5 mm on IVUS. They found a correlation between noninvasive PET myocardial flow and invasive coronary flow measures in heart transplant patients; PET demonstrated high diagnostic performance for detecting epicardial intimal disease in CAV. Optimal PET diagnostic cutoffs for CAV were rate-pressure product-adjusted myocardial flow reserve (cMFR) < 2.9, stress MBF < 2.3, and coronary vascular resistance > 55; they showed high sensitivity for IVUS-determined CAV of combined PET assessment for any one abnormal PET cMFR, stress MBF, or CVR parameter, as well as high specificity for any two abnormal parameters. These results supported a highly promising role for Rb-82 PET in noninvasive assessment of CAV [43].

They proposed a diagnostic algorithm with cardiac PET as a discriminant for CAV unlikely, CAV possible or CAV likely and subsequent indication to consider or perform coronary angiography and IVUS.

Comparable results were found in the same year by Bravo *et al.* who evaluated 94 HT recipients (prognostic cohort), including 66 who underwent invasive coronary angiography and PET within 1 year (diagnostic cohort). They demonstrated that multiparametric cardiac PET evaluation including quantification of MBF provides improved detection and gradation of CAV severity over standard myocardial perfusion assessment and is predictive of major adverse events [44].

4. Conclusion

In conclusion, cardiac allograft vasculopathy, including epicardial arteries disease and coronary microcirculation impairment, remains to date one of the main challenges in long term management of HT patients causing reduction of their life expectancy. Proper early diagnosis is substantial, in order to provide closer follow-up and therapeutic changes. Epicardial coronary arteries involvement is just of the possible manifestation of CAV; microvascular dysfunction is indeed

an early and prognosis-correlated aspect of the disease. As discussed previously, non-invasive assessment of coronary microcirculation with different methods has been proved to be important in CAV diagnosis and prognosis evaluation, in order to provide close and accurate follow-up in HT recipients.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

3DE	Three-dimensional echocardiography
CAV	Cardiac Allograft Vasculopathy
CE-TTE	Contrast-Enhanced Transthoracic Echocardiography
CFR	Coronary Flow Reserve
CMR	Cardiac Magnetic Resonance
CT	Computer tomographic
FFR	fractional flow reserve
GR	Graft Rejection
HF	Heart Failure
HLAs	Human Leukocyte Antigens
HT	Heart Transplantation
IDFW	Intracoronary Doppler Flow Wire
IVUS	Intravascular Ultrasound
LGE	Late Gadolinium Enhancement
LV	Left Ventricle
LVEF	Left Ventricular Ejection Fraction
MBF	myocardial blood flow
MIT	Maximal Intimal Thickness
MPR	myocardial perfusion reserve
MRPI	magnetic resonance perfusion imaging
OCT	Optical Coherence Tomography
PET	Positron Emission Tomography
PRA	Panel-Reactive antibody
RS	Rejection Scores
SPECT	single photon emission computed tomography
VAD	Ventricular Assist Device