The international society of heart and lung transplantation guidelines for the care of heart transplant recipients

Task Force 2: Immunosuppression and Rejection (Nov. 8, 2010)

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Topic 1: Rejection Surveillance

Mechanisms and Clinical Manifestations of Acute rejection

Hyperacute Rejection

Hyperacute allograft rejection occurs within minutes to hours of graft reperfusion due to the presence of preformed recipient antibodies usually directed against human leukocyte antigen (HLA) class I molecules constitutively expressed on the donor vascular endothelium.^{1,2} HLA class II molecules are not usually expressed on the donor vasculature, but they can be induced by inflammation and trauma associated with graft procurement and preservation. Lastly, non-HLA endothelial antigens may also lead to hyperacute rejection.³

Hyperacute rejection is initiated by the binding of a large amount of preformed antibodies to donor antigens which causes fixation of complement throughout the graft vasculature, resulting in cell death, inflammatory cell recruitment, platelet accumulation, and thrombosis.⁴ These processes quickly lead to diffuse graft ischemia and necrosis and are almost uniformly fatal.

Acute Cellular Rejection

Acute cellular rejection (ACR) is most common in the first 6 months after heart transplantation (HT) and is predominantly T-cell mediated Approximately 20% to 40% of HT recipients will experience at least 1 episode of ACR in the first postoperative year.⁵ The recipient immune system can recognize the donor heart as foreign by direct allorecognition, during which the donor's antigen presenting cells (APC) migrate from the allograft to the recipient lymphoid tissue and present donor HLA molecules to the recipient's T-cells, and by indirect allorecognition, during which the recipient's APCs present fragments of donor HLA to the recipient's T-cells.¹

T-cells are stimulated by the APCs through a multi-signal pathway. Signal 1 is through the recognition and binding of alloantigens on the APC by the T-cell receptor-CD3 complex and its co-receptor (CD8 for MHC [major histocompatibility complex] class I or CD4 for MHC class II peptides). However, this signal alone is insufficient to activate T-cells in the absence of a co-stimulation signal (signal 2). Signal 2 predominantly involves the interaction of B7 (CD80 and CD86) on the APC with CD28 of the T-cell.^{1, 6} After signals 1 and 2 there is activation of a tyrosine kinase ZAP-70 which then triggers 3 pathways leading to upregulation of gene expression in the T-cell: 1) the calcium-calcineurin pathway, 2) the nuclear factor-kappa B pathway, and 3) the mitogenactivated protein kinase pathway.7 Activation of these pathways results in the production of cytokines (interleukin [IL]-2 and IL-15) and molecules (CD25 and CD154) which bind to T-cell surface receptors.⁸ Signal 3 occurs after cytokines such as IL-2 binds to the IL-2 receptor and initiates cell proliferation through the target of rapamycin (TOR) pathway.

Activated T-cells migrate from the lymphoid system and across the vascular endothelium of the heart allograft which subsequently becomes infiltrated by effector T-cells, macrophages, B-cells, and plasma cells. The hallmark of ACR is the presence of lymphocytes in the myocardium, with more severe rejection being associated with greater myocardial injury. Immune cell-mediated myocyte injury can occur such through mechanisms as cell lysis by perforin/granulolysin and the Fas/FasL pathway.^{1, 2} The update of ACR grading reflects this continuum of cell infiltration and injury.9 Mononuclear cells infiltration without or with only one focus of myocyte damage is classified as Grade 1R, whereas an infiltrate plus the presence of multifocal myocyte damage is Grade 2R. An infiltrate with diffuse myocyte damage and/or associated edema, hemorrhage or vasculitis is classified as Grade 3R.

Acute Antibody-Mediated Rejection

Acute antibody-mediated rejection (AMR) is less common than ACR, occurring in approximately 10% of patients in conjunction with hemodynamic instability.¹⁰ Allosensitized HT recipients are at greatest risk for AMR. Acute AMR has B-cell predominance with antibodies directed against donor vascular endothelial antigens. However, alloreactive T-cells drive the production of the antibody response. The B-cell receptor binds the donor antigen leading to B-cell activation, proliferation, and maturation into antibody-secreting plasma cells and attachment of circulating complement to the endothelium, which in turn leads to direct cell injury, recruitment of inflammatory cells and phagocytemediated cell death.¹¹ This antibody-mediated injury to the endothelium leads to endothelial dysfunction, microvascular coagulation, myocardial ischemia and allograft dysfunction.

Early histopathology consists of arteriolar, venular and capillary endothelial cell swelling, nuclear enlargement and intracapillary infiltration of macrophages that may occur without lymphocytic infiltration.¹¹ Importantly, both ACR and AMR can coexist in up to 25% of acute rejection episodes.¹² Antibody binding and complement activation is followed by recruitment of neutrophils, interstitial edema, and myocyte injury.¹³ intravascular thrombus and The immunohistochemical evidence of AMR is based on the presence of immunoglobulin (IgG, IgM or IgA), complement fragments (C3d, C4d, C1q) or of CD68 positive cells (macrophages), as well as the appearance of circulating de novo anti-donor HLA antibodies.11, 14-16

Symptoms of Rejection

Because most patients are asymptomatic with early rejection, surveillance endomyocardial biopsies (EMB) are needed to detect and treat rejection before it produces symptomatic allograft dysfunction.

The inflammation and cell death associated with acute rejection, initially leads to myocardial edema and hence increased myocardial stiffness and diastolic dysfunction, but will eventually lead to systolic dysfunction if left untreated.¹⁷ Initially the symptoms may be non specific (fatigue, malaise, nausea or emesis, and fever).¹⁸ As the intracardiac filling pressures increase, congestive symptoms develop (exertional dyspnea, orthopnea or paroxysmal nocturnal dyspnea). Symptoms of right ventricular (RV) dysfunction (edema, abdominal distension, and early satiety) can be secondary to left ventricular (LV) failure or due to direct effects of rejection on the RV. Palpitations, or less commonly syncope, may result from arrhythmias triggered by myocardial inflammation.

Rejection can also be associated with bradyarrhythmias and atrioventricular (AV) block. Pericardial inflammation can produce a friction rub or a pericardial effusion. With worsening rejection low cardiac output symptoms (lethargy, somnolence, oliguria and hypotension with frank cardiogenic shock) may ensue. Rejection may also present with sudden cardiac death before the onset of symptoms of allograft dysfunction.¹⁹

Considerations for Pediatric Recipients

The mechanisms of acute rejection in pediatric HT recipients are similar to those occurring in adults. Developmental/maturational changes account for differences in the incidence of acute rejection. While infants and young children have lower acute rejection rates, adolescents have the highest acute rejection rates.^{20, 21} Many episodes \geq Grade 2R are asymptomatic and detected only by surveillance EMB.²² Symptomatic rejection in older children and adolescents is similar to that in adults. In infants and young children, there may be a history of poor feeding, irritability, lethargy, and fever. Rejection should be suspected in pediatric recipients with nonspecific symptoms in the absence of other obvious causes. Because the clinical diagnosis of rejection is so challenging in children, it is not rare for it to be complicated by even severe hemodynamic compromise.²³ The latter is a common occurrence in medically noncompliant adolescents.

Role of the Endomyocardial EMB in Diagnosis of Acute Rejection

For more than 20 years EMBs have been performed with the re-usable "Stanford-Caves" bioptome.²⁴ Currently, various disposable bioptomes are also employed.

The EMBs are usually done with cannulation of the right internal jugular vein and less often with the femoral, left internal jugular or subclavian venous approaches.²⁵

Risks of Endomyocardial Biopsy

Complications from EMBs occur in approximately 3% of the cases²⁶ and can be either access- or EMB-related. Cannulation of the neck veins can be associated with inadvertent arterial puncture, pneumothorax, local hematoma, and recurrent laryngeal nerve irritation associated with temporary hoarseness. EMB-related complications can be mild (self-limited ventricular ectopy or atrial arrhythmias) triggered by bioptome contact with the myocardium, or more serious (injury to the tricuspid valve causing tricuspid regurgitation or pericardial tamponade due to perforation of the right ventricle or other cardiac structure).²⁶

The risk of procedural complications decreases with operator experience. Since the heart allograft is denervated,

occasional pain associated with EMB originates from innervated pericardial and mediastinal tissues. Sudden sharp pain should raise suspicion of cardiac perforation. In this case echocardiography may demonstrate a new pericardial effusion. Whether tamponade physiology is present can also be determined by echocardiography. A right heart catheterization can quickly diagnose or confirm pericardial tamponade.²⁷ In this case pericardiocentesis should be immediately done under fluoroscopic or echocardiographic guidance. A drainage catheter is usually left in the pericardial cavity to prevent reaccumulation. Rarely surgical evacuation and the opening of a pericardial window is required.²⁸

Damage to the tricuspid valve, generally caused by severance of the chordae tendinae by the bioptome, may result in long-term morbidity. This complication, recognized for many years, continues to occur despite improvements in equipment and techniques.^{29, 30} The incidence of significant tricuspid regurgitation has been correlated with the number of EMBs.^{31, 32} Chordal tissue has been identified in EMB specimens.^{33, 34}

Risks of Endomyocardial Biopsy in Children

The accurate and timely diagnosis of acute rejection is critical as it remains one of the leading causes of death beyond initial hospital discharge after pediatric heart transplantation.³⁵ In children, deep sedation or general anesthesia is generally required to achieve safe vascular access and to perform the EMB. The procedure is particularly challenging in very small infants. Overall risk of serious complications with EMB in children is 0.6%.36 Nonetheless, tricuspid valve damage. cardiac perforation, coronary-right ventricular fistulae, pneumothorax, hemothorax and transient arrhythmias all may occur.³⁶ Damage to, and loss of, vascular access also occurs. especially in small children. The rates of these complications may be higher in the children than in adults. There are also additional small risks associated with sedation or anesthesia. A 3 French bioptome should be considered in infants and small children, although at times the small myocardial samples may be nondiagnostic. Use of the right internal jugular rather than the right femoral vein in children may also reduce morbidity.³⁶ In small children echocardiogram can help to safely guide the bioptome across the tricuspid valve and towards the apical portion of the RV septum, away from the RV outflow tract.

Evaluation and Grading of Rejection by Endomyocardial Biopsy

The first uniform histological classification of heart allograft rejection was published in 1990 and it included 7 grades:0 = no rejection; 1A and 1B = mild rejection, 2 = focal

moderate rejection; 3A and 3B = moderate rejection, and 4 = severe rejection.³⁷

Due to intra- and interobserver variability in the determination of the different grades of mild or moderate rejection and the observation that grades 1 and 2 were mostly self-limited,³⁸⁻⁴² a revised heart allograft rejection grading system was published in 2005.⁹ Grade 0 (no cellular rejection) was now named grade 0R ('R' added to reflect the revised 2005 scale). The intermediate grades of 1A, 1B, and 2 were re-classified as grade 1R, or mild acute cellular rejection. Grades 3A was re-classified as grade 2R, moderate acute cellular rejection, and grade 3B and 4 were re-classified as grade 3R, severe acute cellular rejection. In addition, AMR was recognized as a clinical entity, and recommendation was issued for determination of its presence (AMR1) or absence (AMR0).⁹

Indications for Endomyocardial Biopsy in Heart Transplant Recipients: Adult

Pre-transplant

Before transplantation, if there is a suspicion of an infiltrative or restrictive cardiomyopathy, EMB can be helpful, especially if the suspected disorder could lead to recurrent disease in the allograft. Examples include hemochromatosis, amyloidosis, sarcoidosis, as well as Chagasic cardiomyopathy and giant cell myocarditis. A recent AHA/ACC/ESC consensus document covers the use of EMB in a broad population of patients.⁴³

Post-transplant: Surveillance

The standard of care in adult HT recipients is to perform serial EMB to detect acute rejection before symptoms occur. There is no consensus on the optimal frequency of surveillance EMB, and EMB schedules are highly variable between HT centers. The frequency of EMB is typically highest in the first 3 postoperative months with a tapering frequency thereafter up to 1 year. This schedule is based on the observation that the risk of allograft rejection is highest in the first 6 months and decreases sharply after 12 months.⁴⁴ The usefulness of surveillance EMB in all patients later than 1 year after transplant is subject of debate.^{45, 46}

If the patient manifests a clinical picture consistent with allograft rejection, then it is appropriate to perform EMB, as the results may dictate changes in therapy. See the full Acute Rejection Guideline for further information.

Indications for Endomyocardial Biopsy in Heart Transplant Recipients: Pediatric Considerations

Conflicting data exist on the diagnostic yield and need for surveillance EMB in pediatric recipients. In single center

studies, the rates of acute rejection on surveillance EMB ranges from 0.3% to 14% in the first year post-transplant and from 0% to 10% thereafter. ^{22, 47-50} Due to very low rates of rejection on surveillance EMB beyond 5 years, there is increasing consensus that EMB beyond 5 years have little usefulness in asymptomatic patients. The right heart catheterization (RHC) may still be valuable late after transplantation because the finding of restrictive physiology may indicate the presence of cardiac allograft vasculopathy (CAV).⁵¹

Given the increased risk of complications in pediatric recipients, many centers minimize the number of surveillance EMB in very small children and avoid them altogether in infants, while at a few pediatric centers no routine surveillance EMB are performed in pre-adolescents due to the opinion that echocardiography is sufficient for rejection surveillance in asymptomatic patients.

Recommendations for Rejection Surveillance by Endomyocardial Biopsy in Heart Transplant Recipients:

Class IIa:

1. It is reasonable to utilize EMB in a HT candidate suspected of having an infiltrative cardiomyopathy or an inflammatory process, such as giant cell myocarditis, amyloidosis or sarcoidosis.

Level of Evidence: C.

2. The standard of care for adult HT recipients is to perform periodic EMB during the first 6 to 12 postoperative months for surveillance of HT rejection.

Level of Evidence: C.

3. The standard of care in adolescents should be similar to that in adults, including surveillance EMB for heart allograft rejection for 6 to 12 months after HT. In younger children, especially infants, it is reasonable to utilize echocardiography as a screening tool to reduce the frequency of EMB.

Level of Evidence: C.

4. After the first postoperative year, EMB surveillance for an extended period of time (e.g., every 4-6 months) is recommended in HT recipients at higher risk for late acute rejection, to reduce the risk for rejection with hemodynamic compromise, and the risk of death in African-American recipients.

Level of Evidence: C.

Class IIb:

4. The use of routine EMB later than 5 years after HT is optional in both adults and children, depending on clinical judgment and the risk for late allograft rejection.

Level of Evidence: C.

Noninvasive Monitoring for Acute Rejection

Although clinical assessment is an essential component of rejection monitoring, due to its overall poor sensitivity and specificity, numerous adjunct methods have been evaluated.⁵

Electrophysiological Parameters

Although simple and inexpensive, electrocardiogram (ECG) rejection monitoring of QRS amplitude is unreliable.⁵² Signal averaged ECG (SAECG), heart rate variability and QT dispersion analysis are also inadequate.^{53, 54} More recently, ventricular evoked responses (VER) have been shown to have high negative predictive accuracy (97%) and prognostic value.^{55, 56} However due to conflicting results and lack of sufficient prospective data,⁵⁷ the routine use of this tool for rejection screening cannot be recommended.

