European Heart Journal – Cardiovascular Imaging (2015) **16**, 919–948
doi:10.1093/ehjci/jev139

RECOMMENDATION PAPER

European Association of Cardiovascular Imaging/ Cardiovascular Imaging Department of the Brazilian Society of Cardiology recommendations for the use of cardiac imaging to assess and follow patients after heart transplantation

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Received 30 April 2015; accepted after revision 2 May 2015; online publish-ahead-of-print 2 July 2015

The cohort of long-term survivors of heart transplant is expanding, and the assessment of these patients requires specific knowledge of the surgical techniques employed to implant the donor heart, the physiology of the transplanted heart, complications of invasive tests routinely performed to detect graft rejection (GR), and the specific pathologies that may affect the transplanted heart. A joint EACVI/Brazilian cardiovascular imaging writing group committee has prepared these recommendations to provide a practical guide to echocardiographers involved in the follow-up of heart transplant patients and a framework for standardized and efficient use of cardiovascular imaging after heart transplant. Since the transplanted heart is smaller than the recipient's dilated heart, the former is usually located more medially in the mediastinum and tends to be rotated clockwise. Therefore, standard views with conventional two-dimensional (2D) echocardiography are often difficult to obtain generating a large variability from patient to patient. Therefore, in echocardiography laboratories equipped with three-dimensional echocardiography (3DE) scanners and specific expertise with the technique, 3DE may be a suitable alternative to conventional 2D echocardiography to assess the size and the function of cardiac chambers. 3DE measurement of left (LV) and right ventricular (RV) size and function are more accurate and reproducible than conventional 2D calculations. However, clinicians should be aware that cardiac chamber volumes obtained with 3DE cannot be compared with those obtained with 2D echocardiography. To assess cardiac chamber morphology and function during follow-up studies, it is recommended to obtain a comprehensive echocardiographic study at 6 months from the cardiac transplantation as a baseline and make a careful quantitation of cardiac chamber size, RV systolic function, both systolic and diastolic parameters of LV function, and pulmonary artery pressure. Subsequent echocardiographic studies should be interpreted in comparison with the data obtained from the 6-month study. An echocardiographic study, which shows no change from the baseline study, has a high negative predictive value for GR. There is no single systolic or diastolic parameter that can be reliably used to diagnose GR. However, in case several parameters are abnormal, the likelihood of GR increases. When an abnormality is detected, careful revision of images of the present and baseline study (side-by-side) is highly recommended. Global longitudinal strain (GLS) is a suitable parameter to diagnose subclinical allograft dysfunction, regardless of aetiology, by comparing the changes occurring during serial evaluations. Evaluation of GLS could be used in association with endomyocardial biopsy (EMB) to characterize and monitor an acute GR or global dysfunction episode. RV size and function at baseline should be assessed using several parameters, which do not exclusively evaluate longitudinal function. At follow-up echocardiogram, all these parameters should be compared with the baseline values. 3DE may provide a more accurate and comprehensive assessment of RV size and function. Moreover, due to the

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unpredictable shape of the atria in transplanted patients, atrial volume should be measured using the discs' summation algorithm (biplane algorithm for the left atrium) or 3DE. Tricuspid regurgitation should be looked for and properly assessed in all echocardiographic studies. In case of significant changes in severity of tricuspid regurgitation during follow-up, a 2D/3D and colour Doppler assessment of its severity and mechanisms should be performed. Aortic and mitral valves should be evaluated according to current recommendations. Pericardial effusion should be serially evaluated regarding extent, location, and haemodynamic impact. In case of newly detected pericardial effusion, GR should be considered taking into account the overall echocardiographic assessment and patient evaluation. Dobutamine stress echocardiography might be a suitable alternative to routine coronary angiography to assess cardiac allograft vasculopathy (CAV) at centres with adequate experience with the methodology. Coronary flow reserve and/or contrast infusion to assess myocardial perfusion might be combined with stress echocardiography to improve the accuracy of the test. In addition to its role in monitoring cardiac chamber function and in diagnosis the occurrence of GR and/or CAV, in experienced centres, echocardiography might be an alternative to fluoroscopy to guide EMB, particularly in children and young women, since echocardiography avoids repeated X-ray exposure, permits visualization of soft tissues and safer performance of biopsies of different RV regions. Finally, in addition to the indications about when and how to use echocardiography, the document also addresses the role of the other cardiovascular imaging modalities during follow-up of heart transplant patients. In patients with inadequate acoustic window and contraindication to contrast agents, pharmacological SPECT is an alternative imaging modality to detect CAV in heart transplant patients. However, in centres with adequate expertise, intravascular ultrasound (IVUS) in conjunction with coronary angiography with a baseline study at 4–6 weeks and at 1 year after heart transplant should be performed to exclude donor coronary artery disease, to detect rapidly progressive CAV, and to provide prognostic information. Despite the fact that coronary angiography is the current gold-standard method for the detection of CAV, the use of IVUS should also be considered when there is a discrepancy between non-invasive imaging tests and coronary angiography concerning the presence of CAV. In experienced centres, computerized tomography coronary angiography is a good alternative to coronary angiography to detect CAV. In patients with a persistently high heart rate, scanners that provide high temporal resolution, such as dual-source systems, provide better image quality. Finally, in patients with insufficient acoustic window, cardiac magnetic resonance is an alternative to echocardiography to assess cardiac chamber volumes and function and to exclude acute GR and CAV in a surveillance protocol.

Keywords heart transplantation • echocardiography • three-dimensional echocardiography • Doppler echocardiography • tissue Doppler imaging • myocardial deformation imaging • cardiac allograft rejection • cardiac allograft vasculopathy • stress echocardiography • coronary flow reserve • endomyocardial biopsy

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Abbreviations

GR	graft rejection
CAV	cardiac allograft vasculopathy
CFR	coronary flow reserve
CMR	cardiac magnetic resonance
CT	computed tomography
DTI	Doppler tissue imaging
EACVI	European Association of Cardiovascular Imaging
EF	ejection fraction
EMB	endomyocardial biopsy
FFR	fractional flow reserve
GLS	global longitudinal strain
HT	heart transplantation
HHT	heterotopic heart transplantation
ISHLT	International Society for Heart and Lung Transplantation
IVRT	isovolumetric relaxation time
IVUS	intravascular ultrasound
LV	left ventricle/ventricular
MPI	myocardial performance index
OHT	orthotropic heart transplantation
RV	right ventricle/ventricular
SR	strain rate
STE	speckle tracking echocardiography
TAPSE	tricuspid annular plane systolic excursion

Introduction

Heart transplantation (HT) represents the mainstream therapy of end-stage heart failure, providing a 90% 1-year survival after surgery.[1,2] More than 4000 HT are performed each year in over 300 countries.[3] However, despite advances in surgical techniques, diagnostic approaches, and immunosuppressive strategies, survival after HT continues to be limited by the development of acute/chronic graft rejection (GR) and cardiac allograft vasculopathy (CAV), which represent the leading causes of morbidity and mortality in these patients.[4,5] Since GR is usually an asymptomatic, rapid onset condition bearing a poor prognosis, regular surveillance of HT patients is mandatory, particularly in the first year after HT. In contrast with the rather rapid course of acute GR, CAV is a progressive process that develops over the years, usually without symptoms. Currently, the reference modalities to detect acute GR and CAV are endomyocardial biopsy (EMB) and coronary angiography, respectively.[4] However, both modalities are invasive and expensive, and are associated with non-negligible risk. Although uncommon, EMB complications, including myocardial perforation, pericardial tamponade, arrhythmias, access-site complications, and iatrogenic tricuspid valve injury leading to significant regurgitation, may occur at a rate of 0.5–1.5%.[6–8] Moreover, EMB may not detect GR in up to 20% of patients, due to sampling errors (related to the patchy nature of GR), variability in the interpretation of histological findings, and lack of routine screening for antibody-mediated rejection.[9] Routine coronary angiography used for detecting CAV carries a small, but not negligible risk of complications, such as stroke, heart perforation, coronary artery dissection, and allergic reactions to intravenous contrast.[10] Furthermore, routine coronary angiography should preferably not be performed in patients with moderate to severe chronic kidney disease because of the risk of acute kidney injury (relative contraindication in patients with renal dysfunction).[11]

During the last decade, many efforts have been made in an attempt to create a new non-invasive strategy to detect GR and CAV. Several non-invasive cardiovascular imaging modalities and bio-molecular medicine techniques have been tested, including echocardiography, radionuclide imaging, cardiac magnetic resonance (CMR), intramyocardial electrogram recording, immunologic monitoring, gene expression profile, and biohumoral factors. Nonetheless, current guidelines for the management of heart transplant patients[4] state that no alternative strategy either based on imaging (e.g. echocardiography, CMR) or biomarkers (e.g. natriuretic peptides, cardiac troponins, and C-reactive protein) can be recommended as an alternative to EMB for GR monitoring (Class III; level of evidence: C).

Nevertheless, echocardiography remains the most useful imaging modality to assess and monitor HT patients, as it is widely available, cheap, can be easily and rapidly performed, is safe for both operators and patients, well tolerated, and not associated with the risks of the invasive procedures. Moreover, recent development of new echocardiographic techniques has increased the likelihood of detecting graft dysfunction at an early stage.

Accordingly, the European Association of Cardiovascular Imaging (EACVI) and the Brazilian Cardiovascular Imaging Department developed this document to review and summarize the most recent evidence about the non-invasive assessment of patients who underwent HT, the diagnosis of CAV and acute/chronic GR, with the intent to set up a framework for standardized and efficient use of cardiovascular imaging after HT.

Heart transplantation

Surgical techniques and outcome

Orthotopic heart transplantation

Currently, the bicaval technique (five anastomoses) is the most frequently used surgical technique to perform orthotopic heart transplant (OHT), followed by the standard technique and the total OHT technique. The three techniques were used in 62, 34.7, and $<$3% of the OHT performed in 2007, respectively.[12,13]

The standard technique, also known as biatrial technique, was the first surgical approach used for OHT.[12,13] It entailed simple anastomoses at the mid-level of the left and right atria in addition to the aortic and pulmonary artery anastomoses just above the semilunar valves[14,15] (*Figure 1A*). In addition, the atrial appendages are removed to decrease the risk of post-operative thrombus formation. However, since the right atrial incision is usually close to the donor sinoatrial node, the necrosis of the sinoatrial node with post-operative sinus node dysfunction is a frequent complication. Moreover, with the standard technique, atrial geometry is grossly distorted, resulting in enlarged atria with a 'snowman' shape due to redundant atrial tissue[16] (*Figure 2*).

With the bicaval technique[17–19] (*Figure 1B*), the surgeon performs separate superior and inferior vena cava anastomoses instead of the right atrial anastomosis. The left atrial incision is carried to the base of the left atrial appendage, which is removed leaving a small margin of the atrial cuff around the four pulmonary veins. The

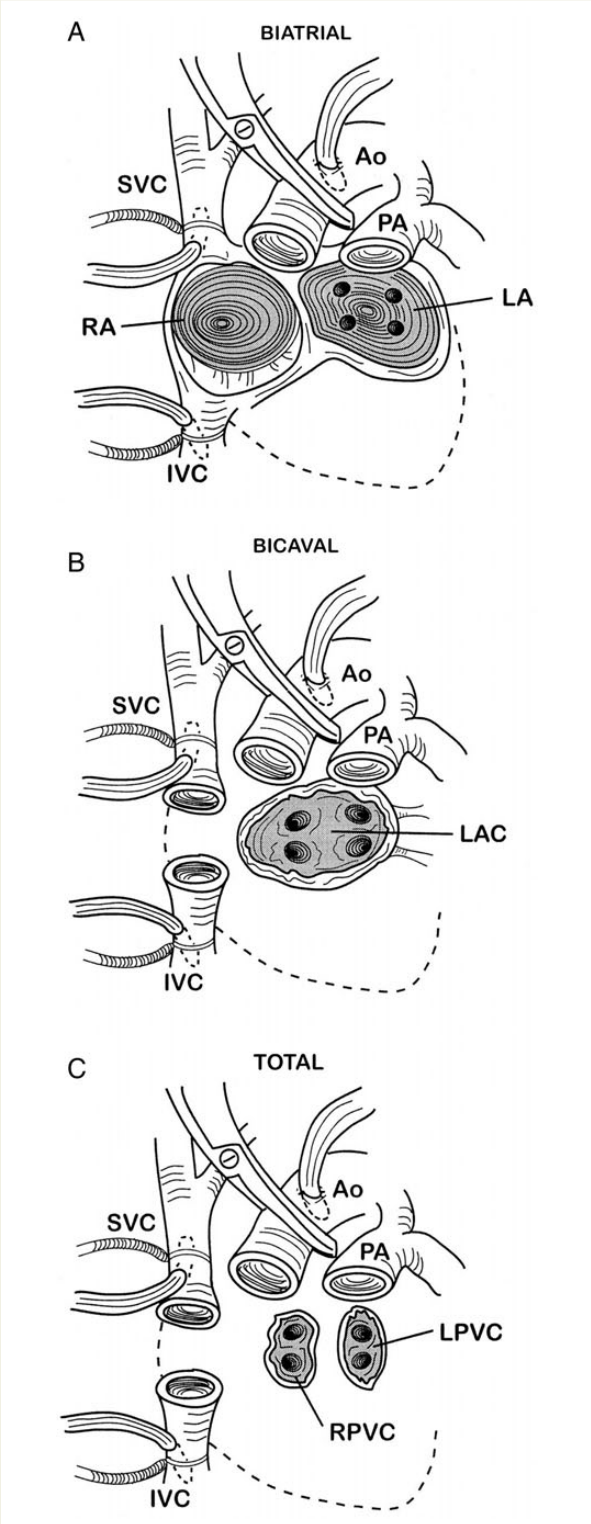


Figure 1 Surgical techniques used in orthotopic cardiac transplantation. Ao, aorta; IVC, inferior vena cava; LAC, left atrial cuff; LPVC, left pulmonary veins cuff; PA, pulmonary artery; RA, right atrium; RPVC, right pulmonary veins cuff; SVC, superior vena cava.

main advantage of the bicaval technique is to retain normal shaped atria, which may preserve atrial and sinus node function.

The total OHT technique (*Figure 1C*) is a complete atrioventricular cardiac transplantation with separate caval and pulmonary vein anastomoses.[20] This technique, while carrying similar advantages to the bicaval operation, presents many technical issues, as bleeding from the suture lines of pulmonary veins and reduced patency of pulmonary veins due to twisting or stenosis at the level of the anastomoses, that may prolong the procedure and increase operative complication rates.[12] Therefore, it is seldom employed today.

Outcome of OHT performed with the different techniques has been compared in several studies with conflicting results. Most data, however, indicate that the bicaval and the total OHT techniques are more physiological than the biatrial method. The first mentioned methods preserve sinoatrial node function and require less pacemaker implants.[13,21,22] Tricuspid valve regurgitation is also significantly lower with these techniques, whereas no effect has been demonstrated on the incidence of mitral regurgitation.[21,22] However, a true comparison between techniques is difficult, as the haemodynamic measurements were recorded at various intervals ranging from days to years post-operatively.

Specific complications for each technique have been documented. Standard technique may trigger arrhythmias and also promote atrial thrombi formation,[21,23] whereas in patients who underwent bicaval or total OHT techniques, superior vena cava stenosis may occur with an overall incidence of 2.4%.[24]

Overall, there is a general consensus about the superiority of the bicaval technique due to the presence of normal right and left atrial sizes, lower right heart filling pressures, and almost normal flows in the caval veins post-operatively.[21,25,26]

Heterotopic heart transplantation

Heterotopic heart transplant (HHT) refers to the placement of a donor heart without recipient cardiectomy.[27] Anatomically, the allograft is placed to the right of the native heart in the right chest to avoid compression by the sternum and at an angle close to 90° to the native heart to allow for the widest possible connection between the native and donor atria. The donor's superior vena cava is attached to the recipient's right atrium so that blood from the body now flows to both hearts. A graft from one of the donor's blood vessels connects the donor's and recipient pulmonary arteries, allowing both hearts to send blood to the lungs. Donor's and recipient's left atria are connected so that blood from the lungs travels to both hearts. The donor heart is attached to the recipient's aorta to transport blood from both hearts out to the body (*Figure 3*).

There are several recognized complications related to HHT. An early post-operative complication is compression of the right middle and right lower lobes of the lung by the allograft, leading to atelectasis, infection, and impaired ventilation. Premature deterioration of the recipient heart is often observed.[28,29] Due to frequent dysrhythmias and different flow conditions, thromboembolic events may occur at an increased rate by leaving the often dilated native heart in place.[30,31]

Compared with OHT, preservation of the 'preconditioned' native right ventricle (RV) seemed to offer better survival to recipients with severe pulmonary hypertension.[32,33] Despite being rarely used today, the heterotopic approach remains a valuable option in recipients with high irreversible transpulmonary pressure gradients and expands the donor pool through use of undersized or

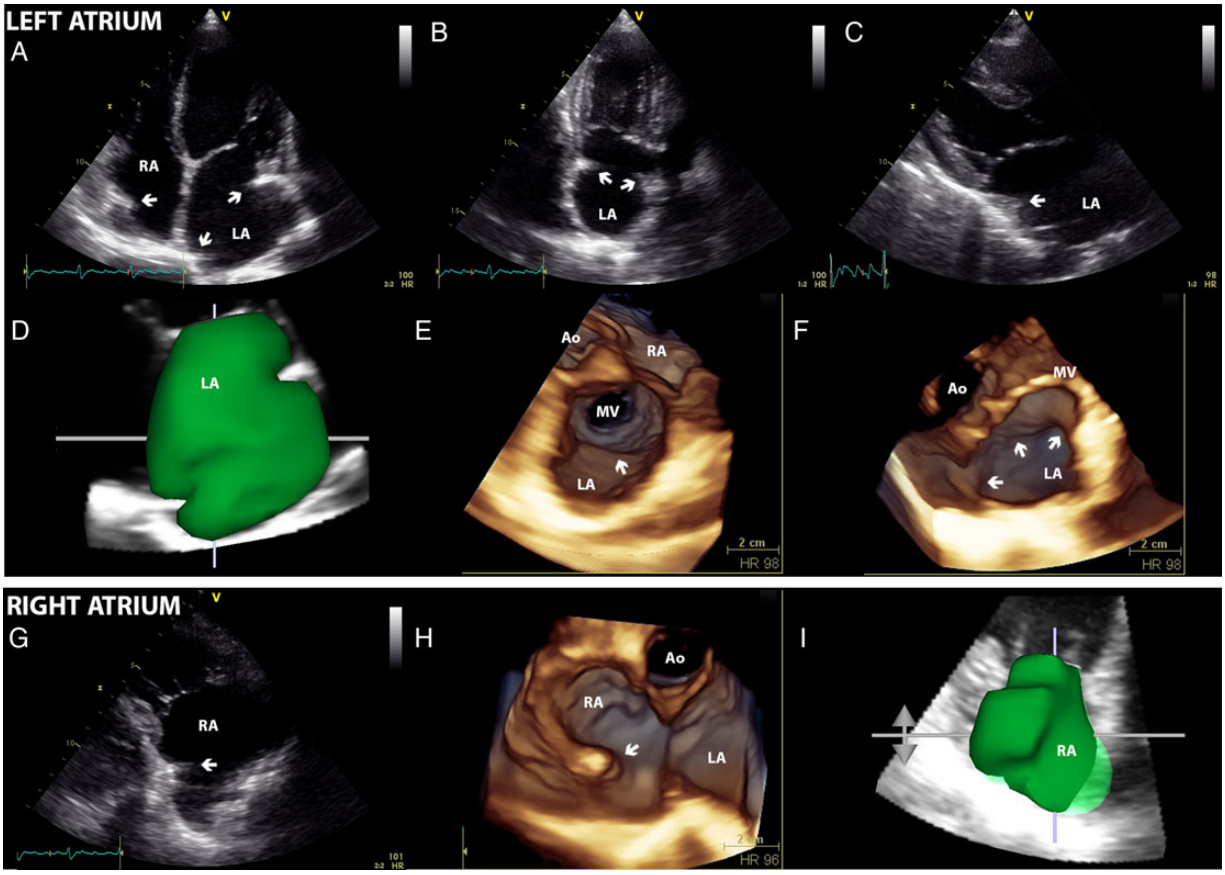


Figure 2 Two- and three-dimensional echocardiography acquisitions in a patient who underwent heart transplant using the standard technique. Both the left and right atria are grossly enlarged and the atrial sutures (arrows) are visualized giving the left atrium the typical 'snowman' shape. Arrow, suture lines, Ao, aorta, LA, left atrium; LV, left ventricle; MV, mitral valve; RA, right atrium.

otherwise compromised allografts.[34,35] However, survival is poorer in HHT recipients, and with improving results in continuous flow ventricular assist devices, many patients can be bridged to normalization of pulmonary artery pressures, allowing OHT.[36]

Heart–lung transplantation

Heart–lung transplantation (cardiopulmonary transplantation) recipients receive an 'en bloc' harvested heart and lung allograft, performing tracheal, right atrial, and aortic anastomosis using cardiopulmonary bypass support during surgery. Care is taken to preserve the donor phrenic nerves and to address the bronchial artery circulation.

