ABSTRACT

Clinical features of allograft injury are often unreliable, and context within the transplant journey is key. In the setting of post-transplant allograft dysfunction, the choice of initial investigation depends on clinical assessment and history. One of the major considerations is the time post transplantation in helping to decide a likely cause for allograft injury. Immediately post transplantation, it is important to consider donor factors (including donor demographics as well as immunological match), ischaemic times, surgical issues as well as early rejection. Clinical suspicion needs to remain high with variable presentations, including haemodynamic instability, arrhythmia, as well as left ventricular dysfunction. Symptoms of allograft dysfunction may include dyspnoea, exertional intolerance, dizziness / lightheadedness, palpitations, as well as right or left heart failure. In the coming weeks and months, endomyocardial biopsy and blood-based biomarkers may be helpful including high sensitivity troponin and donor-derived cell-free DNA. Molecular markers for rejection are hopeful, and may also be useful in non-ischaemic causes of allograft dysfunction. Screening remains important late post heart transplant due to variety of signs associated with rejection (early) and lack of typical anginal symptoms (later). New imaging modalities - especially cardiac magnetic resonance imaging, have been shown to be useful for assessing cause of allograft dysfunction including ischemia, infarction and rejection.

Keywords: Transplantation; Troponin; Cell free DNA; Magnetic resonance imaging; Endomyocardial biopsy

INTRODUCTION

Heart transplantation is an established and successful therapy in the management of end stage heart failure in selected individuals. In the current era, improved immunosuppression regimens, along with improved management of both infection and malignancy, have resulted in excellent medium term outcomes and survival—up to15 years in recent Australian and New Zealand Cardiothoracic Organ Transplant Registry. As well as improvements in the management of patients, there has been corresponding progress in the selection of patients, with increased awareness of the importance of pre-transplantation frailty, peri-transplant donor management, including donation after circulatory death, and identification of
early graft rejection. Presentation with graft dysfunction can be variable, and a high degree of clinical suspicion is required. As well as evident of cardiac dysfunction, there may be non-specific symptoms of fatigue, exertional intolerance, palpitations or light-headedness. This review will examine the role of clinical assessment, as well as imaging, laboratory and genomic techniques in the assessment of cardiac allograft injury post transplantation.

INJURY IN THE CONTEXT OF THE TRANSPLANT JOURNEY

Allograft injury needs to be considered in the context of the potential injury specific to the timeframe of the transplantation (Figure 1). At each time point, symptoms of graft rejection and dysfunction—often non-specific—should be sought. These include symptoms of dyspnoea, fatigue, palpitations, and even rarely, arthralgias. Classical angina is frequently absent due to surgical denervation at transplantation, underscoring the importance of clinical and imaging surveillance. Signs of heart failure may or may not be present, even in the presence of significant rejection, and again a high degree of suspicion is required, with active rejection surveillance assessments particularly in the first-year post transplantation.

Pre-transplant considerations

Allograft injury occurs initially within the donor, at the time of brain death, due to the well-recognised phenomenon of autonomic storm. This can be associated with an acute deterioration in systolic function, which may well be reversible in younger individuals. We, and others, have demonstrated improvement in pre-transplant cardiac function with a regimen of hormonal resuscitation including triiodothyronine (T3), steroids and vasopressin. Older donors may warrant coronary angiography to exclude underlying baseline allograft coronary disease and with standardised donor guidelines developed according to local practice. Early graft dysfunction (within the first few weeks) may warrant early post-transplant angiography to exclude previously unrecognised primary atherosclerotic or thrombotic disease.

Significant factors can influence short- and medium-term outcomes in heart transplantation. In combination with donor age, ischaemic duration has a significant impact on both immediate and medium-term outcomes. Recent developments with ex vivo perfusion devices including TransMedics Organ Care System (OCS), and XVIVO Perfusion technology allow more distant procurement, which is very important in geographically isolated.

Figure 1. Injury in the context of the transplant journey.
BD = brain death; DCD = donation after circulatory death.
transplant centres such as those in Australia and New Zealand. These technologies may allow ischaemic times to be extended up to 10 hours or even beyond.\(^7\)

The donation pathway is also relevant in the assessment of graft function. Improvements in donor preservation solution and management, has allowed re-introduction of donation after circulatory death (DCD) in selected donors.\(^3\) Despite initial concerns of non-immunologic injury post DCD, early outcomes have been as good as, if not better, than contemporaneous donation after routine brain death pathway. The DCD pathway is facilitated by the TransMedics OCS with serial monitoring of serum lactate reflecting active extraction or graft deterioration.