Imaging Modalities

Among the many imaging modalities studied, echocardiography and magnetic resonance imaging (MRI) seem to have attracted the greatest interest. The wide availability and ease of use and versatility of echocardiography make it an appealing screening technique, especially when compared serially. As such, it is commonly used as an adjunct clinical tool to help identify patients with acute rejection. Numerous parameters have been studied including increased wall thickness and echogenicity, presence of pericardial effusion, diastolic function variables including change in E-wave peak velocity, left ventricular doppler inflow and tissue doppler parameters.^{17, 58} However, despite some promising data, there is still a significant lack of consistent positive results and reproducibility between studies As such, at least currently, echocardiography appears to lack both sufficient sensitivity and specificity to be a viable alternative to routine biopsies as a screening method.

Early studies of MRI showed significant correlation between higher T2 relaxation times and acute rejection.^{59, 60} Newer contrast agents, gadolinium enhancement and diastolic and twisting mechanics parameters may increase the usefulness of this modality for rejection diagnosis.⁵⁹ However, limited availability and studies with small sample sizes have limited the application of this tool in the diagnosis of rejection. The use of gadolinium for MRI may also limit the use of MRI in HT recipients with renal insufficiency.

Biochemical and Inflammatory Markers

Some small studies have identified a strong correlation between B-type natriuretic peptide levels (BNP) and rejection^{61, 62} and have shown that troponin levels have an excellent negative predictive value (NPV) in excluding more severe rejection.⁶³ The results of other studies, including those looking at other markers such as C-reactive protein (CRP), have not confirmed these findings.⁶⁴⁻⁶⁷

The measurement of markers of T-cell activation (interleukins, tumor necrosis factor-alpha [TNF- α], interferongamma [IF- γ]-induced chemokines, and adhesion molecules) have also yielded inconsistent results and therefore have limited usefulness as tolls for rejection screening.⁶⁸⁻⁷¹

Gene Expression Profiling

An attractive approach to rejection screening may be the evaluation of the transcription of genes mediating the immune processes presumed underlying acute rejection and myocardial injury. Early studies showed increased transcription of cytokine associated genes (IL-6 and transforming growth factor-beta [TGF- β]) in patients with acute allograft rejection. Subsequently, multiple additional genes were found to be differentially expressed during rejection.⁷² Although some concerns remain on whether peripheral blood analysis is representative of intra-graft rejection processes,⁷³ there is support for the validity of the methodology.⁷³⁻⁷⁵

Assessment of the expression of groups rather than of single genes may better represent of pathophysiological processes underlying acute allograft rejection. The Cardiac Allograft Gene Expression Observational Study (CARGO) evaluated Gene Expression Profiling (GEP) in the diagnosis acute cardiac allograft rejection from gene transcription analysis of peripheral blood mononuclear cells (PBMC).⁷⁶ After identifying and validating a group of 11 discriminator genes, a diagnostic algorithm was developed to generate a score from 0 to 40 and applied to 281 samples obtained later than 1 year after transplantation. The predictive value for significant rejection (Grade 2R/3A) was then calculated for each score. A very high NPV was seen for lower scores, but the positive predictive value (PPV) of high scores was low. Therefore GEP is useful in identifying patients at low risk of rejection in whom surveillance EMB may be avoided. The predictive value of the AlloMap score (XDx, Brisbane, CA) varies by time after transplant (2-6 months vs. 6-12 months vs. > 12 months) so that the same scores correspond to different levels of risk at different post-transplant intervals. A "threshold" score is selected based upon the time posttransplant, the NPV of the test, and patient characteristics. Scores below this threshold represent a low risk of significant rejection. EMB may be avoided whereas higher scores should trigger an EMB. The following thresholds have been suggested: < 20 (3-6 months), < 30 (6-9 months) and < 34 (> 12 months).⁷⁷

The AlloMap test was approved by the US Food and Drug Administration (FDA) in 2008. This diagnostic rejection tool is not indicated for acutely symptomatic patients, those with recurrent rejection, those < 2 months post-transplant, are receiving ≥ 20 mg of daily oral prednisone doses or received high-dose intravenous (IV) corticosteroids (CSs) or myeloablative therapy in the past 21 days, received blood products or hematopoietic growth factors in the past 30 days, are pregnant, or < 15 years old. However, while Allomap is FDA approved in the US for use after 2 months posttransplant, the clinical trials data included patients beyond 6 months, therefore, its utility between 2 and 6 months posttransplant is unclear. The IMAGE trial, a multicenter, noninferiority trial of patients > 6 months post-transplant who are randomized to either GEP-based rejection surveillance strategy or routine EMB is ongoing.⁷⁸ The study showed that the use of GEP in combination with clinical and echocardiographic assessment was not associated with increased serious adverse events when compared with routine biopsy surveillance (HR 1.04, CI 0.67-1.68), while decreasing the number of biopsies per patient.⁷⁹ The initial score used to trigger a biopsy was > 30, later changed to > 34, largely in keeping with above suggested thresholds. However, few rejection episodes were diagnosed prior to clinical development of allograft dysfunction by either surveillance technique (biopsy or AlloMap) raising the question of whether either is useful in low risk recipients.

Considerations for Pediatric Recipients

The challenge of performing repeated surveillance EMB in small children emphasizes the importance of developing noninvasive methods of rejection diagnosis in this population, especially infants. However, there are no published studies of adequate size or design to conclusively establish the role of non-invasive rejection surveillance across the wide range of pediatric age groups and extrapolation of adult data is insufficient for making pediatric-specific recommendations.⁸⁰

Electrophysiologic Parameters

A decrease in QRS complex voltage on surface 12-lead ECG may be seen in acute rejection in children, but is insufficiently sensitive or specific to be used alone for rejection surveillance. It is controversial whether a decrease in total QRS voltage on an intramyocardial ECG is indicative of acute rejection in pediatric recipients.⁸¹ A study evaluating the association between SAECG parameters and acute allograft

rejection in children⁸² showed a significant increase in the filtered QRS duration and presence of late potentials in association with EMB-proven rejection.

New onset of arrhythmia, including high-grade atrial and ventricular ectopy, atrial tachycardia (notably atrial flutter), ventricular tachycardia, and AV block should raise the suspicion of rejection and trigger an EMB.^{83, 84}

Imaging Modalities

Echocardiographic variables cannot accurately predict all acute rejection episodes. Studies have also not focused on the early post-transplant period when acute rejection is most likely to occur. Parameters that have been evaluated include LV fractional shortening and wall thickness, percentage wall thickening, velocity of posterior wall thinning, tissue Doppler patterns, 3-dimensional torsion, myocardial performance index, and others.^{47, 48, 80} Multiple echocardiographic parameters have been used in a scoring system to predict the likelihood of rejection but measurements cannot be easily reproduced.⁴⁷ Although echocardiography may be useful in raising suspicion for rejection, especially in infants, it is unlikely to replace EMB as the primary modality of rejection surveillance. There is minimal experience with cardiac MRI for rejection diagnosis in children, and this, combined with the expense, reduced availability, and need for sedation in small children, make this tool currently unsuitable for rejection surveillance.

Biochemical and Inflammatory Markers

Limited data exists on the use of biochemical markers in pediatric recipients. Studies of BNP have demonstrated significant elevations in BNP associated with EMB-proven rejection. ^{62, 85-87} One study demonstrated that a BNP value > 700 pg/mL was 100% sensitive and 92% specific for detecting acute rejection. ⁶²

Gene Expression Profiling

The CARGO study included 105 pediatric recipients. However, the number of acute rejection episodes captured in this pediatric cohort was too small to enable the assessment of the value of GEP for the diagnosis of acute rejection in children.

Recommendations for the Non-Invasive Monitoring of Acute Heart Transplant Rejection:

Class IIa:

1. In centers with proven expertise in VER monitoring, intramyocardial electrograms recorded non-invasively

with telemetric pacemakers can be used for rejection surveillance in patients at low risk for rejection.

Level of Evidence: C.

2. Gene Expression Profiling (Allomap) can be used to rule out the presence of ACR of grade 2R or greater in appropriate low-risk patients, between 6 months and 5 years after HT.

Level of Evidence: B.

Class IIb:

1. Use of echocardiography as primary monitoring modality for acute heart allograft rejection in infants can be considered as an alternative to surveillance EMB.

Level of Evidence: C.

Class III:

1. The routine clinical use of electrocardiographic parameters for acute heart allograft rejection monitoring is not recommended.

Level of Evidence: C.

2. The use of echocardiography as an alternative to EMB for rejection monitoring is not recommended.

Level of Evidence: C.

3. The routine clinical use of MRI for acute allograft rejection monitoring is not recommended.

Level of Evidence: C.

4. The use of BNP, troponin I or T, or CRP levels for acute heart allograft rejection monitoring is not recommended.

Level of Evidence: C.

5. The use of systemic inflammatory markers for acute heart allograft rejection monitoring is not recommended.

Level of Evidence: C.

 Routine use of non-invasive testing modalities (electrocardiographic, imaging or biomarkers) is not recommended as the primary method for acute heart allograft rejection surveillance in older children and adolescents.

Level of Evidence: C.

Table 1Drugs That Affect the Levels of Tacrolimus, Cyclosporine,Sirolimus, or Everolimus

Decrease immunosuppression levels	Increase immunosuppression levels
Anti-epilectics	Anti-microbials
Carbamazepine	Clarithromycin
Fosphenytoin	Erythromycin
Phenobarbital	Metronidazole and tinidazole
Phenytoin	Quinupristin/dalfopristin
	Levofloxacin
Anti-microbials	Anti-fungals
Caspofungin	Clotrimazole
Nafcillin	Itraconazole
Rifabutin	Ketoconazole
Rifampin	Fluconazole
Rifapentine	Posaconazole
	Voriconazole
Anti-retroviral therapy	Anti-retroviral therapy
Efavirenz	Protease inhibitors (general)
Etravirine	Amprenavir
Nevirapine	Atazanavir
	Darunavir
	Fosamprenavir
	Indinavir
	Nelfinavir
	Ritonavir
	Saquinavir
	Tipranavir
Others	Cardiovascular
Antacids containing	Amiodarone
magnesium, calcium, or	Diltiazem
alumnium (tacrolimus	Verapamil
Deferasirox	
Modafinil	
St. John's wort	
Thalidomide	
Ticlopidine	
Troglitazone	
	Nutraceuticals
	bitter orange
	grape fruit juice
	Others
	Rilonacent
	Theophylline
	Cimetidine
	Fluvoxamine
	Glipizide
	Glyburide
	Imatinib
	Nefazodone

Topic 2: Monitoring of Immunosuppressive Drug Levels

Pharmacology/Pharmacokinetics of Immunosuppressive Agents

A detailed description of the pharmacology and pharmacokinetics of the various immunosuppressive agents can be found in a multitude of sources.^{7, 88} This section will focus on the role of therapeutic drug monitoring and relevant drug-drug interactions.

Calcineurin Inhibitors

Cyclosporine (CYA) and tacrolimus (TAC) bind to a specific immunophilin to form a complex which interacts with intracellular calcineurin and inhibits the expression of genes coding for pro-inflammatory cytokines (such as IL-2). Reduced cytokine production prevents T cells activation and proliferation, up-regulation of adhesion molecules, and reduces downstream inflammatory molecules.

Cyclosporine

Use of CYA in HT began in the early 1980s and initial trials revealed the addition of CYA significantly increased 1and 5-year survival compared to therapy of azathioprine (AZA) and CSs.⁸⁹

Compared to the oil-based compound, CYA microemulsion has better gastrointestinal (GI) absorption and a more reliable pharmacokinetic profile.⁹⁰ A randomized trial of the 2 CYA formulations showed that the microemulsion preparation was associated with a significant reduction in rejection episodes requiring antilymphocyte antibody therapy (6.9 vs. 17.7%, p = 0.002), lower CS dose (0.37 vs. 0.48 mg/kg/day, p = 0.034) and less treatment failures (3.7 vs. 9.4%, p = 0.037) at 24 months.⁹¹

CYA is absorbed mainly in the upper portion of the GI tract. Because the renal excretion of CYA is only 6%, the drug is not appreciably removed by hemodialysis. Metabolism of CYA occurs via the cytochrome P-450 (CYP) enzyme system to at least 30 metabolites and multiple drugs interacting with the CYP-450 enzyme system may alter CYA concentrations. Conversely CYA inhibits CYP3A4 enzymes and alters the metabolism of other drugs. Drugs that alter CYA concentrations are shown in Table 1. Monitoring of CYA levels and renal function with appropriate dose adjustments at the time of initiation and discontinuation of these agents is essential.

Measurement of 12-hour trough CYA concentrations remains the standard approach for monitoring CYA therapy despite evidence that it may underestimate total CYA

exposure. Determination of CYA trough levels is clinically practical and maintenance of therapeutic drug levels has been associated with favorable allograft and patient outcomes.^{89, 91} Evaluation of 2-hour post-dose concentrations (C2) in de novo and stable HT recipients has yielded variable results. In some studies, C2 levels identified patients at risk of receiving inappropriately high doses of CYA and thus are more likely to experience drug toxicity.⁹² Maintenance of a low C2 level in HT recipients given antibody therapy was associated with preserved renal function without increased risk of acute rejection or compromise of heart allograft and recipient survival.⁹³ Compared to 28 historical controls monitored only with CYA trough levels, 28 HT recipients monitored with both C2 and trough levels had a slight reduction of EMBproven rejection (21 vs. 39% p = ns), a significant reduction in 3A rejection (5 vs. 11% p < 0.002) and a lower glomerular filtration rate (GFR).⁹⁴

The specific formulation of CYA used may affect the usefulness of C2 levels monitoring.⁹⁵ For example the C2 level of a commercially available generic CYA failed to accurately estimate drug exposure (area under the plasma concentration time curve [AUC]) that was better represented by the 6-hour post-dose levels. In general, regulatory agencies around the world do not require generic immunosuppressive agents to undergo bioequivalence testing in transplant recipients.⁹⁵ This is problematic since the pharmacokinetics of these generic drugs may be altered in patients with comorbidities or concurrently taking medications affecting their absorption or metabolism.

Tacrolimus

Both the rate and extent of TAC absorption is variable and in special populations such as African Americans, bioavailability may be greatly reduced. This drug also undergoes extensive metabolism via the CYP3A system and several drugs prescribed in transplant recipients may alter its metabolism (see Table 1) Few studies in HT recipients have shown an acceptable correlation between trough concentrations and 12-hour AUC $(r^2 = 0.74)$.⁹⁶ Although some small studies have demonstrated that 2 to 4 hour post-dose levels are more representative of TAC exposure than measurement of trough levels, data correlating this TAC monitoring method with heart allograft outcomes are lacking.97

In several countries TAC is available as an extended release once-daily product. This TAC formulation should be taken in the morning because evening administration has been associated with reduced drug exposure.⁹⁸ In renal and liver transplant recipients, conversion from original twice-daily

TAC to the once-daily preparation was associated with unchanged drug pharmacokinetic profile, safety and allograft outcomes at 2 years after conversion.^{99, 100} Liver, but not renal transplant recipients experienced a 4.7% increase in new onset diabetes or need for insulin use.

