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SUMMARY

Background: Physio-pathological correlation between erectile dysfunction (ED) and cardiovascular diseases are well known; in the Heart Transplantation Centre in Padua, male patients undergone to heart transplant in different periods, show higher prevalence of erectile dysfunction, compared to normal population. Furthermore, patients showing erectile dysfunction, present a multi-district vascular disease, concerning peripheral (femoral and epi-aortic) and cardiac circulation .The mechanism responsible of this multi-district process in heart transplanted patients has not been investigated. Endothelial progenitors cells (EPCs) dysfunction leading to endothelial dysfunction can be the shared mechanisms; furthermore, EPCs are involved in CAV (cardiac allograft vasculopathy).

Aim of the study is to analyze the presence of erectile dysfunction, peripheral vasculopathy and cardiac allograft vasculopathy (CAV) in heart transplanted patients and their correlation with levels of progenitor cells (PC), EPC and OCN+-EPC (osteocalcin-positive).

Methods and Materials: From march 2009 to June 2010, in Padua heart transplant centre, 77 consecutive male heart transplanted patients, aged between 18 and 80 years, have been enrolled to undergo to the following exams: cardiac angiography, Doppler Echocardiography, CFR (coronary flow reserve), penile-doppler and pharmacologic duplex ultrasonography, carotid and femoral doppler ultrasound.and biochemical, haematological, hormonal and lipid blood tests.

Patients were divided depending on the presence of coronaropathy and DE.

Results: Prevalence of ED is higher in the Htx pts (67% after Htx vs 24% before Htx). From this study, it is demonstrated that ED significantly correlates with the development of CAV (25.53% vs 8.7%, $p<0.05$), but also with peripheral vasculopathy. Levels of PC are reduced in pts with ED (1200,13 ±477,19 vs 929,7 ±559,71; $p<0,04$). In pts affected by coronaropathy,

circulating OCN+-EPCs are higher (29,79 ±23,87 vs 14,32 ±18,43 ; p<0.003), while testosterone is decreased (0,27 ±0,08 vs 0,31 ±0,09 ; p<0.05). The coronaropathy correlates significantly also with coronaric flow reserve (2,44 ±0,74 vs 3,05 ±1 ; p<0.02)

Conclusions: Erectile dysfunction in male heart transplanted-patients is associated with a more frequent development of CAV and peripheral vasculopathy. ED might represent an early marker of endothelial dysfunction, associated with the development of CAV and vasculopathy. Levels of EPCs and testosterone may be involved in the development of the process. For these reasons, we suggest an early diagnosis of ED, as a praecox marker of CAV, in order to improve the quality of life of transplanted patients.

RIASSUNTO

E’ noto in letteratura che pazienti con malattie cardiovascolari spesso riferiscono disfunzione erettile; presso il Centro Trapianti di Padova, pazienti di sesso maschile sottoposti a trapianti cardiaco presentano una maggiore incidenza di disfunzione erettile in confronto alla popolazione normale. Questo fenomeno è, inoltre, parte di un processo generalizzato e multi-distrettuale di vasculopatia, che riguarda sia la circolazione periferica (femorale e carotidea) sia quella coronarica. Il meccanismo fisio-patologico alla base della vasculopatia nel paziente cardio-trapiantato non è noto. Una riduzione e una disfunzione delle cellule endoteliali progenitrici (EPCs) alla base di una disfunzione endoteliale, possono essere il fattore comune ai danni della micro-e macro-circolazione in questa categoria di pazienti. Tale processo è inoltre anche implicato nell’insorgenza della vasculopatia dell’allograft, ancora oggi una delle principali cause di morte dopo trapianto.

Scopo dello studio è analizzare l’esistenza di una correlazione fra disunzione erettile, vasculopatia periferica e vasculopatia dell’*allograft* (CAV) nei pazienti di sesso maschile sottoposti a trapianto cardiaco; inoltre, non meno importante, tale studio ha il fine di valutare il ruolo delle cellule endoteliali progenitrici e della loro sotto-popolazione osteocalcina positiva. (PC, EPC e OCN+-EPC) nello sviluppo della vasculopatia.

