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Value of radionuclide studies in cardiac transplantation

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Effective noninvasive evaluation of acute and chronic allograft rejection remains an important challenge in patients with cardiac transplantation. Radionuclide studies have demonstrated utility because of their ease of use, giving relevant information about the pathophysiology of the transplanted heart, along with valuable diagnostic and prognostic indicators. This article focuses on reviewing the pathophysiological changes of the transplanted heart and implications for radionuclide studies.

Key words: cardiac transplantation, radionuclide imaging of necrosis and apoptosis, myocardial perfusion scintigraphy, positron emission tomography, radionuclide imaging of sympathetic innervation

THE SUCCESS of cardiac transplantation has improved over the past 25 years because of advances in immunosuppression (beginning with cyclosporine in 1980) and the early recognition and treatment of the long-term complications. Current 1- and 5-year survival rates are 85% and 70%, respectively. Thus, cardiac transplantation has consolidated as the treatment of choice for patients with terminal heart failure and low probability of survival in the next 6–12 months, with more than 31,000 cardiac transplants and several thousand combined heart and lung transplants worldwide performed to date. However, lack of organ donors has led to strict criteria for the optimal selection of transplant recipients. The causes for cardiac transplantation are cardiomyopathy (50%), coronary artery disease (CAD) (43%), valvular disease (4%) and congenital heart disease (2%). Successful revascularization in patients with CAD and hibernated myocardium yields an improvement of ventricular function and/or survival, with no need for heart transplantation. Different positron emission tomography (PET) and single-photon emission computed tomography (SPECT) studies have broadly shown their usefulness in this area.

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Acute transplant rejection still represents the leading cause of graft failure during the first year following the surgical procedure. Chronic immunologic responses appear to be the main cause of the occlusive long term vascular changes in the cardiac transplant, diversely referred to as chronic rejection, graft arteriosclerosis, accelerated arteriosclerosis, vascular allograft disease or transplant vasculopathy (TV). The latter designation reflects most properly the pathological changes, which involve arteries and veins of the allograft, preserving extracardiac vessels, and thus differing from atherosclerosis.[1,2] The different types of rejection, time frame after transplant and associated immune mechanisms are listed in Table 1. If the cascade of immune events is not interrupted, cellular necrosis results. In addition, since the process of rejection is at first usually clinically silent (not including hyperacute rejection), its early identification is crucial to limit the extension of necrosis. Noninvasive and invasive imaging techniques have played important roles in improving outcomes for transplant patients. Echocardiography grants an accurate assessment of graft and valvular function. Angiography has been the principal method for evaluating TV, but intravascular ultrasound (IVUS) continues to grow in use and has improved understanding of TV. Cardiac magnetic resonance (CMR) offers promise, but nuclear cardiology provides important information about the pathophysiology of the transplanted heart, along with helpful diagnostic and prognostic indicators.

Table 1 Different types of rejection, development after transplantation and immune mechanisms

Type of rejection	Development after transplant	Trigger mechanism	Target structures	Effector mechanism
Hyperacute (rare)	Minutes–hours	Antibody-mediated; pre-formed, "natural" IgM antibodies of ABO blood group system	ABO blood group determinants on vascular endothelial cells	Complement fixation and activation by antibodies, apoptosis and necrosis of endothelial cells; thrombosis of graft vessels
Acute cellular (very common)	2 weeks–many years (most frequent in first year)	Cellular; alloreactive CTL, DTH reaction	MHC alloantigens primarily on cardiomyocytes, direct and indirect recognition	Cell lysis by perforin/ granzyme or Fas ligand, toxic cytokines; cardiomyocyte and endothelial damage
Acute vascular (uncommon, but maybe underdiagnosed)	2 weeks–18 months	Antibody-mediated + cellular component; IgG "immune" antibodies formed after transplantation, alloreactive T lymphocytes	MHC alloantigens (and others?) on vascular endothelial cells	Complement fixation and activation by antibodies, cell lysis by CTL, apoptosis and necrosis of endothelial cells; vasculitis
Chronic (transplant vasculopathy) (very common)	6 months–many years	Unclear: alloreactive T lymphocytes?, antiendothelial CTL?, DTH reaction?, alloantibodies	MHC alloantigens on cardiomyocytes/endothelial cells, exacerbation by atherosclerotic risk factors or CMV infection	Lymphocyte-mediated endothelial damage and repair; chronic growth factors DTH, secretion of neointima formation, vascular occlusion

CMV: cytomegalovirus; CTL: cytotoxic T lymphocytes; DTH: delayed type hypersensitivity, through $CD4^+$ T cell-activated macrophages; HLA: human lymphocyte antigens; MHC: major histocompatibility complex.
Modified from reference 2.