Mycophenolate

Mycophenolic acid (MPA), the active metabolic form of mycophenolate mofetil (MMF) and mycophenolate sodium preparations, is a reversible blocker of inosine monophosphate dehydrogenase (IMPDH), an enzyme which inhibits de novo purine guanosine synthesis.¹⁰¹ Lymphocytes are vulnerable to MPA because of their inability to utilize the salvage pathway for purine synthesis and the preferential blockade by MPA of the type II form of IMPDH, which is upregulated during lymphocytes activation. Inhibition of T- and B-cell proliferation results in diminished cytotoxic T-cell responses and antibodies formation against the allograft.

Mycophenolate is well absorbed in the GI tract and rapidly hydrolyzed in the liver to its active form, MPA. In turn, MPA is metabolized by the uridine 5'-diphospho (UDP)glucuronosyltransferase enzyme in the liver and intestine to an inactive metabolite 7-O-mycophenolic acid glucuronide (MPAG) and undergoes renal and biliary excretion. After hydrolysis and enterohepatic recirculation MPAG reenters the circulation as MPA.¹⁰¹

Mycophenolate is generally administered as a fixed dose regimen adjusted to mitigate side effects. Factors that alter MPA levels include a decrease in protein binding as it occurs with hypoalbuminemia, elevated BUN levels and renal and hepatic dysfunction.¹⁰² The dose-dependent induction of UDPglucuronosyltransferase activity by CS is most apparent early after transplantation when CS doses are highest and attenuated when CS doses are tapered and stabilized. While CYA may alter the biliary excretion of MPAG and thus decrease levels, TAC does not. In one study, acute rejection episodes, survival, and adverse events were evaluated in 60 HT recipients randomized to CYA or TAC in combination with MMF and CS. All patients had MPA trough levels measured and doses adjusted to maintain a concentration in the range of 1.5 to 4.0 µg/mL. The results of this study suggest that the CYA-treated patients needed significantly higher MMF doses to maintain MPA levels within the desired range.¹⁰³ Magnesium and aluminum compounds dramatically decrease MPA absorption and should therefore be given at least 4 hours before or after MMF.

Black renal transplant recipients required higher MMF doses than patients of other ethnicities (3 grams vs. 2 grams) to have comparable rejection rates.¹⁰⁴

The utility of MPA levels to optimize MMF therapy remains uncertain. In some studies, high MPA levels were correlated with the occurrence of leukopenia. A recent study of HT recipients, comparing standard versus 12-hour AUC-guided MMF dosing showed that both dosing strategies achieved the target AUC in 2 weeks and were associated with similar rejection, infection or adverse effects rates.¹⁰⁵

In 902 calcineurin inhibitor (CNI)-treated renal transplant recipients randomized to fixed dose MMF or abbreviated AUC (concentration measurements pre-dose and 30 and 120 minutes post-dose)-guided MMF dosing, 12-month biopsy-proven rejection rates and treatment failures were similar for the 2 dosing strategies. There was, however, a significant relationship between the third day MPA-AUC and biopsy-proven rejection at 1 month and 1 year.¹⁰⁶ The Opticept Trial compared fixed dose MMF versus concentration-guided MMF dosing plus CNI with a third arm of reduced dose CNI plus concentration controlled MMF dosing in 720 renal transplant recipients. Despite a trend toward decreased biopsy-proven acute rejection rates, graft loss and death at 1 year were similar in the 2 groups (23% vs. 28%, p = 0.18).¹⁰⁷

The correlation between MPA trough concentration and 12-hour AUC is poor. A retrospective evaluation of 215 HT recipients with MMF trough levels available at the time of scheduled EMB revealed that grade 3A rejection rates were lower in patients with a MMF trough level $\geq 2 \text{ mg/L}$ compared to those with a level < 2 mg/L.¹⁰⁸ Because the enteric-coated MMF preparation has a delayed peak plasma concentration that may cause higher trough levels, the data summarized above cannot be extrapolated to this formulation.¹⁰⁵

Proliferation Signal Inhibitor or Mammalian Target of Rapamycin Inhibitors

Two proliferation signal inhibitors (PSIs) are currently used in HT recipients, sirolimus (SRL) and everolimus (EVL), but their regulatory approval varies between countries. The PSIs inhibit cytokine-mediated proliferation in T-, B-, and mesenchymal cells, including smooth muscle cells, by initially forming a complex with the immunophyllin FK506 binding protein 12, which then combines with the mammalian target of rapamycin (mTOR), inhibiting IL-2 dependent proliferation via cell-cycle arrest in the G1 to S phase.¹⁰⁹ Although both PSIs undergo hepatic metabolism via the CYP-P450 system, have similar drug interactions and adverse effect profiles, several differences deserve mention. Compared to EVL, SRL has a longer half-life (62 vs. 28 hours) and stronger affinity for FKBP-12.¹⁰⁹ Microemulsion CYA and SRL, given in combination, mutually increase drug exposure due to competitive binding of P-glycoprotein.¹¹⁰ Although this interaction is significantly stronger when the drugs are administered simultaneously, it still occurs when the 2 drugs are given 4 hours apart. This interaction has not been demonstrated with TAC.^{111, 112} Notably CYA, but not TAC, reduces EVL exposure, necessitating determination of PSI concentration when the CYA dose is changed.^{113, 114}

Recent studies have evaluated the outcomes of CNI-free immunosuppressive strategies consisting of the combined use of a PSI with MMF. With this regimen target PSI trough concentrations have generally been higher than those required in the presence of a CNI. Although CNI-free immunosuppression may be associated with favorable renal function and allograft outcomes, its usefulness has been limited by high drug discontinuation rates due to adverse side effects of the PSI.¹¹⁵ At this time, the appropriate target levels of PSI drugs in a CNI-free regimen have not been fully established.

Antilymphocyte Antibody Therapy

Currently, anti-thymocyte globulin (ATG) and anti-IL-2 receptor antibodies are the agents most often used for induction therapy in HT. While having comparable effects on rejection, monoclonal and polyclonal antibodies are associated with different adverse effects and types of infection. Generally data on outcomes beyond 6 months to 1 year are lacking. Monitoring and dosing of individual patients are also different.¹¹⁶ Antibodies can also be associated with protracted leucopenia.

Therapy with rabbit anti-thymocyte globulin (RATG) given either for induction of immunosuppression or the treatment of rejection refractory to CSs, has evolved from a standard dosing strategy to dose adjustment based upon CD3 or CD2 counts. The latter approach was initially reported in 41 high-risk kidney or combined kidney-pancreas transplant recipients receiving RATG for induction therapy. Administration of RATG for CD3 counts > 20 cells/mm³ was associated with an acceptable rejection rates and safety profile.¹¹⁷ In thoracic transplantation the CD3 count-guided approach has yielded similar results and has permitted a 60% reduction in dose and it has resulted in lower adverse events rates.^{118, 119} In HT recipients many clinicians choose to obtain absolute lymphocyte counts rather than CD2 or CD3 counts, because of a lower cost. Therapy with ATG usually lasts for 3 to 7 days.

Basiliximab (a chimeric human/murine) and daclizumab (humanized) monoclonal antibody binds to the IL-2 receptor (CD25) on activated T lymphocytes and thus prevent their clonal expansion. Both monoclonal antibodies are currently

administered at fixed doses. Data on altered dosing strategies and on the utility of CD25 saturation monitoring are lacking.

Significant Drug-Drug Interactions

The CYP3A and gastrointestinal P-glycoprotein systems play key roles in the metabolism of many of the immunosuppressive drugs such as CYA, TAC, SRL, and EVL. Therefore, drugs that either induce or inhibit CYP3A or decrease P-glycoprotein activity cause, respectively, a decrease or increase in immunosuppressive drugs levels.

А potentially life-threatening adverse event. rhabdomyolysis, can occur with the combined use of CYA and a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (lovastatin or simvastatin), particularly when clopidogrel or gemfibrozil are concomitantly used.^{120, 121} All 3 drugs are metabolized via the CYP3A4 enzyme, a process that is inhibited by CYA. The addition of clopidogrel to an otherwise safe drug combination may lead to an increase in plasma concentration of the statin due to competition for the remaining receptor sites. This effect does not occur when CYA and clopidogrel are used in combination with pravastatin because this drug is not metabolized through the CYP 3A pathway. In contrast, fibrate therapy alone is associated with rhabdomyolysis and the incidence increases dramatically when combined with statins or other drugs with the potential for drug-drug interactions.

Human immunodeficiency virus (HIV)-infected patients are undergoing solid organ transplants and transplant recipients may develop acquired immune deficiency syndrome (AIDS). The need to continue antiviral therapy along with immunosuppression presents a challenge since many of the essential drugs needed to treat HIV interfere with the CYP3A system. As this therapy changes, heightened attention to therapeutic drug monitoring of the CNI and PSI agents is essential.

Considerations for Pediatric Recipients

The guidelines outlined above are also applicable to children. As with adults, race/ethnicity, body size, time from transplant, drug-drug interactions, and intercurrent illnesses may all influence immunosuppressive drug blood levels. There may also be differences between liquid preparations (required in small children) and pill forms of the same agents. It is also known that pharmacogenomic variables (e.g., genetic polymorphisms in the CYP3A family and in the gene ABCB1 encoding the membrane pump P-glycoprotein) play an important role in determining immunosuppressant drug pharmacokinetic and pharmacodynamic profiles and efficacy in children.^{122, 123} Developmental/maturational stage may strongly influence pharmacokinetic and pharmacodynamic profiles. Drug metabolism in newborns is influenced by prematurity and postnatal age. Infancy and puberty are associated with the most rapid growth rates in childhood. Failure to maintain adequate dosing during rapid growth may cause drug levels to drop below acceptable therapeutic levels. This is most likely to occur during late follow-up when patients are under less intense surveillance. This may coincide with adolescence, a developmental stage strongly associated with nonadherence. These factors all place the patient at increased risk of allograft loss and emphasize the need for ongoing monitoring of drug concentrations.

Intercurrent illnesses (mostly viral) are very common in early childhood. Gastroenteritis frequently leads to increased TAC levels. In addition, dehydration associated with vomiting and diarrhea may exacerbate renal dysfunction due to afferent vasoconstriction produced by high TAC levels. For this reason, TAC levels and renal function should be closely monitored during any protracted diarrheal illness in childhood or when there is evidence of dehydration. Other primary viral infections that may occur post-transplantation include Epstein-Barr virus (EBV), cytomegalovirus (CMV), and adenovirus. All may cause a hepatitis, further impairing metabolism of drugs that are metabolized by the CYP3A system. This may increase CNI levels and further impair cytotoxic T cell responses required to control the viral infection. When these infections are suspected or confirmed, liver function tests and CNI levels should be closely monitored.

CYA therapy should be monitored with trough levels. As in adults the usefulness of C2 monitoring is also unclear in children. In a study of children ranging in age between 6 months and 14 years, 50% of the participants did not achieve the target C2 level during conversion from C0 to C2 monitoring. The variability in absorption capabilities and metabolic rate at different ages in the pediatric population may account for the difficulty seen.¹²⁴ In another study, C2 monitoring seemed superior to C0 monitoring in identifying children at risk for rejection.¹²⁵ Despite these findings, C0 monitoring has remained the gold standard, perhaps in part due to the logistical challenges of coordinating accurate timing of blood sampling in the clinical setting.

As in adults, TAC levels are measured at C0. Target levels are usually comparable to those in adults. However, because infants experience less acute rejection, slightly lower target TAC levels than in older children or adolescents are acceptable. There is very little experience with once daily TAC dosing in pediatric solid organ transplant recipients.¹²⁶

MMF is the agent most commonly used in conjunction with a CNI in pediatric HT recipients.³⁵ Typically, recommended doses are 40 mg/kg/day, or 1200 mg/m²/day in 2 divided doses. Dose titration is primarily driven by GI adverse events, or bone marrow suppression.¹²⁷ As with adults, controversy exists over the role of therapeutic drug monitoring. There is marked inter-patient variability in levels achieved for a given dose with poor correlation between dose and MPA level.¹²⁸⁻¹³⁰ Levels of MPA are higher with TAC use than with CYA. Smaller children tend to require higher doses.¹²⁹ "Standard" dosing is frequently associated with "subtherapeutic levels" and this may be associated with more graft rejection.¹²⁹ Although evidence is lacking that late graft outcomes in children are improved by routine drug monitoring, the variability in doses required to achieve therapeutic levels and the need for large doses in small children, has led many pediatric centers to perform intermittent MPA level monitoring. Dose adjustment may be most beneficial when low MPA levels (e.g., < 1.5 ng/mL) are associated with rejection episodes.

There is a paucity of experience with use of mTOR inhibitors in pediatric HT recipients.¹³¹ The adverse events profile is similar to adults. Pediatric-specific drug concentration targets have not been established and adult targets are generally used. Once-daily dosing is commonly used. However, the finding that half-life in children may be as short as 12 hours suggests that in some children twice-daily dosing with C0 monitoring at 12 hours may be preferable.¹³²

In children, there is no evidence to suggest that polyclonal antibodies should be dosed to achieve a specific T-cell count. Thymoglobulin®, is generally given at a fixed dose of 1.5 mg/kg/day for 3 to 7 days (most commonly 5 days). Because thrombocytopenia may require dose adjustments, platelets should be measured daily during therapy.

Recommendations for the Monitoring of Immunosuppressive Drug Levels:

(See Table 1)

Class I:

1. The use of the microemulsion formulation of CYA is recommended since it is associated with more favorable pharmacokinetic features compared to the oil-based compound.

Level of Evidence: B.

Class IIa:

1. At present, 2-hour post-dose (C2) levels should not replace 12-hour trough (C0) concentrations for routine

monitoring of CYA exposure in most patients, but may be useful in selected patients in whom a better characterization of the pharmacokinetic profile of CYA is desired.

Level of Evidence: B.

2. Measurement of 12-hour trough CYA concentration is the recommended form of therapeutic drug monitoring for routine clinical use. The target levels are dependent upon the method used (high-performance liquid vs. enzyme chromatography [HPLC] multiplied immunoassay technique [EMIT] vs. cloned enzyme donor immunoassay method [CEDIA]), concomitant immunosuppression, toxicity risks and time after HT. In general, when used in conjunction with AZA or an MPA preparation, the average CYA trough concentration target using the Abbot TDX assay (or equivalent) is 325 ng/mL (range 275-375 ng/mL) for the first 6 post-operative weeks, 275 ng/mL (range 200-350 ng/mL) for weeks 6 to 12, 225 ng/mL (range 150-300 ng/mL) for month 3 to month 6; and 200 ng/mL (range 150-250 ng/mL) from month 6 onward.

Level of Evidence: C.

3. At present, CYA trough concentration targets when CYA is used in combination with PSIs and mTOR inhibitor agents have not been adequately determined.

Level of Evidence: C.

4. Measurement of 12-hour trough concentration for twicedaily TAC and a 24-hour trough concentration for oncedaily TAC is the recommended drug monitoring method for routine clinical use. The therapeutic range of TAC levels varies depending on concomitant drugs, toxicity concerns and time after HT. In general, when used in conjunction with AZA or an MPA preparation, TAC trough concentration targets range between 10 and 15 ng/mL during the early post-operative period (Days 0 – 60); between 8 and 12 ng/mL for the next 3 to 6 months; and between 5 and 10 ng/mL in stable patients 6 months after HT.

Level of Evidence: C.

5. At this time, target therapeutic TAC trough concentrations when TAC is used in combination with PSI (mTOR inhibitors) agents have not been adequately determined.