The follow-up of heart–lung transplant recipients is similar to that of double and single lung transplant patients. The majority of the post-operative complications, including acute and chronic GR, and infections are related to the lung allograft, not to the cardiac allograft. Isolated acute GR of the heart, however, is infrequent and much less common than after single HT.[37–39] Therefore, most centres do not recommend EMB for routine heart–lung transplantation surveillance after 4–6 months follow-up.[40] However, recommended non-invasive surveillance protocol for acute and chronic cardiac GR in heart–lung transplant is the same as for single HT. The 1-year survival rate after a heart–lung transplant is 65%; the

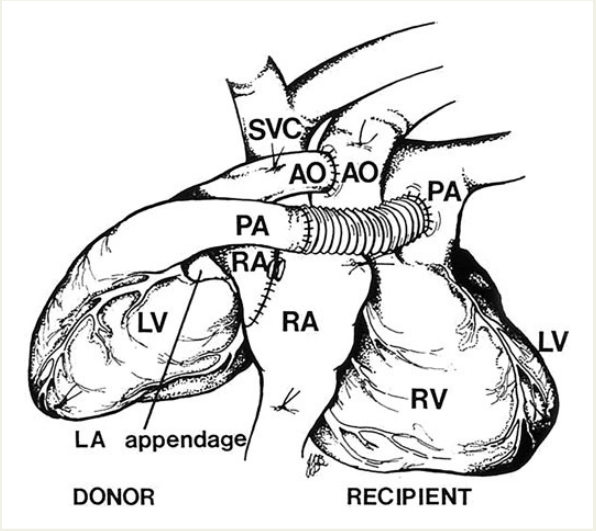


Figure 3 Schematic drawing of the connections between the native and the donor heart in heterotopic heart transplantation. AO, aorta; LA, left atrial; LV, left ventricle; PA, pulmonary artery; RV, right ventricle; SVC, superior vena cava.

5-year survival rate is 40%.[41] Early mortality is secondary to surgical losses and acute allograft failure. The late attrition is due to obliterative bronchiolitis (the chronic GR process of the lung) and rejection.

Physiology of the transplanted heart

Despite the donor's heart function is usually normal, the particular cardiovascular physiology of cardiac allograft (afferent and efferent allograft denervation) and surgical complications (myocardial injury and maladaptation that occur at the time of organ harvest, subsequent rejection injuries) determine the peculiar haemodynamic conditions observed in HT recipients. In addition, pre-existing, undetected, donor cardiac pathologies may also affect transplanted heart function.

Cardiac denervation is an inevitable consequence of HT, as the cardiac plexus is divided in the donor, resulting in a denervated donor heart.[42] With the standard technique, the atrial remnant of the recipient remains innervated, but no impulse will cross the suture line. As a result, the donor atrium is responsible for heart beat generation in the implanted hearts independent on the surgical technique, and it beats at a higher intrinsic rate (90–110 bpm) and shows reduced heart rate variability. Normal heart rate responses to postural changes and heart rate variations in response to stimuli such as the Valsalva manoeuvre and carotid sinus massage are reduced. Drugs or manoeuvres that act via autonomic nerve fibres are also ineffective. However, the heart retains its responsiveness to direct acting agents such as isoproterenol, epinephrine, norepinephrine, dopamine, and dobutamine.

Intrinsic cardiac functions such as impulse formation and conduction and Frank–Starling mechanism are preserved. Therefore, the initial response to Frank–Starling in a denervated heart is an increase in stroke volume, which is critically dependent on an adequate left ventricular (LV) end-diastolic volume. The increase in contractility secondary to increased heart rate is a secondary effect and is dependent on circulating catecholamines. The transplanted heart is, therefore, critically preload dependent, and higher filling pressures are needed to maintain a normal stroke volume.

In patients who underwent OHT using the standard technique, atrial mechanics is impaired, apparently due to the mid-atrial anastomoses between the donor and the recipient hearts. Assessment of LV diastolic function in the transplanted heart is challenging, because sinus tachycardia of the denervated heart often induces merging of E and A waves. In addition, after standard technique, the sinus nodes of the donor and recipient remain intact, with two P waves present on the electrocardiogram up to 3 weeks after surgery, and both donor and the remaining recipient atria may trigger mechanical activity provoking important variations in the transmitral E and A velocities. Moreover, adequate transthoracic recording of pulmonary venous flow is technically demanding after HT, and pulmonary vein flow velocities are often altered by residual recipient atrial tissue contraction that usually occurs at early ventricular filling decreasing the systolic flow component. Finally, end-diastolic atrial contraction will increase pulmonary vein atrial reversal wave (Ar wave) velocity. As a result, the atrial contribution to net stroke volume is generally reduced compared with normal subjects. Bicaval and total HT techniques should provide better atrial mechanics and function, achieving greater left atrial emptying force and more physiological atrioventricular coupling.[26]

Usually, HT patients show restrictive physiology early after the transplant operation, which tends to improve during the follow-up.[43,44] However, early haemodynamic studies conducted in 'healthy' OHT patients reported normal intracardiac pressures at rest, but dramatic increase in LV end-diastolic pressure during exercise, suggesting an occult rather than absent restrictive pattern.[45] As a further confirmation, the first sign of the onset of acute GR is often overt restrictive physiology.[46]

Early allograft failure

Early allograft failure is the main cause of death in the first 30 days after HT and remains an important cause of death throughout the post-transplant period.[5] Main features of early allograft failure are LV or biventricular dysfunction with hypotension, low cardiac output, and high filling pressures. Graft failure is defined as primary in the absence of obvious anatomic or immunologic cause, or as secondary when it can be attributed to reperfusion injury, unresponsive pulmonary hypertension, immunologic cause, or hyperacute rejection.[5,47,48] Echocardiographic evaluation demonstrate reduced global myocardial function [LV ejection fraction (EF) $<$ 45%], loss of contractile reserve, and increased RV volume with systolic dysfunction [tricuspid annulus plane systolic excursion (TAPSE) $<$ 15 mm or a RV EF $<$ 45%].

Reperfusion injury during surgery results from prolonged cold ischaemia time and/or reperfusion ischaemia. It may be transient (myocardial stunning), lasting 12–24 h after HT or may trigger early allograft failure after surgery.[49] Prolonged cold ischaemia time (longer than 5 h) has been associated with higher incidence of allograft dysfunction and is a significant cause of early allograft failure.[50,51]

Hyperacute rejection is an extremely rare condition that occurs within the first 24 h after HT. The hyperacute rejection is the most ominous cause of perioperative LV dysfunction. It is initiated by preformed recipient antibodies (IgG or IgM) that cross-react with endothelial epitopes on the allograft, promoting widespread endothelial damage leading to global ischaemia and catastrophic allograft failure.[52] Hyperacute rejection is most often observed after implantation of an ABO-mismatched heart, or in highly sensitized patients, such as multiparous women or patients who underwent multiple blood transfusions.[52]

Isolated RV failure occurring in the operating room or detected by echocardiography performed during the first 48 h after surgery is defined by the presence of a TAPSE $<$ 15 mm or a RV EF $<$ 45% alongside normal or near-normal LV systolic performance, in the absence of other obvious causes of graft dysfunction triggering severe haemodynamic instability. RV failure accounts for 50% of all cardiac complications and 19% of death in the early period after HT.[53–55] Acute changes in haemodynamics after HT mainly affect RV function. Several factors may influence donor RV function after HT: (i) organ preservation, affecting donor RV contractility; (ii) pre-existing and underestimated pulmonary hypertension of the recipient; and (iii) cardiopulmonary bypass, which may increase pulmonary vascular resistances particularly in patients with abnormal pre-operative values.

Acute graft rejection

Acute graft rejection is the leading cause of mortality during the first year after HT. Its incidence is around 20–40% and is responsible for

Table 1 International Society of Heart and Lung Transplantation classification of acute allograft rejection

1990 Classification		2005 Revised classification	
Grade 0	**No rejection**	**Grade 0 R**	**No rejection**
Grade 1 (mild)		Grade 1 R (mild)	Interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage
1A: Focal	Focal perivascular and/or interstitial infiltrate without myocyte damage		
1B: Diffuse	Diffuse infiltrate without myocyte damage		
Grade 2 (moderate)	One focus of infiltrate with associated myocyte damage		
Grade 3 (moderate)		Grade 2R (moderate)	Two or more foci of infiltrate with associated myocyte damage
3A: Focal	Multifocal infiltrate with myocyte damage		
3B: Diffuse	Diffuse infiltrate with myocyte damage	Grade 3R (severe)	Diffuse infiltrate with multifocal myocyte damage. Oedema, haemorrhage, and vasculitis may be present
Grade 4 (severe)	Diffuse, polymorphous infiltrate with extensive myocyte damage. Oedema, haemorrhage, and vasculitis may be present		

Adapted from Stewart *et al.*[58]

~12% of all fatalities.[5] Acute GR is caused by a recipient alloimmune-dependent process directed against donor major histocompatibility complex antigens, or peptides presented by dendritic cells. Acute GR is categorized into acute cellular or antibody-mediated rejection.[56,57] Acute cellular rejection is well characterized and graded according to established histological criteria—International Society for Heart and Lung Transplantation (ISHLT) classification of body specimen, established in 1990 and then revised in 2004 (*Table 1*).[58] Antibody-mediated rejection is less well characterized; the diagnosis is made on a number of histological and/or immunopathological features without standardized diagnostic criteria. Recently, the ISHLT has addressed this issue and a new grading system has been proposed.[59] However, further work is required to test its consistency and reproducibility.

Most cases of acute cellular rejection are diagnosed by routine surveillance 'protocol' of frequent EMB, even if the patient is asymptomatic and LV EF remains in the normal range.[5,54] Sampling error associated with the patchy nature of acute rejection, variability in the interpretation of histological findings, and non-routine screening for antibody-mediated rejection may result in underestimation of the severity or miss the diagnosis of acute cellular rejection. As a result, the absence of pathologic evidence for severe rejection in the presence of otherwise unexplained LV dysfunction, heart failure, or shock should not prevent treatment for rejection. Symptoms usually develop only when the heart damage is extensive.

Chronic graft rejection

Chronic rejection is mainly determined by CAV and is characterized by a fibro-proliferative process affecting cardiac blood vessels, resulting in concentric narrowing and obliteration of coronary vessels. CAV has been reported to occur in 20% of patients after 3 years, 30% at 5 years, and up to 50% after 10 years.[5] Recurrent acute antibody-mediated GR increases the risk of CAV. Initially, CAV is a diffuse process affecting the large epicardial coronary arteries, the coronary veins, and the microcirculation; later, CAV may also cause focal luminal stenosis.[60] Histologically, CAV is characterized by concentric fibrous intimal hyperplasia and smooth muscle cell proliferation. ISHLT has defined CAV based on visual coronary angiographic stenosis parameters along with LV EF plus LV diastolic function assessment (*Table 2*).[61]

Diagnosis of CAV is usually made by coronary angiography and echocardiographic assessment of allograft function. However, angiography may provide a gross underestimation of this diffuse and concentric vasculopathy. Because of these limitations, alternative imaging modalities, such as intravascular ultrasound (IVUS) and fractional flow reserve (FFR), have been proposed to improve the sensitivity for CAV detection. However, even if IVUS is considered the most sensitive technique for detecting the anatomic features of CAV, its application is limited by its costs and lack of widespread expertise with this imaging technique.

Cardiac interstitial fibrosis, which leads to ventricular stiffness and diastolic dysfunction of the graft, is typically monitored by repeated EMBs.[62] However, this invasive procedure often fails to detect patchy cardiac interstitial fibrosis at an early stage of its evolution. Of note, in some patients, chronic GR may also be triggered by recurrent, chronic immune response against the transplanted heart, which gradually impairs myocardial function by replacing myocardial cells with fibrous tissue.

Graft dysfunction due to other aetiologies

Other causes of graft dysfunction, mimicking GR and CAV, have been reported. The most common of them are cytomegalovirus, *Toxoplasma gondii* and coxsackievirus infections, as well as *Chagas disease* (*Trypanosoma cruzi*) reactivation and/or myocarditis. No cardiac imaging finding has been found to be diagnostic of a specific aetiological cause. In case of a graft dysfunction, serological and

Table 2 International Society of Heart and Lung Transplantation (ISHLT) nomenclature for cardiac allograft vasculopathy

Classification	Findings description
ISHLT CAV0 (not significant)	No detectable angiographic lesion
ISHLT CAV1 (mild)	Angiographic left main stenosis of <50%, or primary vessel with maximum lesion of <70%, or any branch stenosis <70% (including diffuse narrowing) without allograft dysfunction
ISHLT CAV2 (moderate)	Angiographic left main stenosis of ≥50%; a single primary vessel ≥70%, or isolated branch stenosis ≥70% in branches of two systems, without allograft dysfunction
ISHLT CAV3 (severe)	Angiographic left main stenosis of ≥50%, or two or more primary vessels ≥70% stenosis, or isolated branch stenosis ≥70% in all three systems; or ISHLT CAV1 or CAV2 with allograft dysfunction (defined as LVEF ≤ 45%, usually in the presence of regional wall motion abnormalities) or evidence of significant restrictive physiology (which is common but not specific)

Definitions:
(1) A 'primary vessel' denotes the proximal and middle 33% of the left anterior descending artery, the left circumflex, the ramus, and the dominant or co-dominant right coronary artery with the posterior descending and posterior branches;
(2) A 'secondary branch vessel' includes the distal 33% of the primary vessels or any segment within a large septal perforator, diagonals and obtuse marginal branches, or any portion of a non-dominant right coronary artery;
(3) Restrictive cardiac allograft physiology is defined as symptomatic heart failure with echocardiographic *E* to *A* velocity ratio >2 (>1.5 in children), shortened isovolumetric relaxation time (<60 ms), shortened deceleration time (<150 ms), or restrictive haemodynamic values (right atrial pressure >12 mmHg, pulmonary capillary wedge pressure >25 mmHg, cardiac index <2 l/min/m^2)

Adapted from Mehra *et al.*[61]

specific immunohistochemistry testing is necessary for accurate aetiological diagnosis.[4,63,64]

Infective endocarditis in heart transplant patients

There are limited data about the incidence and prognosis of infective endocarditis after HT. Even in the ESC guidelines on prevention, diagnosis, and treatment of infective endocarditis,[65] this condition is not mentioned. However, it has been reported that the incidence of infective endocarditis among HT recipients was 50/110-fold higher than in the general population.[66,67] Possible explanations for this markedly increased incidence include catheter-related and other nosocomial blood stream infections, LV assist device-related mediastinitis, donor heart contamination, deep wound infections following transplant, EMB, and suppression of cell-mediated immunity. Forty to fifty per cent of patients had tricuspid valve infection. This rate of tricuspid involvement approaches that in intravenous drug users with endocarditis. Aziz *et al.*[68] reported a 0% bacteraemia rate just prior to EMB, but a 70% rate of coagulase-negative *Staphylococcus* bacteraemia in atrial blood immediately following the biopsies. Despite the fact that it seems that increased numbers of EMB were associated with tricuspid valve infection, the true role of EMB (as opposed to catheter-related and other blood stream infections) as a predisposition to infective endocarditis remains undefined. In the reported series, the most common pathogens were *Staphylococcus aureus* (40% of cases) and *Aspergillus fumigatus* (30% of post-transplant endocarditis). All patients who developed *A. fumigatus* endocarditis following HT had antecedent *Cytomegalovirus* viremia. This finding suggests that patients with *A. fumigatus* infection had heightened immunosuppression prior to infective endocarditis. In literature, mortality of HT patients with infective endocarditis ranges from 22 to 80%, with a peak rate of 100% endocarditis-related mortality in *Aspergillus* infective endocarditis following HT.[66]

The prevalence of endocarditis is high in HT recipients, and it should be treated promptly because of a high mortality rate. Therefore, a high score of suspicion should be used in this specific population. Echocardiography should be performed rapidly to exclude the diagnosis and even repeated when initial echocardiographic study is negative, but the clinical suspicion remains moderate or high.

Conventional echocardiographic evaluation

Echocardiography is the first line imaging modality to assess HT patients, providing accurate information about graft anatomy and function, which is part of all serial evaluations during post-transplant follow-up.[4] The ISHLT Guidelines for Heart Transplant Recipients do not specify the timing of echocardiographic evaluations and do not recommend echocardiography as an alternative to serial EMB in rejection monitoring.[4] Nevertheless, echocardiography is commonly used when there is a high clinical suspicion of acute GR despite negative EMB findings and to monitor LV function during confirmed GR episodes.

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 GR (*Figure 4*), CAV (*Figure 5*), and to monitor pulmonary artery systolic pressure. A main technical issue is that echocardiographic parameters are more variable in HT patients than in the general population. This fact makes it difficult to define 'normal' transplanted heart morphology and function and to identify appropriate cut-off values for the different echocardiographic parameters to detect allograft dysfunction.[69]

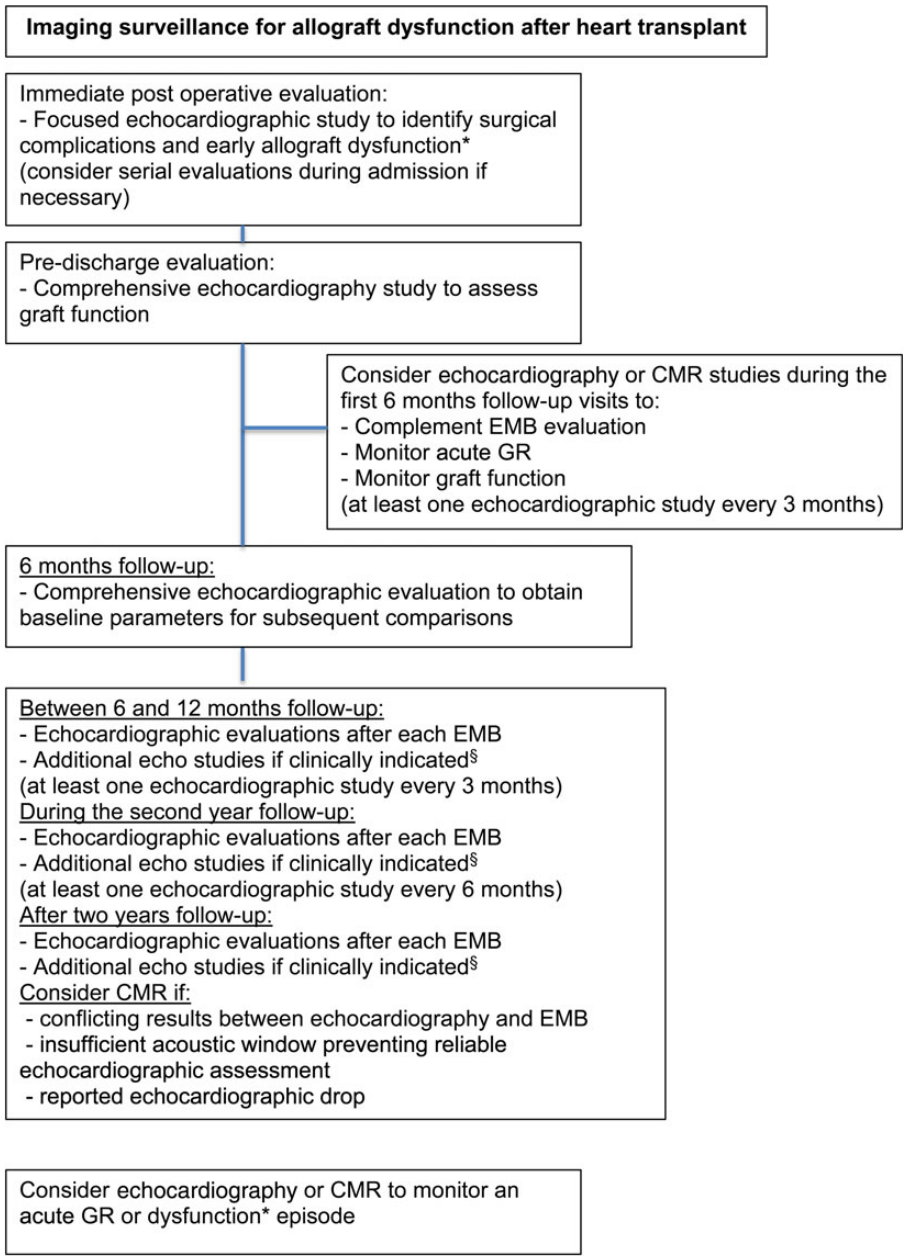


Figure 4 Flow chart summarizing the timing and imaging modalities used to monitor allograft dysfunction after heart transplant. CMR, cardiac magnetic resonance; EMB, endomyocardial biopsy; GR, graft rejection *Allograft dysfunction: a confirmed, reported echocardiographic evaluation, drop of the left ventricular ejection fraction of more than 10 percent points to a value less than 50% compared to 6th month baseline evaluation §Patients with suspected or confirmed allograft dysfunction: new symptoms of possible cardiac dysfunction; ECG changes.

Therefore, in this special population, having a comprehensive individual baseline echocardiographic study available for comparison during the serial follow-up studies is more useful than the absolute value of each measurement.[70] Such a baseline echocardiographic assessment should be performed after at least 6 months from the HT procedure. Earlier after HT, the adaptation of the new heart to the thoracic space, its different positioning in the chest compared with the native heart, and the presence of confounders such as early allograft dysfunction, pericardial effusion, and other co-morbidities (e.g. sepsis, mechanical complications of EMB, and multiple organ dysfunction) may affect the recorded echocardiographic parameters and decrease the sensitivity of echocardiography to detect acute GR during follow-up.[71]

Timing of echocardiographic assessment and acquisition protocol

Due to the lack of evidences about the optimal timing of echocardiographic studies in HT patients, this writing committee reached a consensus on recommending echocardiographic evaluations as described in *Figures 4* and *5*.