**Peri transplant considerations**

Graft function may also be impacted by the extent and complexity of surgery. “Clean-skin” recipients (those without prior cardiac surgery) with standard anatomic configuration are expected to proceed more easily than re-do complex congenital heart disease candidates who may require independent vascular repairs and redirections. The recipient baseline pulmonary vascular resistance is also important in contributing to the recovery of the newly transplanted right ventricle. As it is known that cardiopulmonary bypass is associated with significant pulmonary vasoconstriction, current practice may involve inhaled pulmonary vasodilators, such as inhaled nitric oxide or iloprost post heart transplantation in the setting of elevated pre-operative transpulmonary gradient or pulmonary hypertension.\(^9\) The ability to rest the new heart with extra-corporeal membrane oxygenation (ECMO) also provides support in the setting of autonomic storm or massive transfusion.\(^9\) Recipients with longstanding post-capillary pulmonary hypertension due to underlying cardiac disease may need a larger donor heart with consideration of the impact of ischaemic time on surgical outcome.

Cross matching has also developed past simple cell-derived cytotoxicity (CDC) matching based on blood group. Matching is increasingly performed using virtual flow cross matching, allowing assessment of recipient HLA matching and donor specific antibodies (DSA) expression at the time of transplantation. High titre DSA may result in acute antibody mediated rejection (AMR), even in the absence of a positive CDC crossmatch.\(^10\) Progress has been made here also, with the introduction of sensitised protocols involving plasmapheresis and intravenous gammaglobulin in addition to induction therapy according to local practice. These techniques improve immediate outcomes, but require ongoing close surveillance for rejection to avoid ongoing allograft injury. Standardisation of the diagnostic criteria for the severity of cellular and AMR was updated in 2004, ranging from grade 0 (no evidence of rejection) to grade 3R (severe acute cellular rejection) and presence or absence of antibody mediated rejection (AMR 1).\(^11\)

**Post-transplant considerations: short- and medium-term**

Early allograft dysfunction immediately following transplantation is usually termed primary graft dysfunction (PGD) with clear diagnostic criteria created based on international consensus.\(^12\) Causes for secondary graft dysfunction need to be considered beyond the first week. Most transplant programs use regular surveillance endomyocardial biopsies from the first week after heart transplantation. Local guidelines involve biopsies weekly for the first 6 weeks, then fortnightly to 3 months, monthly to 6 months and then second or third monthly until the first year post transplant. The role of these is to exclude significant acute cellular rejection (ACR) or AMR, and provide guidance in the gradual reduction of the intensive immunosuppressive regimen started on the day of transplant. Most units will also commence functional surveillance with echocardiography at the first week, then month, then 3 and 6 months, before going onto annual echocardiography thereafter.
Evidence for vasculopathy is typically sought annually with angiography at the first year and, in our practice, every 5 years thereafter. This is usually with computed tomography coronary angiography. Functional assessment in the intervening years is usually performed with stress echocardiography or nuclear medicine scintigraphy to minimise the nephrotoxicity associated with intravenous or intracoronary contrast agents.

**ASSESSMENT OF MYOCARDIAL INJURY: IMAGING OPTIONS**

Causes of allograft dysfunction may be apparent on initial imaging. Ventricular impairment, with thickened walls may suggest rejection, or severe valvular regurgitation may explain fluid retention, and regional wall motion abnormalities suggesting obstructive coronary artery disease. A diagnosis of non-specific graft dysfunction can only be made after exclusion of significant rejection, coronary disease (primary or secondary to allograft vasculopathy), giant cell myocarditis recurrence, significant haemodynamic valvular disease, drug or toxin associated myocardial impairment or underlying non-cardiac conditions.

**Echocardiography**

Bedside echocardiography is often the initial test of choice. While algorithms based on electrocardiogram (ECG) have been proposed to diagnose cardiac rejection in the setting of transplantation, they are very non-specific on an individual patient, and only helpful in when there has been a clear change from previous recordings. In the New-Heart study, changes in QRS duration and QT interval were only seen in patients with moderate-severe rejection, making early diagnosis of rejection by ECG alone, difficult. Changes seen in echocardiography include contractile changes (fractional shortening, tissue Doppler with global longitudinal strain) and unexpected increase in left ventricular wall thickening. A composite rejection score, using these parameters has been suggested, with excellent sensitivity, but is limited by a specificity of 70% in the presence of significant rejection.

Changes in filling parameters, both systolic and diastolic have been suggested, but are still only usually present in the more severe rejection groups. More recent studies, using speckle tracking have been suggested. In the meta-analysis and review by Elkaryoni and colleagues, the overall sensitivity and specificity for global longitudinal strain was only 78% and 68% respectively (Figure 2). A combination approach of troponin changes with echocardiography increased sensitivity in one study.