Level of Evidence: C.

6. Therapeutic drug monitoring for PSIs using trough concentration levels is recommended for SRL and EVL. Levels should be measured at least 5 days after adjustment of the dose, when a new steady state is

achieved. When used in combination with CYA, the optimal trough target levels range for EVL between 3 and 8 ng/mL. The corresponding optimal trough level range for SRL is 4 to 12 ng/mL.

Level of Evidence: B.

7. In pediatric HT recipients, TAC and CYA should be monitored using C0 levels, when twice-daily dosing is used. Target levels are comparable to those in adults, but slightly lower targets may be used in low risk patients such as non-sensitized infant HT recipients.

Level of Evidence: C.

8. There is insufficient data to support routine monitoring of MPA levels in pediatric recipients. However, intermittent monitoring is reasonable when there is ongoing rejection, doubts about adequacy of dosing (e.g., infants and young children), and to assess medical compliance.

Level of Evidence: C.

Class IIb:

1. At this time replacement of twice-daily TAC with oncedaily TAC dosing cannot be recommended in HT recipients. Should a patient require the once-daily formulation, appropriate monitoring should be used to ensure maintenance of appropriate levels and preserved heart allograft function.

Level of Evidence: C.

2. In patients with a therapeutic 12-hour trough concentration for twice-daily TAC but evidence of potential drug-related toxicity or reduced efficacy (rejection), a 3-hour post-dose level (C3) may help to adjust TAC doses.

Level of Evidence: C.

3. In selected situations (rejection, infection, renal failure, malnutrition, and certain ethnic populations) where it is suspected that altered MMF exposure contributes to heart allograft dysfunction, measurement of trough MPA levels may be used to guide drug dosing. In such cases, a MPA level of < 1.5 mg/L is considered to be subtherapeutic.</p>

Level of Evidence: C.

4. Dose adjustments and frequency of therapy with polyclonal antibodies (e.g., ATG) used as induction therapy can be monitored with daily measurement of CD3 or CD2 counts with the goal of maintaining the CD2 or CD3 count between 25 and 50 cells/mm³ or absolute total lymphocyte counts < 100 to 200 cells/mm³.

Level of Evidence: C.

5. In pediatric HT recipients, CYA C2 monitoring may be performed instead of C0 in centers with extensive experience with this form of monitoring.

Level of Evidence: C.

6. As in adults, routine monitoring of SRL and EVL at C0 is recommended also in children.

Level of Evidence: C.

Class III:

1. Routine therapeutic drug monitoring of MPA levels to adjust MMF doses cannot be recommended at this time.

Level of Evidence: C.

2. Measuring CD 25 saturation to adjust the dose of antiinterleukin-2 receptor antibodies remains experimental and its routine clinical use cannot be recommended.

Level of Evidence: C.

Recommendations for the Monitoring of Immunosuppressive Drug Levels for Pediatric Heart Transplant Recipients:

Class IIa:

1. TAC and CYA should be monitored using C0 levels, when twice daily dosing is used. Target levels are comparable to those in adults, but slightly lower targets may be used in low risk patients such as non-sensitized infant recipients.

Level of Evidence: C.

2. There is insufficient data to support routine monitoring of MPA levels. However, intermittent monitoring is reasonable when there is ongoing rejection, doubts about adequacy of dosing (e.g., infants and young children) and to assess medical compliance.

Level of Evidence: C.

Class IIb:

1. CYA C2 monitoring may be performed in lieu of C0 in centers with extensive experience with this form of monitoring.

Level of Evidence: C.

2. As in adults, routine monitoring of SRL and EVL at C0 is recommended also in children.

Level of Evidence: C.



Figure 1 Schematic of mechanisms of action of immunosuppressive drugs. T-cell proliferation results from activation after presentation of donor antigen by antigen-presenting cells in conjunction with the major histocompatibility complex class II and B7 complex. This mechanism results in activation of calcineurin, which leads to production of IL-2. Autocrine stimulation by IL-2 results in cell proliferation by a pathway involving target of ramapycin and cyclin/cyclin-dependent kinase. Immunosuppressive agents exert their effects on a number of different targets to prevent T-cell proliferation. G1 (first growth phase), S (synthesis of DNA), G2 (second growth phase) and M (cell division) represent the phases of the cell cycle.

APC, antigen presenting cell; CDK-cyclin-dependent kinase; IL-2, interleukin-2; IL-2R, interleukin-2 receptor; IL-2R Ab, interleukin-2 receptor antibody; MHC, major histocompatibility complex; MMF, MMF; mRNA, messenger RNA; NFAT, nuclear factor of activated T cells; TCR, T-cell receptor; TOR, target of rapamycin protein.

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Topic 3: Principles of Immunosuppression and Recommended Regimens

Most immunosuppressive regimens employed in HT recipients consist of a combination of agents that affect different pathways in the activation of the T-cell (Figure 1).¹³³

Corticosteroids are a key component of HT immunosuppression, and are the first-line of therapy during episodes of acute cellular rejection. Their immunosuppressive and anti-inflammatory actions are due to effects on the transcriptional regulation of a number of genes that affect leukocyte function.^{134, 135} Data from the latest International Society of Heart and Lung Transplantation (ISHLT) Registry show that 73% of HT recipients remain on CSs at 1 year.

Several studies indicate that it is both feasible and safe to wean most patients from CSs by 6 to 12 months after HT.¹³⁶ Reduction and discontinuation of CSs is desirable because it lowers the long-term adverse effects of these drugs. This practice has not been tested in randomized trials.

By blocking purine synthesis, AZA inhibits leukocytes proliferation. Because the actions of AZA are not confined to T-cells, AZA-treated patients are at higher risk for opportunistic infections, bone marrow suppression and hepatotoxicity.

The CNIs are the mainstay of immunosuppression in HT. Adverse effects of CYA therapy include hypertension, renal insufficiency, hepatotoxicity, gingival hyperplasia, hypertrichosis, tremor, and increased risk of malignancy.

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Author (year)	Study	No.	Follow-up	Survival	Rejection	CAV by IVUS
Kobashigawa ¹³⁷ (1998)	MMF vs. AZA	650	3 years	MMF = higher survival ^a	MMF = less rejection	NS; MMF = less CAV at 1 year ^b
Reichart ¹⁴⁸ (1998)	TAC vs. CYA	82	1 year	NS	NS	
Taylor ¹³⁹ (1999)	TAC vs. CYA	85	1 year	NS	NS	
Eisen ¹⁴⁰ (2003)	EVL vs. AZA	634	1 year	NS	EVL groups = less rejection	EVL groups = less CAV
Keogh ¹⁴¹ (2004)	SRL vs. AZA	136	2 years	NS	SRL groups = less rejection at 6 months	SRL groups = less CAV
Grimm ¹⁴² (2006)	TAC vs. CYA	314	1.5 year	NS	TAC = less rejection at 6 months	
Kobashigawa ¹⁴³ (2006)	TAC/MMF vs. TAC/SRL vs. CYA/MMF	343	1 year	NS	NS; TAC groups = lower any treated rejection	
Baran ¹⁴⁴ (2007)	TAC/MMF vs. TAC	58	1 year	NS	NS	NS
Lehmkuhl ¹⁴⁵ (2008)	EVL/rd-CYA vs. MMFsd-CYA	176	1 year	NS	NS	

 Table 2
 Significant Differences in Primary Endpoints between Study Groups from Major Clinical Trials

CAV, cardiac allograft vasculopathy; CYA, cyclosporine; EVL, everolimus; IVUS, intravascular ultrasound; MMF, mycophenolate mofetil; NS, not stated; rd, repeated dose; sd, single dose; SRL, sirolimus; TAC, tacrolimus.

^aTreated-patient population (see text).

^bRe-analysis of MMF IVUS data.¹⁵¹

Tacrolimus is a CNI with a mode of action and adverse effects similar to those of CYA. Compared to CYA, TAC is associated with a smaller incidence of hypertension, gingival hyperplasia and dyslipidemia but higher incidence of diabetes.^{138, 139, 142, 148}

The major side-effects of MMF are GI intolerance and leucopenia which occasionally necessitate dose reductions.

The PSIs, SRL and EVL, have been associated with hyperlipidemia, thrombocytopenia, peripheral edema, apthous ulcers, and GI problems. Proteinuria and delayed wound healing have occurred in SRL-treated patients.¹⁴⁶ An increased risk of nephrotoxicity exists when either SRL or EVL are used in conjunction with standard doses of CNIs.^{141, 142}

Induction therapy with poly- or monoclonal antibodies is currently used in approximately 40% of HT recipients and it is discussed in a separate section of these guidelines.¹⁴⁷

Review of the Major Randomized Clinical Trials in Heart Transplantation

The major randomized clinical trials of immunosuppression in heart transplantation are listed in Table 2. Intent-to-treat analyses have shown that various

immunosuppressive regimens are not associated with differential effects on survival. This is true also for the 1998 multicenter MMF trial in which MMF did not improve 1-year survival compared to AZA. However, in this study, randomization occurred pre-operatively and 11% of recipients never received study drug. When the analysis was restricted to patients who received at least 1 dose of MMF (treated-patient analysis), 1-year survival was greater in the MMF than in the AZA group (6.2% vs. 11.4%; p = 0.031). Trials of new immunosuppressive drug combinations have yielded conflicting results. In the early European and US TAC- and CYA-based immunosuppression trials, similar rejection rates were found, whereas a separate trial revealed significantly lower 6-month rejection rates in TAC-treated HT recipients compared to CYA-treated ones.^{139, 148} More recently, a 3-Arm Trial¹⁴³ comparing regimens of TAC/MMF, TAC/SRL, and CYA/MMF showed that both TAC-based regimens were associated with significantly lower 6-month rates of anytreated rejection than the CYA/MMF regimen. Furthermore, TAC/MMF-treated patients had lower rates of cellular rejection (ISHLT grade > 3A) and of any-treated rejection than the CYA/MMF-treated subjects. Of interest, the TICTAC (Tacrolimus in Combination, Tacrolimus Alone Compared)

Trial demonstrated that TAC monotherapy was associated with rejection rates comparable to those observed with TAC/MMF.¹⁴⁴ In a recent study, EVL combined with reduced-

dose CYA was associated with 1-year rejection rates similar to those occurring in patients treated with MMF and standard CYA doses.¹⁴⁵

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Author (year)	Study	No.	Renal function	Infections	Cholesterol & triglycerides	Hypertension
Kobashigawa ¹³⁷ (1998)	MMF vs. AZA	650		MMF = more any opportunistic infection		
Reichart ¹⁴⁸ (1998)	TAC vs. CYA	82	NS	NS		CYA = more hypertension
Taylor ¹³⁹ (1999)	TAC vs. CYA	85	NS	NS	CYA = higher chol & tri	CYA = more hypertension
Eisen 2003 ¹⁴⁰	EVL vs. AZA	634	EVL groups = worse renal function	EVL groups = lower viral/CMV but more bacterial infections	EVL groups = higher chol & tri	NS
Keogh ¹⁴¹ (2004)	SRL vs. AZA	136	SRL groups = worse renal function	SRL groups = lower CMV but more pneumonia	VNS for chol; SRL groups = higher trig	NS
Grimm ¹⁴² (2006)	TAC vs. CYA	314	NS	NS	CYA = higher chol & tri	CYA = more hypertension
Kobashigawa ¹⁴³ (2006)	TAC/MMF vs. TAC/SRL vs. CYA/MMF	343	TAC/MMF = best renal function	TAC/SRL = lower viral but more fungal infections	NS for chol; 5 TAC/MMF = lower trig	NS
Baran ¹⁴⁴ (2007)	TAC/MMF vs. TAC	58	NS	TAC/MMF = more hospitalized infections		
Lehmkuhl ¹⁴⁵ (2008)	EVL/rd-CYA vs. MMFsd- CYA	176	NS	EVL = Less CMV infections		

CAV, cardiac allograft vasculopathy; CYA, cyclosporine; EVL, everolimus; IVUS, intravascular ultrasound; MMF, mycophenolate mofetil; NS, not stated; rd, repeated dose; sd, single dose; SRL, sirolimus; TAC, tacrolimus.

Several of the recent randomized immunosuppressive trials showed that MMF, EVL, and SRL reduced the incidence and severity of CAV, as assessed by intravascular ultrasound (IVUS), compared to AZA-based immunosuppression. The EVL study was the most robust in demonstrating first-year benefit in terms of several IVUS variables (intimal area, volume and index along with maximal intimal thickness [MIT] > 0.5 mm). The MMF study showed that compared to AZA-MMF therapy was associated with less CAV if the threshold of normal intimal thickness was set at < 0.3 mm intimal thickening, but difference were no longer significant if the value was increased to 0.5 mm. In the SRL study, IVUSderived intimal thickness was lower at 6 months in SRL- than in AZA-treated patients. A single-center randomized angiographic study suggested that SLR may attenuate progression of established CAV (Table 3A and Table 3B).¹⁴⁶ Compared to AZA, the mTOR inhibitors, EVL and SRL, are associated with greater renal dysfunction, higher lipid levels, poorer wound healing, more anemia, thrombocytopenia, diarrhea, and mouth ulcers when combined with standard

CYA doses. In contrast, EVL combined with reduced-dose CYA was associated with 1-year renal function similar to observed with MMF combined with standard CYA doses.¹⁴⁵ The results of nonrandomized studies suggest that conversion from CNI- to SRL-based immunosuppression results in improved renal function.^{149, 150} A recent multicenter randomized trial in late HT recipients with renal insufficiency has demonstrated that conversion to CNI-free immunosuppression (MMF, SRL) is associated with greater improvement in renal function than CNI-reduced immunosuppression.¹¹⁵ Compared to the AZA-treated patients those given EVL and SRL also had a lower incidence of CMV infections. MMF-treated patients tend to have more opportunistic infections, diarrhea, and esophagitis than AZAtreated patients.¹³⁷ In trials comparing TAC with CYA, CYAtreated subjects had higher cholesterol and triglyceride levels, and more hypertension, cholelithiasis, gingival hyperplasia and hirsutism than TAC-treated patients.^{139, 142, 148} The latter, however, had more diabetes mellitus, tremor, and anemia. From the 3-Arm Trial, the regimen of TAC/MMF was associated with the best renal function and lowest triglyceride levels.¹⁴³ The TAC/SRL group had a higher incidence of poor wound healing and the greatest number of patients requiring insulin.

Selection of immunosuppression after HT appears to be based on experience and interpretation of the randomized

clinical trials. In addition, individualization of immunosuppression is practiced throughout the HT community, according to patient characteristics and perceived risks for complications. The multicenter, randomized immunosuppression trials provide valuable information that can be used by clinicians to individualize immunosuppression and thus optimize outcomes.