Materiali e metodi: da marzo 2009 a giugno 2010, previo ottenimento del consenso informato, 77 pazienti di sesso maschile, sottoposti a trapianto cardiaco, di età compresa fra i 18 e gli 80 anni sono stati arruolati. I pazienti sono stati sottoposti ad i seguenti esami: cateterismo cardiaco sinistro con coronarografia e ventricolografia, Ecocardio-color Doppler, CFR (coronary flow reserve), Eco-Doppler penieno, femorale e carotideo, esami del sangue con esame emocromocitometrico, profilo biochimico, lipidico, proteico ed ormonale. I

pazienti sono stati suddivisi in base alla presenza o assenza di disfunzione erettile e di coronaropatia.

Risultati: l'incidenza di disfunzione erettile è risultata essere molto maggiore nei pazienti sottoposti a trapianto cardiaco, indipendentemente dall'epoca del trapianto (67% dopo trapianto versus 24% prima del trapianto). Questo studio ha dimostrato che la disfunzione erettile correla positivamente con lo sviluppo della vasculopatia dell'*allograft* (25.5% vs 8.7%, $p<0.05$), ma anche con la vasculopatia periferica. I livelli di cellule progenitrici (PC) sono ridotti nei pazienti con disfunzione erettile (1200,13 ±477,19 vs 929,7 ±559,71; $p<0,04$), mentre, nei pazienti affetti da coronaropatia, i livelli di cellule endoteliali progenitrici osteocalcina positive (OCN+-EPC) sono aumentati (29,79 ±23,87 vs 14,32 ±18,43 ; $p<0.003$), con livelli di testosterone ridotti (0,27 ±0,08 vs 0,31 ±0,09 ; $p<0.05$). Infine la coronaropatia mostra una correlazione positiva anche con la riduzione della riserva coronarica (CFR) (2,44 ± 0,74 vs 3,05 ±1 ; $p<0.02$).

Conclusioni: la disfunzione erettile nei pazienti sottoposti a trapianto cardiaco risulta essere associata ad un maggiore sviluppo di vasculopatia dell'*allograft* e periferica. La disfunzione erettile può rappresentare un marker precoce di disfunzione endoteliale. I livelli di EPCs e di testosterone possono essere coinvolti nello sviluppo di tale processo multri-distrettuale. Per questi motivi, noi propoiamo una diagnosi precoce di disfunzione erettile come marker precoce di vasculopatia dell'allograft e con l'obiettivo finale di migliorare la qualità di vita dei pazienti sottoposti a trapianto cardiaco.

BACKGROUND

HEART TRANSPLANTATION

Heart transplantation is actually a common surgical procedure in humans; almost 85,000 operations have been performed worldwide over the past 40 years (1), in more than 200 centres. Cardiac transplantation remains the primary therapeutic choice for most patients under 65 years of age with advanced heart failure who remain symptomatic despite maximal medical therapy, with a 1-year graft survival rates of around 85% . Because of the donor shortage cardiac transplantation should be reserved for those patients most likely to benefit in terms of both life expectancy and quality of life. Patients with multiple comorbidities have inferior survival and might be considered for alternative therapies. A basic tenant of organ allocation embraces two axioms that are at times contradictory: equity (equal access of all patients to donor organs), with priority given to patients ‘‘closest to death’’; and utility (an allocation policy for organs that maximizes patient and graft survival). Application of these principles requires that a lower limit of acceptable expected post-transplant survival be determined and that secure patient-specific information about midterm survival with current therapy for subsets of advanced heart failure is available.

Clearly, the major foci of postoperative care must be directed at assessment of cardiac function, surveillance of rejection, titration of immunosuppression, and identification and treatment of the adverse effects related to immunosuppression.