ACUTE REJECTION

During the first postoperative year, with the exception of the first 2–3 weeks when mortality is principally due to right heart failure secondary to vascular lung disease, infections and acute rejection are the leading causes of mortality. Roughly, 50%–70% of cardiac transplant recipients experience at least one significant episode of acute cellular rejection during this period, particularly during the first three months. Currently these figures may be lower with modern immunosuppressive therapy. The correct diagnosis of acute rejection is crucial to correctly adjust the immunosuppressive therapy without increasing the risk of opportunistic infections.

Endomyocardial biopsy of the right ventricle is the "gold standard," for the detection and follow-up of acute rejection. It is a percutaneous and transvenous technique, rapidly performed with local anesthesia, guiding the bioptome through fluoroscopy or echocardiography. However, it is expensive and invasive, and complications may occur in up to 1% of patients, including pneumothorax, vascular damage, tricuspid regurgitation, air embolus, arrhythmias, right ventricular perforation and tamponade, bleeding and infection.[3] Furthermore, despite the relatively diffuse nature of rejection, the technique is subject to sampling error if the number of specimens is inadequate. Therefore, 4–6 specimens are needed to reduce this error.[4] With one specimen, sampling error is nearly 80% decreasing to <5% with 5 specimens. An added limitation of the technique is the sampling of previously biopsied sites, resulting in interpretation difficulties due to the presence of contraction bands, inflammatory phenomena and collagen formation. Moreover, endomyocardial specimens may include confounding histopathologic changes related to events inherent to the transplantation itself (e.g. cardiotomy, freezing, ischemic time and reperfusion of the allograft) soon after the surgical procedure, or concurrent myocardial infections at the time of the biopsy (e.g. cytomegalovirus or toxoplasmosis). EMB is useless in the diagnosis of TV, since it rarely includes coronary arteries.

Because treatment of rejection is based on the results of EMB and because of the high frequency of episodes of acute rejection during the first postoperative year (specially the first 3 months), surveillance biopsies are performed at short intervals during that time. About 12

biopsies are performed the first year posttransplantation: once per week the first month, once per two weeks the second month, once per month for months 3–6, and once per three months thereafter. Mild rejection (i.e. mononuclear infiltrate with no cardiomyocyte necrosis) is usually not treated, but a follow-up biopsy should be performed within a week. On the other hand, moderate or severe rejection (i.e. presence of cardiomyocyte necrosis) should be treated because the conducting system is often involved and fatal arrhythmias can occur. Follow-up biopsy should be performed 10 days after the end of therapy, repeating treatment if active myocardial damage is documented. The regular biopsy program is resumed when the myocardial damage abates.

Noninvasive means for identification of rejection have been based on detection of activated circulating lymphoblasts, changes of electrocardiographic (ECG) voltage, morphologic and functional parameters, and assessment of myocardial energy by magnetic resonance spectroscopy (MRS). Quantification of different lymphocyte populations is neither sensitive nor specific, and changes in the helper/suppressor ratio have limitations in differentiating acute rejection from certain infections, while changes in immunosuppressive therapy can also alter lymphocyte subpopulations. A decrease in the amplitude of the QRS complex on ECG is not a specific finding. Although observed when there is myocardial and pulmonary edema, this is also true in cases of lung infiltration and pericardial effusion. Additionally, reduction of myocardial edema with cyclosporine therapy decreases even more the sensitivity of this finding.