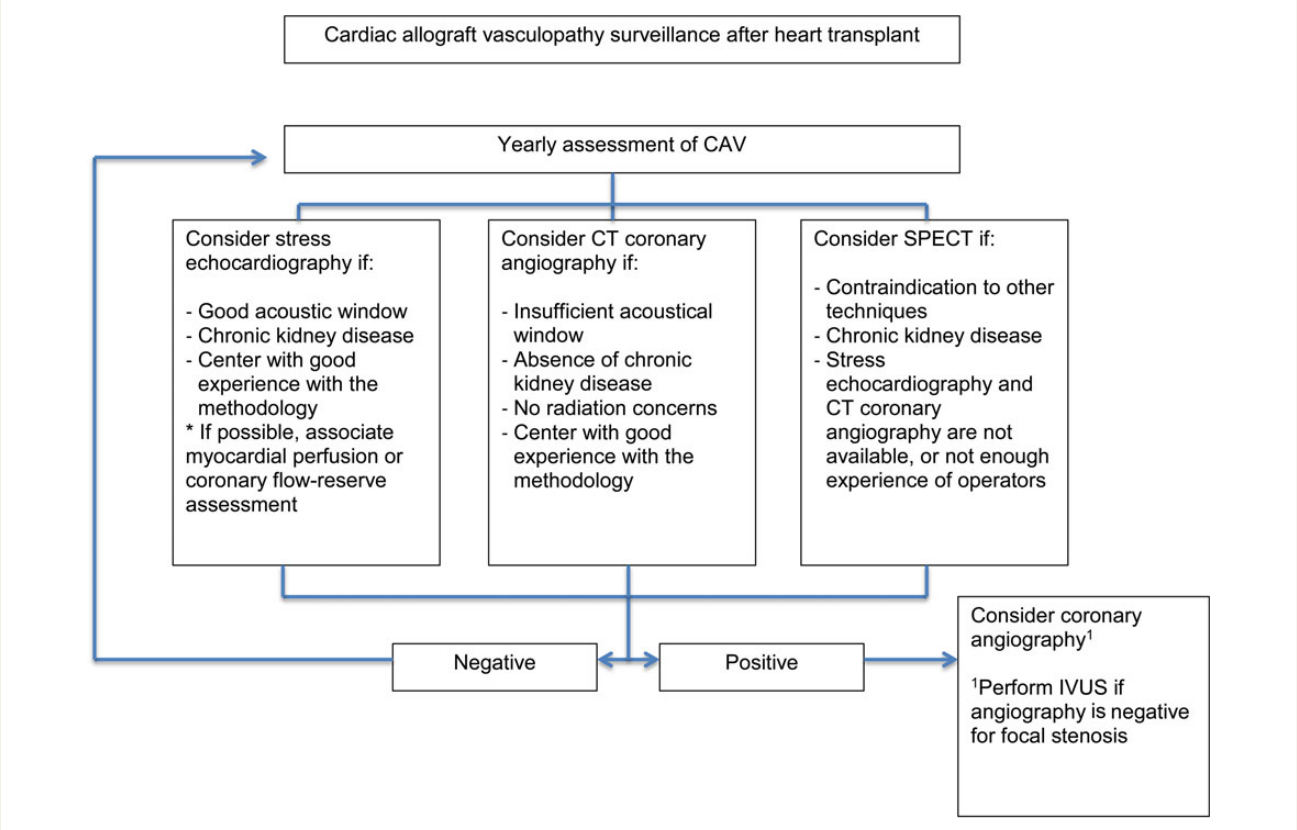


Figure 5 Flow chart summarizing the timing and imaging modalities used to monitor cardiac allograft vasculopathy after heart transplant. CAV, cardiac allograft vasculopathy; CT, computerized tomography; IVUS, intravascular ultrasound; SPECT, Single Photon Emission Computerized Tomography.

As the donor heart is normal in size, it is smaller than the original recipient dilated heart; therefore, it is located more medially in the mediastinum and tends to be rotated clockwise. Due to this rotation and medial displacement in some patients, standard transthoracic views are obtained from non-standard transducer positions, with variability from patient to patients.

Standard echocardiographic evaluation protocol should include two-dimensional (2D) as well as spectral and colour Doppler imaging (*Tables 3* and *4*). In case of abnormalities of graft geometry or function, additional views and acquisitions are often required. In all studies, it is necessary to measure the size of the four cardiac chambers and great vessels, assess LV and RV function, assess heart valve function, estimate pulmonary artery systolic pressure, and describe the pericardium (*Table 5*). To address specific clinical questions and to improve sensitivity in detecting morpho-functional changes in the transplanted hearts, laboratories that have the techniques and expertise in using them can employ advanced echocardiographic techniques (*Table 6*).

Cardiac chamber morphology and function

LV geometry and systolic function

During the first month after HT, the LV morphology is characterized by an increase in LV mass and in wall thickness,[72] that is likely to be caused by inflammatory cell infiltration and graft oedema. LV wall thickness usually tends to decrease after 3 months, probably due to liquid reabsorption.

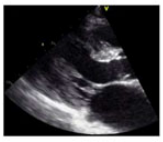
During long-term follow-up, a secondary increase in LV mass and wall thickness may occur as a consequence of many factors such as repetitive rejection episodes, chronic tachycardia, and systemic hypertension, usually induced by immunosuppressive agents.[73] The increase in LV mass and wall thickness has also been described during acute GR episodes[74] probably secondarily to myocardial inflammation which induces cellular oedema. However, several reports demonstrate a low sensitivity of this parameter because of the fluctuation of wall thickness related to scattered acute rejection cellular oedema pattern combined with immunosuppressive therapy antiedemigen effect.[75,76] The recommendation is to monitor wall thickness during follow-up. Sudden and evident changes in LV mass and/or wall thickness should raise suspicion of acute GR.

Usually, in patients with uncomplicated HT, LV dimensions remain within the normal range, as demonstrated in a 10- to 15-year follow-up study.[73]

LV pump function is usually normal after HT. Despite being the strongest predictor of outcome in HT patients,[77] LV EF is not an early indicator of graft dysfunction and usually does not correlate with the grade of rejection found at the EMB. Moreover, LV EF is an insensitive marker of acute GR.[78] Late reduction of LV EF is often associated with progression of CAV and carries a poor prognosis.[73]

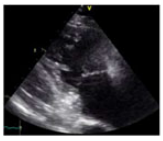
Table 3 Two-dimensional echocardiography views, acquisition techniques, and parameters to be routinely assessed in patients who underwent heart transplant

Parasternal long-axis of LV



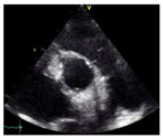
Acqusition techniques
2D loop + M-mode + color Doppler for MV and AV
Subject to assess and parameters to measure
- Left ventricle morphology, diameters, wall thickness, mass, function and motion abnormalities
- Left atrium morphology and anterior-posterior diameter
- Aortic and mitral valve morphology and function
- Pericardium

Parasternal long-axis of RV inflow



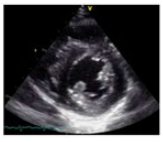
Acqusition techniques
2D loop + color Doppler for TV + CW Doppler
Subject to assess and parameters to measure
- Tricuspid valve morphology and function
- Retrograde and anterograde flow pattern and velocities
- Right atrium morphology

Parasternal short-axis of bifurcation of the PA



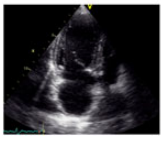
Acqusition techniques
2D loop + color Doppler for PV and PA + CW and PW Doppler for PV
Subject to assess and parameters to measure
- Pulmonary valve function and morphology. Anterograde and retrograde flow velocities
- Mean and diastolic pulmonary artery pressure
- Assessment of pulmonary stenosis at suture line

Parasternal short-axis at papillary muscle level



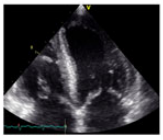
Acqusition techniques
2D loop + M-mode
Subject to assess and parameters to measure
- LV wall tickness
- LV global andLV regional systolic function - mid-ventricular segments
- IVS morphology and movement
- Pericardium

Apical 2-chamber



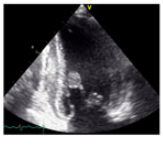
Acqusition techniques
2D loop + color Doppler for MV + CW and PW for MV
Subject to assess and parameters to measure
- Left atrium morphology and volume
- Mitral valve morphology and function. Anterograde and retrograde flow

LV focused apical 4-chamber



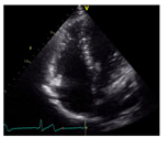
Acqusition techniques
2D loop
Subject to assess and parameters to measure
- LV volumes and function
- LV regional systolic function
- IVS evaluation

LV focused apical 2-chamber



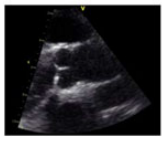
Acqusition techniques
2D loop
Subject to assess and parameters to measure
- LV volumes and function
- LV regional systolic function

RV focused apical 4-chamber



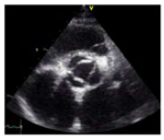
Acqusition techniques
2D loop + M-mode
Subject to assess and parameters to measure
- Right ventricle morphology, diameters and motion abnormalities

Parasternal long-axis of aorta



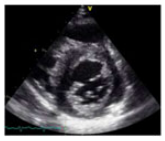
Acqusition techniques
2D loop + color Doppler for AV
Subject to assess and parameters to measure
- Aorta diameters and morphology
- Aortic valve morphology and function
- Suture line
- LV outflow diameter

Parasternal short-axis at aortic valve level



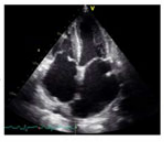
Acqusition techniques
2D loop + color Doppler for AV and TV + CW Doppler for TV
Subject to assess and parameters to measure
- Aortic valve morphology and function
- Tricuspid valve morphology and function. Anterograde and retrograde flow velocities
- RV outflow diameter

Parasternal short-axis at mitral valve level



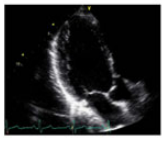
Acqusition techniques
2D loop + M-mode + color Doppler for MV
Subject to assess and parameters to measure
- Mitral valve morphology and function
- LV regional systolic function - basal segments
- Pericardium

Apical 4-chamber



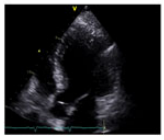
Acqusition techniques
2D loop + color Doppler for MV + CW and PW for MV
Subject to assess and parameters to measure
- Left atrium morphology and size
- Pulmonary vein flow pattern and velocities
- Mitral and tricuspid valve morphology and function. Anterograde and retrograde flow
- IAS evaluation

Apical 3-chamber



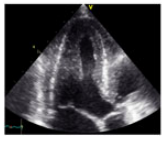
Acqusition techniques
2D loop + color Doppler for MV and AV
Subject to assess and parameters to measure
- Left atrium morphology
- Mitral valve morphology and function. Anterograde and retrograde flow
- Aortic valve morphology and function. Anterograde and retrograde flow velocities

LV focused apical 5-chamber



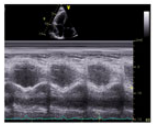
Acqusition techniques
2D loop
Subject to assess and parameters to measure
- LV regional systolic function
- IVS evaluation

LV focused apical 3-chamber



Acqusition techniques
2D loop
Subject to assess and parameters to measure
- LV function
- LV regional systolic function

TAPSE

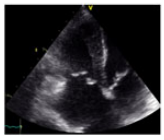


Acqusition techniques
M-mode
Parameters to measure
- Tricuspid annulus displacement

Continued

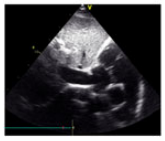
Table 3 *Continued*

RA focused apical 4-chamber



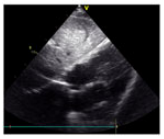
Acqusition techniques
2D loop + color Doppler for TV + CW Doppler
Subject to assess and parameters to measure
- RA diameters and area
- Tricuspid valve morphology and function. Anterograde and retrograde flow

Subcostal short-axis of basal RV



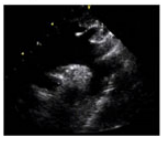
Acqusition techniques
2D loop + color Doppler
Subject to assess and parameters to measure
- IVC diameters
- Hepatic vein flow pattern

RV subcostal 4-chamber



Acqusition techniques
2D loop + color Doppler
Subject to assess and parameters to measure
- IAS evaluation
- RV thickness

Suprasternal aortic arch long-axis



Acqusition techniques
2D loop + color Doppler + CW Doppler
Subject to assess and parameters to measure
- Aorta morphology, diameters and flow

AV, aortic valve; CW, continuous wave; IAS, inter-atrial septum; IVC, inferior vena cava; IVS, interventricular septum; LV, left ventricular; MV, mitral valve; PW, pulsed wave; RA, right atrium; RV, rightventricle; TV, tricuspid valve.

New wall motion abnormalities have been reported to be associated with the presence of CAV yielding a low sensitivity but a high specificity (69–100%).[79] Detection of new wall motion abnormalities at rest should raise suspicion of progression of CAV, encouraging further tests to rule out this hypothesis. Side-by-side visualization of the current echocardiographic study with a previous one (or baseline one) will increase both sensitivity and specificity of new wall motion abnormality detection.

LV diastolic function

Changes in LV diastolic function are a more sensitive marker of acute GR than the reduction of LV EF. Early investigators found a clear relationship between severe impairment of diastolic function and development of graft failure.[46] Evidences that have been collected so far suggest that rejection episodes may alter the diastolic dynamics of the heart earlier than the EF.[80] This is probably caused by myocardial oedema or by initial fibrosis, which stiffens the LV myocardium. Impairment of diastolic function has been reported during episodes of acute GR with a subsequent improvement after effective treatment.[81,82] A stiffer heart may also result from chronic GR. Moreover, diastolic dysfunction with a preserved LV EF has been associated with worse long-term prognosis, development of heart failure symptoms, higher number of rejection episodes and cumulative immune-mediated graft damage.[46] Unfortunately, studies that attempted to correlate indexes of diastolic function (including pulmonary vein flow pattern, transmitral velocity of propagation) and myocardial performance index (MPI) with acute GR episodes have shown conflicting results. *Table 7* summarizes the accuracy of different echocardiographic techniques to detect acute GR.

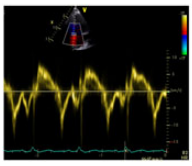
Doppler indices of mitral inflow have been the most widely investigated parameters. The filling pattern is usually more preserved in the bicaval technique surgery, because with this technique atrial contraction is kept more physiologic. Mena *et al*[92] performed a systematic review and found 19 good quality studies from 1985 to 2005 about the use of transmitral Doppler diastolic indexes (E wave, A wave, E/A ratio, deceleration time, isovolumic relaxation time) in predicting acute GR. They were unable to demonstrate any significant correlation between variations of LV filling parameters and proved acute GR. Other studies evaluated pulmonary vein flow parameters and mitral inflow propagation velocity, but again they were unable to demonstrate any clear correlation between these parameters and acute GR severity.[71,90] Moreover, these parameters have been reported to be abnormal even in some 'healthy' HT patient.[46] Unfortunately, the assessment of LV filling is affected by many variables, including pre-load conditions, atrial dynamics and morphology (dissociation of recipient and donor atrial contraction), LV compliance and contractility, end-systolic volume, and heart rate. Therefore, diastolic function may be impaired by different reasons and is therefore not specific to rejections. Finally, high heart rate, usually present in the denervated heart, makes the assessment of diastolic function more difficult due to the frequent fusion of E and A waves. Nevertheless, we recommend to continue evaluating diastolic parameters, because the occurrence of diastolic dysfunction carries significant prognostic value[93] in any case and because the diagnosis of acute GR becomes increasingly accurate when additional echocardiographic parameters are impaired.

Based on the concept that GR affects both LV and RV diastolic and systolic function simultaneously, MPI has been proposed as an early marker of rejection in HT patients. At present, the accuracy of MPI to detect acute GR in HT patients is controversial.[88,94,95] Tona *et al.*[96] evaluated the role of MPI as a marker of long-term allograft dysfunction in 154 patients and found a progressive increase in MPI during long-term follow-up in HT patients with preserved LV systolic function. MPI resulted higher in patients with multiple rejection episodes but no correlation was found with the occurrence of CAV.

Usually, DTI parameters are useful to improve the accuracy of the assessment of LV diastolic function.[97,98] However, in HT patients the role of myocardial velocities should be interpreted with caution, because velocities may be affected by the exaggerated translation motion of the transplanted heart.[99] Similarly to transmitral LV filling parameters, several studies have looked into the ability of DTI parameters to predict acute GR. In normal HT patients, LV e′ and s′ wave velocities tend to be low in the first weeks after transplantation and

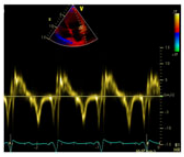
Table 4 Doppler tracings and measurements to be routinely performed in patients who underwent heart transplant

Septal Mitral Annulus



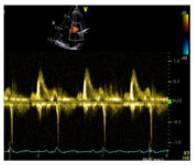
Acqusition techniques
PW Tissue Doppler
Parameters to measure
- Mitral annulus displacement velocities

Lateral Mitral Annulus



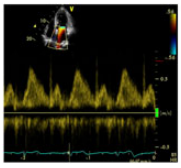
Acqusition techniques
PW Tissue Doppler
Parameters to measure
- Mitral annulus displacement velocities

Mitral Anterograde Flow



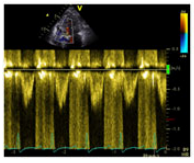
Acqusition techniques
PW Doppler
Parameters to measure
- E and A wave velocities
- E wave deceleratiom time

Pulmonary Vein Flow



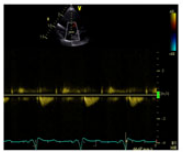
Acqusition techniques
PW Doppler
Parameters to measure
- Ar wave peak velocity and duration

LVOT Flow



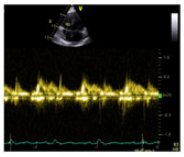
Acqusition techniques
PW Doppler
Parameters to measure
- LVOT anterograde flow velocities and VTI

Aortic Anterograde Flow



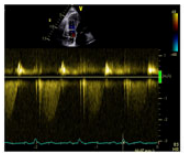
Acqusition techniques
CW Doppler
Parameters to measure
- Aortic anterograde flow velocities and VTI
- Aortic gradients

Tricuspid Anterograde Flow



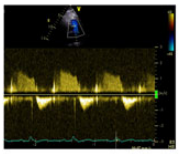
Acqusition techniques
PW Doppler
Parameters to measure
- E wave velocities

Tricuspid Regurgitant Flow



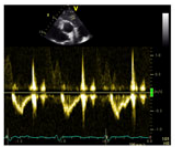
Acqusition techniques
CW Doppler
Parameters to measure
- Maximum regurgitant flow velocitie

Pulmonary Regurgitant Flow



Acqusition techniques
CW Doppler
Parameters to measure
- Pulmonary regurgitant flow velocities
- Pulmonary artery pressures

Pulmonary Anterograde Flow



Acqusition techniques
PW Doppler
Parameters to measure
- Pulmonary anterograd flow aceleration time
- Pulmonary artery pressure

CW, continuous wave; LVOT, left ventricular outflow tract; PW, pulsed wave; TVI, time-velocity integral.

then they increase gradually. After 1 year, DTI velocities in HT patients tend to be lower compared with the normal population.[72] Dandel *et al.*[87] and Puleo *et al.*[83] found a strong association between the reduction of e′ wave velocity and episodes of acute GR and CAV, whereas Stengel *et al.*[86] failed to confirm this finding. Due to high specificity of DTI parameters, severe rejection could be safely excluded in the presence of a mitral annular a′ wave velocity >9 cm/s or <10% reduction in diastolic mitral annular motion velocities.[86,87]

Similarly, Sun *et al.*[71] described a large cohort of HT patients (264 patients with >400 echocardiographic studies performed) followed up to 3 years after HT. Based on EMB results, patients were divided into no rejection (ISHLT grade 0 and 1a) and rejection (ISHLT grade 1B or higher) groups. The authors found that several echocardiographic parameters were statistically different between the two groups. Patients in the rejection group showed more pericardial effusion (but with a high prevalence of pericardial effusion in both groups), shorter IVRT (<90 ms), and greater peak velocity and duration of Ar wave and a′ wave at both septal and lateral LV walls. However, the authors could not confirm the previous results[83,87] on e′ wave reduction as a marker of acute GR, probably because of the different definition of the rejection group (in Dandel's study, acute GR was defined as more than Grade 2 ISHLT rejection score). Notably, there was a significant correlation between the number of abnormal echocardiographic parameters and rejection grade. On the other hand, a completely normal echocardiographic examination provided a high negative predictive value for detecting acute GR at EMB. Mankad *et al.*[84] combined peak systolic wave Doppler (s′ wave) together with peak diastolic e′ wave velocity (evaluated at lateral mitral annulus) in a new index, the peak-to-peak mitral annular velocity. They found that values equal or higher than 135 mm/s have a sensitivity of 93%, specificity of 71%, and a negative predictive value of 98% in excluding acute GR (defined as greater than grade 1B

Table 5 Recommended measurements to be reported (recommended acquisition technique) in echocardiographic studies obtained from heart transplant patients

Cardiac structure	Mandatory measurements	Optional measurements
Left ventricle	End-diastolic, end-systolic volumes (2D biplane) Ejection fraction (2D biplane) Interventricular septum and infero-lateral wall thicknesses (2D or 2D guided M-mode) Lateral s and e′ wave velocities (DTI) Global longitudinal strain (STE)	End-diastolic, end-systolic volumes (3D) Ejection fraction (3D) Mass (3D) Myocardial performance index (Doppler)
Right ventricle	TAPSE (M-mode) Fractional area change (2D) Free wall thickness Free wall s wave velocity (DTI) Free wall longitudinal strain	End-diastolic, end-systolic volumes (3D) Ejection fraction (3D) Myocardial performance index
Mitral valve	E and A wave velocities (PW Doppler) Semi and quantitative assessment of severity of regurgitation (vena contracta diameter, E wave velocity, PW pulmonary veins, PISA)	Mitral annulus (3D)
Left/right atrium	Volume (2D) Pulmonary vein velocity (PW)	Volume (3D)
Aorta	Root diameter (2D) Diameter at the suture line (2D) Ascending aorta diameter (2D) Aortic arch diameter (2D)	
Tricuspid valve	Semi-quantitative assessment of severity of regurgitation (vena contracta, PISA radius) Systolic atrioventricular gradient (CW Doppler)	Morphology (3D) Annulus size (3D)
Inferior vena cava	Expiratory diameters and respiratory collapse (2D)	
Pericardium	Presence and semi-quantitative assessment of severity and extent of effusion	

2D, two-dimensional echocardiography; 3D, three-dimensional echocardiography; CW, continuous-wave Doppler; DTI, Doppler tissue imaging; PW, pulsed wave; STE, speckle tracking echocardiography; PISA, proximal isovelocity surface area; TAPSE, tricuspid annular plane systolic excursion.