Heart transplant Kaplan-Meier Survival: comparison between our centre and the International Society of Heart and Lung Transplantation Registry

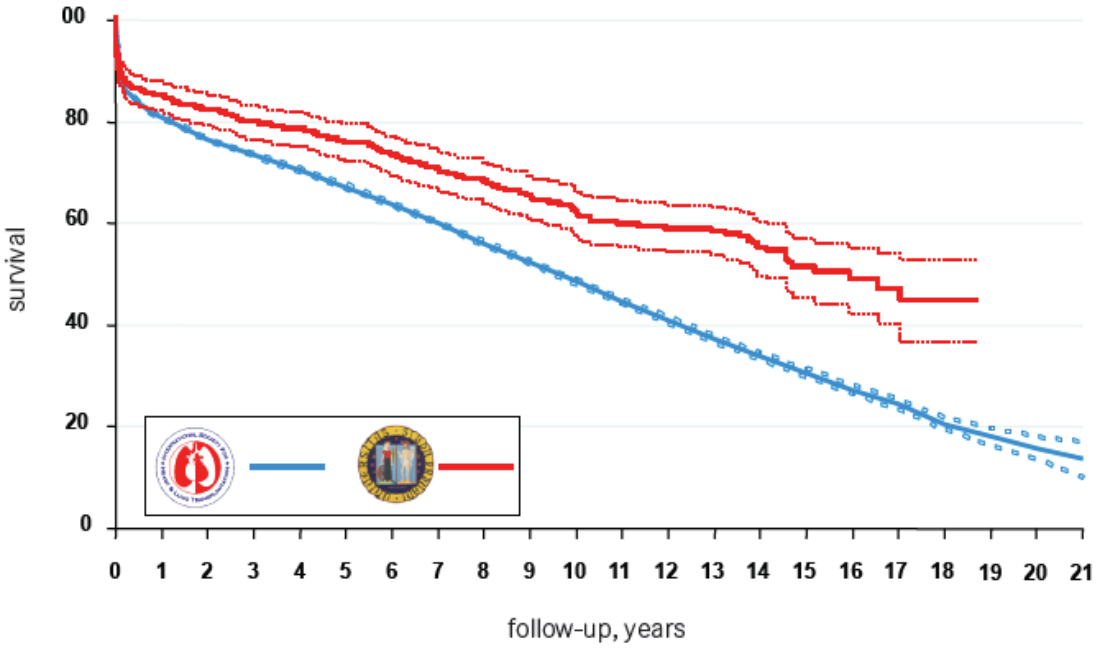

Fig. 1 - NUMBER OF HEART TRANSPLANTS REPORTED BY YEAR
Heart Transplant Registry - ISHLT vs Padua Cardiac Surgery
Kaplan-Meier survival estimate, overall

IMMUNSUPPRESSANT THERAPY:

An increasing number of immunosuppressive agents are available and these target different steps of the immunological response to an allograft. All cause non-specific immunosuppression and each has their own agent-specific side-effects.

See in the table below, the immunuppressant drugs actually used in organ solid transplantation.

Immunosuppressive agents used in solid organ transplantation

Class of agent	Agent
Corticosteroid	Prednisolone Prednisone Methyl prednisolone
Anti-proliferative	Azathioprine Mycophenolate mofetil Mycophenolate sodium
Calcineurin inhibitor	Cyclosporine Tacrolimus
TOR inhibitor	Sirolimus Everolimus
Polyclonal anti-lymphocyte antibodies	ALG ATG ALS
Monoclonal antibodies	Muromonab-CD3 Basiliximab Daclizumab