Reduction in LV systolic function, increase in LV mass and parameters of diastolic dysfunction are all potentially useful noninvasive markers of rejection. However, LV systolic function usually remains normal with mild to moderate rejection, becoming abnormal only with severe rejection. For this reason, systolic function cannot be used to detect or rule out most episodes of rejection. LV mass and wall thickness increase during rejection episodes and resolve with treatment. Nevertheless, chronic cyclosporine therapy minimizes these changes, which have become less sensitive markers of rejection. Finally, indices of diastolic dysfunction are more useful for the detection of rejection, but are more difficult to interpret when baseline diastolic dysfunction is abnormal without rejection. Additionally, individual changes in some of these indices are often within the range of spontaneous variation observed in the absence of rejection. Thus, the filling pattern in an individual patient should be documented in the absence of rejection, and a subsequent intrapatient comparison made to detect abnormalities. Overall, the sensitivity of altered diastolic function is poor to fair, and the specificity is moderate. Moreover, none these morphologic and functional parameters are useful for detection of rejection in the early postoperative period, since they are usually abnormal due to the myocardial edema resulting from reperfusion ischemic injury during organ preservation. Significant right ventricle systolic dysfunction after transplantation should raise suspicion of rejection, inadequate organ preservation, or prolonged warm ischemic times. Pericardial effusion is present in up to 50% of posttransplant patients but usually resolves spontaneously, since typically it is secondary to postoperative bleeding or mismatches between donor heart size and recipient pericardial size. It should be noted that in the early postoperative period transthoracic echocardiography is sometimes severely limited by poststernotomy changes, hyperinflation of the lungs and chest tubes, with transesophageal echocardiography and radionuclide ventriculography being substitutes.[5,6]

Magnetic resonant spectroscopy (MRS) relies on the detection of high-energy phosphates (ATP, phosphocreatinine) that are critical to tissue metabolism. A decrease in these organic phosphates and an increase in inorganic phosphates suggest cellular damage. In animal studies, phosphate MRS correlates with EMB results and may differentiate between ischemic injury and rejection, although the data available are limited.[7]

Scintigraphy with ^{111}In-labeled murine monoclonal antimyosin Fab antibody fragments (R11D10-Fab) has shown high sensitivity and specificity for the detection of cardiomyocyte necrosis. Uptake of ^{111}In-antimyosin by the myocardium only occurs when the integrity of the sarcolemma is lost as a result of cell damage and the antibody fragments bind to the exposed intracellular heavy chain of cardiac myosin to which they have a selective affinity.[8–11] The small dimensions of antimyosin Fab (65 × 35 Å) make it able to enter the membrane gaps created by complement membrane attack complexes or by inflammatory reactions that may subsequently result in myocyte necrosis.[12]

Myocardial uptake of ^{111}In-antimyosin can be semiquantified by calculating the heart-to-lung count density ratio (HLR) by dividing average counts per pixel in the cardiac region of interest by average counts per pixel in an area of interest set at the right lung on the anterior view.[13] HLR has been shown to correlate directly with the severity of myocardial damage as assessed by EMB.[14,15] This method provides a means for assessing both the presence and intensity of myocardial damage *in-vivo*, allows comparison between different groups of patients, and may be used to follow the course of cell damage in individual patients.[14,15]

^{111}In-antimyosin studies have revealed a direct correlation between antibody uptake and EMB score, with high sensitivity (nearly 100%) for detection of rejection. Moreover, rejection is barely detected during long-term follow-up in patients with normal scans. On the contrary, when there is abnormal uptake, rejection appears at some stage during follow-up, with an increased probability of detecting rejection at biopsy with increasing intensities of the antibody uptake, thus providing a powerful tool for risk

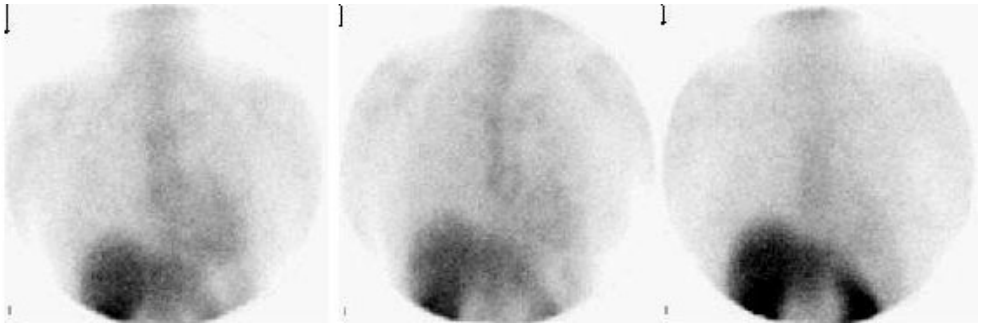

Fig. 1 Serial ^{111}In-antimyosin scans obtained in the anterior view after 1, 2 and 3 months (*from left to right*) of orthotopic cardiac transplantation with no evidence of rejection. Notice gradual decrease of diffuse myocardial uptake, supporting the absence of severe rejection-related complications during the first year.