Table 3B Significant Differen	ces in Adverse Events	s from the Major Clinical Trials
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Author (year)	Study	Ν	Hematologic	GI Disorders	Other
Kobashigawa ¹³⁷ (1998)	MMF vs. AZA	650	AZA = more leukopenia	MMF = more diarrhea and esophagitis	NS for hyperglycemia treatment
Reichart ¹⁴⁸ (1998)	TAC vs. CYA	82			NS for glucose intolerance
Taylor ¹³⁹ (1999)	TAC vs. CYA	85	NS		
Eisen ¹⁴⁰ (2003)	EVL vs. AZA	634	NS	NS	NS for wound infection
Keoghx ¹⁴¹ (2004)	SRL vs. AZA	136 S	RL groups = more anemia & thrombocytopenia	AZA = more nausea; SRL groups = more diarrhea	AZA = more arrhythmia and atrial fibrillation; SRL groups = more mouth ulcers & abnormal healing
Grimm ¹⁴² (2006)	TAC vs. CYA	314	TAC = more anemia	CYA = more cholelithiasis	TAC = more diabetes mellitus & tremor; CYA = more gum hyperplasia & hirsutism
Kobashigawa ¹⁴³ (2006)	TAC/MMF vs. TAC/SRL vs. CYA/MMF	343	NS		TAC/SRL = more insulin therapy & impaired wound healing; NS for diabetes mellitus
Baran ¹⁴⁴ (2007)	TAC/MMF vs. TAC	58	NS		NS for malignancy
Lehmkuhl ¹⁴⁵ (2008)	EVL/rd-CYA vs. MMFsd- CYA	176	MMF = more leukopenia		

CAV, cardiac allograft vasculopathy; CYA, cyclosporine; EVL, everolimus; GI, gastrointestinal; IVUS, intravascular ultrasound; MMF, mycophenolate mofetil; NS, not stated; rd, repeated dose; sd, single dose; SRL, sirolimus; TAC, tacrolimus.

Great caution should be used in the interpretation of immunosuppression trials. It is unclear if TAC trough levels of 5 to 10 ng/mL are equivalent to CYA trough levels of 100 to 200 ng/mL. Outcomes may be influenced more by the chosen drug combination than by the individual drugs. Adverse effects may be due to drug-drug interactions and not to the specific immunosuppressive drug. For example, EVL and SRL are not nephrotoxic alone but augment the nephrotoxicity of the CNI. Comparison of studies outcomes is hampered by the lack of standardized post-operative care. The "control" drug is frequently AZA, which is currently seldom used for HT immunosuppression. High-risk individuals, including with renal older patients, those insufficiency or allosensitization have generally been excluded. None of the studies summarized above were powered to detect differences in survival. In contrast, there were significant differences between regimens in terms of rejection, CAV, and adverse events. For example, TAC-based regimens may be associated

with lower rejection rates than CYA-based regimens even when the 2 CNIs are given in conjunction with MMF.^{142, 143} Diabetes mellitus appears more prevalent with TAC than with CYA. The third arm of the 3-Arm Trial, demonstrated that TAC/SRL-treated patients had lower rejection rates but greater renal dysfunction and poorer wound healing than those treated with TAC/MMF.¹⁴³ Recently, EVL with reduced-dose CYA was shown to have similar rejection rates and less renal dysfunction than MMF combined with standard CYA doses.¹⁴⁵ The regimen of EVL and reduced CYA dose has not been compared to TAC/MMF in a randomized trial.

The beneficial effects of MMF, EVL, and SRL on CAV assessed by IVUS support the inclusion of these drugs in contemporary immunosuppressive regimens.^{140, 141, 151} However, the renal dysfunction reported in trials of EVL and SRL combined with standard-dose CYA dampens the enthusiasm for the use of this drug regimen. Of note, there are

differences in IVUS study design and results. The multicenter IVUS validation study (using first-year IVUS MIT > 0.5 mm as an endpoint) applies only to the IVUS results of the EVL trial in terms of the association with improved outcomes including 5-year survival, freedom from nonfatal-MACE and CAV. This does not diminish the CAV benefits from the IVUS data in the MMF and SRL trials. Currently, a multicenter trial using first-year IVUS comparing EVL with reduced dose CYA versus MMF with standard dose CYA is ongoing.

The various adverse events observed in the randomized clinical trials further underscore the need for individualization of immunosuppression. For example, patients at high risk for CMV infection may benefit from EVL or SRL-based immunosuppression; patients with gingival hyperplasia may benefit from a TAC-based regimen; patients with tremors, peripheral neuropathy or pre-transplant diabetes mellitus may be better served by CYA-based regimens (Table 3A and Table 3B).

Considerations for Pediatric Recipients

No Phase 3, randomized, controlled trials of any immunosuppressive regimens have been conducted in pediatric thoracic transplant recipients. In single-center trials and registry data, TAC-based immunosuppression is associated with less rejection,^{35, 152} less hyperlipidemia,¹⁵³ and improved cosmetic outcomes.¹⁵² The impact of choice of CNI on incidence of post-transplant diabetes mellitus and lymphoproliferative disorders is unknown in children. Over the last decade, there has been a steady increase in the proportion of children receiving TAC.

Although many children may be successfully managed with long-term CNI monotherapy (generally with TAC¹⁵²), the evidence in adults that use of adjunctive therapies (notably MMF) improves outcome, has led most pediatric centers to routinely use MMF with a CNI. Although use of AZA is declining in children, a significant number of pediatric HT recipients,¹²⁷ particularly infants, are intolerant of MMF. When MMF is discontinued due to adverse events, there are no data on whether this agent should be replaced by AZA or an mTOR inhibitor. If the patient has experienced recurrent rejection, or is considered at high immunologic risk, replacement with another agent seems prudent.

There is very limited experience with use of mTOR inhibitors in pediatric HT recipients.^{131, 154} These drugs have mostly been used when patients are intolerant of MMF, there is evidence of graft CAV, or late CNI minimization (or discontinuation) is sought for complications, notably renal

insufficiency. Only a few pediatric centers are using mTOR inhibitors from the time of HT.

Early CS weaning or complete avoidance is actively sought in pediatric HT recipients. Many centers using polyclonal antibody induction therapy have practiced CS avoidance for more than 2 decades. Maintenance CSs are only commenced for severe or recurrent rejection episodes. Avoidance of CS is aimed at minimizing the long-term complications of CSs including osteoporosis, impaired linear growth, obesity, hyperlipidemia, hypertension, and diabetes mellitus. Both early CS weaning¹⁵⁵ and complete avoidance¹⁵⁶.

Role of Antilymphocyte Induction Therapy

Concept of Induction

The use of intense immunosuppression in the perioperative HT period (induction) is based on the empirical observation that more powerful immunosuppression is required to prevent early acute rejection. Induction therapy mainly consists of early post-transplant use of polyclonal or monoclonal antibodies. Whether prophylactic monoclonal or polyclonal antibody therapy results in lower rejection and mortality rates, or facilitates development of tolerance to the allograft remains unclear. Furthermore, the long-term effects of induction agents are incompletely understood. Recommendations about the use versus avoidance of induction immunosuppression should be interpreted with caution because the data upon which they are based is largely derived from retrospective analyses, given the paucity of controlled clinical trial in this area.

Classification of Induction Antibodies

Currently, about half of the centers worldwide use antibody-based induction therapy.¹⁵⁸ The use of OKT3 has declined from 22% in 1995 to 4% in 2007.^{158, 159} The monoclonal antibody OKT3 has largely been supplanted by anti-IL-2 receptor blockers that in 2007 were used in 27% of HT recipients. Although the use of polyclonal antibodies has remained approximately 22% during the past 12 years, new preparations (thymoglobulin, ATG-F) have replaced the antibodies used in the past (ATGAM, Minnesota-ATG).

Polyclonal Antibodies

Heterologous antibody preparations derived from immunized animals have been used in transplantation since the 1960s, both as induction and rescue therapies. Polyclonal antibodies induce dose-dependent T-cell depletion in blood and peripheral lymphoid tissues, most likely due to complement-dependent cell lysis and activation-associated apoptosis. Given their broad spectrum of activity it is believed that their anti-rejection properties are mediated by mechanisms other than T cell depletion, including costimulation blockade, adhesion molecule modulation, and Bcell depletion.¹⁶⁰⁻¹⁶³ This broad spectrum of activity is also responsible for the antibodies' toxicities including thrombocytopenia and leucopenia.

Induction with ATG has been linked in some studies to higher rates of post-transplant lymphoproliferative disorder (PTLD).¹⁶⁴ In contrast, data from a registry that included 25,000 transplant patients failed to reveal such association.¹⁶⁵ Moreover, ATG may have a protective effect against PTLD if antiviral prophylaxis is used after induction therapy.¹⁶⁴ Three polyclonal preparations are currently used for induction: 2 rabbit-derived antibody preparations, F-ATG (Fresenius-ATG, Fresenius) and R-ATG (Thymoglobuline by Genzyme), and 1 horse derived product (ATGAM, Upjohn).



Figure 2 Various maintenance immunosuppression regimens after heart transplantation at 1- and 5-year follow-up. Reproduced with permission from the Journal of Heart and Lung Transplantation 2008.¹⁵⁸

OKT-3

OKT3 (Muromonab, Orthoclone, Ortho Biotech) is a murine monoclonal antibody that binds to the CD3 molecule causing internalization of the T-cell receptor and simultaneous T cell activation and depletion. OKT-3 was the first monoclonal antibody approved for clinical use in transplantation. Early studies showed a protective effect against early acute rejection¹⁶⁶ but no survival benefit. Its toxicity includes a cytokine-release syndrome that manifests as fevers, rigors, hypotension, and pulmonary edema. In addition, because this is a murine product, anti-mouse antibodies may develop, and, in such cases, repeat

administration months to years later may be associated with anaphylaxis or therapeutic failure. The prolonged use of OKT3, for prophylaxis of acute rejection after heart transplantation is also associated with a higher risk for PTLD.¹⁶⁷ However, newer reports have shown a significant reduction in lymphoma incidence.¹⁶⁵

IL-2 Receptor Antagonists

Two CD25 (IL2R) – specific monoclonal antibodies, daclizumab and basiliximab, are currently used as induction agents. These antibodies have been designed to reduce the limitations of nonhuman antibodies. The inclusion of human

proteins prevents the destruction of the therapeutic antibodies by the recipients' anti-mouse antibodies (daclizumab: 10% murine, 90% human protein; basiliximab: 30% murine, 70% human protein) and the development of serum sickness associated with mouse, rabbit or horse, derived proteins. Furthermore, the activity of monoclonal antibodies is more consistent than that of polyclonal antibodies, whose potency varies from batch to batch. Importantly, because they target a receptor unique to activated T cells, they are less likely to decrease overall immunocompetence. Their mechanism of action is thought to be primarily related to their steric inhibition of the binding of IL-2 to its CD25 receptor.

Alemtuzumab

Alemtuzumab is a humanized anti-CD52 monoclonal antibody that rapidly depletes CD52 expressing lymphocytes in central and peripheral lymphoid tissues. It might combine the potent depleting capabilities of polyclonal antibodies with the benefits of humanized monoclonal antibodies including ease of administration, consistent activity and safety. However there exist no published trial data on the use of alemtuzumab in HT recipients.¹⁶⁸⁻¹⁷⁰ Alemtuzumab has been associated with prolonged leukopenia in renal and lung transplant recipients.

Clinical Trials and Studies with Antibody Induction Therapy

A total of 23 prospective trials and retrospective studies, published over the last 20 years, have been examined as the source of recommendations for these guidelines.

In 10 trials, an induction antibody was compared to no antibody induction. In 13 trials, different antibody-induction protocols were compared with each other. Fifteen studies used IL-2R blockers, 14 involved polyclonal antibodies, and OKT3 was used in 9 trials.

Polyclonal Antibodies

One retrospective analysis showed that, compared to recipients not given induction therapy, RATG-treated patients had less rejection episodes and a trend towards less graft vasculopathy.¹⁷¹ Two studies comparing different polyclonal antibodies have shown different results. One study showed less rejection in 342 patients treated with thymoglobulin compared to 142 patients treated with Fresenius-ATG.¹⁷² However, no difference between these 2 antibodies was seen in a 50-patient prospective randomized trial.¹⁷³

OKT3 has not been tested against the new polyclonal formulations (thymoglobulin, Fresenius-ATG).^{174, 175}

In recent years, investigators have evaluated a shorter ATG course (5 vs. 7 days) or adjustment of ATG dose to

achieve a lymphocyte count below < $100/\mu$ L.^{176, 177} Shorter duration of ATG therapy was associated with higher rejection rates. In contrast, adjustment of ATG doses according to T-cell counts was associated with lower rejection rates as well as lower or fewer ATG doses.

Delay of CNI therapy under the protection of polyclonal antibodies was examined in 2 studies. Both showed improvement of renal function with delay of CNI initiation between 5 and 12 days. Acute rejection incidence was not increased.^{178, 179}

ОКТЗ

As noted above, the use of OKT3 as an induction therapy has decreased during the last decade and its availability in the future is uncertain. Comparison of OKT3 versus no induction was described mostly in the 1990s and showed no influence on rejection or survival. A 9-year experience with 85 patients given OKT3, and 29 who did not receive induction therapy, found no differences between groups.¹⁸⁰ A review of the literature up until 1992 by Carrier et al concluded that the use of OKT3 was not associated with any mortality benefit in heart transplantation.¹⁸¹

Interleukin-2 Receptor Antagonists

Trials comparing IL-2 receptor antagonists with no induction have yielded contradictory results. Beniaminovitz reported the results of daclizumab induction in a 55-patient prospective, randomized, open-label pilot trial. Although rejection was decreased during the first 3 months after transplantation, there was no difference in rejection and survival at 1 year.¹⁸² This small trial was followed by a 434-patient prospective, randomized, double-blinded, multicenter trial showing significantly less acute rejection episodes at 12 months post-transplantation (35.6% vs. 47.7%) with daclizumab.¹⁸³ The use of cytolytic antibody to treat rejection was associated with a higher risk of death from infection in the daclizumab group.

A multicenter, prospective, double-blind, randomized trial of basiliximab induction versus placebo in 56 patients failed to show significant differences between treatment groups in terms of adverse events.¹⁸⁴ A retrospective comparison of 25 patients with renal insufficiency treated with basiliximab and a CNI delay of 4 days and 33 patients without induction demonstrated similar survival and rejection rates.¹⁸⁵

Two retrospective studies compared the use of an IL-2 receptor antagonist with OKT3 and reported conflicting results. One study showed less allograft rejection in the IL-2 receptor blocker group, while in the other there were no differences in rejection between groups.^{186, 187} In 2 prospective

trials with daclizumab and basiliximab, survival and rejection incidence were similar in IL-2 receptor blocker- and OKT3-treated groups. However, safety was significantly better with IL2-receptor antagonists.^{188, 189}

A total of 5 trials compared thymoglobuline with basiliximab. All studies showed less rejection episodes in thymoglobuline groups.^{178, 190-193} However infection rates (bacterial, CMV-infection) were lower in the basiliximab groups. In all studies, survival was similar between groups. A prospective comparison of thymoglobulin with daclizumab failed to detect differences in survival, rejection or infection rates.¹⁹⁴

Summary

After more than 40 years of clinical HT, the use of induction therapy is still controversial. Although there is a fair amount of data showing acceptable efficacy and tolerability, there is poor evidence that it is superior to not using induction therapy. General guidelines are difficult to formulate unless or until more trials have been performed. Some patient populations seem to have an early benefit from induction therapy, but it is still speculative whether this will result in better survival in the long term.