**Cardiac magnetic resonance imaging (CMRI)**

Magnetic resonance imaging has enhanced spatial resolution compared to echocardiography with lower test variability providing a solid platform for longitudinal studies in single patients. As well as resting cardiac structure, tissue characterisation allows assessment of myocardial oedema (T1 and T2 mapping), focal (late gadolinium enhancement, LGE) and diffuse (extracellular volume) interstitial oedema expansion. Functional assessment of tissue strain and dyssynchrony is further available. Previous studies have confirmed the sensitivity of T1-mapping in diagnosis of significant rejection, with further studies showing improved accuracy with T2 mapping, confirmed in meta-analysis. Evidence of acute reversal of changes in tissue characteristics in response to anti-rejection therapy has been confirmed. A clinical utility study comparing a strategy of CMRI based surveillance to routine endomyocardial biopsy in the first year post transplantation, has shown equivalent...
outcomes in terms of episodes of treated rejection and immunosuppression burden, suggesting non-invasive imaging may supplant biopsies in the future.\(^{23}\)

Importantly, in that study, the cost of CMRI was significantly less than that associated with the invasive strategy. The diagnostic accuracy using a combination of T1 and T2 was very high, Figure 3. While CMRI is promising, remnant ICD lead fragments post-transplantation or extreme claustrophobia limit its clinical utility across the entire transplant population and alternate methods of rejection diagnostics will be always be required. The ability to image wall motion, scar, function as well as rejection markers, makes CMRI very appealing for screening for the cause of allograft dysfunction.

Figure 2. Sensitivity and specificity of strain in the detection acute rejection episodes.\(^{17}\) CI = confidence interval.

outcomes in terms of episodes of treated rejection and immunosuppression burden, suggesting non-invasive imaging may supplant biopsies in the future.\(^{21}\)

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Figure 3. Receiver operator characteristic curves for individual and combined tissue characterisation (T1, T2) according to severity of acute rejection.\(^{23}\) AMR = antibody mediated rejection; AUC = area under the curve.
Coronary artery vasculopathy (CAV) screening

Because of the frequent absence of ischaemic symptoms, despite obstructive coronary disease, routine annual surveillance for coronary artery vasculopathy is warranted. This is most commonly with exercise stress echocardiography, although some centres prefer dobutamine stress echocardiography (DSE) on an annual basis. Whether early disease can be detected with DSE alone has been questioned, although in low-risk transplant patients defined by an initial invasive confirmatory test such as intravascular ultrasound, it has been shown to be reasonable, avoiding risks associated with recurrent invasive angiography. A meta-analysis of DSE shows limited sensitivity, but good specificity for post-transplant CAV. Determining which patient is low risk is not always straightforward. As shown in a series of 50 patients imaged with optical coherence tomography at the time of surveillance invasive coronary angiography at 1 and 12 months, younger transplant recipients, and those with higher baseline total and low-density lipoprotein (LDL) cholesterol were more likely to demonstrate progression of intimal thickening. Significantly, cytomegalovirus CMV status, rejection severity and immunosuppression were not predictors of intimal thickening progression.

ASSESSMENT OF MYOCARDIAL INJURY: LABORATORY ASSESSMENTS

Troponin

While troponin is accepted as a marker for myocardial injury, it is not used extensively in transplant rejection assessment. This is due to low sensitivity in the setting of anything less than severe rejection. While the positive predictive value of high-sensitivity cardiac troponin (hs-cTn) is low, the negative predictive value is acceptable, suggesting there may be a role as a “rule out” test for severe rejection (Figure 4). The low sensitivity of convention troponin makes its use questionable. The value of a negative hs-cTn in avoiding endomyocardial biopsy remains to be proven in a prospective trial.

Cell free DNA

Donor-derived cell-free DNA (ddcfDNA) is detectable in urine and serum of transplant patients. Quantification of cell-free donor-specific (cfdDNA) relies detection of single nucleotide polymorphisms (SNPs) distinguishing donor from recipient DNA (Figure 5). A key step is determining the SNPs for the donor at the time of transplant, to allow detection later on. At each timepoint, the number of sequencing reads is measured from serum and those specific to the donor are quantified. As can be seen in Figure 6, the fraction of cfDNA from the donor decreases rapidly post transplantation, only to increase during times of acute rejection or myocardial injury. Studies examining this new technique typically exclude serum from the first month post-transplant to avoid this confounder. In the initial longitudinal study, successful treatment of acute cellular rejection (as judged by endomyocardial biopsy), was also associated with a rapid decline in ddcfDNA.