stratification. Discrepancies between positive ^{111}In-antimyosin scans and negative biopsies likely represent biopsy sampling error.[16] However, Narula et al.[12] in a study of 40 patients with recent onset of dilated cardiomyopathy and positive ^{111}In-antimyosin scan but lacking right ventricular biopsy evidence of myocarditis, established that biopsy sampling error cannot be the only reason for the scan-biopsy discordance. They found that in the absence of histologically identifiable myocarditis and myocyte necrosis, myofibrillarlysis (often called vacuolar degeneration or myocytolysis) was the only biopsy finding observed frequently in patients with cardiomyopathy and positive scan as compared with those with negative scan. The myofibrillarlytic myocyte population is presumed to combine myocytes at different stages of injury based on the sarcolemmal integrity and can comprise a broad continuum between reversibly damaged (sarcolemmal integrity preserved) and necrotic myocytes (with tiny sarcolemmal breaches, small enough to permit ^{111}In-antimyosin access but not to permit light microscopy identification). Therefore, necrotic myocytes within myofibrillarlytic population may also play a role in the ^{111}In-antimyosin scan-biopsy discordance.

The great sensitivity of ^{111}In-antimyosin scintigraphy for myocardial damage precludes its use as the only diagnostic test for patient management during the first year after transplantation, when 80% of patients show a positive result. During this period, aggressive treatment is based upon evidence of cell damage in EMB. After this time, patients can be withdrawn from biopsy, with individual management adjusted on the basis of the ^{111}In-antimyosin study.[17] ^{111}In-antimyosin studies in combination with biopsies during the first trimester after the operation have been shown to provide additional information to that contained solely in biopsies. A decrease of antibody uptake during this early period appears to be related to an absence of severe rejection-related complications during the first year (Fig. 1), whereas a persistent ^{111}In-antimyosin uptake alerts against the presence of such complications during that interval.[16] Additionally, the sensitivity and non-invasiveness of ^{111}In-antimyosin imaging allow its use as a research tool in the assessment of the efficacy of new immunosuppressive regimens or drugs. Consequently, ^{111}In-antimyosin imaging may provide the most sensitive means to detect tolerance to the graft once the first few months after heart transplant have elapsed, optimizing patient management.[17] ^{111}In-antimyosin was recently approved by the FDA for commercial marketing, despite which, approved indications have limited its use to ischemic heart disease, and commercial production has ceased at the present time.

CHRONIC REJECTION (transplant vasculopathy, TV)

TV is the most vexing and difficult long-term complication of cardiac transplant patients, being the main cause of mortality beyond the first postoperative year. The incidence of TV has increased with survival improvement of cardiac transplantation, along with time from transplant. Angiographic studies have reported a prevalence up to 50% after 5 years of transplantation, with an annual incidence of 10–15%, which has not been modified with the introduction of cyclosporine.[18] TV is a diffuse process of concentrically intimal thickening involving the whole vessel from mid portions (angiographic lesions type B and C). Initially it does not affect the luminal diameter at the epicardial vessel level, and flow is limited in vessels not seen on angiography, which therefore may be undetected. Probably TV has a multifactorial nature, with both recipient (chronic immunologic responses, white race, cytomegalovirus infection and triglyceride levels) and donor properties (age, undersize and smoking history) playing important roles. Its clinical diagnosis is also difficult, due to the inability to perceive pain in patients with denervated hearts. Therefore, initial symptoms may be ventricular arrhythmias, heart failure or sudden death secondary to infarcts in major epicardial vessels or ischemia in the microcirculation. The process may be combined with

typical atheromatous lesions (type A lesions, excentrical, focal and occasionally proximal), in relation with donor CAD. Revascularization treatment is also disappointing, and retransplantation is the ultimate solution when TV is in advanced stage. Therefore, early diagnosis of TV is crucial for prompt control of risk factors and changes in treatment, which may include an intensification of the immunosuppressive regimen.[19,20]

Although the detection of epicardial CAD correlates with decreased survival rates (50% in two years), since the advent of IVUS, angiography is no longer the "gold standard" for the detection of TV. A normal angiogram does not indicate lack of TV. In fact, IVUS is capable of demonstrating and assessing lesion thickness in "normal" angiographic vessels. However, as angiography IVUS is expensive, invasive and has similar risks to those of angiography (bleeding, coronary dissection, and coronary spasm).[21]