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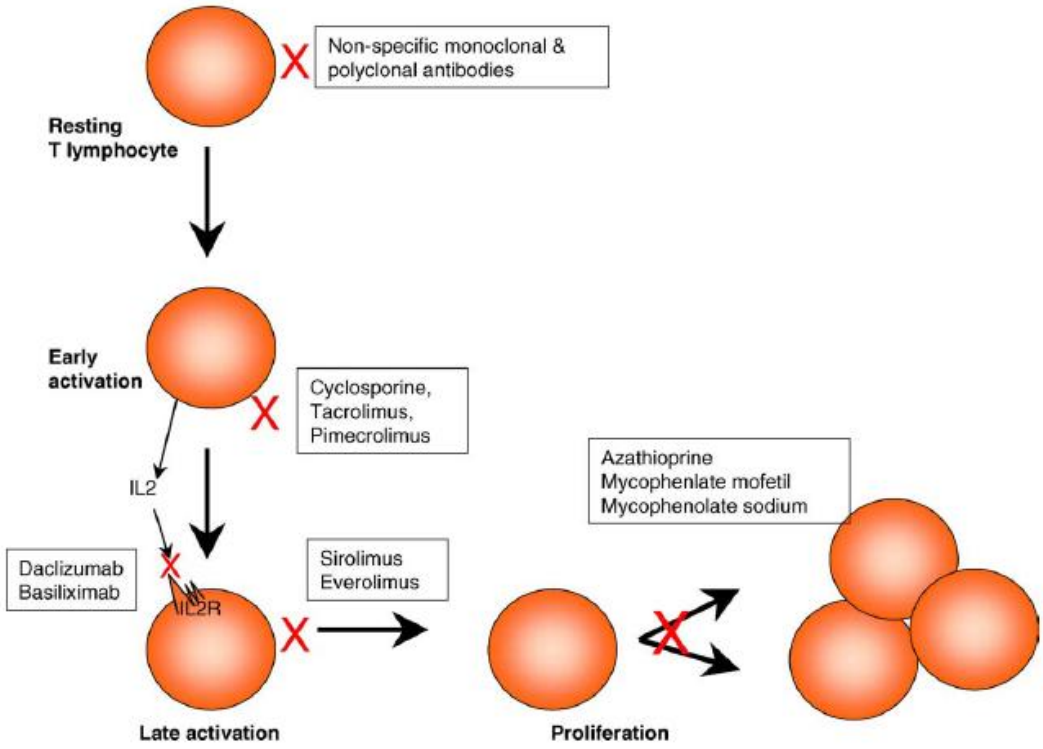
Site of action of common immunosuppressants:



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Corticosteroids

Corticosteroids are still widely considered an important component of most immunosuppressive regimens and are almost universally used as first-line treatment for acute allograft rejection. The two main corticosteroids used for the prevention of allograft rejection are prednisolone (used mainly in Europe) and prednisone (used mainly in the U.S.A.). Both have predominantly glucocorticoid effects with minimal mineralocorticoid effects. First line treatment of acute allograft rejection in most centres comprises high-dose intravenous steroids, usually methylprednisolone

Corticosteroids have a variety of anti-inflammatory and immunomodulatory effects. These include stabilisation of lysosomal membranes, suppression of prostaglandin synthesis, reduction of histamine and bradykinin release and lowering of capillary permeability. Corticosteroids cross into

the cytoplasm and bind to glucocorticoid receptors, anchored in the cytoplasm by a complex of heat shock proteins. Binding permits release of heat shock proteins allowing the corticosteroid/ glucocorticoid receptor complex to translocate to the cell nucleus where it influences gene transcription, including transcription of the nuclear activating factor family of genes. These genes are important in activating the transcription and production of several pro-inflammatory cytokines and the net result is a decrease in the inflammatory response through reduced production of cytokines, including IL-1, IL-2, IL-6, IFN-γ and TNF-α. Corticosteroids also impair monocyte/macrophage function and decrease the number of circulating CD4+ T cells.

The side effects of steroid treatment are numerous and well known. Metabolic effects include diabetogenesis due to altered carbohydrate metabolism, fat redistribution from the extremities leading to plethoric face and central obesity, and protein loss from skeletal muscle resulting in proximal weakness. Fluid retention is a consequence of mineralocorticoid activity, with hypokalaemia and hypertension. Long-term steroid therapy results in adrenal suppression and, eventually, adrenal atrophy. Psychosis, cataracts and glaucoma, peptic ulceration, abdominal wall striae and purpura, avascular necrosis of the femoral head and osteoporosis, and impaired wound healing are other common problems.

In Padua Heart Transplant Centre, according to the worldwide experience, corticosteroids are withdrawn usually within the first year after transplantation; sometimes corticosteroids at low dosagehave to be re.introduced, to reduce calcineurin inhibitors dosages (CNI sparing protocol), avoiding renal insufficiency related to them.