A recent multicentre prospective study (entitled GRAfT) has extended these findings to demonstrate excellent accuracy (beyond the first month), with area under the curve (AUC) of 0.92 for combined cellular and AMR, with almost 10-fold higher levels seen in the latter (Figure 7). Interestingly the ddcfDNA was already increased up to months before clinical rejection episodes and was superior to the gold standard endomyocardial biopsy for the detection of graft dysfunction (Figure 8). As described by the authors of this important
study, the next step in this technique will be a clinical utility study assessing whether endomyocardial biopsies can be avoided entirely.

**ASSESSMENT OF MYOCARDIAL INJURY: MOLECULAR DIAGNOSTICS**

Building on experience from renal transplant genomic signatures of rejection, a new technique of the ‘molecular microscope diagnostic’ (MMDx) system has been described.\(^{33}\) This requires endomyocardial biopsies and uses cluster analysis (machine learning) to assign scores according to upregulation of rejection associated transcripts. By comparing the different patterns of gene expression, individual biopsies can be assigned not only to normal pattern (no upregulation), cellular rejection or AMR, but also unexplained variance, possibly due to ischaemic injury, giving further insights, particularly early in the post-transplant course (Figure 9).\(^{34}\) New biopsies are compared against a database of specimens to assign a probability of the likelihood of injury causation (Figure 10).\(^{35}\)

By analysing the transcriptome associated with AMR, it can be seen that the distinct pattern of injury relates to upregulation of endothelium activation, microcirculatory inflammation by monocytes and macrophages and natural killer cells.\(^{36}\) The ability to distinguish between underlying drivers for allograft injury allows more targeted therapies—for example plasmapheresis and immunoglobulin for AMR or pulse steroids for ACR. Demonstration of a non-immunological cause for graft dysfunction also allows for decrease of immunosuppressive therapies, where appropriate.
OVERALL COMMENTARY ON THE VALUE OF ASSESSMENT TECHNIQUES FOR MYOCARDIAL INJURY

One of the recurring themes in the various techniques outlined in this review, is the sensitivity and specificity for assessment of severe (≥2R ACR and/or AMR). As seen in the assessment of cfdDNA, the diagnostic performance is excellent distinguishing ≥2R ACR/AMR from no rejection (AUC, 0.95), but only reasonable for distinguishing severe from mild ACR (AUC, 0.75) and not helpful in distinguishing mild ACR from non-rejection.
Similar comments can be made for echocardiography and cardiac magnetic resonance imaging. The additive value for the assessment technique needs to be considered when planning diagnostics. In this regard, the ability of MRI to further distinguish between infarction and inflammation, and to accurately assess left and right ventricular volumes and wall thickness, makes this an excellent choice, where available. A caveat needs to be recognised that the gold standard against which all assessments are tested—endomyocardial biopsy, has significant interobserver variability and variable sensitivity.

Given promising results with ddcfDNA, this has the potential to become the gold standard for rejection testing in the future. Routine screening for allograft injury remains important with vigilance for assessment rejection episodes, given the variability in presentation both for early rejection as well as for coronary vasculopathy later, frequently without classical symptoms of angina. The choice of initial investigation depends on clinical assessment and history, with an awareness of the clinical context and contributors to injury at different time points throughout the transplant journey.
Figure 8. Threshold for detection of ACR and AMR according to percentage of ddcfDNA. It can be seen the threshold is crossed significantly earlier in AMR, even when endomyocardial biopsy are still registering absence of rejection (0).\textsuperscript{21}

ACR = acute cellular rejection; AMR = antibody mediated rejection; ddcfDNA = donor-derived cell-free DNA.

Figure 9. Principal component analysis for 889 heart transplant biopsies based on the normalized expression of rejection associated transcripts.\textsuperscript{34} Gene expression microarrays were analyzed using a machine learning algorithm to assign a molecular score based on transcripts associated with the highest expression of genes on interferon-\gamma-inducible effector T-cells, mononuclear cells (including macrophages, monocytes, dendritic cells), natural killer cells, and human umbilical vein endothelial cells. Combination of the independent endomyocardial biopsy assigned histological diagnosis (S1: normal, S2: TCMR, S3: AMR, S4: UV) with the variation in transcript regulation allows clusters to become evident (A1: normal, A2: TCMR, A3: AMR, A4: UV).

TCMR = T-cell mediated rejection; AMR = antibody mediated rejection; UV = unassigned variation; PC1, 2, 3 = variance from principal component analysis.
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