Electron-beam computed tomography (EBCT) is a promising noninvasive test for the detection of coronary artery calcification as an index of CAD in cardiac transplant recipients. Reported diagnostic accuracy for detecting stenosis as compared with coronary angiography (>50% coronary artery stenoses) and IVUS has been reported, with sensitivity, specificity and positive and negative predictive values of 94%, 79%, 43%, 99%, respectively.[22] Diagnostic and prognostic value of dobutamine stress echocardiography (DSE) for noninvasive assessment of TV has also been assessed. Resting 2D echocardiography detected TV defined by IVUS and angiography with a sensitivity of 57% (specificity 88%). DSE increased the sensitivity to 72%. DSE identified patients at risk for events and facilitates monitoring of TV (normal DSE predicted an uneventful clinical course, which justified postponement of invasive studies). The prognostic value of DSE was comparable to that of IVUS and angiography.[23]

Because of its high spatial resolution, magnetic resonance first-pass perfusion imaging (MRFP) allows for the discrimination of perfusion across layers in the same myocardial segment. Transmural perfusion analysis has been found useful in TV.[24] MRFP may be capable of detecting perfusion abnormalities isolated to the endocardium, discern transmural flow gradients, and identify reductions in flow reserve that are undetectable with radionuclide imaging. Furthermore, the accuracy of CMR imaging in the diagnosis of CAD has recently been reported to be 72%. However, these magnetic resonance modalities remain technically demanding with respiratory and cardiac motion superimposed to the limited vessel wall thickness (0.5–2 mm), tortuous course and close proximity to epicardial fat, coronary blood and myocardium. Additionally, the safety of pharmacological stress testing with dobutamine infusion is controversial in CMR imaging, particularly with regard to access to the patient in the case of emergency.

Myocardial perfusion scintigraphy has been reported to have poor sensitivity to detect TV.[25] It is possible that when scintigraphy is performed long after transplantation, diffuse and chronic involvement of the coronary arteries is already established, and regional perfusion defects are not detected due to balanced ischemia in various coronary territories. However, Lamich et al.[20] detected 91% of the episodes of TV by ^{201}Tl SPECT, indicating that detection of early TV is feasible by serial myocardial perfusion scans. Sixty-five percent of detected episodes of TV were not apparent on coronary angiograms, suggesting that perfusion abnormalities may precede angiographic evidence of the disease. Therefore, these authors advocate that more frequent scintigraphic studies be performed during the first year of transplantation in order to detect (and consequently treat) TV in its early phases. Likewise, there is reasonably convincing evidence that a myocardial perfusion SPECT without reversible defects virtually excludes lesions suitable for coronary artery revascularization. Moreover, a normal scan one-year after cardiac transplantation is an important predictor of 5-year survival. Thus, the high negative predictive value of the test indicates that patients who demonstrate normal perfusion by this method may be excluded from further invasive studies.[26–29]

PET studies have shown an increased baseline myocardial blood flow during the first year posttransplantation, together with an attenuated maximal microcirculatory dilation capacity during the first three months. One year after the transplantation both baseline myocardial blood flow and microvascular function tend to normalize. At three years of transplantation, the hyperemic response to vasodilator stress becomes impaired, despite normal baseline myocardial blood flow, probably due to endothelial dysfunction, rejection and TV.[30–34] PET studies have also shown increased ^{18}F-fluorodeoxyglucose uptake in the LV of allografts with no concomitant evidence of rejection, not explained by changes in blood flow or cardiac work. Increased blood levels of catecholamines or inefficient use of glucose have been suggested.[35] There are studies supporting a metabolic switch from free fatty acids to glucose under conditions of denervation.[36] Myocardial efficiency has been shown to be improved in transplant recipients compared with failing hearts, and comparable to normal hearts under resting conditions.[37]

INNERVATION OF THE TRANSPLANTED HEART

During orthotopic heart transplantation, the entire recipient heart is excised except for the posterior atrial walls, to which the donor atria are anastomosed. During the process, the allograft becomes completely denervated. Lack of autonomic nerve supply is associated with major physiologic limitations. The inability to perceive pain does not allow symptomatic recognition of TV, and heart transplant patients often develop acute ischemic events or LV