ISHLT rejection score at EMB). Palka *et al*[90] tested the hypothesis that during acute GR, there is an alteration of early diastolic untwist mechanisms of the LV and that this alteration may affect the RV too. Thus, they tested the differences in diastolic movements of LV and RV walls, by measuring timing differences between onset of early diastolic mitral inflow velocity (*E*) and onset of e′ velocity at lateral annulus, at septal annulus (e′ sep), and at tricuspid annulus (e′ tri). They found that increased time differences between the onset of *E* and e′ septal annulus waves and between the onset of e′ of RV and LV lateral wall waves could help to discriminate patients. However, none of the evaluated parameters were significant enough to be used as a surveillance variable.

Finally, Bader *et al.*,[94] in a prospective study involving 54 HT patients did not find any echocardiographic parameters to be able to reliably predict acute GR assessed by EMB, and this result was confirmed by another smaller study.[100] In conclusion, constant DTI velocities (e.g. e′ change $<10\%$ compared with baseline) and high DTI velocities (e.g. e′ >16 cm/s) seem to have good accuracy to exclude (more than detect) acute GR, with a negative predictive value of 92%. However, these parameters need further validation.

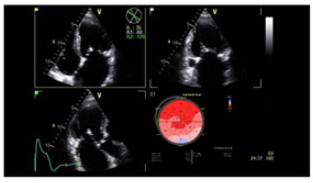
Echocardiographic evaluation of LV systolic and diastolic function is of great importance to detect GR. However, there is large discordance among the different studies. Alterations of diastolic and/or systolic function may be due to rejection, or other cardiac conditions like ischaemia, hypoxia, and sepsis.[101] Therefore, the presence of alterations in LV function has an important role in the assessment of prognosis in HT patients, but it is not an accurate marker of GR or CAV. Some groups are proposing echocardiographic scores to rule out active rejection;[102] however, there is a need for more extensive studies with larger sample sizes before these scores could be implemented in clinical practice.

Recommendations

Obtain a comprehensive echocardiographic study at 6 months from the cardiac transplantation as a baseline and make a careful quantitation of cardiac chamber size, RV systolic function, both systolic and diastolic parameters of LV function, and pulmonary artery pressure. Subsequent echocardiographic studies should be interpreted in comparison with the data obtained from the 6-month study. An echocardiographic study, which shows no change from the baseline study, has a high negative predictive value for GR. There is no single systolic or diastolic parameter that can be reliably used to diagnose GR. However, in case several parameters are abnormal the likelihood of GR increases. When an abnormality is detected, careful revision of images of the present and baseline study (side-by-side) is highly recommended.

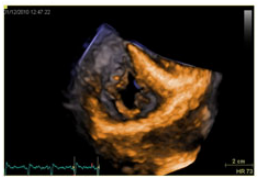
Table 6 Advanced methodologies that may be useful during follow-up of patients who underwent heart transplant

Left Ventricle Strain



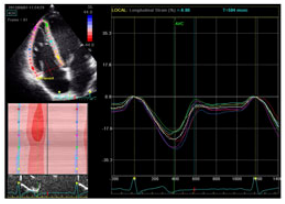
Echocardiographic technique
Speckle Tracking
Parameters to measure
- Global longitudinal strain

Tricuspid Valve Rendering



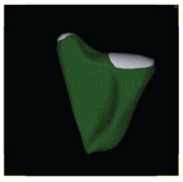
Acquisition techniques
3D echocardiography
Parameters to evaluate
- Leaflets dynamics
- Annular dimensions and function

Right Ventricle Strain



Acquisition techniques
Speckle Tracking
Parameters to measure
- Global longitudinal strain

Right Ventricle 3D Volume



Acquisition techniques
3D echocardiography
Parameters to measure
- Systolic and diastolic volumes
- Ejection fraction

RV geometry and function

Right heart failure is a common and much feared complication after HT, being the single most important cause of death in the early post period, together with acute GR.[5,103,104] Immediately after HT, the RV cavity size increases due to afterload mismatch with the relatively high pulmonary pressures of the recipient. Indeed, normalization in RV cavity size is expected the following weeks along with the progressive reduction of pulmonary resistances.[105] Impaired RV longitudinal systolic function measured by TAPSE, DTI, and RV fractional area change[106] is also present in all HT patients in the early weeks after HT, and two-thirds of HT patients show a partial recovery of RV longitudinal function during the first year, even if TAPSE remains significantly lower compared with normal.[107] The incomplete recovery of RV systolic function after HT can be explained by pre-transplant pulmonary pressures, increased post-transplant pulmonary gradient, significant tricuspid regurgitation, and prolonged ischaemia time. However, it is well established that the RV longitudinal function is not a sensitive parameter of global RV function after cardiac surgery.[108] Recent studies on HT patients suggest that TAPSE and DTI may be reduced due a distorted anatomy in the context of a normal overall RV function and EF.[109,110]

Recommendations

Assess RV size and function at baseline using several parameters, which do not exclusively evaluate longitudinal function. At follow-up echocardiogram, all these parameters should be compared with the baseline values. 3D echocardiography may provide a more accurate and comprehensive assessment of RV size and function.

Atrial geometry and function

Atrial geometry and function of the transplanted heart are directly related to the surgical technique. In patients who underwent HT using the standard technique, a unique morphological shape is visualized by echocardiography (best seen in the apical four-chamber view) as an enlargement of the long-axis dimension of the atria with a ridge at the site of anastomosis (*Figure 2A* and *B*). In patients who underwent HT using the bicaval or total orthotopic HT techniques, the atrial geometry and function are better preserved.[21,22,26]

Recommendations

Due to the unpredictable shape of atria in transplanted patients, atrial volume should be measured using the discs' summation algorithm (biplane algorithm for the left atrium) or 3D echocardiography.

Cardiac valves

Tricuspid valve

Tricuspid valve regurgitation is the most common single valve disease after HT. It may result from multiple pathophysiological mechanisms. In the first weeks after HT, tricuspid regurgitation is usually secondary to pulmonary hypertension. Its severity often decreases spontaneously as pulmonary resistance decreases. Other frequent causes of tricuspid regurgitation are persistent high pulmonary pressures, tricuspid annulus enlargement secondary to RV dilation, lesion of valve apparatus during EMB, acute GR, papillary muscle dysfunction, and alterations in right atrial contraction with functional impairment of the valvular apparatus.[111] The surgical technique used for HT seems to influence the occurrence of tricuspid regurgitation because of the alteration of right atrial morphology.[112]

Aortic and mitral valves

Structural alterations of left-sided valves are uncommon after HT, and changes in morphology and dimension of these valve structures have not been associated with acute GR. Rates of mitral regurgitation are slightly higher than the aortic regurgitation (attributed to

Table 7 Accuracy of different echocardiography techniques to detect acute graft rejection

Study	Patients (prevalence of acute GR)	Method (parameter cut-off value)	Gold standard (EMB score)	Sensitivity (%)	Specificity (%)	NPV
Angermann *et al.*[75]	52 (18%[a])	Integrated backscatter	Grade ≥ 1B			
		(PW 2D-IB increase >1.5 dB)		88	89	97
		(Septal 2D-IB increase >1.5 dB)		83	85	96
Puleo *et al.*[83]	121 (13%[a])	PW-DTI (e′ of inferior wall <16 cm/s)	Grade ≥ 3A	76	88	96
Mankad *et al.*[84]	78 (18%[a])	Colour-coded TDI	Grade ≥ 1B			
		(combined peak MV systolic and peak		93	71	98
		diastolic velocity <135 mm/s)		91	61	97
		(posterior wall tissue Doppler				
		diastolic time-gradient integral <0.26)				
Moidl *et al.*[85]	94 (20%[a])	Automated border detection	Grade ≥ 2			
		(peak filling rate <4.0 EDV/s)		100	70	100
		(peak filling rate <4.0 EDV/s and		100	95	100
		>18% reduction)				
Stengel *et al.*[86]	41 (39%[a])	PW-DTI	Grade ≥ 3A	82	53	82
		(Aa of lateral MV annulus <8.7 cm/s)				
Dandel *et al.*[87]	408 (39.9%[a])	Sm reduction >10%	Clinically relevant[b]	88.3	94	93
		TSm reduction >10%		83.3	94	90.5
		Sm/TSm reduction >10%		86.6	96	92.4
		Em reduction >10%		91.6	92	94.8
		TEm reduction >10%		93.3	95	96
		Em/TEm reduction >10%		91.6	94	95
Vivekananthan *et al.*[88]	40 (50%)	MPI increase ≥20% from baseline evaluation	Grade ≥ 3A	90	90	
Dandel *et al.*[89]	190 (17%[a])	PW-DTI	Clinically relevant[b]			
		(Sm of basal posterior wall		88	95	97
		reduction ≥10%)		89	97	98
		(Ea of basal posterior wall				
		reduction ≥10%)				
Palka *et al.*[90]	44 (27%[a])	PW and colour M-mode TDI	Grade ≥ 3A			
		(e′ of septal MV annulus <12 cm/s)		69	46	80
		(peak late IVR MVG[c] >0.1/s)		88	58	93
		(onset E wave – onset		81	84	92
		Emed[d] > −35 ms)		81	84	92
		(onset ETric – onset EMitr[e] >15 ms)				
Sun *et al.*[71]		2D and standard Doppler	Grade ≥ 1B			
	223 (37%[a])	(≥2 among: PE, IVRT <90 ms		57	54	68
	183 (27%[a])	E/A >1.7)		60	93	86
	264 (29%)	Post-OHT ≤ 6 months		67	49	78
		Post-OHT > 6 months				
		PW-DTI (Aa of septal/lateral				
		MV annulus <0.9 cm/s)				
Marciniak *et al.*[91]	31 (32%[a])	Colour DTI	Grade ≥ 1B			
		(mild-LVPW radial peak systolic		85	90	93
		strain ≤30%)		80	86	90
		(mild-LVPW radial peak systolic				
		SR < 3.0 s^{-1})				

Aa, peak late diastolic velocity; acute GR, acute graft rejection; dB, decibels; Ea, peak early diastolic velocity; EDV, end-diastolic volume; Em, peak early diastolic wall motion velocity; EMB, endomyocardial biopsy; OHT, orthotropic heart transplantation; IVRT, isovolumetric relaxation time; LVPW, left ventricle posterior wall; MPI, myocardial performance index; MV, mitral valve; NPV, negative predictive value; PE, pericardial effusion; PW 2D-IB, pulsed-wave 2-dimensional-integrated backscatter; Sm, peak radial systolic velocity; SR, strain rate; TDI, tissue Doppler imaging; TEm, early diastolic time—from onset of second heart sound to the peak of the early diastolic wave Em; TSm, systolic time—from onset of first heart sound to the peak of the systolic wave Sm.

[a]Prevalence of acute GR based on the percentage of biopsies with acute GR defined by the EMB score.

[b]Clinically relevant acute GR defined as EMB grade >2 plus Grades 1A and 1B when accompanied by clinical symptoms.

[c]Peak late isovolumic relaxation myocardial velocity gradient of the LV posterior wall.

[d]Timing difference between onset of mitral early diastolic velocity (E wave) and early diastolic septal MV annulus velocity (EMed).

[e]Timing difference between onset of early diastolic velocity, a lateral tricuspid (ETric) annulus, and an LV early diastolic lateral MV annulus velocity (EMitr).

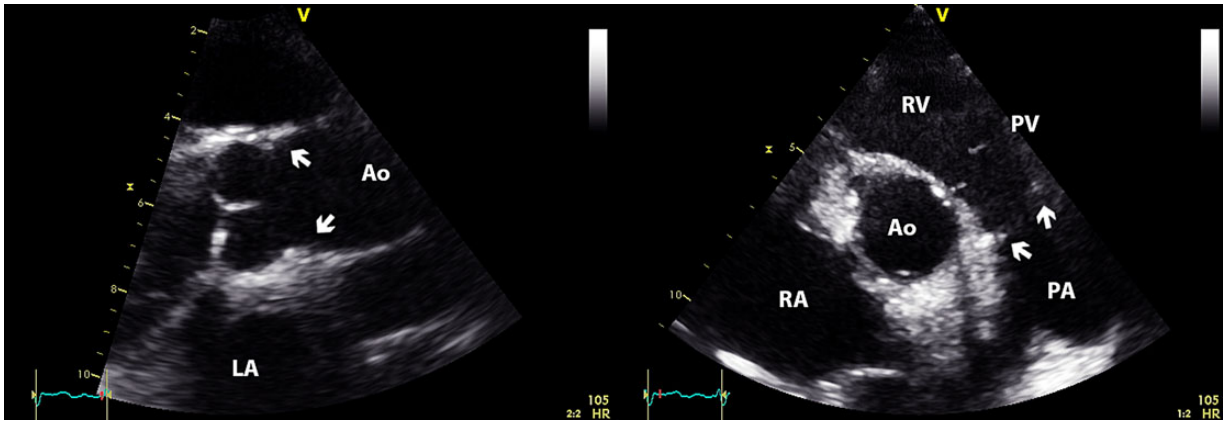


Figure 6 Two-dimensional echocardiography acquisitions focused on the ascending aorta and on the pulmonary artery. The suture lines could be easily identified. Arrow, suture lines; Ao, aorta; LA, left atrium; PA, pulmonary artery; PV, pulmonary valve; RA, right atrium; RV, right ventricle.

oedema of the papillary muscles) but tend to decrease over time and it is usually mild mitral regurgitation.[73,113]

Recommendations

Tricuspid regurgitation should be looked for and properly assessed in all echocardiographic studies. In case of significant changes in severity of tricuspid regurgitation during follow-up, a 2D/3D and colour Doppler assessment of its severity and mechanisms should be performed. Aortic and mitral valves should be evaluated according to current recommendations.

Aorta and pulmonary artery

The anastomoses of the donor heart to the aorta and pulmonary artery can be visualized (*Figure 6*, left panel). In the normal transplanted heart, Doppler flow velocities at the aortic and pulmonic level are usually normal. Occasionally, there is a mismatch between the diameter of the donor and recipient proximal pulmonary arteries and the suture line in the proximal pulmonary artery offers an aspect of 'pseudo-narrowing'. However, no significant gradient is usually detected by Doppler. The aortic anastomosis may also be a site of potential surgical complications. An echocardiographic evaluation of the aorta assessing diameters and potential wall thinning or leaking are recommended. Few reports have described the occurrence of aortic rupture, pseudoaneurysms, aneurysms, or dissection related to compliance mismatch, flow turbulence, and systemic hypertension.[114]

Superior and inferior vena cavae

Special attention should be paid to the superior vena cava, particularly in patients operated with non-standard surgical technique, since stenosis at the level of the surgical anastomosis has been described in 2.4% of these cases.[24]

Pericardium

Documentation of new pericardial effusion has been reported to be associated with GR.[115] However, the presence of pericardial effusion *per se* is not due to GR and its high prevalence in HT patients, as an early response to surgical 'injury' or as compensation for differences in volumes between the recipient and the donor heart size, results in low sensitivity and specificity to detect GR.[71] It is seen in approximately two-thirds of patients at 3 months after HT, and in 25% of patients at 6 months, independent of GR status.[71]

A localized intrapericardial haematoma may occur early or late after open heart surgery and not uncommonly is localized anterior and lateral to the right atrium making the diagnosis with transthoracic echocardiography challenging. If the haematoma is large or if it is an expanding one, it may compress the right atrium and cause haemodynamic impairment (e.g. low output state) resembling acute GR.

Recommendations

Pericardial effusion should be serially evaluated regarding extent, location, and haemodynamic impact. In case of newly detected pericardial effusion, GR should be considered taking into account the overall echocardiographic assessment and patient evaluation.

Advanced echocardiography

Deformation imaging (speckle tracking and Doppler TISSUE imaging)

Strain and strain rate (SR) are parameters of myocardial deformation. First assessment of strain and SR was derived from DTI velocity data, and several studies evaluated diagnostic accuracy of these parameters in OHT patients.[91,116,117] Overall, the majority of the studies found that, even if conventional echocardiographic examination (including DTI) and right heart catheterization did not reveal any significant changes compared with previous studies, global longitudinal peak systolic strain (GLS) was reduced in patients with histologically proven acute GR. Moreover, segmental longitudinal strain was reduced in LV segments, which showed inducible

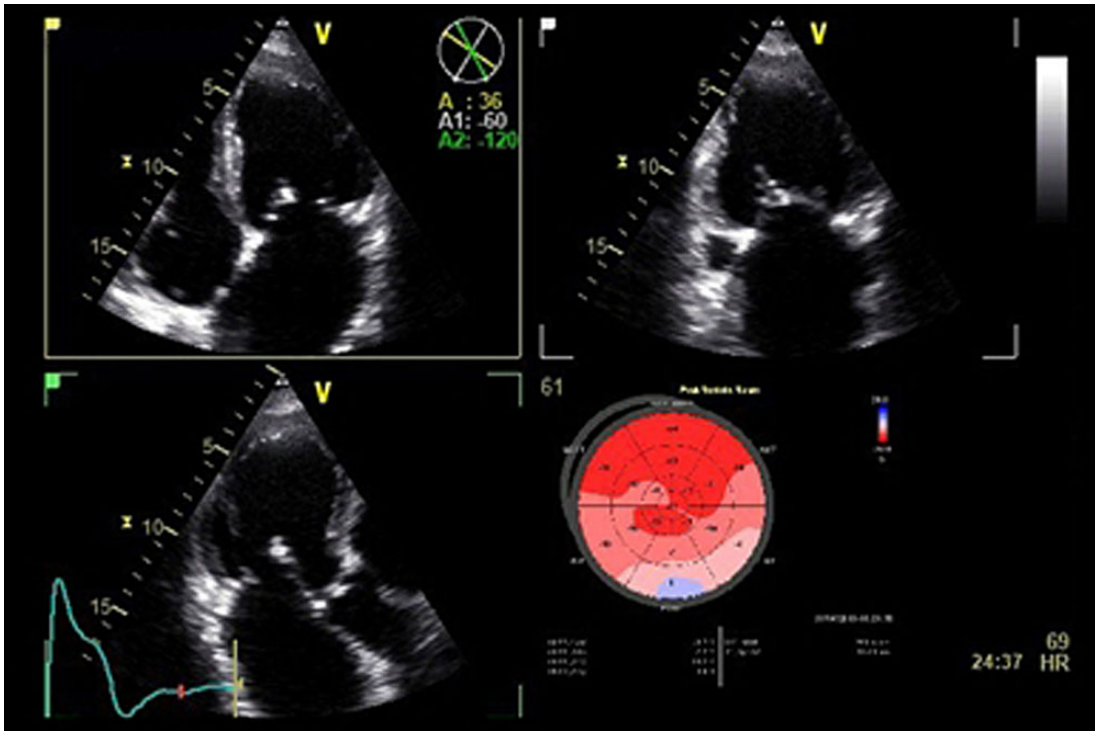


Figure 7 Computation of peak LV GLS by using the speckle tracking technique on three conventional apical views: four-chamber (left, upper panel), two-chamber (right, upper panel), and apical long axis (left, lower panel). LV segmental values of longitudinal strain are displayed both as numbers and as parametric colorization on a bull's eye display (right, lower panel).

wall motion abnormalities during stress test, and strain values could predict CAV. This may be explained by the fact that the regional changes induced by patchy rejection may not be large enough to alter global LV function indices, as rejection can be a non-uniform process.[91] This also explains the relative insensitivity in detecting <Grade IIB rejection using global LV function parameters. In the study by Marciniak *et al.*,[91] the authors postulated that subclinical myocardial modifications secondary to early acute GR are best detected by techniques that do not rely on reference points external to the heart to be able to detect regional changes in systolic function. They found that radial peak systolic strain was significantly reduced in the group with EMB-proved rejection. However, only regional strain/SR from the LV lateral wall was predictive with acute GR, and not the strain/SR from the septum, probably due to paradoxical septal motion that is common after cardiac surgery.