Special Situations

Calcineurin Inhibitor Delay Due to Renal Insufficiency

Patients with severe peri-operative renal dysfunction may benefit from induction therapy because it allows for delay of CNI initiation by 4 to 12 days. Use of ATG seems to be associated with lower rejection rates than basiliximab.¹⁷⁸

Calcineurin Inhibitor-Free Immunosuppression

In a pilot trial, 8 de novo HT recipients treated with SRL, MMF, and CS received r-ATG antibody induction for 4 days in the immediate post-operative period.¹⁹⁵ Over a 3- to 12-month follow-up period, patient survival and freedom from rejection were, respectively, 100%, and 75%. Mean creatinine levels initially decreased and stabilized thereafter. Adverse events included pericardial and pleural effusions (38%), peripheral edema (50%), and poor wound healing (50%).

Of 20 patients with severe pre-transplant renal insufficiency and treated with SRL/EVL, MMF, and CS, 45% received induction therapy with either daclizumab or basiliximab. Compared to untreated patients, those given induction therapy had significantly lower acute rejection rates (33% vs 73%).¹⁹⁶

Both studies suggest that CNI-free protocols are associated with higher rejection rates and that use of antibody induction therapy could potentially reduce rejection rates in these protocols. However, at this time, CNI-free protocols cannot be considered a standard of care in de novo HT.

Patients with a High Risk for Acute Rejection

Allosensitized HT recipients have longer waiting times and lower survival to transplantation, and a higher rejection rates.¹⁹⁷ Despite desensitization therapies patients with panel reactive antibody (PRA) levels \geq 11% have earlier and more severe rejection with significantly lower postoperative survival, even with a negative donor-specific crossmatch.^{198,} ¹⁹⁹ A 2008 consensus conference recommended the use of induction therapy with thymoglobulin in HT recipients with preformed anti-donor antibodies who had required preoperative desensitization therapy.²⁰⁰

The Cardiac Transplant Research Database (CTRD) investigated the impact of induction therapy on the outcomes of 6,553 recipients undergoing HT between 1990 and 2001.²⁰¹ Patients that survived beyond 48 hours were stratified based on no induction therapy (63%) or induction with OKT3 or with anti-thymocyte preparations (37%). The analysis identified 4 characteristics of patients at high risk for fatal rejection: young age, black race, ventricular assist device for more than 6 months (a surrogate for allosensitization that offten complicates long durations of support), and 4-to-6 HLA antigen mismatches.²⁰¹ Patients with a combination of these risk factors benefit from cytolytic induction therapy.

Patients with Primary Graft Dysfunction (especially if immunological causes are suspected)

Another indication to use cytolytic induction therapy may be for patients who have acute graft failure where there is a question of an immune mechanism, such as hyperacute rejection or AMR. Moreover, graft dysfunction is often associated with acute renal failure and in those patients delay of CNI might be beneficial.

Consideration for Pediatric Recipients

As with adult transplantation, the role of induction therapy in pediatric HT recipients remains controversial. According to the latest ISHLT pediatric report,³⁵ induction therapy was utilized in 37% of patients in 2001 and in 60% in 2008. Although the greatest increase has been in the use of polyclonal antibody preparations, partially due the greater availability of the rabbit anti-thymocyte globulin product Thymoglobulin®, treatment with of IL2 receptor antagonists has also risen.

Small, nonrandomized reports have described single center experiences with polyclonal antibodies, monoclonal T cell depleting antibodies and IL2 receptor antagonists.^{188, 202-205} There has been almost no experience with use of alemtuzumab

in pediatric recipients.¹⁷⁰ The combined data suggests that these agents may be useful when CNI initiation must be delayed, as in the presence of perioperative renal failure. Data from the Pediatric HT Study group shows that rabbit anti-thymocyte serum (ATS) induction was associated with a lower rate of all-cause and rejection-related mortality when compared to no induction or use of OKT3 without an increase in infectious mortality, or rates of malignancy.²⁰⁶ Because ATS and OKT3 are no longer routinely used, conclusions relevant to contemporary practice cannot be drawn.

There is one major divergence in practice between adult and pediatric centers that is worthy of consideration. CSs have serious adverse effects in children, including the potential to stunt growth. Therefore, pediatric transplant physicians have strived to minimize or completely avoid CS use. Centers prescribing CS-free immunosuppression have typically utilized antibody-induction therapy. In general, polyclonal antibody induction therapy has been used since it is unknown whether IL-2R antagonists will have similar benefit if CS maintenance is avoided.¹⁵⁶

Role of Statins as Adjunctive Immunosuppressive Agents and Cardiac Allograft Vasculopathy Prophylaxis

Three-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (HMG-CoA reductase inhibitors or statins) function as both lipid-lowering and immunomodulating agents.²⁰⁷ The immunomodulatory mechanisms of statins, which are partially independent of cholesterol-lowering properties may favorably impact survival by reducing episodes of acute rejection²⁰⁷ and the progression of CAV.²⁰⁸

Mechanisms

Increased lipid levels may contribute to atherosclerosis progression, partially mediated by an immune process. Oxidized low-density lipoproteins (LDL) lead to activation of macrophages and endothelial cells, which, in turn, mediate the oxidation of LDL and upregulation of cytokines and growth factors.²⁰⁹ Statins appear to block activation of natural killer cells by repressing interferon-gamma induced MHC-II expression.²¹⁰ This effect is dose-dependent and specific for inducible, but not for constitutive forms of MHC-II (e.g., dendritic cells or B cell lymphocytes).²¹¹ Studies with mRNA revealed that the specific mechanism of inhibition of MHC-II induction by statins is due to the selective repression of promoter IV on the MHC-II transactivator (CIITA) gene. The observation that this effect was abolished in the presence of Lmevalonate suggests that the utilization of the statin was the responsible mechanism. Statin therapy is also associated with

blockade of β -2 intergrin and leukocyte function antigen-1 (LFA-1). Inhibition of LFA-1-mediated adhesion to the intercellular adhesion molcecule-1 (ICAM-1) and costimulation of lymphocytes is, in part, mediated by statins.²¹² Statins also reduce mevalonate in the cholesterol biosynthetic pathway, which results in the reduction of isoprenylation of signaling molecules like ras, rho and other G-protein signaling molecules which have a pivotal role in T-cell activation and effector function²¹³ while other G-protein molecules may influence NO-mediated vasodilatation, blood pressure, and cell survival. Other mechanisms responsible for statins' benefit include attenuation of antibody-mediated responses (decreased IgG alloantibody levels), intimal proliferation, and favorable alteration of coagulation by influencing platelet function and fibronectin.^{214, 215}

Trials/Outcomes

Multiple clinical trials in non transplant patients have shown a mortality benefit from statin use.^{216, 217} Additionally, in HT recipients elevated LDL cholesterol levels are correlated with the development of CAV at 1 and 3 years.^{218, 219}

In HT recipients early initiation of statins results in significantly lower rates of first-year rejection complicated by hemodynamic compromise and of IVUS-detected CAV.^{207, 220} The ability of statins to decrease NK cell activity has been substantiated in a 10-year follow-up study in which a sustained survival benefit and lower CAV rates were demonstrated.^{208, 221-223} The combination of CNI and statins may increase the serum levels of the statins and thus potentiate their immuno-modulatory effects. Notably, patients with delayed initiation of statins had less benefit than patients given statins from the early postoperative period.²²⁴ Statins are currently a key component of the therapy of HT recipients irrespective of cholesterol levels.

Doses and Complications/Side effects/Drugs Interactions

The most serious adverse effect of statins in HT recipients is myositis complicate by rhabdomyolysis and consequent renal failure. The risk of the adverse effect is higher for the more lipophilic than hydrophilic statins due to greater muscle penetration. The risk of rhabdomyolysis is greater in HT recipients because concomitant use of CNI raises the blood level of statins. In addition, it is critical to avoid other drugs that may further increase blood levels of statins, including fibrates, azole antifungal agents, macrolide antibiotics, and nondihydropyridine calcium channel blockers (diltiazem and verapamil) (Table 4).^{207, 208, 225-227}

Considerations for Pediatric Recipients

Although CAV is less frequent in pediatric than in adult recipients it still is the major cause of late graft failure and death.²²⁸⁻²³⁰ Studies in pediatric recipients suggest that post-transplant hyperlipidemia is common in children of all ages and is influenced by the immunosuppressive regimen used.^{153, 231-234} The evidence for routine use of statins in children, especially the very young, is weaker than in adults.

Safety and Efficacy

In children with familial hypercholesterolemia, statins effectively lower LDL cholesterol with minimal short-term side effects and decrease the rate of carotid intimal thickening.^{235, 292} Concerns regarding the long-term use of statins are raised by their unknown effects on cognitive development, endocrine maturation, skeletal growth, bone mineral accretion, and long-term renal and hepatic effects.²³⁷ The US Food and Drug Administration has approved pravastatin for the treatment of dyslipidemia in children ages \geq 8 years, regardless of pubertal status.²³⁸

Single center studies of statin use in pediatric transplant recipients have also shown that in the short term statins are safe and effective in improving the lipid profile.^{231, 239-242} The number of infants and very young children in these studies has been small. Although relatively infrequent, the most common side effects are myositis, elevation of liver enzymes, and potentiation of CNI toxicity. In one study concomitant use of CNI was associated with a 10-fold increase in pravastatin levels, which may increase the risk of statin-related side effects.²⁴⁰

In non-randomized studies, the use of pravastatin or atorvastatin has been associated with significant reductions in CAV rates.^{241, 242} Data on whether statins reduce pediatric rejection rates are contradictory.^{239, 242} Routine use of statins in all pediatric HT recipients, especially in infants and young children, remains controversial. Less controversy exists on statin use in the adolescents with hyperlipidemia, or in those at high risk for CAV.

Pediatric Dosing

Pravastatin dosing by age and weight has been 5 mg to 20 mg (0.2-0.3 mg/kg/day) in young pediatric recipients and 20 mg to 40 mg in older pediatric recipients.^{231, 232, 240, 241} Atorvastatin dosing has ranged from 2.5 mg to 10 mg based on age and weight (~0.2 mg/kg/day).^{239, 242} Very limited data is available on the pharmacokinetic profiles of statins when combined with CNI in pediatric transplant recipients.

Table 4	Recommendation for Statin Doses in Heart Transplant
Patients ²	25-227
1 40101105	

Drug	Dose	Risks
Pravastatin	20-40 mg	Myositis (lower)
Simvastatin	5-20 mg >20 mg not recommended	Myositis (higher)
Atorvastatin	10-20 mg	Myositis (higher)
Fluvastatin	40-80 mg	Myositis (lower)
Lovastatin	20 mg	Myositis (higher)
Rosuvastatin	5-20 mg	Myositis

Recommendations on the Principles of Immunosuppressive Regimens in Heart Transplant Recipients:

(See Table 2, Table 3A, Table 3B, and Table 4)

Class I:

1. Maintenance therapy should include a CNI in all pediatric HT recipients.

Level of Evidence: C.

2. In adults, the use of statins beginning 1 to 2 weeks after HT is recommended regardless of cholesterol levels. Due to pharmacologic interactions with CNI and risk for toxicity, initial statin doses should be lower than those recommended for hyperlipidemia.

Level of Evidence: A.

3. Creatinine kinase levels should be monitored in all children receiving statins.

Level of Evidence: C.

Class IIa:

1. Calcineurin inhibitor-based therapy remains the standard in immunosuppressive protocols used after HT.

Level of Evidence: B.

2. MMF, EVL, or SRL as tolerated, should be included in contemporary immunosuppressive regimens because therapies including these drugs have been shown to reduce onset and progression of CAV as assessed by IVUS.

Level of Evidence: B.

3. Immunosuppressive induction with polyclonal antibody preparations may be beneficial in patients at high risk of renal dysfunction when used with the intent to delay or avoid the use of a CNI.

Level of Evidence: B.

4. In pediatric HT recipients routine use of induction therapy with a polyclonal preparation is indicated when complete CS avoidance is planned after HT.

Level of Evidence: C.

5. Routine use of statins is recommended for all pediatric patients with evidence of hyperlipidemia, CAV or following retransplantation.

Level of Evidence: C.

6. TAC is the preferred CNI for pediatric HT recipients considered at high immunologic risk (e.g., sensitized recipients with evidence of donor-specific antibody [DSA]).

Level of Evidence: C.

7. CS avoidance, early CS weaning or very low dose maintenance CS therapy are all acceptable therapeutic approaches.

Level of Evidence: B.

8. If used, CS weaning should be attempted if there are significant CS side effects and no recent rejection episodes (e.g., within 6 months).

Level of Evidence: C.

9. Pediatric recipients with pre-formed alloantibodies and a positive donor-specific cross-match should receive induction therapy, and TAC-based "triple therapy" with CSs and either MMF or an mTOR inhibitor.

Level of Evidence: C.

Class IIb:

1. The results of clinical trials suggest that TAC-based regimens may be associated with lower rejection rates but not with superior survival after HT than CYA-based regimens.

Level of Evidence: B.

2. The adverse events of immunosuppressive drugs observed in randomized clinical trials underscore the need for individualization of immunosuppression according to the characteristics and risks of the individual HT recipient.

Level of Evidence: C.

3. Most children should receive adjunctive therapy with an anti-metabolite or a PSI.

Level of Evidence: C.

4. If a child is intolerant of adjunctive therapy, the decision whether or not to replace it with another agent should be made following review of the patient's rejection history and immunologic risk. TAC monotherapy is acceptable in patients with a benign rejection history.

Level of Evidence: C.

5. For children diagnosed with CAV, the addition of an mTOR inhibitor should be strongly considered.

Level of Evidence: C.

6. Routine use of immunosuppressive induction in all patients has not been shown to be superior to immunosuppressive regimens that do not employ such therapy.

Level of Evidence: B.

7. Immunosuppressive induction with anti-thymocyte globulin (ATG) may be beneficial in patients at high risk for acute rejection.

Level of Evidence: C.

8. Routine use of statins is recommended for adolescents and selected younger children with at an increased risk of rejection or CAV.

Level of Evidence: C.

Topic 4: Management of Acute Cellular Rejection

Acute Cellular Rejection

Approximately 20% to 40% of patients experience moderate or severe acute cellular rejection in the first year after transplantation.¹⁵⁸ The number and severity of acute rejection episodes during this time period has been correlated with development of CAV and mortality. Acute cellular rejection may be diagnosed in a patient presenting with symptoms and signs of graft dysfunction or the diagnosis may be made on routine surveillance EMB in an asymptomatic patient.

Symptomatic Acute Cellular Rejection

Symptoms accompanying acute cellular rejection are caused by graft dysfunction. Acute rejection complicated by hemodynamic compromise (hypotension, low cardiac output, marked elevation of pulmonary capillary wedge pressure) is associated with significantly increased short- and long-term morbidity and mortality and often irreversible myocardial damage.²⁴³ Prompt institution of therapy in symptomatic acute cellular rejection is necessary to reverse allograft dysfunction. If suspicion for rejection is high, EMB should be urgently performed and therapy initiated immediately. The patient with

symptomatic acute cellular rejection should be admitted to the hospital and treated in an intensive care unit (ICU) if hemodynamic compromise is present. Table 5 summarizes commonly used therapies for ACR.