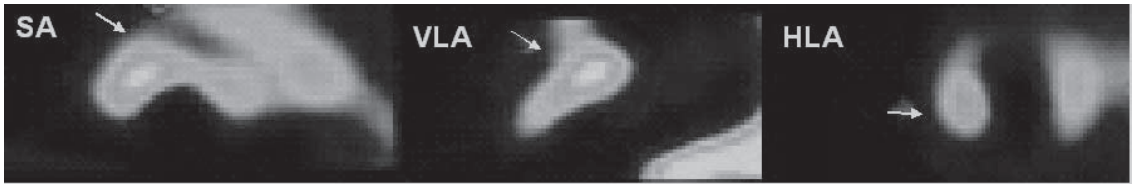

Fig. 2 Representative slices (short axis, SA; vertical long axis, VLA; and horizontal long axis, HLA) of ^{123}I-MIBG SPECT imaging in a patient 10 years after cardiac transplantation. Myocardial uptake indicating reinnervation can be observed in anterior, anteroseptal and anterolateral regions, while it is defective in the inferior wall and apex.

dysfunction or die suddenly. In addition, denervation of the sinus node does not allow adequate acceleration of heart rate during stress and efficient increase in cardiac output. Furthermore, loss of vasomotor tone may adversely affect the physiologic alterations in blood flow, produce altered hemodynamic performance at rest and during exercise, and decrease exercise capacity.

The denervated transplanted heart constitutes an exceptional model by which to assess the specificity of tracers for presynaptic innervation. Soon after the surgical procedure, myocardial tracer uptake must be a consequence of nonspecific mechanisms. Thereafter, the progressive sympathetic reinnervation provides a model for studying intraindividually the effects of innervation and denervation on myocardial biology.

Scintigraphic studies with ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) and ^{11}C-hydroxyephedrine (^{11}C-HED), analogues of norepinephrine that are taken up by myocardial sympathetic nerves, support the concept of spontaneous reinnervation taking place after transplantation.[38–40] SPECT studies with ^{123}I-MIBG allow semiquantification of tracer retention and washout from early (15 minutes) and delayed (4 hours) images. Heart-to-mediastinum ratio is calculated by dividing mean counts per pixel in a region of interest over the myocardium by mean counts per pixel in a region of interest over the mediastinum, in the anterior view. PET studies with ^{11}C-HED provide absolute quantification of tracer retention in the terminal nerves by acquiring images as a dynamic set and applying complex mathematical models.

Reinnervation is likely to be a slow process and occurs only after 1 year post-transplantation. Sympathetic reinnervation progressively increases with time after transplantation, with a positive correlation between tracer retention and time after transplantation. Serial ^{123}I-MIBG and ^{11}C-HED studies over time show that reinnervation begins from the base of the heart and spreads towards the apex. Tracer uptake is seen primarily in the anterior, anterolateral and septal regions. Uptake is usually not apparent in the inferior myocardial wall (Fig. 2). Complete reinnervation of the transplanted heart is not seen on scintigraphic studies, even up to 13 years post-transplantation. Clinical determinants of reinnervation may relate to donor and recipient age, duration and complexity of transplant surgery, and frequency of allograft rejection. Early vasculopathy may inhibit the process of sympathetic reinnervation of the transplanted heart. The relationship between reinnervation status and graft vasculopathy deserves further investigation and may help to characterize subsets of transplant patients with different clinical outcomes.[41,42]

Di Carli et al.[43] in a PET study using ^{13}N-ammonia and ^{11}C-HED evaluated the blood flow responses to adrenergic stimulation in reinnervated and denervated coronary territories of transplanted recipients. They assessed myocardial blood flow at rest, in response to sympathetic stimulation induced by cold pressor testing (as an index of endothelial-dependent vasodilation), and during adenosine-induced hyperemia (as a combined index of endothelial-dependent and –independent vasodilation). They found a significant improvement in flow response to cold pressor in innervated compared with denervated vascular territories, demonstrating the relevance of sympathetic innervation for regulation of vascular reactivity and supporting the physiologic importance of reinnervation for transplant recipients.