2D-Speckle tracking echocardiography (STE) is an echocardiographic technique that overcomes several limitation of DTI derived strain/SR (*Figures 7* and *8*). This technique has been proved to be accurate for the quantification of myocardial deformation, and it has already been validated in special subset of patients as an early marker of myocardial dysfunction.[118] The first studies of STE in HT have been conducted in rats in which both global strain and peak systolic or diastolic strain were significantly reduced in graft with alloimmune rejection, while LV EF and fractional shortening did not show any difference between hearts with and without rejection.[119] The study by Eleid *et al.*[120] was the first one to use STE to assess myocardial function in HT patients. During a 3-year follow-up of 51 HT patients, they were able to show that all patients showed a reduced GLS immediately after HT, but those who did not improve their GLS during follow-up experienced higher incidence of death and cardiac events. These results were independent from biopsy-detected acute GR, suggesting that STE is able to detect early and subtle alteration of LV systolic function, which may carry a poor prognosis even in the absence of detectable acute GR.

Many studies demonstrated that strain/SR measurements are abnormal in many clinical settings with preserved LV EF; thus, it is uncertain whether the lower GLS values found in transplanted heart recipients represent normal values of this population or are the first subtle alterations that consequently lead to myocardial dysfunction.[121–123] Nevertheless, these studies confirm that longitudinal strain values remain stable (even if lower in absolute values compared with general population) over the years and therefore a reduction over time of such parameters must be interpreted as pathological. Accordingly, Lisi *et al.* described a case of biopsy-proven acute GR associated with marked reduction in longitudinal, radial, and circumferential strain, with no alteration of other echocardiographic parameters, and complete recovery of myocardial deformation parameters after appropriate immunosuppressive therapy and regression of acute GR at the biopsy.[124] *Table 8* summarizes the results of the different studies which tested the accuracy of STE in detecting acute GR.

In 167 patients studied with STE during the first weeks after transplantation, GLS has been reported to be an independent predictor of 1-year mortality. Using a cut-off value of −9%, the sensitivity was

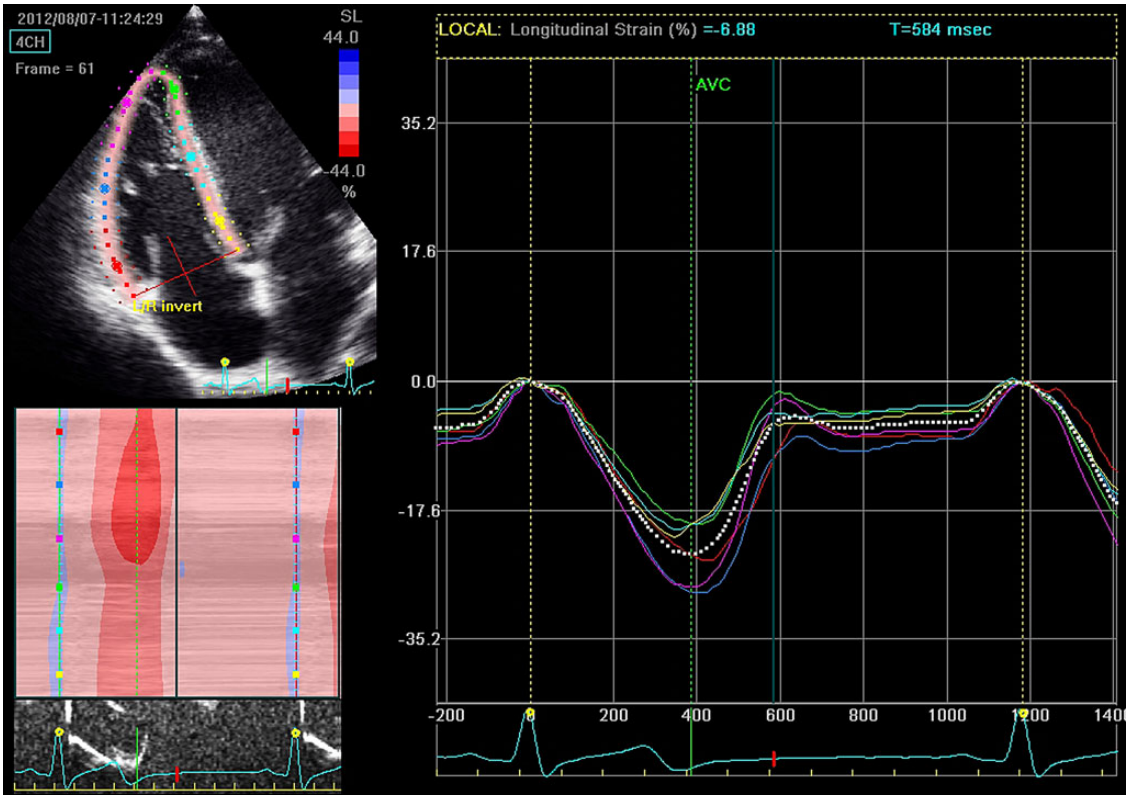


Figure 8 Computation of peak RV GLS by using the speckle tracking technique on a conventional four-chamber apical view (left, upper panel). The regional strain values are displayed both as regional strain/time curves (right panel) and in an M-mode parametric colourization (left, lower panel).

Table 8 Accuracy of the different parameters obtained with speckle tracking echocardiography to detect acute graft rejection

Study	Number of biopsies (prevalence of acute GR)[a]	STE method (parameter cut-off value)	Gold standard (EMB score)	Sensitivity (%)	Specificity (%)	Predictive accuracy (%)
Sato *et al.*[125]	301 (8.9%)	25% reduction in LV torsion values compared with baseline values	Grade $\geq$ 2	73.7	95.1	92.9
Kato *et al.*[117]	396 (11.3%)	Systolic ε -27.4% Diastolic SR $-2.8\ s^{-1}$	Grade $\geq$ 1b[b]	82.2 75.6	82.3 74.9	82.3 75
Marciniak *et al.*[91]	106 (16.9%)	LV PW radial $\varepsilon \leq 30\%$ SR $< 3.0\ s^{-1}$	Grade $\geq$ 1b[b]	85 80	90 86	93 89

E, strain; GR: graft rejection; LV, left ventricle; PW, posterior wall; SR, strain rate; STE, speckle tracking echocardiography.
[a]Prevalence of acute GR based on the percentage of biopsies with acute GR defined by the EMB score.
[b]ISHLT 1990 classification criteria for acute GR.[1]

73% and the specificity was 91%. However, because the causes of death in the cohort were various (from acute GR to CAV, HIT, and sepsis), no suggestion can be made on the relation between low GLS and the presence of acute GR and/or CAV at autopsy. Moreover, there were no differences in GLS, early after HT, among patient who experienced rejection within the first year and in those who did not.[126] Therefore, from this study, GLS can be regarded as a predictor of poor clinical outcome but not as a sensitive marker of rejection.

Recently, an echocardiographic rejection score was proposed considering a multiparametric evaluation according to the formula [(PWT + LVMI) − (Lat-ε + Sep-TS)] where PWT (posterior wall thickness measured in mm); LVMI (LV mass index in g/m^2); Lat-ε (lateral peak systolic strain); and Sep-TS (septal time to systole

Table 9 Accuracy of stress echocardiography for the diagnosis of CAV

Study	Patients (prevalence of CAV)	Stress	Reference for CAV	Sensitivity (%)	Specificity (%)
Collings *et al.*[128]	51 (27%)	Exercise	Angiography[a]	29	82
Mairesse *et al.*[129]	37 (11%)	Exercise	Angiography[b]	0	97
Cohn *et al.*[130]	51 (51%)	Exercise	Angiography[a]/IVUS[c]	33/15	85
Ciliberto *et al.*[131]	80 (31%)	Dipyridamole	Angiography[a]	100	72
Ciliberto *et al.*[132]	68 (37%)	Dipyridamole	Angiography[a]	100	87
Akosah *et al.*[133]	41 (51%)	Dobutamine	Angiography[a]	100	41
Herregods *et al.*[134]	28 (50%)	Dobutamine	Angiography[b]	50	71
Akosah *et al.*[135]	45 (53%)	Dobutamine	Angiography[b]	96	52
Derumeaux *et al.*[127]	41 (38%)	Dobutamine	Angiography[a]	100	77
Derumeaux *et al.*[136]	64 (47%)	Dobutamine	Angiography[a]	100	NR
Spes *et al.*[137]	46 (26%)	Dobutamine	Angiography[b]/IVUS[d]	83/79	56/83
Akosah *et al.*[138]	22 (32%)	Dobutamine	Angiography[a]	100	59
Derumeaux *et al.*[139]	37 (46%) 37 (70%)	Dobutamine	Angiography[b]	65 92	95 73
Spes *et al.*[140]	109 (46%)	Dobutamine	Angiography[b] and/or IVUS[d]	72	88
Bacal *et al.*[141]	39 (38%)	Dobutamine	Angiography[a]	64	91
Eroglu *et al.*[116]	42 (19)	Dobutamine	Angiography[b]	63[e] 88[f]	88[e] 85[f]
Rodrigues *et al.*[142]	35 (29%)	Contrast-enhanced	Angiography[a]	70	96
Tona *et al.*[143]	73 (47%)	Contrast-enhanced	Angiography[a]	82	87

CAV, cardiac allograft vasculopathy; IVUS, intravascular ultrasound.
[a]Coronary stenosis >50% in at least 1 vessel.
[b]Any angiographic abnormalities including luminal irregularities.
[c]Stanford classification Grade III to IV.
[d]Approximating to Stanford classification Grade III–IV; angiographic luminal irregularities or intravascular ultrasound (IVUS) severity approximating to Stanford classification Grade III–IV.
[e]Conventional regional wall motion analysis.
[f]Regional peak systolic strain rate analysis.

in ms).[102] This score has been proved to be useful to discard an acute GR episode (with 100% negative predictive value), when the result is 0.[102] Further studies are needed to prove the utility of this score as a screening tool to rule out the presence of acute GR and avoid an EMB.

Recommendations

GLS is a suitable parameter to diagnose subclinical allograft dysfunction, regardless of aetiology, by comparing the changes occurring during serial evaluations. Evaluation of GLS could be used in association with EMB to characterize and monitor an acute GR or a global dysfunction episode.

Stress echocardiography

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 (*Table 9*).[127] Due to the diminished heart rate response to exercise, related to cardiac denervation state, exercise protocols have a limited sensitivity of 15–33%.[128,130] When intimal thickening by IVUS is used as the gold standard, dobutamine stress echocardiography increases its specificity up to 88%[137,140] Even considering non-focal and non-significant stenotic disease, the sensitivity of dobutamine stress echocardiography remains high.[127,133] Deterioration between serial stress echocardiography tests yields an increased risk of events, compared with no deterioration, with a relative risk of 7.3.[140] A positive dobutamine test was found to be an independent predictor of cardiac events or death in a 4-year follow up study by Bacal *et al.*[141] On the other hand, a negative stress test indicates a low likelihood of a prognostically relevant CAV and low rate of occurrence of major adverse cardiac event at 1 year.[61,140] Quantitative analysis of segmental LV function, considering a peak systolic longitudinal strain rate increase of <0.5/s at peak stress test as a pathological response, improves the sensitivity to 88% for detecting any angiographic abnormalities and with negative predictive value of 92%.[116] A post-systolic strain index >34% at peak stress was reported to be the best parameter to detect CAV.[116] Combination of myocardial contrast echocardiography with quantitative assessment of myocardial perfusion during dobutamine stress test has been reported to increase the sensitivity from

71 to 86% with a 91% specificity.[144] Adding contrast infusion to assess myocardial perfusion during dobutamine stress test was confirmed to be moderately sensitive (70%) and highly specific (96%) for the presence of ≥50% coronary stenosis at angiography.[142] However, correlation of detected perfusion defects with coronary territory was poor except for the left anterior descending artery, in addition, the test failed to identify multivessel disease. The main contribution of stress echocardiography in the management of the post-transplant patients is to assess their prognosis. Indeed, patients with negative dobutamine stress echocardiography have <3% risk of experiencing major adverse cardiac events over the following year.

In theoretically unsuitable heart donors with brain death, pharmacologic stress echocardiography (either dipyridamole or dobutamine) has been used to exclude occult coronary artery disease or cardiomyopathy to extend the numbers of heart donors among the elderly, patient with stunned hearts or those with previous history of cardiac disease with a normal test result.[145–147]

Quantitative myocardial perfusion by contrast echocardiography

An advantage of quantitative contrast myocardial perfusion echocardiography is that it provides both structural (rBV—the vascular density relative to the surrounding tissue) and functional (B—microvascular conductance index) parameters, which actually constitute the microvasculature at the arteriolar and capillary level.[148] An rBV value <0.14 at rest can accurately detect severe CAV with a sensitivity of 90% and specificity of 75%.[149] However, because quantitative analysis is a time-consuming procedure, requiring specialized trained personnel and because the software analysis is not widespread, myocardial perfusion imaging is currently assessed semi-quantitatively by visual analysis.

Echocardiographic evaluation of coronary flow reserve

Direct measurement of coronary blood flow velocity at rest and during adenosine stress in the distal left anterior descending artery, using transthoracic Doppler echocardiography, could be applied and used to calculate the ratio between peak test velocity/baseline velocity which correlates with invasively measured coronary flow reserve (CFR). A CFR ≤ 2.7 by transthoracic echocardiography has demonstrated good accuracy (87% specific and 82% sensitive) for detecting CAV.[143] In addition, echocardiographic CFR has been reported to have prognostic value for CAV-related major cardiac events (3.3 relative risk of death, myocardial infarction, congestive heart failure, or need for percutaneous intervention at a mean of 19 months).[150] A CFR < 2.9 can detect a maximal intimal thickness of ≥0.5 mm by IVUS with 80% sensitivity, 100% specificity, and 89% negative predictive value.[151]

Recommendations

Dobutamine stress echocardiography might be a suitable alternative to routine coronary angiography to assess CAV at centres with adequate experience with the methodology. CFR and/or contrast infusion to assess myocardial perfusion can be combined with stress echocardiography to improve the accuracy of the test.

Integrated backscatter

Integrated backscatter is an echocardiographic technique based on myocardial acoustic properties, which aims to characterize myocardial tissue. Initial studies reported higher values of end-diastolic integrated backscatter measured at the LV infero-lateral wall in Grade 1B, 2, or 3 acute GR, compared with Grade 0. Backscatter signal measurements in the LV infero-lateral wall were more discriminatory than those measured in the anterior septum, with a 88% sensitivity and 89% specificity for detecting ≥1B grade acute GR (1.5 dB increased backscatter signal), and 92% sensitivity and 90% specificity for detecting ≥3 grade (5.5 dB increased backscatter signal).[75]

Recommendations

Integrated backscatter is highly dependent on acoustic window, it is technically demanding and with high inter- and intra-observer variability. Therefore, integrated backscatter is not recommended for clinical purposes.

Three-dimensional echocardiography

There are two studies evaluating the role of three-dimensional echocardiography (3DE) in HT patients.[110,152] Assessment of LV mechanical dyssynchrony by 3DE (time to minimum systolic volume adjusted by R–R interval length in a 16-segment model of LV) showed a 95% sensitivity and 73% specificity for predicting acute GR.[152] In another study, 3DE has been used to assess RV geometry and EF, suggesting that it is a more accurate methodology to perform a functional evaluation.[110]

According to recent studies, 3DE should 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 LV and RV volumes, LV mass, and atrial volumes. Moreover, during stress echocardiography, 3DE may improve the assessment of regional wall motion, possibly improving the accuracy of acute GR and CAV screening.[153,154]

Recommendations

In echocardiography laboratories equipped with 3DE scanners and specific expertise with the technique, 3DE may be a suitable alternative to conventional 2D echocardiography to assess the size and the function of cardiac chambers. 3DE measurement of LV and RV size and function are more accurate and reproducible than conventional 2D calculations. Cardiac chamber volumes obtained with 3DE cannot be compared with those obtained with two-dimensional echocardiography.

Echocardiography to guide EMB

Traditionally, fluoroscopy has been used to guide EMB, but this imaging modality has a number of limitations, including cumulative radiation exposure, limited portability, and the limited area of access (interventricular septum) for biopsy. In contrast, echocardiography provides greater portability, eliminates radiation exposure, provides

important information about cardiac function, and safely allows biopsy of any area of the RV, including the free wall and apex.[155] Several observational studies demonstrated the benefits of echocardiography over fluoroscopy in guiding EMB: (i) more adequate positioning of the bioptome against the septum;[155–157] (ii) higher percentage of adequate biopsy samples;[158] (iii) less biopsy-related complications;[158,159] and (iv) less radiation exposure.[155–157,159] However, there is no randomized study to prove the superiority of echocardiography over fluoroscopy.

Recommendations

In experienced centres, echocardiography might be an alternative to fluoroscopy to guide EMB, particularly in children and young women, since it avoids repeated X-ray exposure and permits visualization of soft tissues and safer performance of biopsies of different RV regions.

Role of other imaging modalities

Invasive imaging

Coronary angiography

Coronary angiography remains the main screening tool for CAV in most centres, also being able to guide management, and to predict adverse events in HT recipients. The latest guidelines for management of HT recipients consider coronary angiography as the gold-standard method to detect CAV (Class I, evidence C). The Stanford classification system is used to describe the morphology of coronary lesions from a discrete atherosclerosis to concentric arterial obliteration.[160,161] However, coronary angiography can underdiagnose both the prevalence and extent of CAV due to the vascular remodelling, involving the entire coronary tree, which in an early stage does not necessarily reduce the luminal diameter.[162,163] Therefore, angiograms should be interpreted serially as new and concentric lesions may be missed on one-time angiograms.

Invasive CFR

Evaluation of invasive CFR may provide additional information about the presence and severity of CAV, and may identify early CAV in patients without angiographically evident narrowings.[164,165] However, there is little evidence that invasive CFR is of any prognostic relevance.[61] A possible explanation may be related to the uniformly high resting heart rate, which impairs the reliability of the CFR assessment in detecting microvascular impairment. Furthermore, the presence of LV hypertrophy and higher donor ages independently contribute to a reduced CFR in patients after HT.[166] In HT patients, the reduction in CFR is due to elevated baseline flow velocities rather than to a change in hyperaemic flow velocities and should be taken into account for the test interpretation.

Recently, the instantaneous wave-free ratio has been proposed as an alternative invasive pressure-only index of coronary disease severity.[167] Because instantaneous wave-free ratio is calculated under baseline coronary haemodynamics—precluding induction of hyperaemia[168]—it emerges as a potential tool to investigate stenosis in transplanted patients. Instantaneous wave-free ratio could circumvent the limitations of FFR in such sub-population of patients with known microvascular disease and variable response to coronary vasodilators.[169]

Table 10 Stanford classification of CAV severity on IVUS

	Class I	Class II	Class III	Class IV
Severity	Minimal	Mild	Moderated	Severe
Intimal thickness	<0.3 mm	<0.3 mm	0.3–0.5 mm	>1.0 mm
Extent of plaque	<180	>180	>0.5 mm, <180	>0.5 mm, >180

CAV, cardiac allograft vasculopathy; IVUS, intravascular ultrasound.
Reproduced from St Goar FG *et al.*[175]

Intravascular ultrasound

IVUS has emerged as the gold standard for early detection of CAV due to high-resolution images of the cross-section of the vessel. It allows the accurate quantitative assessment of lumen size, intimal thickening, vessel wall morphology, and composition.[162,170] Numerous studies have demonstrated that even in the presence of a normal coronary angiogram, IVUS findings are predictive of CAV and are reliable prognostic markers of subsequent mortality and non-fatal major adverse cardiac events at 1 and 5 years after HT.[171–173] Indeed, an intimal thickness measured by IVUS correlated with microvascular impairment, even when FFR and angiograms were normal.[174] Therefore, the guidelines for the management of heart transplant patients state that 'IVUS in conjunction with coronary angiography with a baseline study at 4–6 weeks and at 1 year after HT is an option to exclude donor coronary artery disease, to detect rapidly progressive CAV, and provide prognostic information', giving a class of recommendation IIa and level of evidence B (*Table 10*).

Optical coherence tomography

Optical coherence tomography is an optical signal acquisition and processing method, typically employing near-infrared light, which captures micrometer-resolution, 3D images from within optical scattering media (e.g. biological tissue). The use of relatively long wavelength light allows it to penetrate into the scattering coronary wall providing an even higher spatial resolution than IVUS and, in theory, an earlier detection of morphological changes in the coronary wall. Good correlation with IVUS for measurement of maximal intimal thickness and luminal area has been demonstrated, with a lower inter-observer variability.[176]

Recommendations

Currently, coronary angiography is the gold-standard method for the detection of CAV.