Azathioprine

Azathioprine is metabolised to 6-mercaptopurine (6-MP) through reduction by glutathione, and then converted to 6- thiouric acid, 6-methyl-MP, and 6-thioguanine (6TG). These compounds are incorporated into replicating DNA and halt replication. They also block the de novo pathway of purine synthesis by formation of thio-inosinic acid. The principle side effect of azathioprine is dose-related bone marrow suppression but it may also cause occasional liver impairment and cholestatic jaundice; hepatic venoocclusive disease has also been reported. In addition, a number of hypersensitivity reactions, usually manifesting as a rash, have been reported.

Mycophenolic acid

Mycophenolate mofetil (MMF) and mycophenolate sodium (MPS) are rapidly converted in the liver to mycophenolic acid which is the active compound. The target of mycophenolic acid is inosine monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in the de novo synthesis of guanosine nucleotides, themselves essential for DNA synthesis. Most cell types can generate guanosine nucleotides by two pathways, the IMPDH pathway and a salvage pathway. Lymphocytes do not possess such a salvage pathway, hence blockade of the IMPDH pathway results in relatively selective blockade of lymphocyte proliferation. There are two isoforms of the IMPDH enzyme, the type I isoform being found predominantly on resting cells and the type II isoform being induced and expressed on activated lymphocytes. Mycophenolic acid preferentially inhibits the type II isoform of IMPDH, expressed on the activated lymphocyte population. The drug-specific side effects of MMF and MPS are

similar. The most common dose limiting adverse effect is diarrhoea, but other gastrointestinal side effects such as nausea, vomiting and abdominal pain are also common. Marrow suppression also occurs. In addition there is a suggestion from some of the clinical trials of an increased incidence of viral infections such as cytomegalovirus compared to placebo or azathioprine; candida and herpes simplex are also more common.

Calcineurin inhibitors (CNIs): cyclosporine and tacrolimus (FK506)

The molecular mechanisms whereby CNIs inhibit T cell activation are well understood. T cell receptor engagement with donor MHC/peptide normally triggers calcium dependent intracellular signalling resulting in activation of the calcium/calmodulin-dependent phosphatase calcineurin. This leads to the de-phosphorylation of NF-AT allowing translocation into the nucleus where it enhances binding of transcription factors to genes encoding for pro-inflammatory cytokines such as IL-2, IL-3, IL-4, IFN-γ and TNF-α. After entering the cytoplasm, CNIs form complexes with their immunophilins. Cyclosporine binds to cyclophilin and tacrolimus binds to the 12 kDa FK506- binding protein (FKBP-12). The CNI-immunophilin complexes inhibit calcineurin activity, and hence prevent nuclear translocation of NF-AT and cytokine gene transcription. The net result is that CNIs block the production of cytokines such as IL-2 and inhibit T cell activation and proliferation.

CNIs are associated with a range of agent-specific side effects. Many of the important side effects of CNIs are dose dependent and relate to the sites where calcineurin concentrations are highest, notably in the brain and the kidney. Cyclosporine and tacrolimus share many dose-related side effects but there are also important differences. Both drugs are associated with nephrotoxicity and this is one of the most important side effects particularly after renal

transplantation. It is due in part due to severe vasoconstriction of the afferent arteriole, with concomitant reduction in renal blood flow and glomerular filtration rate; these changes are reversible with discontinuation of the CNI. In the longer-term CNIs cause chronic non-reversible changes that are characterised by interstitial fibrosis and obliterative arteriolar changes due to fibrous intimal thickening.. Hypertension is a common consequence of CNI treatment, in part secondary to the renal effect and may be less common with tacrolimus. The neurotoxicity of CNIs may manifest in many ways, is more common with tacrolimus than cyclosporine, and is exacerbated in the presence of low serum magnesium concentration. Headache and tremor may occur, worse one to two hours following administration when the plasma concentration of the drugs are highest. Insomnia is also common. Agitation, convulsions, psychosis, hallucinations, encephalopathy and impaired consciousness are less common The metabolic effects of CNIs include diabetogenesis, which is two to four times more common with tacrolimus than cyclosporine, and may also reflect different sensitivity to the diabetogenic effects of corticosteroids. Hyperkalaemia, hyperuricaemia and hyperlipidaemia are the other common metabolic side effects and the latter may occur less frequently with tacrolimus than cyclosporine. Gingival hyperplasia and hypertrichosis are drug specific side effects of cyclosporine while alopecia may accompany tacrolimus use.