Sympathetic reinnervation assessed by ^{11}C-HED was found to be associated with improved responses of heart rate and global as well as regional contractile function to exercise, supporting the functional importance of sympathetic restoration and suggesting a clinical benefit for the transplant recipient through improved exercise capacity.[44]

NEW DEVELOPMENTS

Recently, the feasibility of noninvasive detection of acute rejection by ^{111}In-pentetreotide scintigraphy has been described by targeting the activated lymphocytes of the inflammatory infiltrate with this somatostatin analogue.[45] Uptake of ^{111}In-pentetreotide may predict impending rejection at least 1 week before the EMB becomes positive. The late appearance of diagnostic EMB may reflect a lag-time between lymphocytic activation and induction of myocyte damage. Furthermore, somatostatin receptor imaging at 4 hours may in any case allow earlier intervention in the event of rejection, given the time required for histological processing of endomyocardial biopsy.

Recent knowledge of expression of other types of receptors in different vascular disorders opens further new frontiers of research as well.

Although apoptosis was thought not to occur in terminally differentiated tissues such as myocardium, it has been reported in a variety of heart disorders. Programmed cell death is important in the spectrum of myocardial damage, since it is becoming gradually more apparent that cell death may begin as apoptosis and not as necrosis depending on the intracellular ATP contents. In fact, insults of whatever origin (ischemic, inflammatory or toxic) not sufficiently severe to induce necrosis can force the cardiomyocyte to follow with apoptotic death.[46] Apoptosis of myocytes is frequently observed during cardiac allograft rejection, even in the absence of histologic rejection activity, but with ^{111}In-antimyosin uptake, which suggests that apoptosis could be an additional mechanism of transplant-associated myocardial damage.[47] Although characteristic morphologic changes of apoptosis are essentially intracellular, it also leads to abnormalities of phospholipid distribution within the cell membrane bilayer, with random distribution of phospholipids, and expression of phosphatidylserine (PS) on the outer leaflet. Annexin V is an endogenous intracellular human protein with high affinity for anionic phospholipids bound to the cell membrane.[48] Binding to PS is reversible and comparable to many ligand-receptor systems.[49] Its affinity for PS is significantly higher than that for phosphatidylethanolamine. Radiolabeling of annexin V by direct ^{99m}Tc reduction with stannous ion has revealed clinical feasibility and safety for non-invasive detection of transplant rejection, thus reducing the need for surveillance endomyocardial biopsies.

Narula et al.[50] performed ^{99m}Tc-annexin V SPECT imaging in 18 recently (<1 year) heart transplanted patients. Five patients showed variable degrees of ^{99m}Tc-annexin V uptake in the LV, which correlated with at least moderate transplant rejection and immunohistochemical evidence of apoptosis in biopsy. All 13 patients without histologic evidence of rejection had a negative scan. Kown et al.[51] have also reported the initial experience in this field at Stanford University Medical Center. Five of 10 cardiac transplant patients had ≥2 areas of ^{99m}Tc-annexin V uptake in the right ventricle on SPECT images. EMB was positive in two of these patients and showed a low grade of acute rejection in the other 3 patients. Additional five patients had either one or zero hot spot areas and corresponding negative biopsies. These results suggest a potential role for annexin imaging in the area of heart transplant monitoring, which will increase understanding of allograft pathophysiology and lead to new effective therapies for increasing salvage of myocardium in cardiovascular disease.

Although the localization of ^{99m}Tc-annexin V seems to depend on the occurrence of apoptosis, experimental work suggests that physiological stress may produce transient and reversible PS expression, not specific to apoptosis, which may be visualized following the injection of annexin. However, prolongation of the stressor action actually leads to cell death by apoptosis. These observations suggest that *in-vivo* ^{99m}Tc-annexin V imaging may reflect both cells undergoing apoptosis and cells that are severely injured but still capable of surviving. On the other hand, ^{99m}Tc-annexin V accumulation has also been observed in predominantly necrotic areas, probably due to either binding to PS on the inner plasma membrane leaflet of cells with irreversible membrane damage or failure of energy-dependent cellular systems to restrict PS to the inner leaflet, thus allowing the passive redistribution of PS from the inner to outer leaflet of the plasma membrane.[52] These findings reveal the likelihood of cell death mixtures in acute myocyte injury. The different targets and imaging windows of ^{99m}Tc-annexin V and ^{111}In-antimyosin could be possibly combined to identify dead cells from not-so-dead cells and differentiate reversible myocardial damage in clinical settings with ongoing chronic diffuse myocardial damage.

In conclusion, there is no perfect invasive or noninvasive test for the detection of rejection. The course of rejection after the operation is different in every patient, and patients may undergo rejection years after transplantation. Therefore, a wise combination of various tests at different stages after transplantation is necessary to diagnose rejection and manage patients.

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