High-dose IV CSs should be first-line therapy for symptomatic acute cellular rejection of ISHLT grades 1R, 2R and 3R. A commonly used dose is methylprednisolone, 1000 mg daily given for 3 consecutive days.²⁴⁴ This may or may not be followed by gradual weaning of the CS dose. There is no consensus on the need or schedule for CS weaning and clinical practices range from immediate return to pre-rejection CS doses to progressive reduction of CS doses over several days or weeks.²⁴⁵

In a patient with hemodynamic compromise, IV inotropic agents and vasopressors may be required to maintain adequate cardiac output and systemic blood pressure.

Cytolytic immunosuppressive therapy should be considered in addition to CS in acute cellular rejection complicated by hemodynamic compromise. If clinical improvement does not occur within 12 to 24 hours, polyclonal anti-thymocyte antibodies or, less commonly the monoclonal antibody OKT3, have been used.²⁴⁶ These agents are administered daily for 3 to 10 days. Premedication with CS, antihistamines and antipyretics is recommended. The interleukin-2 receptor antagonists basiliximab and daclizumab should not be used in the setting of acute cellular rejection.

Alemtuzumab (campath-1H) has been used for treatment of acute cellular rejection in kidney transplantation, however, the experience with its use for treatment of acute cellular rejection in HT remains limited and therefore it is not routinely used in this setting.²⁴⁷

During and following high-dose CS and cytolytic therapy, antibacterial prophylaxis against opportunistic infections should be administered.

In addition it is important to determine the likely cause of the rejection episode and adjust maintenance immunosuppression accordingly. If acute rejection is a result of noncompliance, re-establishing the prior maintenance immunosuppressive regimen and ensuring compliance may be sufficient. If noncompliance is excluded, the following changes to baseline immunosuppression should be considered:

(1) Increase of the dose of current immunosuppressive medications. Examples include slowing the rate of CS weaning in the first post-transplant weeks/months, aiming for a higher target serum level of CNI, and/or increasing the dose of MMF.^{129, 248, 249}

- (2) Addition of an agent. For example, CS can be restarted in a patent who had been weaned off but later developed rejection. Introduction of MMF or a PSI (EVL, SRL) may be considered in patients on double therapy with a CNI and CS.
- (3) Conversion to a different maintenance regimen. Conversion from CYA to TAC²⁵⁰⁻²⁵² and AZA to MMF²⁵³ has been shown to decrease the risk of recurrent rejection.²⁵³ Conversion from AZA or MMF to a PSI is another possible approach, although conclusive evidence of the effectiveness of this approach is lacking.

Institution of treatment for acute cellular rejection usually results in progressive resolution of symptoms and in partial or complete recovery of heart allograft function. An EMB should be performed 1 to 2 weeks after initiation of therapy to assess resolution of histological changes of acute cellular rejection. Serial echocardiographic evaluation of myocardial function can help to assess the response to therapy and guide decisions on the timing of follow-up EMB and CS weaning (Table 5).

Recommendations for Treatment of Symptomatic Acute Cellular Rejection:

Class I:

1. An EMB should be performed as early as possible if there is suspicion of symptomatic acute heart allograft rejection.

Level of Evidence: C.

2. The HT recipient with symptomatic acute cellular rejection should be hospitalized. Patients with hemodynamic compromise should be treated in the ICU.

Level of Evidence: C.

3. High-dose IV CS should be first-line therapy for symptomatic acute cellular rejection irrespective of ISHLT EMB grade (1R, 2R or 3R).

Level of Evidence: C.

4. Cytolytic immunosuppressive therapy with antithymocyte antibodies should be administered in addition to IV CS if hemodynamic compromise is present, and especially if there is no clinical improvement within 12 to 24 hours of IV CS administration.

Level of Evidence: C.

5. IV inotropes and vasopressors should be used as necessary to maintain adequate CO and systemic blood pressure until recovery of heart allograft function occurs.

Level of Evidence: C.

6. Antimicrobial prophylaxis against opportunistic infections should be administered when high-dose CS and/or cytolytic therapy are used for the treatment of rejection.

Level of Evidence: C.

7. Appropriate adjustments of maintenance immunosuppressive therapy should be made to decrease the risk of recurrent rejection. These can include ascertainment of compliance with current therapy, increase in the dose of current immunosuppressive agent(s), addition of new agent(s) or conversion to different agent(s).

Level of Evidence: C.

8. Follow-up EMB should be done 1 to 2 weeks after initiation of therapy for acute cellular rejection.

Level of Evidence: C.

9. Serial echocardiograms should be used to monitor changes in heart allograft function in response to anti-rejection therapy.

Level of Evidence: C.

10. In a patient with low-grade acute cellular rejection and hemodynamic compromise, the possibility of AMR should also be entertained (see AMR section).

Level of Evidence: C.

11. IL-2 receptor blockers should not be used to reverse acute cellular rejection.

Level of Evidence: C.

Asymptomatic Acute Cellular Rejection

The majority of acute cellular rejection episodes are diagnosed by surveillance EMB in asymptomatic patients.²⁴³ The rationale for treatment of asymptomatic rejection is to prevent its further progression and associated graft dysfunction. The likelihood of progression to symptomatic rejection depends on patient characteristics, such as the time from transplant, rejection history, etc. An isolated rejection episode can be self-limited and resolve without treatment.^{39, 42} The aggressiveness of therapy will depend on its expected benefits, and the risks of withholding treatment. Therapy for severe (ISHLT 3R) acute cellular rejection without hemodynamic compromise should consist of high-dose IV CS (methylprednisolone, 1000 mg daily given for 3 consecutive days). This may or may not be followed by a gradual weaning of the CS dose. Addition of cytolytic immunosuppressive agents is usually unnecessary and is reserved for patients who do not demonstrate histological resolution of rejection with CS, or who have evidence graft dysfunction despite the absence of symptoms.

Therapy for moderate (ISHLT 2R) acute cellular rejection should consist of either high-dose IV CS (methylprednisolone, 250 - 1000 mg/day for 3 days) or a lower-dose oral CS pulse (1-3 mg/kg of prednisone daily for 3-5 days, with or without CS taper). Therapy for mild acute cellular rejection (ISHLT 1R) detected on surveillance EMB in an asymptomatic patient should be guided by the patient's risk. Most episodes of asymptomatic mild rejection are self-limited especially when they occur later than one year after transplantation. The occurrence of 1R rejection associated with a more diffuse infiltrate should prompt reassessment of maintenance immunosuppression and target drug levels. A follow-up EMB should be done 2 to 4 weeks after the EMB diagnosis of rejection deemed to be more than mild.

Recommendations for the Treatment of Asymptomatic Acute Cellular Rejection:

Class I:

1. Severe acute cellular rejection (ISHLT 3R) diagnosed by surveillance EMB should be treated even in the absence of symptoms or evidence of heart allograft dysfunction.

Level of Evidence: C.

2. High dose IV CS should be given for asymptomatic severe (ISHLT 3R) acute cellular rejection.

Level of Evidence: C.

3. Asymptomatic moderate acute cellular rejection (ISHLT 2R) can be treated with either IV or oral CS.

Level of Evidence: C.

4. Adjustment of maintenance immunosuppressive therapy should be done in patients with asymptomatic moderate (ISHLT 2R) or severe (ISHLT 3R) acute cellular rejection. This can include an increase of the dose of current medications, addition of an agent or conversion to a different maintenance regimen.

Level of Evidence: C.

5. Antimicrobial prophylaxis against opportunistic infections should be administered when high-dose CSs and/or cytolytic therapy are used for treatment of rejection.

Level of Evidence: C.

Class IIa:

1. The performance of a follow-up EMB should be considered 2 to 4 weeks after initiation of therapy for asymptomatic moderate or severe acute cellular rejection.

Level of Evidence: C.

2. Cytolytic immunosuppressive therapy can be considered if there is no histological resolution of rejection on the follow-up EMB.

Level of Evidence: C.

3. Asymptomatic mild cellular rejection (ISHLT 1R) does not require treatment in the vast majority of cases.

Level of Evidence: C.

Class IIb:

1. Asymptomatic moderate cellular rejection (ISHLT 2R), especially if occurring later than 12 months after HT, may not require treatment. Close surveillance (clinical, echocardiographic, and follow-up EMB) is strongly suggested if no treatment is administered in this setting.

Level of Evidence: C.

Recurrent/Resistant Cellular Rejection

In a small number of patients, rejection will persist after the above described treatment (resistant rejection), or will recur soon after therapy is completed (recurrent rejection). In addition to the strategies described above, further immunomodulatory approaches can be considered:

- Administration of an additional course of cytolytic therapy. Polyclonal anti-thymocyte antibody is preferred to OKT3 because development of anti-OKT3 antibodies and increased incidence of AMR have been attributed to protracted OKT3 use.²⁵⁴
- Photopheresis can be considered for treatment of recurrent or resistant rejection because it has been shown to resolve severe rejection and decrease the risk of recurrent hemodynamically compromising rejection.^{255, 256}

Photopheresis treatments are usually administered twice weekly and repeated every 1 to 3 weeks.

- Total lymphoid irradiation^{257, 293} use in adult patients has been limited in recent years as pharmacotherapeutic approaches have similar efficacy and are safer.
- Pulse therapy with methotrexate (2.5 to 20 mg once weekly) has also been shown to lead to resolution of rejection resistant to standard therapy.²⁵⁸⁻²⁶⁰

Recommendations for Treatment of Recurrent or Resistant Acute Cellular Rejection:

Class I:

1. For recurrent or CS-resistant acute cellular rejection, cytolytic immunosuppressive therapy with anti-thymocyte antibodies should be considered.

Level of Evidence: C.

2. Maintenance immunosuppression should be re-evaluated in patients with recurrent/resistant HT rejection (see above).

Level of Evidence: C.

3. Frequent surveillance of heart allograft function (e.g., by echocardiography) is recommended in patients with recurrent/resistant rejection, even if persistently asymptomatic.

Level of Evidence: C.

Class IIb:

1. Additional approaches that can be considered for recurrent or resistant acute cellular rejection include methothrexate pulse therapy, photopheresis and total lymphoid irradiation.

Level of Evidence: B.

2. Evaluation of EMB specimens for concomitant AMR (see the Recommendations for Treatment of Antibody Mediated Rejection) and determination of the presence of anti-HLA antibodies in the HT recipient's serum is also suggested.

Level of Evidence: C.

Topic 5: Treatment of Antibody Mediated Rejection

The most dramatic and now infrequent clinical presentation of AMR is hyperacute rejection-acute graft injury occurring within minutes or hours after HT triggered by preformed antibodies against ABO- or HLA antigens. More frequently, AMR presents in the first weeks and months after transplantation and is associated with symptoms and signs of graft injury. The significance of histological changes suggestive of AMR without graft dysfunction is uncertain.

Hyperacute Form of Antibody Mediated Rejection

The term hyperacute rejection is used when immunemediated acute graft dysfunction manifests within minutes or hours after HT. The severe graft injury results from high titers of antibodies directed against donor antigens which are present in the recipient's serum at the time of transplantation. Hyperacute rejection is rare, as sera of transplant candidates are routinely screened for the presence of anti-HLA antibodies.

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Medication	Dose	Duration
Corticosteroids		
Methylprednisolone (high-dose)	250-1000 mg/day IV	3 days ^a
Prednisone	1–3 mg/kg/day PO	3–5 days ^a
Polyclonal anti-thymocyte antibody		
Thymoglobulin ^b	0.75–1.5 mg/kg/day	5–14 days
ATGAM ^b	10 mg/kg/day	5–14 days
ATG-Fresenius ^b	3 mg/kg/day	5–14 days
Monoclonal antibody	5 mg/day	5–14 days
Muromonoab-CD3 (OKT3) ^b		

ATG, anti-thymocyte gamma-globulin-fresenius; ATGAM, anti-thymocyte gamma-globulin; IV, intravenous; PO, oral (per os).

^aCorticosteroid taper can be considered.

^bPremedicate with CS, anti-histamine, and anti-pyretic.

Treatment, which must be initiated immediately, may include IV inotropes and vasopressors, high-dose IV CS, plasmapheresis, and cytolytic agents. A CNI (CYA or tacrolimus) and metabolic cycle inhibitors (MMF or cyclophosphamide) should also be initiated promptly. Temporary biventricular may be necessary as the full effect of the immunosuppressive therapies may not occur for hours or days. If these measures do not sufficiently improve graft function, consideration should be given to urgent retransplantation, with the caveat that when this procedure is performed < 1 year after the first HT it has been consistently associated with a high mortality risk.²⁶¹⁻²⁶⁴

Recommendations for the Treatment of Hyperacute Rejection:

Class I:

 Treatment for hyperacute rejection should be initiated as soon as the diagnosis is made, preferably when the HT recipient is still in the operating room. Treatments that should be considered include: (1) high-dose IV CS; (2) plasmapheresis; (3) IV Ig; (4) cytolytic immunosuppressive therapy; (5) IV CNI (CYA, TAC) and metabolic cycle inhibitors (MMF); (6) IV inotropes and vasopressors; (7) mechanical circulatory support.

Level of Evidence: C.

1. Intraoperative myocardial EMB should be obtained to confirm the diagnosis of hyperacute heart allograft rejection.

Level of Evidence: C.

Class IIb:

1. Urgent retransplantation may be considered if the above measures do not result in restoration of acceptable heart

allograft function, but repeat HT in the setting of hyperacute rejection is associated with high mortality.

Level of Evidence: C.

Acute Antibody Mediated Rejection

The management of AMR starts with its prevention. Exposure of prospective HT recipients to alloantigens should be minimized; nonessential blood product transfusions should be avoided and when transfusion is needed, leukocyte-depleted and CMV-negative products should be used.²⁶⁵ In allosensitized transplant candidates prospective serological crossmatch or virtual crossmatch should be done to ascertain donor immunocompatibility.

Once AMR develops, the therapy is directed at removal of circulating alloantibodies and reduction of the synthesis of additional alloantibodies. The selection of individual therapies and their duration should guided by the symptoms severity. Patients presenting with hemodynamic compromise are at the highest risk of both short- and long-term morbidity and mortality and should be aggressively treated.

Initial therapy, especially when hemodynamic alterations are present should include high-dose IV CS (methylprednisolone, 1000 mg daily given for 3 consecutive days) and cytolytic therapy. Polyclonal antilymphocytic antibodies are preferred to OKT3, as the latter has been associated with development of antibodies against OKT3 and subsequent increased risk of AMR.^{266, 267}

Plasmapheresis, immune apheresis (immunoadsorption) and IV immunoglobulin decrease the impact of circulating antibodies.^{14, 268-270}

Plasmapheresis removes alloantibodies from the recipient's plasma. There is no consensus on the number or

frequency of plasmapheresis sessions; common protocols range from 1 to 5 times per week for 1 to 4 weeks (Table 6).

Immune apheresis (immunoadsorption) can also be used to remove circulating antibodies. As compared to plasmapheresis, it is less efficient in removing circulating cytokines but is more specific in removal of antibodies, and poses significantly less hemodynamic stress. Immune apheresis is less widely available than plasmapheresis and therefore is less commonly used.^{271, 272}

Administration of IV immunoglobulin at various doses and intervals is used in the treatment of AMR (Table 6). Immunoglobulin therapy is believed to decrease production of antibodies and to modify the immune reactivity of antibodies that are already in circulation. Cyclophosphamide had been used for this purpose, but its role with current immunosuppressive protocols is unclear.