The TOR inhibitors sirolimus and everolimus

Sirolimus and everolimus belong to the group of immunosuppressive agents called mammalian target of rapamycin (mTOR) inhibitors. Sirolimus (SRL) and everolimus (EVL) bind to the12 kDa intracellular immunophilin FK506 binding protein (FKBP12) but, unlike tacrolimus, do not inhibit calcineurin activity. Instead the SRL/FKBP12 and EVL/FKBP12 complexes are highly specific inhibitors of mammalian target of rapamycin (mTOR) . MTOR

is a serine/ threonine kinase involved in the phosphatidylinositol 3- kinase (P12K)/AKT (protein kinase B) signalling pathway.

Inhibition of mTOR has a profound effect on the cell signalling pathway required for cell-cycle progression and cellular proliferation. The net effect is blockade of T cell activation by preventing progression of the cell cycle from the G1 to the S phase. In addition to their immunosuppressive effects, mTORinhibitors inhibit fibroblast growth factors required for tissue repair, which can result in wound healing problems. The same effects on smooth muscle proliferation probably underlie the effects observed on the development of intimal proliferation following angioplasty.

Everolimus and sirolimus have similar side effects, as might be expected from their similarity of structure and mode of action. The side effects are best categorised as metabolic, haematological, dermatological effects and effects related to growth factor inhibition. Principal among the metabolic effects are increases in serum cholesterol and triglycerides. Other effects include reduction in uric acid, and elevation in liver function tests. Suppression of all three blood elements, leucocytes, erythrocytes and platelet count is common, with anaemia being particularly troublesome in the presence of renal impairment and responding to supplementary erythropoietin. Skin rashes, particular acne, and mouth ulcers are more common in patients converted to mTOR inhibitors than those patients started de novo. The mouth ulcers often manifest like herpes simplex. Growth factor inhibition may well underlie the effects of sirolimus on the bone marrow. Inhibition of epithelial growth factor might contribute to the occurrence of mouth ulceration, while inhibition of fibroblast growth factor accounts for impaired wound healing. Peripheral oedema, diarrhoea and lymphocoele formation post renal transplant are also well recognised complications. Everolimus in cardiac transplantation has shown to be associated with a reduction in the incidence and severity of the coronary artery vasculopathy, as assessed by intravascular ultrasound.

Polyclonal antibodies

Polyclonal antibodies (ATG, ALS and ALG)) prepared by inoculating rabbits or horses with human lymphocytes or thymocytes are still widely used in solid organ transplantation. The purified IgG fraction contains antibodies directed against many different cell-surface molecules expressed on T lymphocytes, B cells, NK cells and macrophages .

Administration of anti-lymphocyte polyclonal antibodies results in rapid and profound lymphopaenia in the majority of patients, probably due to complement mediated cell lysis and uptake by the reticulo-endothelial system of opsonised T cells. In addition to depletion, polyclonal antibodies may cross-link the TCR, causing partial T cell activation and blockade of T cell proliferation. A so-called “first dose reaction” is seen in up to 80% of patients and may be caused by the presence of xenogeneic proteins or the initial activation of T cells following engagement of cell surface receptors and cytokine release. The most common reaction is a febrile episode, which is seen much less frequently on subsequent infusions and is thought to result from pyrogen release due to the initial large lymphocytolysis. Other reactions include skin rash, pruritis, thrombocytopenia and rarely anaphylaxic shock. To minimise these reactions, a combination of steroids, antihistamines and paracetamol are given routinely 30–60 min before starting antibody therapy.

Immunosuppressive drugs; potency and side effects

	Cyclosporine	Tacrolimus	Sirolimus	Azathioprine	Mycophenolate	Corticosteroids
Immunosuppressive potency	+++	+++±	++±	+	++	+
Nephrotoxicity	++	++	–	–	–	–
Neurotoxicity	+	++	–	–	–	–
Hirsutism/hypertrichosis	++	–	–	–	–	++
Skin rash	–	–	+	–	–	–
Diabetogenic	+	++	–	–	–	++
Diarrhoea	–	–	+	–	++	–
Hepatotoxicity	±	±	+	+	–	–
Marrow suppression	–	–	+	+	+	–

Key: –: equals no effect; +: mild (or low incidence) toxicity/potency; ++++: extreme toxicity or potency.