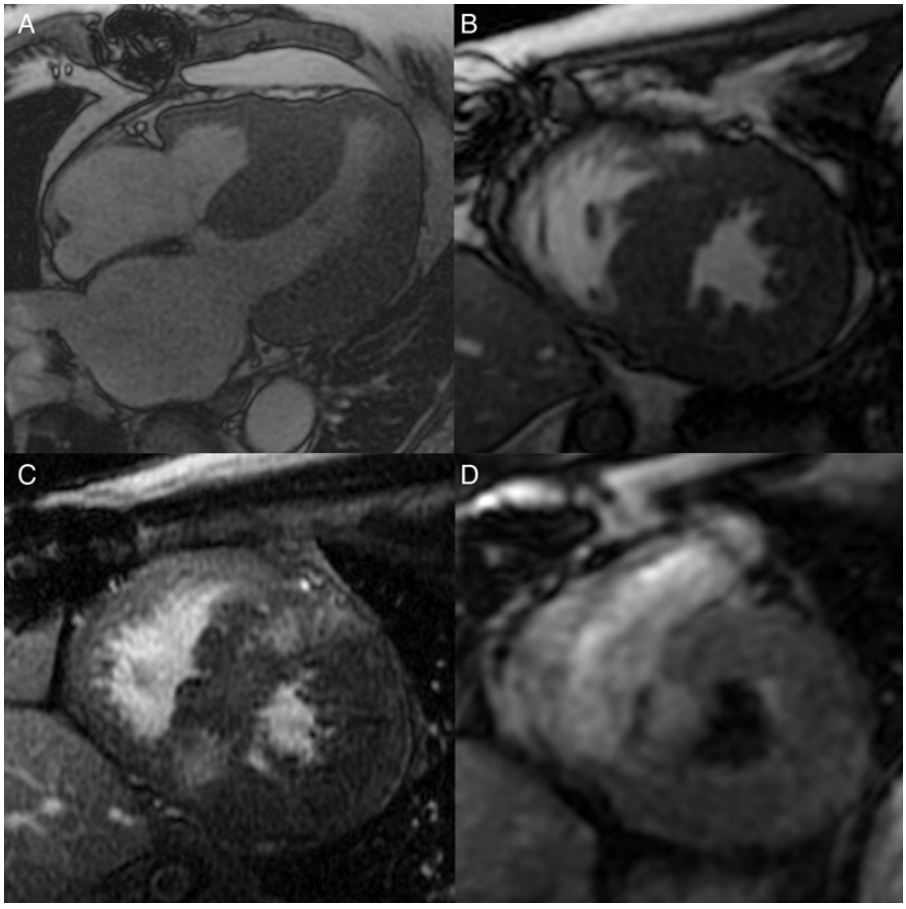


Figure 9 Cardiac magnetic resonance acquisitions using different protocols for quantitating changes in myocardial structure and detect myocardial tissue inflammatory and perfusion alterations.

Nuclear cardiac imaging

Considerable variations in study methodologies (different stressors protocols and tracer agents, time of the evaluation, and variable criteria to diagnose CAV) and results have been found when trying to evaluate the accuracy of nuclear cardiac imaging in detecting CAV.[177–184] Despite dobutamine has been reported to be advantageous as a stressor, recent studies demonstrated comparable accuracy to stress protocols using dipyridamole.[183–186] A large study evaluating dipyridamole-stress Sestamibi SPECT to assess CAV, demonstrated 92% sensitivity and 86% specificity to detect significant vascular disease (luminal narrow $\geq$50%), and only 56% sensitivity to detect angiographic abnormalities of any grade.[186] Prognostic information is also provided from both dobutamine-stress and dipyridamole-stress SPECT protocols, where dobutamine protocol demonstrated higher sensitivity and negative predictive value for major adverse cardiac events.[184,186,187] A range of molecular components of acute GR has been targeted with radionuclide scintigraphy; however, based on the lack of availability of large multicenter trial results, the conflicting results in published studies and the high burden of radiation related to this imaging technique, it can be recommended in clinical practice only in patients unsuitable for stress echocardiography.[188]

Recommendations

In patients with inadequate acoustic window and contraindication to contrast agents, pharmacological SPECT is an alternative imaging modality to detect CAV in HT patients. However, in centres with adequate expertise, IVUS in conjunction with coronary angiography with a baseline study at 4–6 weeks and at 1 year after HT should be performed to exclude donor coronary artery disease, to detect rapidly progressive CAV, and to provide prognostic information. IVUS should also be performed when there is discrepancy between non-invasive imaging tests and coronary angiography concerning the presence of CAV.

Computed tomographic coronary angiography

Computer tomographic (CT) coronary angiography can be employed to exclude relevant CAV. One possible limitation of this method is the difficulty to reach the appropriate heart rate necessary to obtain good quality images in OHT patients, since transplanted hearts are denervated and beat at high heart rate. Scanners that provide high temporal resolution, such as dual-source systems, provide

Table 11 Suggested echo techniques and imaging modalities during planned follow-up visits of heart transplant patients

Imaging modality	Technique	Class	Annotations
Echocardiography	Conventional TEE	Recommended	
	3D	Reasonable	In centres with specific expertise, to assess LV and RV geometry and function in patients with good acoustic window
	Speckle tracking	Reasonable	In centres with specific expertise, in patients with good acoustic window
	Contrast LVO	Reasonable	In patients with insufficient acoustic window
	TOE	Not recommended	
	Dobutamine stress echo	Recommended	In centres with specific expertise, to detect CAV
	Coronary flow reserve	Reasonable	In centres with specific expertise, to detect CAV
	Contrast MCE	Not recommended	
	Integrated backscatter	Not recommended	
Cardiac magnetic resonance		Reasonable	In patients with inadequate acoustic window despite contrast
CT		Reasonable	In patients with contrandication to CMR
Coronary angiography		Recommended	To detect CAV
IVUS		Reasonable	When there is discrepancy between coronary angio and non-invasive tests about the presence of CAV
Invasive coronary flow reserve		Questionable	To detect CAV when there is discrepancy between coronary angio and non-invasive tests about the presence of CAV
Optical coherence tomography		Reasonable	In centres with specific experience
Pharmacological SPECT	Either dobutamine or dipyridamole	Reasonable	In patients with inadequate acoustic window and contraindications to contrast agents
CT coronary angiography		Not recommended for routine clinical use	

3DE, three-dimensional echocardiography; CAV, cardiac allograft vasculopathy; CT, computed tomography; IVUS, intravascular ultrasound; LVO, left ventricular opacification; MCE, myocardial perfusion contrast echocardiography; SPECT, single-photon emission computed tomography; TOE, transoesophageal echocardiography; TTE, transthoracic echocardiography.

more guarantee for sufficient image quality in patients with a persistently high heart rate. The use of CT in the evaluation of CAV has been reported in the guidelines for the management of HT patients as a Class IIb recommendation (Level of Evidence: C). 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. CT coronary angiography can detect up to 50% more coronary segments with increased wall thickness than conventional coronary angiography.[189,190] Although relatively high sensitivities (70–100%) and specificities (81–100%) have been reported in the detection of significant coronary stenosis, comparative studies with IVUS or optical coherence tomography to assess its true sensitivity are scarce.[61,189–191] Its high negative predictive value to exclude coronary stenosis makes this technique a possible screening test before undergoing coronary angiography.[190,192–194] Although concerns regarding the exposure to ionizing radiation and nephrotoxic contrast remain, exposure generally does not exceed nuclear imaging or invasive coronary angiography, and contemporary scanner technology allows coronary imaging at ever decreasing radiation doses.

In addition, using CT, calcium is detected more frequently than would be suggested by studies using intravascular ultrasound. It is associated with the presence of angiographic disease and with some conventional risk factors for coronary disease. At follow-up, the presence of coronary calcium was associated with an adverse clinical outcome.[195]

CT is a powerful imaging technique for undisturbed interrogation of the cardiovascular morphology, which is particularly effective in various acute conditions, and when echocardiography cannot be performed adequately. In more exceptional situations, cardiac CT can be employed to quantify global LV and RV function. Dynamic myocardial perfusion imaging during pharmacological vasodilation allows quantification of myocardial perfusion and may provide a non-invasive alternative to PET imaging for the detection of microvascular disease in the future.[196–198]

Recommendations

In experienced centres, CT coronary angiography is a good alternative to coronary angiography to detect CAV. In patients with a persistently high heart rate, scanners that provide high temporal resolution, such as dual-source systems, provide better image quality.

Cardiac magnetic resonance

CMR provides accurate measurements of LV and RV diastolic and systolic volumes and hence LV and RV EF in addition to its unique ability to quantitate changes in myocardial structure and detect myocardial tissue alterations (oedema and fibrosis) secondary to acute GR and

CAV (*Figure 9A* and *B*). T_2 relaxation time is the most widely investigated parameter for acute GR screening, showing a significant positive correlation with rejection severity by histology and *ex vivo* myocardial water content.[199–201] A longer T_2 relaxation time (≥56 ms) had a 97% negative predictive value for detecting significant acute GR (≥ ISHLT grade 2), but only 35% positive predictive value.[201,202] Shortening of T_2 relaxation time during immunosuppressive therapy has also been reported.[200] Data about myocardial signal intensity analysis with T_2-weighted imaging to assess acute GR are inconsistent. Most of the studies have been performed using technology which were inferior to current standards in scanner hardware and sequence design; this may explain why some studies failed to demonstrate significant difference relative myocardial signal intensity.[203]

Myocardial inflammatory changes can be detected with the use of gadolinium (*Figure 9C*). An increase in relative myocardial contrast uptake has been identified in patients with myocardial necrosis on EMB.[204] Estep *et al.*[205] reported that areas of hyper enhancement are detected in acute GR, and these areas appear to be reduced in extent after immunosuppressive treatment. However, the presence and extent of hyper enhancement was not related to EMB ISHLT grade.[202,206] A multi-sequential CMR protocol, combining 'short tau inversion recovery' sequence for calculation of oedema ratio and a T_1-weighted spin-echo sequence for assessment of global relative enhancement, has a potential to exclude GR with a negative predictive value of 96% but with a low specificity (57%) and a low positive predict value (23%).[207]

Stress perfusion CMR with adenosine (*Figure 9D*) was tested in two studies, demonstrating reduced myocardial blood flow in patients with CAV compared with the normal population, allowing stratification of vascular disease severity.[208,209]

Recommendations

In patients with insufficient acoustic window, CMR is an alternative to echocardiography to assess cardiac chamber volumes and function and to exclude acute GR and CAV in a surveillance protocol.

Future directions in research

Single-centre studies have shown the potentialities of different imaging modalities to diagnose GR and CAV and to predict outcome in HT patients. However, these results need to be confirmed by multicenter outcome studies involving large numbers of patients from and different institutions. The role of new echo technologies (e.g. speckle tracking myocardial deformation imaging, 3D echocardiography, non-invasive CFR, and myocardial perfusion echocardiography), as well as the role of CMR and CT coronary angiography, in diagnosing GR and CAV seems to be particularly promising and should be tested in a specifically designed multicentre trial. This type of trial should not only test the accuracy of different multimodality protocols to assess HT patients, but also their safety and cost-effectiveness.

Conclusions

The cohort of long-term survivors of HT is expanding, and the assessment of these patients requires specific knowledge of the surgical techniques employed to implant the donor heart, the physiology of the transplanted heart, complications of invasive tests routinely performed to detect GR, and the specific pathologies that may affect the transplanted heart. A joint EACVI/Brazilian cardiovascular imaging writing group committee has prepared these recommendations to provide a practical guide to echocardiographers involved in the follow-up of HT patients and a framework for standardized and efficient use of cardiovascular imaging after HT (*Table 11*).

Conflict of interest: None declared.

References

1. Nishimura K, Okajama H, Inoue K, Saito M, Nagai T, Suzuki J *et al.* Visualization of traumatic tricuspid insufficiency by three-dimensional echocardiography. *Circ J* 2010;**55**:143–6.
2. Arora S, Aukrust P, Andreassen A, Simonsen S, Gude E, Grov I *et al.* The prognostic importance of modifiable risk factors after heart transplantation. *Am Heart J* 2009;**158**:431–6.
3. Taylor DO, Edwards LB, Boucek MM, Trulock EP, Aurora P, Christie J *et al.* Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult heart transplant report–2007. *J Heart Lung Transplant* 2007;**26**:769–81.
4. Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S *et al.* The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant* 2010;**29**:914–56.
5. Stehlik J, Edwards LB, Kucheryavaya AY, Aurora P, Christie JD, Kirk R *et al.* The Registry of the International Society for Heart and Lung Transplantation: twenty-seventh official adult heart transplant report–2010. *J Heart Lung Transplant* 2010;**29**:1089–103.
6. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U *et al.* The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. *Circulation* 2007;**116**:2216–33.
7. Yilmaz A, Kindermann I, Kindermann M, Mahfoud F, Ukena C, Athanasiadis A *et al.* Comparative evaluation of left and right ventricular endomyocardial biopsy: differences in complication rate and diagnostic performance. *Circulation* 2010;**122**:900–9.
8. Baraldi-Junkins C, Levin HR, Kasper EK, Rayburn BK, Herskowitz A, Baughman KL. Complications of endomyocardial biopsy in heart transplant patients. *J Heart Lung Transplant* 1993;**12**(1 Pt 1):63–7.
9. Subherwal S, Kobashigawa JA, Cogert G, Patel J, Espejo M, Oeser B. Incidence of acute cellular rejection and non-cellular rejection in cardiac transplantation. *Transplant Proc* 2004;**36**:3171–2.
10. Ammann P, Brunner-La Rocca HP, Angehrn W, Roelli H, Sagmeister M, Rickli H. Procedural complications following diagnostic coronary angiography are related to the operator's experience and the catheter size. *Catheter Cardiovasc Interv* 2003;**59**:13–8.
11. James MT, Samuel SM, Manning MA, Tonelli M, Ghali WA, Faris P *et al.* Contrast-induced acute kidney injury and risk of adverse clinical outcomes after coronary angiography: a systematic review and meta-analysis. *Circ Cardiovasc Interv* 2013;**6**:37–43.
12. Aziz TM, Burgess MI, El-Gamel A, Campbell CS, Rahman AN, Deiraniya AK *et al.* Orthotopic cardiac transplantation technique: a survey of current practice. *Ann Thorac Surg* 1999;**68**:1242–6.
13. Davies RR, Russo MJ, Morgan JA, Sorabella RA, Naka Y, Chen JM. Standard versus bicaval techniques for orthotopic heart transplantation: an analysis of the United Network for Organ Sharing database. *J Thorac Cardiovasc Surg* 2010;**140**:700–8, 708 e1–2.
14. Lower RR, Stofer RC, Shumway NE. Homovital transplantation of the heart. *J Thorac Cardiovasc Surg* 1961;**41**:196–204.
15. Cass MH, Brock R. Heart excision and replacement. *Guys Hosp Rep* 1959;**108**:285–90.
16. Miniati DN, Robbins RC. Techniques in orthotopic cardiac transplantation: a review. *Cardiol Rev* 2001;**9**:131–6.
17. Stinson EB, Dong E Jr, Iben AB, Shumway NE. Cardiac transplantation in man. 3. Surgical aspects. *Am J Surg* 1969;**118**:182–7.
18. Barnard CN. What we have learned about heart transplants. *J Thorac Cardiovasc Surg* 1968;**56**:457–68.
19. Sarsam MA, Campbell CS, Yonan NA, Deiraniya AK, Rahman AN. An alternative surgical technique in orthotopic cardiac transplantation. *J Card Surg* 1993;**8**:344–9.

20. Dreyfus G, Jebara V, Mihaileanu S, Carpentier AF. Total orthotopic heart transplantation: an alternative to the standard technique. *Ann Thorac Surg* 1991;**52**: 1181–4.
21. Schnoor M, Schafer T, Luhmann D, Sievers HH. Bicaval versus standard technique in orthotopic heart transplantation: a systematic review and meta-analysis. *J Thorac Cardiovasc Surg* 2007;**134**:1322–31.
22. Locali RF, Matsuoka PK, Cherbo T, Gabriel EA, Buffolo E. Should biatrial heart transplantation still be performed?: a meta-analysis. *Arq Bras Cardiol* 2010;**94**: 829–40.
23. Little RE, Kay GN, Epstein AE, Plumb VJ, Bourge RC, Neves J *et al.* Arrhythmias after orthotopic cardiac transplantation. Prevalence and determinants during initial hospitalization and late follow-up. *Circulation* 1989;**80**(5 Pt 2):III140–6.
24. Sze DY, Robbins RC, Semba CP, Razavi MK, Dake MD. Superior vena cava syndrome after heart transplantation: percutaneous treatment of a complication of bicaval anastomoses. *J Thorac Cardiovasc Surg* 1998;**116**:253–61.
25. Peteiro J, Redondo F, Calvino R, Cuenca J, Pradas G, Castro Beiras A. Differences in heart transplant physiology according to surgical technique. *J Thorac Cardiovasc Surg* 1996;**112**:584–9.
26. Dell'Aquila AM, Mastrobuoni S, Bastarrika G, Praschker BL, Aguero PA, Castano S *et al.* Bicaval versus standard technique in orthotopic heart transplant: assessment of atrial performance at magnetic resonance and transthoracic echocardiography. *Interact Cardiovasc Thorac Surg* 2012;**14**:457–62.
27. Barnard CN, Losman JG. Left ventricular bypass. *S Afr Med J* 1975;**49**:303–12.
28. Allen MD, Naasz CA, Popp RL, Hunt SA, Goris ML, Oyer PE *et al.* Noninvasive assessment of donor and native heart function after heterotopic heart transplantation. *J Thorac Cardiovasc Surg* 1988;**95**:75–81.
29. Hildebrandt A, Reichenspurner H, Gordon GD, Horak AR, Odell JA, Reichart B. Heterotopic heart transplantation: mid-term hemodynamic and echocardiographic analysis–the concern of arteriovenous-valve incompetence. *J Heart Transplant* 1990;**9**:675–81; discussion 682.
30. Baumgartner WA. Heterotopic transplantation: is it a viable alternative? *Ann Thorac Surg* 1992;**54**:401–2.
31. Hassoulas J, Barnard CN. Heterotopic cardiac transplantation. A 7-year experience at Groote Schuur Hospital, Cape Town. *S Afr Med J* 1984;**65**:675–82.
32. Vassileva A, Valsecchi O, Sebastiani R, Fontana A, Gamba A. Heterotopic heart transplantation for elevated pulmonary vascular resistance in the current era: long-term clinical and hemodynamic outcomes. *J Heart Lung Transplant* 2013;**32**: 934–6.
33. Butler J, Stankewicz MA, Wu J, Chomsky DB, Howser RL, Khadim G *et al.* Pretransplant reversible pulmonary hypertension predicts higher risk for mortality after cardiac transplantation. *J Heart Lung Transplant* 2005;**24**:170–7.
34. Newcomb AE, Esmore DS, Rosenfeldt FL, Richardson M, Marasco SF. Heterotopic heart transplantation: an expanding role in the twenty-first century? *Ann Thorac Surg* 2004;**78**:1345–50, discussion 1350-1.
35. Wang SS, Chu SH, Ko WJ, Chen YS, Chou NK. Heterotopic heart transplantation for severe pulmonary hypertension. *Transplant Proc* 1998;**30**:3408–9.
36. Marasco SF, Bell D, Lee G, Bailey M, Bergin P, Esmore DS. Heterotopic heart transplant: is there an indication in the continuous flow ventricular assist device era? *Eur J Cardiothorac Surg* 2014;**45**:372–6.
37. Pinderski LJ, Kirklin JK, McGiffin D, Brown R, Naftel DC, Young KR Jr *et al.* Multiorgan transplantation: is there a protective effect against acute and chronic rejection? *J Heart Lung Transplant* 2005;**24**:1828–33.
38. Keenan RJ, Bruzzone P, Paradis IL, Yousem SA, Dauber JH, Stuart RS *et al.* Similarity of pulmonary rejection patterns among heart-lung and double-lung transplant recipients. *Transplantation* 1991;**51**:176–80.
39. Baldwin JC, Oyer PE, Stinson EB, Starnes VA, Billingham ME, Shumway NE. Comparison of cardiac rejection in heart and heart-lung transplantation. *J Heart Transplant* 1987;**6**:352–6.
40. Glanville AR, Imoto E, Baldwin JC, Billingham ME, Theodore J, Robin ED. The role of right ventricular endomyocardial biopsy in the long-term management of heart-lung transplant recipients. *J Heart Transplant* 1987;**6**:357–61.
41. Trulock EP. Lung and heart-lung transplantation: overview of results. *Semin Respir Crit Care Med* 2001;**22**:479–88.
42. Rowan RA, Billingham ME. Myocardial innervation in long-term heart transplant survivors: a quantitative ultrastructural survey. *J Heart Transplant* 1988;**7**:448–52.
43. Greenberg ML, Uretsky BF, Reddy PS, Bernstein RL, Griffith BP, Hardesty RL *et al.* Long-term hemodynamic follow-up of cardiac transplant patients treated with cyclosporine and prednisone. *Circulation* 1985;**71**:487–94.
44. Young JB, Leon CA, Short HD 3rd, Noon GP, Lawrence EC, Whisennand HH *et al.* Evolution of hemodynamics after orthotopic heart and heart-lung transplantation: early restrictive patterns persisting in occult fashion. *J Heart Transplant* 1987; **6**:34–43.
45. Campeau L, Pospisil L, Grondin P, Dyrda I, Lepage G. Cardiac catheterization findings at rest and after exercise in patients following cardiac transplantation. *Am J Cardiol* 1970;**25**:523–8.
46. Valantine HA, Appleton CP, Hatle LK, Hunt SA, Billingham ME, Shumway NE *et al.* A hemodynamic and Doppler echocardiographic study of ventricular function in long-term cardiac allograft recipients. Etiology and prognosis of restrictive-constrictive physiology. *Circulation* 1989;**79**:66–75.
47. Russo MJ, Iribarne A, Hong KN, Ramlawi B, Chen JM, Takayama H *et al.* Factors associated with primary graft failure after heart transplantation. *Transplantation* 2010;**90**:444–50.
48. Jahania MS, Mullett TW, Sanchez JA, Narayan P, Lasley RD, Mentzer RM Jr. Acute allograft failure in thoracic organ transplantation. *J Card Surg* 2000;**15**: 122–8.
49. Appleyard RF, Cohn LH. Myocardial stunning and reperfusion injury in cardiac surgery. *J Card Surg* 1993;**8**(2 Suppl):316–24.
50. Young JB, Naftel DC, Bourge RC, Kirklin JK, Clemson BS, Porter CB *et al.* Matching the heart donor and heart transplant recipient. Clues for successful expansion of the donor pool: a multivariable, multiinstitutional report. The Cardiac Transplant Research Database Group. *J Heart Lung Transplant* 1994;**13**:353–64, discussion 364–5.
51. Jahania MS, Sanchez JA, Narayan P, Lasley RD, Mentzer RM Jr. Heart preservation for transplantation: principles and strategies. *Ann Thorac Surg* 1999;**68**: 1983–7.
52. Trento A, Hardesty RL, Griffith BP, Zerbe T, Kormos RL, Bahnson HT. Role of the antibody to vascular endothelial cells in hyperacute rejection in patients undergoing cardiac transplantation. *J Thorac Cardiovasc Surg* 1988;**95**:37–41.
53. Stobierska-Dzierzek B, Awad H, Michler RE. The evolving management of acute right-sided heart failure in cardiac transplant recipients. *J Am Coll Cardiol* 2001;**38**: 923–31.
54. Hosenpud JD, Bennett LE, Keck BM, Boucek MM, Novick RJ. The Registry of the International Society for Heart and Lung Transplantation: seventeenth official report-2000. *J Heart Lung Transplant* 2000;**19**:909–31.
55. Haddad F, Couture P, Tousignant C, Denault AY. The right ventricle in cardiac surgery, a perioperative perspective: II. Pathophysiology, clinical importance, and management. *Anesth Analg* 2009;**108**:422–33.
56. Benichou G. Direct and indirect antigen recognition: the pathways to allograft immune rejection. *Front Biosci* 1999;**4**:D476–80.
57. Matzinger P. Tolerance, danger, and the extended family. *Annu Rev Immunol* 1994; **12**:991–1045.
58. Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J *et al.* Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant* 2005;**24**:1710–20.
59. Berry GJ, Angelini A, Burke MM, Bruneval P, Fishbein MC, Hammond E *et al.* The ISHLT working formulation for pathologic diagnosis of antibody-mediated rejection in heart transplantation: evolution and current status (2005–2011). *J Heart Lung Transplant* 2011;**30**:601–11.
60. Billingham ME. Histopathology of graft coronary disease. *J Heart Lung Transplant* 1992;**11**(3 Pt 2):S38–44.
61. Mehra MR, Crespo-Leiro MG, Dipchand A, Ensminger SM, Hiemann NE, Kobashigawa JA *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**:717–27.
62. Armstrong AT, Binkley PF, Baker PB, Myerowitz PD, Leier CV. Quantitative investigation of cardiomyocyte hypertrophy and myocardial fibrosis over 6 years after cardiac transplantation. *J Am Coll Cardiol* 1998;**32**:704–10.
63. Bacal F, Silva CP, Pires PV, Mangini S, Fiorelli AI, Stolf NG *et al.* Transplantation for Chagas' disease: an overview of immunosuppression and reactivation in the last two decades. *Clin Transplant* 2010;**24**:E29–34.
64. Bacal F, Neto JD, Fiorelli AI, Mejia J, Marcondes-Braga FG, Mangini S *et al.* II Brazilian Guidelines for cardiac transplantation. *Arq Bras Cardiol* 2010;**94**(1 Suppl): e16–76.
65. Habib G, Hoen B, Tornos P, Thuny F, Prendergast B, Vilacosta I *et al.* Guidelines on the prevention, diagnosis, and treatment of infective endocarditis (new version 2009): the Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the International Society of Chemotherapy (ISC) for Infection and Cancer. *Eur Heart J* 2009;**30**:2369–413.
66. Uip DE, Neto VA, Strabelli TM, Bocchi EA, Pileggi F, Jatene AD *et al.* Infective endocarditis in 100 patients subjected to heart transplantation. *Arq Bras Cardiol* 1996;**66**:1–3.
67. Sherman-Weber S, Axelrod P, Suh B, Rubin S, Beltramo D, Manacchio J *et al.* Infective endocarditis following orthotopic heart transplantation: 10 cases and a review of the literature. *Transpl Infect Dis* 2004;**6**:165–70.
68. Aziz TM, Krysiak P, el-Gamel A, Campbell C, Rahman A, Deiraniya A *et al.* Bacteremia and endocarditis following endomyocardial biopsy. *Transplant Proc* 1998;**30**: 2112–3.