The role of rituximab, an antibody directed against the CD 20 antigen expressed on B-lymphocytes, is being evaluated.^{271,273-275} Table 6 lists rituximab dosing that has been most frequently used in treatment of AMR.

Polyclonal and monoclonal antilymphocytic antibodies, IV immunoglobulin or rituximab should not be given shortly before plasmapheresis or immune apheresis, as they are removed by this process.

When AMR is complicated by hemodynamic compromise, IV inotropic agents and vasopressors and at times mechanical circulatory support (MCS) may be required to maintain adequate organ perfusion until heart allograft function is sufficiently improved.

Systemic anticoagulation can be considered during an episode of AMR. This is aimed to prevent microvascular thrombosis of the allograft coronary vasculature.

While data on the differential effects of various maintenance immunosuppressive regimens on the prevention of recurrence of AMR are scarce, modifications of baseline immunosuppression seem reasonable:

- Increase of the dose of current immunosuppressive medications.
- Addition of an agent. For example, restarting CS, adding an mTOR inhibitor, or adding cyclophosphamide.
- Conversion to a different maintenance regimen. Conversion from CYA to TAC, or from AZA to MMF.^{276,} 277

Splenectomy has been used to treat recurrent AMR in kidney transplant recipients but data regarding its role in HT are lacking.²⁷⁸

Follow-up EMB should be performed 2 to 4 weeks after initiation of therapy for acute AMR. Measurement of serum donor-specific antibodies and changes in their levels in response to therapy should be considered.

 Table 6
 Examples of Therapies for Antibody-Mediated Rejection

Therapeutic modality	Dose	Frequency	Duration
Plasmapheresis	1-2 plasma exchanges	Daily Every other day 3 times per week Once weekly	3-5 days 1-2 weeks 1-4 weeks 2-4 weeks
IV immunoglobulin	100 – 1000 mg/kg	1–3 times per week, often given after each plasmapheresis	1-4 weeks
Rituximab	375 mg/m^2	Once weekly	1-4 weeks

IV Ig, intravenous immunoglobulin.

Based on Grauhan O et al,²⁷⁰ Leech SH et al,²⁷⁹ Michaels PJ et al,¹⁰ Miller LW et al,²⁶⁸ Kaczmarek I et al,²⁷¹ Takemoto SK et al,²⁷⁸ and Bierl C et al.²⁷⁵

Mixed Rejection

The term mixed rejection has been used in circumstances where EMB reveals abnormalities consistent with both cellular rejection and AMR. When hemodynamic compromise is present, aggressive therapy with high-dose IV CS and cytolytic therapy is appropriate. Additional therapies directed at AMR should be considered. In mild forms of mixed rejection without significant symptoms, therapy should in general follow the algorithm for cellular rejection.

Asymptomatic Antibody Mediated Rejection

Histological findings of AMR may be present without graft dysfunction. Some data suggest that AMR, even without heart allograft dysfunction, may lead to increased incidence of CAV and cardiovascular mortality.²⁸⁰⁻²⁸² It is unclear whether or which therapies improve the prognosis of this condition. Currently, when asymptomatic AMR is diagnosed, it is wise to assure that baseline immunosuppression is adequate and the patient is closely monitored.

Considerations for Pediatric Recipients

The principles of acute rejection therapy are comparable to those in adults. In children deterioration is often rapid when any degree of graft dysfunction is present and close monitoring is required. With echocardiographic evidence of severe graft dysfunction, it is prudent to begin inotropes, even if the child does not appear acutely ill. Infants and small children with hemodynamic compromise are often treated empirically without EMB due to the risks of precipitating clinical deterioration with the anesthesia required to perform the procedure. Since the most severe forms of rejection are often reversible, MCS should be instituted if graft failure occurs.

In older children approaching adult size, therapy for moderate or severe acute cellular rejection includes IV methylprednisolone at a daily dose of 1 gram for 3 days. Smaller children are generally treated with IV methylprednisolone 10 to 20 mg/kg daily for 3 days. Therapy is usually given in an inpatient setting, with serial monitoring of blood pressure and glucose. There is less experience with the use of outpatient oral prednisone pulses for the treatment of moderate acute rejection (Grade 2R) in children. This is an option in the absence of an extensive infiltrate, lack of allograft dysfunction or when rejection occurs ≥ 6 months after heart transplantation. Acute rejection associated with graft dysfunction or refractory to IV CS requires the addition of a polyclonal T cell depleting antibody. In the pediatric age group it is not standard practice to observe episodes of Grade 2R rejection without intervention. In general, pediatricians do not treat mild rejection (Grade 1R, old classification grade 1A). However, 1R rejection associated with a more diffuse infiltrate (old classification IB) should lead to reassessment of maintenance immunosuppression and target drug levels, with consideration of intensification of immunosuppression. These findings should also raise suspicion for AMR and the presence of donor-specific alloantibodies.

Many children are highly sensitized due to prior use of homografts for congenital heart disease surgery. Many of these patients are unlikely to receive a donor organ with a negative donor-specific cross-match. Selected patients with very short life expectancy are being transplanted with organs for which the donor-specific cross-match will be positive.^{236,} ²⁸³ These patients require prophylactic intra-operative and early postoperative plasma exchange/plasmapheresis. They should be managed with polyclonal antibody induction therapy, and TAC-based immunosuppression which should include MMF and CS. Duration of plasmapheresis treatment depends upon various factors including pre-transplant antibody concentrations. A process of 'accommodation' to the allograft clearly occurs in most patients and short-term outcomes have been good despite frequent acute rejection episodes.²⁸³ Early graft dysfunction should lead to reintroduction of plasmapheresis if previously discontinued. The role of rituximab and newer monoclonal antibodies directed at plasma cells is not well established. During longerterm follow-up, this population may be at high risk for the development of CAV. A multicenter observational study of children transplanted across a positive cross-match is ongoing.

Recommendations for Treatment of Antibody Mediated Rejection:

Class IIa:

 The following treatments can be used to disrupt the immune-mediated injury of the heart allograft in AMR: (1) high-dose IV CS; (2) cytolytic immunosuppressive therapy.

Level of Evidence: C.

2. The following treatments may be used to remove circulating anti-HLA antibodies or decrease their reactivity: (1) plasmapheresis; (2) immune apheresis (immunoadsorption); 3) IV Ig.

Level of Evidence: C.

3. The following treatments are used to maintain adequate cardiac output and systemic blood pressure: (1) IV inotropes and vasopressors; (2) MCS.

Level of Evidence: C.

4. When AMR is suspected, EMB examination should be expanded to include immunohistochemistry stains for complement split products and possibly antibody.

Level of Evidence: C.

5. Recipient serum should be screened for presence, quantity and specificity of anti-donor (HLA) antibodies.

Level of Evidence: C.

6. Follow-up EMB should be performed 1 to 4 weeks after initiation of therapy and include immunohistochemistry examination.

Level of Evidence: C.

7. Adjustment of maintenance immunosuppressive therapy may be considered. This can include increase in the dose of current immunosuppressive agent(s), addition of new agent(s) or conversion to different agent(s).

Level of Evidence: C.

Class IIb:

1. Systemic anticoagulation may decrease intravascular thrombosis in the heart allograft.

Level of Evidence: C.

2. Emergent retransplantation may be considered if the above measures do not restore acceptable heart allograft function, but outcomes in this situation are unfavorable.

Level of Evidence: C.

Topic 6: Management of Late Acute Rejection

While the vast majority of heart transplantation centers perform surveillance EMBs during the first post-transplant years, subsequent EMB schedules are highly variable. At some centers, all patients undergo routine EMBs every 3 to 6 months, at least up to 2 to 4 years or indefinitely whereas at other centers EMB later than 1 month is performed only when rejection is clinically suspected.²⁸⁴⁻²⁸⁷ A recent multi-institutional study showed that continued EMB surveillance increased the rate of diagnosis of late rejection but 5 year survival was unchanged in the overall population with the exception of the benefit noted in African-American recipients.⁴⁶

Studies on the outcomes of continued surveillance EMB have yielded conflicting results. In some studies the occurrence of late rejection is correlated to increased mortality and CAV progression in pediatric HT recipients.^{20, 288} Other studies have demonstrated that asymptomatic moderate late rejection is often self-limited.²⁸⁹

Various reports over the years identified risk factors for late ACR.^{46, 290, 291} These include younger age, prior history of acute rejection episodes, African-American ethnicity, HLA sensitization, recipient female gender, and rejection events occurring > 6 months after transplantation. Surveillance for late ACR can be tailored to patient risk profile. Subtherapeutic immunosuppressive drug levels and medical noncompliance also increase the risk for late rejection. Because medical compliance and alertness for early recognition of symptoms may decline in long-term recipients, patient education regarding therapy and self-assessment should be continued to prevent late rejection and other longterm complications.

Management of Symptomatic Late Rejection

See prior section on the management of acute rejection.

In patients presenting with acute graft dysfunction late after heart transplantation, additional points deserve consideration:

• Patient perception of symptoms may be delayed, and the diagnostic yield of repeated EMB is reduced, so that a

negative EMB does not always exclude the presence of rejection-mediated graft dysfunction.

- Echocardiography should be performed when symptoms and/or signs of heart failure and graft dysfunction are present or suspected.
- CAV may cause acute/progressive heart failure and myocardial infarction without angina, and should be considered in the differential diagnosis. Levels of CK-MB and troponin must be measured; coronary angiography (and possibly IVUS) should be performed especially if there is objective evidence of graft dysfunction.

Management of Asymptomatic Late Rejection

See prior section on the management of acute rejection

The following considerations apply to late asymptomatic rejection:

- Severe, asymptomatic late rejection is uncommon.
- Late rejection may occur in low-risk patients with therapeutic immunosuppressive drug levels.
- Close rejection surveillance without intensification of immunosuppression can be considered for moderate asymptomatic late rejection.
- Background immunosuppression should be re-evaluated in patients experiencing late ACR despite drug levels within the range appropriate for the post-transplant period.

Considerations for Pediatric Recipients

One large multicenter analysis from the Pediatric HT Study Group has assessed acute rejection occurring later than 1 year after pediatric heart transplantation and showed that recurrent first year rejection, African-American race and adolescent age at transplant are the major risk factors for the development of late acute rejection.²⁰ Among 431 patients surviving for > 1 year, 25% experienced \geq 1 late rejection episodes and 15% of these were associated with severe hemodynamic compromise. Mortality was 1% in children who survived > 1 year without late rejection and 25% in those with late rejection. These findings underscore the severe consequences of late rejection in the pediatric population. Of the deaths, 30% occurred with the first late rejection episode and 70% occurred later, some due to CAV. It seems prudent to recommend selective coronary angiography within 1 year of late acute rejection episodes in children. Some late rejection episodes may be due to medical noncompliance, but subtherapeutic immunosuppressive drug levels due to rapid growth, often occurring when rejection surveillance is relaxed, may also be responsible. These data raise the question whether

many late deaths may be prevented with sustained intense clinical monitoring.

Recommendation for the Management of Late Acute Rejection:

Class I:

1. Maintenance immunosuppression and the intensity of clinical follow-up should be re-evaluated after symptomatic or asymptomatic late acute heart allograft rejection.

Level of Evidence: C.

Class IIa:

1. After the first year, EMB surveillance (e.g., every 4-6 months) for an extended period of time is recommended in patients at higher risk for late acute rejection, to reduce the risk of rejection with hemodynamic compromise, and the risk of death in African-American recipients.

Level of Evidence: C.

2. Repeated education on the critical importance of adherence to treatment and early reporting of symptoms contribute to the prevention and early recognition of late acute rejection.

Level of Evidence: C.

3. Patients at low risk for late rejection do not appear to significantly benefit from indefinite EMB surveillance. The usefulness of long-term routine EMB should be evaluated against the risks and the costs of the procedure. Repeated EMB increase the probability of damage to the TV apparatus and collection of non-diagnostic material.

Level of Evidence: C.

4. In pediatric HT recipients CAV should be considered in the differential diagnosis of late symptomatic or asymptomatic rejection when heart allograft dysfunction is present. Coronary angiography (and possibly IVUS) should be considered in these patients.

Level of Evidence: C.

5. In pediatric HT recipients, late rejection has negative prognostic implications, and may be associated with an increased risk for subsequent development of CAV; consequently, a follow-up coronary angiography may be recommended.

Level of Evidence: C.

Class IIb:

1. In pediatric HT recipients withholding treatment for asymptomatic mild-moderate late heart allograft rejection is reasonable, but it requires close follow-up.

Level of Evidence: C.

ABBREVIATIONS

- ACR = acute cellular rejection
- AIDS = acquired immune deficiency syndrome
- AMR = acute antibody-mediated rejection
- APC = antigen presenting cell
- ATG = anti-thymocyte globulin
- ATS = anti-thymocyte serum
- AUC = area under the plasma concentration time curve
- AV = atrioventricular
- AZA = azathioprine
- BNP = B-type natriuretic peptide level
- CARGO = Cardiac Allograft Gene Expression Observational Study
- CAV = cardiac allograft vasculopathy
- CEDIA = cloned enzyme donor immunoassay method

CMV = cytomegalovirus

- CNI = calcineurin inhibitor
- CRP = C-reactive protein
- CS = corticosteroid
- CTRD = Cardiac Transplant Research Database
- CYA = cyclosporine
- CYP = cytochrome P-450 enzyme system
- DSA = donor-specific antibody
- EBV = Epstein-Barr virus
- ECG = electrocardiogram
- EMB = endomyocardial biopsy
- EMIT = enzyme multiplied immunoassay technique
- EVL = everolimus
- FDA = Food and Drug Administration
- GEP = Gene Expression Profiling
- GFR = glomerular filtration rate
- GI = gastrointestinal
- HIV = Human immunodeficiency virus
- HLA = human leukocyte antigen

HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A

- HPLC = high-performance liquid chromatography
- HT = heart transplant
- ICAM-1 = intercellular adhesion molcecule-1
- ICU = intensive care unit
- IF- γ = interferon-gamma
- IL = interleukin
- IMPDH = inosine monophosphate dehydrogenase
- ISHLT = International Society of Heart and Lung
- Transplantation IV = intravenous
- IV = intravenous
- IVUS = intravascular ultrasound
- LDL = low-density lipoproteins
- LFA-1 = leukocyte function antigen-1

LV = left ventricle

- MCS = mechanical circulatory support
- MHC = major histocompatibility complex
- MIT = maximal intimal thickness
- MMF = mycophenolate mofetil
- MPA = mycophenolic acid
- MPAG = mycophenolic acid glucuronide
- MRI = magnetic resonance imaging
- mTOR = mammalian target of rapamycin
- NPV = negative predictive value
- PBMC = peripheral blood mononuclear cell PPV = positive predictive value
- PRA = panel reactive antibody
- PSI = proliferation signal inhibitor
- PTLD = post-transplant lymphoproliferative disorder
- RATG = rabbit anti-thymocyte globulin
- RHC =right heart catheterization
- RV = right ventricle
- SAECG = signal averaged electrocardiogram
- SRL = sirolimus
- TAC = tacrolimus
- $TGF-\beta$ = transforming growth factor-beta
- TICTAC = Tacrolimus in Combination, Tacrolimus Alone Compared
- TNF- α = tumor necrosis factor-alpha
- TOR = target of rapamycin
- VER = ventricular evoked response
- UDP = uridine 5'-diphospho

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