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The current success of organ transplantation is in very large part attributable to advances in immunosuppressive therapy and very few allografts are now lost as a result of acute rejection. Improvement in surgical technique and in immunosuppressant management have ameliorated quality of life of patients and survival years, but, nevertheless, complications are still present being related to immunological problems or, on the other hand, to side effects of immunosuppressant therapy.

COMPLICATION OF HEART TRANSPLANT

REJECTION

Acute cardiac allograft rejection represents a failure of the immunosuppressive maintenance regimen to prevent activation of immune effector cells. Almost all cardiac transplant recipients will experience episodes of graft rejection at some point postoperatively. The importance of clinical physiological monitoring, therefore, cannot be overemphasized.

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.(2,3) 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.(4)

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 (5). These processes quickly lead to diffuse graft ischemia and necrosis and might be 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.(6) 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.(2)

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.(2, 7). 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.(8). 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

through mechanisms such as cell lysis by perforin/granulolysin and the Fas/FasL pathway.(2,3). 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 (10)

Table1. ISHLT Standardized Cardiac Biopsy Grading: Acute Cellular Rejection (9)

2004		1990	
Grade 0 R[a]	No rejection	Grade 0	No rejection
Grade 1 R, mild	Interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage	Grade 1, mild A—Focal	Focal perivascular and/or interstitial infiltrate without myocyte damage
		B—Diffuse	Diffuse infiltrate without myocyte damage
		Grade 2 moderate (focal)	One focus of infiltrate with associated myocyte damage
Grade 2 R, moderate	Two or more foci of infiltrate with associated myocyte damage	Grade 3, moderate A—Focal	Multifocal infiltrate with myocyte damage
Grade 3 R, severe	Diffuse infiltrate with multifocal myocyte damage ± edema, ± hemorrhage ± vasculitis	B—Diffuse	Diffuse infiltrate with myocyte damage
		Grade 4, severe	Diffuse, polymorphous infiltrate with extensive myocyte damage ± edema, ± hemorrhage + vasculitis

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(11).

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.(12). 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.(12). Importantly, both ACR and AMR can coexist in up to 25% of acute rejection episodes. Antibody binding and complement activation is followed by

recruitment of neutrophils, interstitial edema, and intravascular thrombus and myocyte injury. 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.(12)

Table 2. ISHLT Recommendations for Acute Antibody-Mediated Rejection (AMR) (9)

	2004	1990
AMR 0	Negative for acute antibody-mediated rejection No histologic or immunopathologic features of AMR	
AMR 1	Positive for AMR Histologic features of AMR Positive immunofluorescence or immunoperoxidase staining for AMR (positive CD68, C4d)	Humoral rejection (positive immunoflourescence, vasculitis or severe edema in absence of cellular infiltrate) recorded as additional required information

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 (13). Initially the

symptoms may be non specific (fatigue, malaise, nausea or emesis, and fever) (14). 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.

INFECTION

Infection (eg, mediastinitis, pneumonia, urinary tract infection, or intravenous catheter-induced sepsis) is the leading cause of postoperative death among cardiac transplant recipients (15).- The problem appears to be most troublesome during the first 2 years following transplantation. Bacterial and viral infections are most common (47% and 41 % of infections), with fungi and protozoa accounting for 12% of post-transplant morbidity (15). The most frequent infecting organism is cytomegalovirus (15,16).