69. Valantine HA, Hatle LK, Appleton CP, Gibbons R, Popp RL. Variability of Doppler echocardiographic indexes of left ventricular filling in transplant recipients and in normal subjects. *J Am Soc Echocardiogr* 1990;**3**:276–84.
70. Gorcsan J 3rd, Snow FR, Paulsen W, Arrowood JA, Thompson JA, Nixon JV. Echocardiographic profile of the transplanted human heart in clinically well recipients. *J Heart Lung Transplant* 1992;**11**(1 Pt 1):80–9.
71. Sun JP, Abdalla IA, Asher CR, Greenberg NL, Popovic ZB, Taylor DO *et al.* Noninvasive evaluation of orthotopic heart transplant rejection by echocardiography. *J Heart Lung Transplant* 2005;**24**:160–5.
72. Goland S, Siegel RJ, Burton K, De Robertis MA, Rafique A, Schwarz E *et al.* Changes in left and right ventricular function of donor hearts during the first year after heart transplantation. *Heart* 2011;**97**:1681–6.
73. Wilhelmi M, Pethig K, Wilhelmi M, Nguyen H, Struber M, Haverich A. Heart transplantation: echocardiographic assessment of morphology and function after more than 10 years of follow-up. *Ann Thorac Surg* 2002;**74**:1075–9, discussion 1079.
74. Sagar KB, Hastillo A, Wolfgang TC, Lower RR, Hess ML. Left ventricular mass by M-mode echocardiography in cardiac transplant patients with acute rejection. *Circulation* 1981;**64**(2 Pt 2):II217–20.
75. Angermann CE, Nassau K, Stempfle HU, Kruger TM, Drewello R, Junge R *et al.* Recognition of acute cardiac allograft rejection from serial integrated backscatter analyses in human orthotopic heart transplant recipients. Comparison with conventional echocardiography. *Circulation* 1997;**95**:140–50.
76. Ciliberto GR, Mascarello M, Gronda E, Bonacina E, Anjos MC, Danzi G *et al.* Acute rejection after heart transplantation: noninvasive echocardiographic evaluation. *J Am Coll Cardiol* 1994;**23**:1156–61.
77. Barbir M, Lazem F, Banner N, Mitchell A, Yacoub M. The prognostic significance of non-invasive cardiac tests in heart transplant recipients. *Eur Heart J* 1997;**18**: 692–6.
78. Streeter RP, Nichols K, Bergmann SR. Stability of right and left ventricular ejection fractions and volumes after heart transplantation. *J Heart Lung Transplant* 2005;**24**: 815–8.
79. Thorn EM, de Filippi CR. Echocardiography in the cardiac transplant recipient. *Heart Fail Clin* 2007;**3**:51–67.
80. Yun KL, Niczyporuk MA, Daughters GT 2nd, Ingels NB Jr, Stinson EB, Alderman EL *et al.* Alterations in left ventricular diastolic twist mechanics during acute human cardiac allograft rejection. *Circulation* 1991;**83**:962–73.
81. Valantine HA, Yeoh TK, Gibbons R, McCarthy P, Stinson EB, Billingham ME *et al.* Sensitivity and specificity of diastolic indexes for rejection surveillance: temporal correlation with endomyocardial biopsy. *J Heart Lung Transplant* 1991;**10**(5 Pt 1): 757–65.
82. Amende I, Simon R, Seegers A, Daniel W, Heublein B, Hetzer R *et al.* Diastolic dysfunction during acute cardiac allograft rejection. *Circulation* 1990;**81**(2 Suppl): III66–70.
83. Puleo JA, Aranda JM, Weston MW, Cintron G, French M, Clark L *et al.* Noninvasive detection of allograft rejection in heart transplant recipients by use of Doppler tissue imaging. *J Heart Lung Transplant* 1998;**17**:176–84.
84. Mankad S, Murali S, Kormos RL, Mandarino WA, Gorcsan J 3rd. Evaluation of the potential role of color-coded tissue Doppler echocardiography in the detection of allograft rejection in heart transplant recipients. *Am Heart J* 1999;**138**(4 Pt 1): 721–30.
85. Moidl R, Chevtchik O, Simon P, Grimm M, Wieselthaler G, Ullrich R *et al.* Noninvasive monitoring of peak filling rate with acoustic quantification echocardiography accurately detects acute cardiac allograft rejection. *J Heart Lung Transplant* 1999;**18**:194–201.
86. Stengel SM, Allemann Y, Zimmerli M, Lipp E, Kucher N, Mohacsi P *et al.* Doppler tissue imaging for assessing left ventricular diastolic dysfunction in heart transplant rejection. *Heart* 2001;**86**:432–7.
87. Dandel M, Hummel M, Muller J, Wellnhofer E, Meyer R, Solowjowa N *et al.* Reliability of tissue Doppler wall motion monitoring after heart transplantation for replacement of invasive routine screenings by optimally timed cardiac biopsies and catheterizations. *Circulation* 2001;**104**(12 Suppl. 1):I184–91.
88. Vivekananthan K, Kalapura T, Mehra M, Lavie C, Milani R, Scott R *et al.* Usefulness of the combined index of systolic and diastolic myocardial performance to identify cardiac allograft rejection. *Am J Cardiol* 2002;**90**:517–20.
89. Dandel M, Hummel M, Meyer R, Muller J, Kapell S, Ewert R *et al.* Left ventricular dysfunction during cardiac allograft rejection: early diagnosis, relationship to the histological severity grade, and therapeutic implications. *Transplant Proc* 2002; **34**:2169–73.
90. Palka P, Lange A, Galbraith A, Duhig E, Clarke BE, Parsonage W *et al.* The role of left and right ventricular early diastolic Doppler tissue echocardiographic indices in the evaluation of acute rejection in orthotopic heart transplant. *J Am Soc Echocardiogr* 2005;**18**:107–15.
91. Marciniak A, Eroglu E, Marciniak M, Sirbu C, Herbots L, Droogne W *et al.* The potential clinical role of ultrasonic strain and strain rate imaging in diagnosing acute rejection after heart transplantation. *Eur J Echocardiogr* 2007;**8**:213–21.
92. Mena C, Wencker D, Krumholz HM, McNamara RL. Detection of heart transplant rejection in adults by echocardiographic diastolic indices: a systematic review of the literature. *J Am Soc Echocardiogr* 2006;**19**:1295–300.
93. Ross HJ, Gullestad L, Hunt SA, Tovey DA, Puryear JB, McMillan A *et al.* Early Doppler echocardiographic dysfunction is associated with an increased mortality after orthotopic cardiac transplantation. *Circulation* 1996;**94**(9 Suppl):II289–93.
94. Bader FM, Islam N, Mehta NA, Worthen N, Ishihara S, Stehlik J *et al.* Noninvasive diagnosis of cardiac allograft rejection using echocardiography indices of systolic and diastolic function. *Transplant Proc* 2011;**43**:3877–81.
95. Burgess MI, Bright-Thomas RJ, Yonan N, Ray SG. Can the index of myocardial performance be used to detect acute cellular rejection after heart transplantation? *Am J Cardiol* 2003;**92**:308–11.
96. Tona F, Caforio AL, Piaserico S, Bontorin M, De Simone G, Leone MG *et al.* Abnormal total ejection isovolume index as early noninvasive marker of chronic rejection in heart transplantation. *Transpl Int* 2005;**18**:303–8.
97. Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA *et al.* Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Eur J Echocardiogr* 2009;**10**:165–93.
98. Nagueh SF, Middleton KJ, Kopelen HA, Zoghbi WA, Quinones MA. Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressures. *J Am Coll Cardiol* 1997;**30**:1527–33.
99. Aranda JM Jr, Weston MW, Puleo JA, Fontanet HL. Effect of loading conditions on myocardial relaxation velocities determined by Doppler tissue imaging in heart transplant recipients. *J Heart Lung Transplant* 1998;**17**:693–7.
100. Fabregas RI, Crespo-Leiro MG, Muniz J, Regueiro M, Rodriguez JA, Alvarez N *et al.* Usefulness of pulsed Doppler tissue imaging for noninvasive detection of cardiac rejection after heart transplantation. *Transplant Proc* 1999;**31**:2545–7.
101. Sade LE, Sezgin A, Ulucam M, Taymaz S, Simsek V, Tayfun E *et al.* Evaluation of the potential role of echocardiography in the detection of allograft rejection in heart transplant recipients. *Transplant Proc* 2006;**38**:636–8.
102. Roshanali F, Mandegar MH, Bagheri J, Sarzaeem MR, Chitsaz S, Alaeddini F *et al.* Echo rejection score: new echocardiographic approach to diagnosis of heart transplant rejection. *Eur J Cardiothorac Surg* 2010;**38**:176–80.
103. Leeman M, Van Cutsem M, Vachiery JL, Antoine M, Leclerc JL. Determinants of right ventricular failure after heart transplantation. *Acta Cardiol* 1996;**51**:441–9.
104. Haddad F, Fisher P, Pham M, Berry G, Weisshaar D, Kuppahally S *et al.* Right ventricular dysfunction predicts poor outcome following hemodynamically compromising rejection. *J Heart Lung Transplant* 2009;**28**:312–9.
105. Bhatia SJ, Kirshenbaum JM, Shemin RJ, Cohn LH, Collins JJ, Di Sesa VJ *et al.* Time course of resolution of pulmonary hypertension and right ventricular remodeling after orthotopic cardiac transplantation. *Circulation* 1987;**76**:819–26.
106. Proclemer A, Allocca G, Badano LP, Pavoni D, Baldassi M, Nucifora G *et al.* Permanent atrial fibrillation and heart failure: radiofrequency ablation of atrioventricular junction and cardiac resynchronization therapy: review of the literature and of the new techniques for echocardiographic assessment. *G Ital Cardiol (Rome)* 2007;**8**:215–24.
107. Mastouri R, Batres Y, Lenet A, Gradus-Pizlo I, O'Donnell J, Feigenbaum H *et al.* Frequency, time course, and possible causes of right ventricular systolic dysfunction after cardiac transplantation: a single center experience. *Echocardiography* 2013;**30**:9–16.
108. Tamborini G, Muratori M, Brusoni D, Celeste F, Maffessanti F, Caiani EG *et al.* Is right ventricular systolic function reduced after cardiac surgery? A two- and three-dimensional echocardiographic study. *Eur J Echocardiogr* 2009;**10**:630–4.
109. Raina A, Vaidya A, Gertz ZM, Susan C, Forfia PR. Marked changes in right ventricular contractile pattern after cardiothoracic surgery: implications for post-surgical assessment of right ventricular function. *J Heart Lung Transplant* 2013;**32**:777–83.
110. D'Andrea A, Riegler L, Nunziata L, Scarafile R, Gravino R, Salerno G *et al.* Right heart morphology and function in heart transplantation recipients. *J Cardiovasc Med (Hagerstown)* 2013;**14**:648–58.
111. Aziz TM, Burgess MI, Rahman AN, Campbell CS, Deiraniya AK, Yonan NA. Risk factors for tricuspid valve regurgitation after orthotopic heart transplantation. *Ann Thorac Surg* 1999;**68**:1247–51.
112. Dandel M, Hummel M, Loebe M, Weng Y, Muller J, Buz S *et al.* Right atrial geometry and tricuspid regurgitation after orthotopic heart transplantation: benefits of a modified biatrial surgical technique. *J Heart Lung Transplant* 2001;**20**:246–7.
113. De Simone R, Lange R, Sack RU, Mehmanesh H, Hagl S. Atrioventricular valve insufficiency and atrial geometry after orthotopic heart transplantation. *Ann Thorac Surg* 1995;**60**:1686–93.
114. Vigano M, Rinaldi M, D'Armini AM, Pederzolli C, Minzioni G, Grande AM. The spectrum of aortic complications after heart transplantation. *Ann Thorac Surg* 1999;**68**:105–11.
115. Ciliberto GR, Anjos MC, Gronda E, Bonacina E, Danzi G, Colombo P *et al.* Significance of pericardial effusion after heart transplantation. *Am J Cardiol* 1995;**76**: 297–300.

116. Eroglu E, D'Hooge J, Sutherland GR, Marciniak A, Thijs D, Droogne W *et al.* Quantitative dobutamine stress echocardiography for the early detection of cardiac allograft vasculopathy in heart transplant recipients. *Heart* 2008;**94**:e3.
117. Kato TS, Oda N, Hashimura K, Hashimoto S, Nakatani T, Ueda HI *et al.* Strain rate imaging would predict sub-clinical acute rejection in heart transplant recipients. *Eur J Cardiothorac Surg* 2010;**37**:1104–10.
118. Giatrakos N, Kinali M, Stephens D, Dawson D, Muntoni F, Nihoyannopoulos P. Cardiac tissue velocities and strain rate in the early detection of myocardial dysfunction of asymptomatic boys with Duchenne's muscular dystrophy: relationship to clinical outcome. *Heart* 2006;**92**:840–2.
119. Pieper GM, Shah A, Harmann L, Cooley BC, Ionova IA, Migrino RQ. Speckle-tracking 2-dimensional strain echocardiography: a new noninvasive imaging tool to evaluate acute rejection in cardiac transplantation. *J Heart Lung Transplant* 2010;**29**:1039–46.
120. Eleid MF, Caracciolo G, Cho EJ, Scott RL, Steidley DE, Wilansky S *et al.* Natural history of left ventricular mechanics in transplanted hearts: relationships with clinical variables and genetic expression profiles of allograft rejection. *JACC Cardiovasc Imaging* 2010;**3**:989–1000.
121. Saleh HK, Villarraga HR, Kane GC, Pereira NL, Raichlin E, Yu Y *et al.* Normal left ventricular mechanical function and synchrony values by speckle-tracking echocardiography in the transplanted heart with normal ejection fraction. *J Heart Lung Transplant* 2011;**30**:652–8.
122. Syeda B, Hofer P, Pichler P, Vertesich M, Bergler-Klein J, Roedler S *et al.* Two-dimensional speckle-tracking strain echocardiography in long-term heart transplant patients: a study comparing deformation parameters and ejection fraction derived from echocardiography and multislice computed tomography. *Eur J Echocardiogr* 2012;**12**:490–6.
123. Pichler P, Binder T, Hofer P, Bergler-Klein J, Goliasch G, Lajic N *et al.* Two-dimensional speckle tracking echocardiography in heart transplant patients: three-year follow-up of deformation parameters and ejection fraction derived from transthoracic echocardiography. *Eur Heart J Cardiovasc Imaging* 2012;**13**:181–6.
124. Lisi M. Two-dimensional speckle tracking echocardiography of acute cardiac transplant rejection following pregnancy. *J Clin Ultrasound* 2012;**40**:451–4.
125. Sato T, Kato TS, Kamamura K, Hashimoto S, Shishido T, Mano A *et al.* Utility of left ventricular systolic torsion derived from 2-dimensional speckle-tracking echocardiography in monitoring acute cellular rejection in heart transplant recipients. *J Heart Lung Transplant* 2011;**30**:536–43.
126. Sarvari SI, Gjesdal O, Gude E, Arora S, Andreassen AK, Gullestad L *et al.* Early postoperative left ventricular function by echocardiographic strain is a predictor of 1-year mortality in heart transplant recipients. *J Am Soc Echocardiogr* 2012;**25**:1007–14.
127. Derumeaux G, Redonnet M, Mouton-Schleifer D, Bessou JP, Cribier A, Saoudi N *et al.* Dobutamine stress echocardiography in orthotopic heart transplant recipients. VACOMED Research Group. *J Am Coll Cardiol* 1995;**25**:1665–72.
128. Collings CA, Pinto FJ, Valantine HA, Popylisen S, Puryear JV, Schnittger I. Exercise echocardiography in heart transplant recipients: a comparison with angiography and intracoronary ultrasonography. *J Heart Lung Transplant* 1994;**13**:604–13.
129. Mairesse GH, Marwick TH, Vanoverschelde JL, Baudhuin T, Wijns W, Melin JA *et al.* How accurate is dobutamine stress electrocardiography for detection of coronary artery disease? Comparison with two-dimensional echocardiography and technetium-99 m methoxyl isobutyl isonitrile (mibi) perfusion scintigraphy. *J Am Coll Cardiol* 1994;**24**:920–7.
130. Cohn JM, Wilensky RL, O'Donnell JA, Bourdillon PD, Dillon JC, Feigenbaum H. Exercise echocardiography, angiography, and intracoronary ultrasound after cardiac transplantation. *Am J Cardiol* 1996;**77**:1216–9.
131. Ciliberto GR, Massa D, Mangiavacchi M, Danzi GB, Pirelli S, Faletra F *et al.* High-dose dipyridamole echocardiography test in coronary artery disease after heart transplantation. *Eur Heart J* 1993;**14**:48–52.
132. Ciliberto GR, Parodi O, Cataldo G, Mangiavacchi M, Alberti A, Parolini M *et al.* Prognostic value of contractile response during high-dose dipyridamole echocardiography test in heart transplant recipients. *J Heart Lung Transplant* 2003;**22**:526–32.
133. Akosah KO, Mohanty PK, Funai JT, Jesse RL, Minisi AJ, Crandall CW *et al.* Non-invasive detection of transplant coronary artery disease by dobutamine stress echocardiography. *J Heart Lung Transplant* 1994;**13**:1024–38.
134. Herregods MC, Anastassiou I, Van Cleemput J, Bijnens B, De Geest H, Daenen W *et al.* Dobutamine stress echocardiography after heart transplantation. *J Heart Lung Transplant* 1994;**13**:1039–44.
135. Akosah K, Olsovsky M, Mohanty PK. Dobutamine stress-induced angina in patients with denervated cardiac transplants. Clinical and angiographic correlates. *Chest* 1995;**108**:695–700.
136. Derumeaux G, Redonnet M, Mouton-Schleifer D, Cribier A, Soyer R, Letac B. Value of dobutamine echocardiography in the detection of coronary disease in heart transplant patient. Groupe de Recherche VACOMED. *Arch Mal Coeur Vaiss* 1996;**89**:687–94.
137. Spes CH, Mudra H, Schnaack SD, Klauss V, Reichle FM, Uberfuhr P *et al.* Dobutamine stress echocardiography for noninvasive diagnosis of cardiac allograft vasculopathy: a comparison with angiography and intravascular ultrasound. *Am J Cardiol* 1996;**78**:168–74.
138. Akosah KO, McDaniel S, Hanrahan JS, Mohanty PK. Dobutamine stress echocardiography early after heart transplantation predicts development of allograft coronary artery disease and outcome. *J Am Coll Cardiol* 1998;**31**:1607–14.
139. Derumeaux G, Redonnet M, Soyer R, Cribier A, Letac B. Assessment of the progression of cardiac allograft vasculopathy by dobutamine stress echocardiography. *J Heart Lung Transplant* 1998;**17**:259–67.
140. Spes CH, Klauss V, Mudra H, Schnaack SD, Tammen AR, Rieber J *et al.* Diagnostic and prognostic value of serial dobutamine stress echocardiography for non-invasive assessment of cardiac allograft vasculopathy: a comparison with coronary angiography and intravascular ultrasound. *Circulation* 1999;**100**:509–15.
141. Bacal F, Moreira L, Souza G, Rodrigues AC, Fiorelli A, Stolf N *et al.* Dobutamine stress echocardiography predicts cardiac events or death in asymptomatic patients long-term after heart transplantation: 4-year prospective evaluation. *J Heart Lung Transplant* 2004;**23**:1238–44.
142. Rodrigues AC, Bacal F, Medeiros CC, Bocchi E, Sbano J, Morhy SS *et al.* Non-invasive detection of coronary allograft vasculopathy by myocardial contrast echocardiography. *J Am Soc Echocardiogr* 2005;**18**:116–21.
143. Tona F, Caforio AL, Montisci R, Angelini A, Ruscazio M, Gambino A *et al.* Coronary flow reserve by contrast-enhanced echocardiography: a new noninvasive diagnostic tool for cardiac allograft vasculopathy. *Am J Transplant* 2006;**6**(5 Pt 1):998–1003.
144. Hacker M, Hoyer HX, Uebleis C, Ueberfuhr P, Foerster S, La Fougere C *et al.* Quantitative assessment of cardiac allograft vasculopathy by real-time myocardial contrast echocardiography: a comparison with conventional echocardiographic analyses and [Tc99 m]-sestamibi SPECT. *Eur J Echocardiogr* 2008;**9**:494–500.
145. Leone O, Gherardi S, Targa L, Pasanisi E, Mikus P, Tanganelli P *et al.* Stress echocardiography as a gatekeeper to donation in aged marginal donor hearts: anatomic and pathologic correlations of abnormal stress echocardiography results. *J Heart Lung Transplant* 2009;**28**:1141–9.
146. Bombardini T, Gherardi S, Arpesella G, Maccherini M, Serra W, Magnani G *et al.* Favorable short-term outcome of transplanted hearts selected from marginal donors by pharmacological stress echocardiography. *J Am Soc Echocardiogr* 2011;**24**:353–62.
147. Bombardini T, Gherardi S, Leone O, Sicari R, Picano E. Transplant of stunned donor hearts rescued by pharmacological stress echocardiography: a "proof of concept" report. *Cardiovasc Ultrasound* 2013;**11**:27.
148. Vogel R, Indermuhle A, Reinhardt J, Meier P, Siegrist PT, Namdar M *et al.* The quantification of absolute myocardial perfusion in humans by contrast echocardiography: algorithm and validation. *J Am Coll Cardiol* 2005;**45**:754–62.
149. Rutz T, de Marchi SF, Roelli P, Gloekler S, Traupe T, Steck H *et al.* Quantitative myocardial contrast echocardiography: a new method for the non-invasive detection of chronic heart transplant rejection. *Eur Heart J Cardiovasc Imaging* 2013;**14**:1187–94.
150. Tona F, Caforio AL, Montisci R, Gambino A, Angelini A, Ruscazio M *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):I49–55.
151. Tona F, Osto E, Tarantini G, Gambino A, Cavallin F, Feltrin G *et al.* Coronary flow reserve by transthoracic echocardiography predicts epicardial intimal thickening in cardiac allograft vasculopathy. *Am J Transplant* 2010;**10**:1668–76.
152. Pan C, Wang C, Pan W, Shu X, Chen H. Usefulness of real-time three-dimensional echocardiography to quantify global left ventricular function and mechanical dyssynchrony after heart transplantation. *Acta Cardiol* 2011;**66**:365–70.
153. Badano LP MD, Rigo F, Del Mestre L, Ermacora D, Gianfagna P, Proclemer A. High volume-rate three-dimensional stress echocardiography t assess inducible myocardial ischemia: a feasibility study. *J Am Soc Echocardiogr* 2010;**23**:628–35.
154. Aggeli C, Giannopoulos G, Misovoulos P, Roussakis G, Christoforatou E, Kokkinakis C *et al.* Real-time three-dimensional dobutamine stress echocardiography for coronary artery disease diagnosis: validation with coronary angiography. *Heart* 2007;**93**:672–5.
155. Miller LW, Labovitz AJ, McBride LA, Pennington DG, Kanter K. Echocardiography-guided endomyocardial biopsy. A 5-year experience. *Circulation* 1988;**78**(5 Pt 2):III99–102.
156. Bell CA, Kern MJ, Aguirre FV, Donohue T, Bach R, Wolford T *et al.* Superior accuracy of anatomic positioning with echocardiographic- over fluoroscopic-guided endomyocardial biopsy. *Cathet Cardiovasc Diagn* 1993;**28**:291–4.
157. McCreery CJ, McCulloch M, Ahmad M, deFilippi CR. Real-time 3-dimensional echocardiography imaging for right ventricular endomyocardial biopsy: a comparison with fluoroscopy. *J Am Soc Echocardiogr* 2001;**14**:927–33.

158. Ragni T, Martinelli L, Goggi C, Speziali G, Rinaldi M, Roda G *et al.* Echo-controlled endomyocardial biopsy. *J Heart Transplant* 1990;**9**:538–42.
159. Grande AM, Minzioni G, Martinelli L, Campana C, Rinaldi M, D'Armini AM *et al.* Echo-controlled endomyocardial biopsy in orthotopic heart transplantation with bicaval anastomosis. *G Ital Cardiol* 1997;**27**:877–80.
160. Gao SZ, Alderman EL, Schroeder JS, Silverman JF, Hunt SA. Accelerated coronary vascular disease in the heart transplant patient: coronary arteriographic findings. *J Am Coll Cardiol* 1988;**12**:334–40.
161. Johnson DE, Gao SZ, Schroeder JS, DeCampli WM, Billingham ME. The spectrum of coronary artery pathologic findings in human cardiac allografts. *J Heart Transplant* 1989;**8**:349–59.
162. Nissen S. Coronary angiography and intravascular ultrasound. *Am J Cardiol* 2001; **87**:15A–20A.
163. Rickenbacher PR, Pinto FJ, Chenzbraun A, Botas J, Lewis NP, Alderman EL *et al.* Incidence and severity of transplant coronary artery disease early and up to 15 years after transplantation as detected by intravascular ultrasound. *J Am Coll Cardiol* 1995;**25**:171–7.
164. Wolford TL, Donohue TJ, Bach RG, Drury JH, Caracciolo EA, Kern MJ *et al.* Heterogeneity of coronary flow reserve in the examination of multiple individual allograft coronary arteries. *Circulation* 1999;**99**:626–32.
165. Mazur W, Bitar JN, Young JB, Khalil AA, Vardan S, Short BC *et al.* Progressive deterioration of coronary flow reserve after heart transplantation. *Am Heart J* 1998; **136**:504–9.
166. Klauss V, Spes CH, Rieber J, Siebert U, Werner F, Stempfle HU *et al.* Predictors of reduced coronary flow reserve in heart transplant recipients without angiographically significant coronary artery disease. *Transplantation* 1999;**68**:1477–81.
167. Sen S, Escaned J, Malik IS, Mikhail GW, Foale RA, Mila R *et al.* Development and validation of a new adenosine-independent index of stenosis severity from coronary wave-intensity analysis: results of the ADVISE (ADenosine Vasodilator Independent Stenosis Evaluation) study. *J Am Coll Cardiol* 2012;**59**:1392–402.
168. Sen S, Asrress KN, Nijjer S, Petraco R, Malik IS, Foale RA *et al.* Diagnostic classification of the instantaneous wave-free ratio is equivalent to fractional flow reserve and is not improved with adenosine administration. Results of CLARIFY (Classification Accuracy of Pressure-Only Ratios Against Indices Using Flow Study). *J Am Coll Cardiol* 2013;**61**:1409–20.
169. Miller LW. Coronary microvasculopathy after heart transplantation: a new marker to guide future trials? *Circulation* 2007;**116**:1224–5.
170. Tsutsui H, Ziada KM, Schoenhagen P, Iyisoy A, Magyar WA, Crowe TD *et al.* Lumen loss in transplant coronary artery disease is a biphasic process involving early intimal thickening and late constrictive remodeling: results from a 5-year serial intravascular ultrasound study. *Circulation* 2001;**104**:653–7.
171. Mehra MR, Ventura HO, Stapleton DD, Smart FW. The prognostic significance of intimal proliferation in cardiac allograft vasculopathy: a paradigm shift. *J Heart Lung Transplant* 1995;**14**(6 Pt 2):S207–11.
172. Tuzcu EM, Kapadia SR, Sachar R, Ziada KM, Crowe TD, Feng J *et al.* Intravascular ultrasound evidence of angiographically silent progression in coronary atherosclerosis predicts long-term morbidity and mortality after cardiac transplantation. *J Am Coll Cardiol* 2005;**45**:1538–42.
173. Kobashigawa JA, Tobis JM, Starling RC, Tuzcu EM, Smith AL, Valantine HA *et al.* Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. *J Am Coll Cardiol* 2005;**45**:1532–7.
174. Lee CM, Wu YW, Jui HY, Yen RF, Tzen KY, Chou NK *et al.* Intravascular ultrasound correlates with coronary flow reserve and predicts the survival in angiographically normal cardiac transplant recipients. *Cardiology* 2008;**109**:93–8.
175. St Goar FG, Pinto FJ, Alderman EL, Valantine HA, Schroeder JS, Gao SZ *et al.* Intracoronary ultrasound in cardiac transplant recipients. In vivo evidence of "angiographically silent" intimal thickening. *Circulation* 1992;**85**:979–87.
176. Hou J, Lv H, Jia H, Zhang S, Xing L, Liu H *et al.* OCT assessment of allograft vasculopathy in heart transplant recipients. *JACC Cardiovasc Imaging* 2012;**5**:662–3.
177. Wu YW, Yen RF, Lee CM, Ho YL, Chou NK, Wang SS *et al.* Diagnostic and prognostic value of dobutamine thallium-201 single-photon emission computed tomography after heart transplantation. *J Heart Lung Transplant* 2005;**24**:544–50.
178. Ciliberto GR, Mangiavacchi M, Banfi F, Massa D, Danzi G, Cataldo G *et al.* Coronary artery disease after heart transplantation: non-invasive evaluation with exercise thallium scintigraphy. *Eur Heart J* 1993;**14**:226–9.
179. Rodney RA, Johnson LL, Blood DK, Barr ML. Myocardial perfusion scintigraphy in heart transplant recipients with and without allograft atherosclerosis: a comparison of thallium-201 and technetium 99 m sestamibi. *J Heart Lung Transplant* 1994; **13**:173–80.
180. Ambrosi P, Habib G, Kreitman B, Metras D, Riberi A, Faugere G *et al.* Thallium perfusion and myocardial hypertrophy in transplanted heart recipients with normal or near-normal coronary arteriograms. *Eur Heart J* 1994;**15**:1119–23.
181. Howarth DM, Forstrom LA, Samudrala V, Sinak LJ, McGregor CG, Rodeheffer RJ. Evaluation of 201Tl SPET myocardial perfusion imaging in the detection of coronary artery disease after orthotopic heart transplantation. *Nucl Med Commun* 1996;**17**:105–13.
182. Ciliberto GR, Ruffini L, Mangiavacchi M, Parolini M, Sara R, Massa D *et al.* Resting echocardiography and quantitative dipyridamole technetium-99 m sestamibi tomography in the identification of cardiac allograft vasculopathy and the prediction of long-term prognosis after heart transplantation. *Eur Heart J* 2001; **22**:964–71.
183. Elhendy A, Sozzi FB, van Domburg RT, Vantrimpont P, Valkema R, Krenning EP *et al.* Accuracy of dobutamine tetrofosmin myocardial perfusion imaging for the noninvasive diagnosis of transplant coronary artery stenosis. *J Heart Lung Transplant* 2000;**19**:360–6.
184. Hacker M, Tausig A, Romuller B, Hoyer X, Klauss V, Stempfle U *et al.* Dobutamine myocardial scintigraphy for the prediction of cardiac events after heart transplantation. *Nucl Med Commun* 2005;**26**:607–12.
185. Carlsen J, Toft JC, Mortensen SA, Arendrup H, Aldershvile J, Hesse B. Myocardial perfusion scintigraphy as a screening method for significant coronary artery stenosis in cardiac transplant recipients. *J Heart Lung Transplant* 2000;**19**: 873–8.
186. Ciliberto GR, Ruffini L, Mangiavacchi M, Parolini M, Sara R, Massa D, De Maria R *et al.* Resting echocardiography and quantitative dipyridamole technetium-99 m sestamibi tomography in the indentification of cardiac allograft vasculopathy and the prediction of long-term prognosis after heart transplantation. *Eur Heart J* 2001;**22**:964–71.
187. Elhendy A, van Domburg RT, Vantrimpont P, Poldermans D, Bax JJ, van Gelder T *et al.* Prediction of mortality in heart transplant recipients by stress technetium-99 m tetrofosmin myocardial perfusion imaging. *Am J Cardiol* 2002; **89**:964–8.
188. Miller CA, Chowdhary S, Ray SG, Sarma J, Williams SG, Yonan N *et al.* Role of noninvasive imaging in the diagnosis of cardiac allograft vasculopathy. *Circ Cardiovasc Imaging* 2011;**4**:583–93.
189. Romeo G, Houyel L, Angel CY, Brenot P, Riou JY, Paul JF. Coronary stenosis detection by 16-slice computed tomography in heart transplant patients: comparison with conventional angiography and impact on clinical management. *J Am Coll Cardiol* 2005;**45**:1826–31.
190. Wever-Pinzon O, Romero J, Kelesidis I, Wever-Pinzon J, Manrique C, Budge D *et al.* Coronary computed tomography angiography for the detection of cardiac allograft vasculopathy: a meta-analysis of prospective trials. *J Am Coll Cardiol* 2014;**63**:1992–2004.
191. von Ziegler F, Leber AW, Becker A, Kaczmarek I, Schonermarck U, Raps C *et al.* Detection of significant coronary artery stenosis with 64-slice computed tomography in heart transplant recipients: a comparative study with conventional coronary angiography. *Int J Cardiovasc Imaging* 2009;**25**:91–100.
192. Pichler P, Loewe C, Roedler S, Syeda B, Stadler A, Aliabadi A *et al.* Detection of high-grade stenoses with multislice computed tomography in heart transplant patients. *J Heart Lung Transplant* 2008;**27**:310–6.
193. Rohnean A, Houyel L, Sigal-Cinqualbre A, To NT, Elfassy E, Paul JF. Heart transplant patient outcomes: 5-year mean follow-up by coronary computed tomography angiography. *Transplantation* 2011;**91**:583–8.
194. Barthelemy O, Toledano D, Varnous S, Fernandez F, Boutekadjirt R, Ricci F *et al.* Multislice computed tomography to rule out coronary allograft vasculopathy in heart transplant patients. *J Heart Lung Transplant* 2012;**31**:1262–8.
195. Ludman PF, Lazem F, Barbir M, Yacoub M. Incidence and clinical relevance of coronary calcification detected by electron beam computed tomography in heart transplant recipients. *Eur Heart J* 1999;**20**:303–8.
196. George RT, Silva C, Cordeiro MA, DiPaula A, Thompson DR, McCarthy WF *et al.* Multidetector computed tomography myocardial perfusion imaging during adenosine stress. *J Am Coll Cardiol* 2006;**48**:153–60.
197. Bamberg F, Becker A, Schwarz F, Marcus RP, Greif M, von Ziegler F *et al.* Detection of hemodynamically significant coronary artery stenosis: incremental diagnostic value of dynamic CT-based myocardial perfusion imaging. *Radiology* 2011;**260**: 689–98.
198. Rossi A, Dharampal A, Wragg A, Davies LC, van Geuns RJ, Anagnostopoulos C *et al.* Diagnostic performance of hyperaemic myocardial blood flow index obtained by dynamic computed tomography: does it predict functionally significant coronary lesions? *Eur Heart J Cardiovasc Imaging* 2014;**15**:85–94.
199. Sasaguri S, LaRaia PJ, Fabri BM, Fallon JT, Ayelsworth CA, D'Ambra MN *et al.* Early detection of cardiac allograft rejection with proton nuclear magnetic resonance. *Circulation* 1985;**72**(3 Pt 2):II231–6.
200. Aherne T, Tscholakoff D, Finkbeiner W, Sechtem U, Derugin N, Yee E *et al.* Magnetic resonance imaging of cardiac transplants: the evaluation of rejection of cardiac allografts with and without immunosuppression. *Circulation* 1986;**74**: 145–56.
201. Marie PY, Angioi M, Carteaux JP, Escanye JM, Mattei S, Tzvetanov K *et al.* Detection and prediction of acute heart transplant rejection with the myocardial T2

determination provided by a black-blood magnetic resonance imaging sequence. *J Am Coll Cardiol* 2001;**37**:825–31.
202. Taylor AJ, Vaddadi G, Pfluger H, Butler M, Bergin P, Leet A *et al.* Diagnostic performance of multisequential cardiac magnetic resonance imaging in acute cardiac allograft rejection. *Eur J Heart Fail* 2010;**12**:45–51.
203. Miller CA, Fildes JE, Ray SG, Doran H, Yonan N, Williams SG *et al.* Non-invasive approaches for the diagnosis of acute cardiac allograft rejection. *Heart* 2013;**99**:445–53.
204. Almenar L, Igual B, Martinez-Dolz L, Arnau MA, Osa A, Rueda J *et al.* Utility of cardiac magnetic resonance imaging for the diagnosis of heart transplant rejection. *Transplant Proc* 2003;**35**:1962–4.
205. Estep JD, Shah DJ, Nagueh SF, Mahmarian JJ, Torre-Amione G, Zoghbi WA. The role of multimodality cardiac imaging in the transplanted heart. *JACC Cardiovasc Imaging* 2009;**2**:1126–40.
206. Steen H, Merten C, Refle S, Klingenberg R, Dengler T, Giannitsis E *et al.* Prevalence of different gadolinium enhancement patterns in patients after heart transplantation. *J Am Coll Cardiol* 2008;**52**:1160–7.
207. Krieghoff C, Barten MJ, Hildebrand L, Grothoff M, Lehmkuhl L, Lucke C *et al.* Assessment of sub-clinical acute cellular rejection after heart transplantation: comparison of cardiac magnetic resonance imaging and endomyocardial biopsy. *Eur Radiol* 2014;**24**:2360–71.
208. Rivard AL, Swingen CM, Blake D, Huang AS, Kanth P, Thomsen GF *et al.* A comparison of myocardial perfusion and rejection in cardiac transplant patients. *Int J Cardiovasc Imaging* 2007;**23**:575–82.
209. Muehling OM, Wilke NM, Panse P, Jerosch-Herold M, Wilson BV, Wilson RF *et al.* Reduced myocardial perfusion reserve and transmural perfusion gradient in heart transplant arteriopathy assessed by magnetic resonance imaging. *J Am Coll Cardiol* 2003;**42**:1054–60.