HYPERTENSION

Most cardiac transplant recipients develop cyclosporine related hypertension (17,18). Post-transplant hypertension is most likely the result of cyclosporine-induced renal vasoconstriction superimposed on chronic renal hypoperfusion (as a consequence of congestive heart failure), third-spacing of fluids (as a consequence of extracorporeal

circulation), and an abnormal distribution of blood flow (as a consequence of anesthesia and inotropic medications). Therefore, cardiac transplant recipients' blood pressure should be closely monitored,

CARDIAC ALLOGRAFT VASCULOPATHY (CAV)

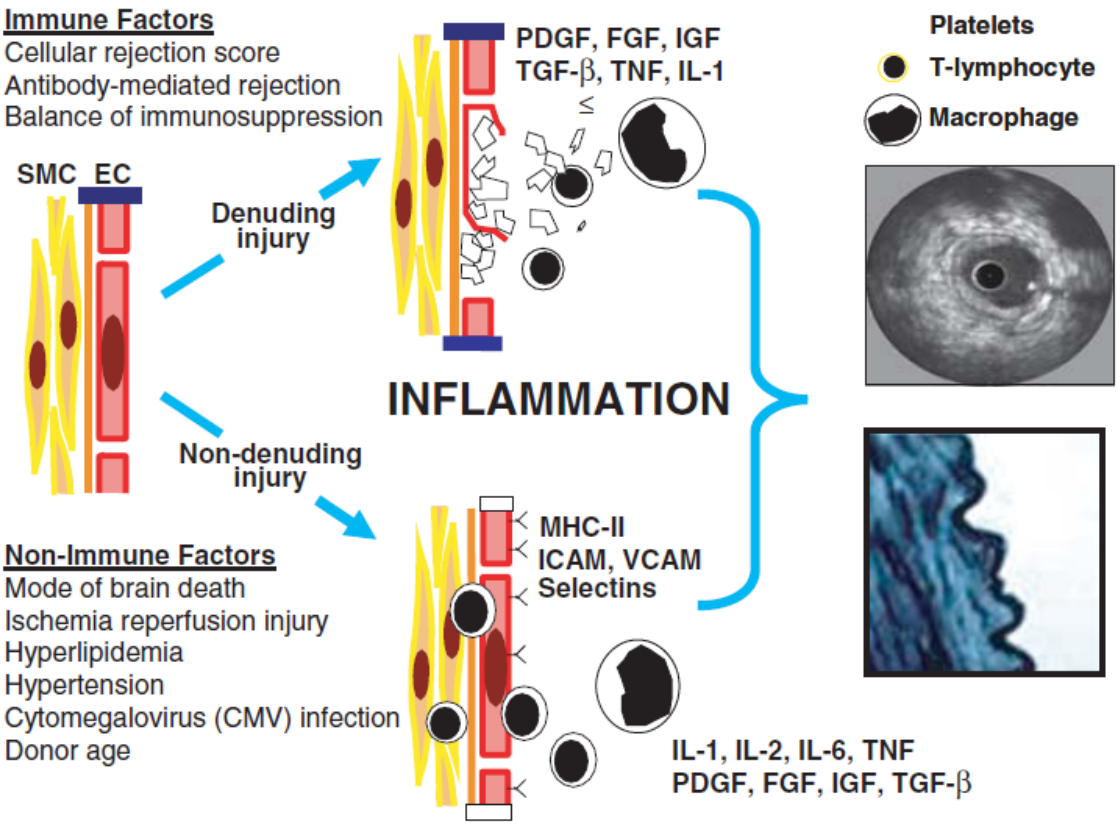
Cardiac allograft vasculopathy (CAV), characterized by a proliferation of the allograft vascular intima, remains the leading cause of death in cardiac transplant recipients at 5 years post-transplant, accounting for up to 30% of deaths (1). Due to the lack of premonitory signs, CAV often presents as sudden death, silent myocardial infarction or severe arrhythmia.

CAV Pathogenesis

CAV is initiated and propagated by both immunological and nonimmunologic insults (Figure 1) (20). Endothelial damage and inflammatory processes contribute to intimal thickening via cytokine-induced myofibroblast proliferation and fibrosis (21). Constrictive remodelling later in the disease process further contributes to the narrowing of vessels (22-24). Vascular lesions similar to those seen in CAV also occur in allografted organs other than the heart (25). This observation, supported by findings that a history of acute rejection is an important risk factor for CAV (25,26), points to the importance of immune mediators early in the post-transplant period while non-immunologic factors play a propagative role in its progression (27). It has been proposed that endothelial damage is a primary precipitating event in CAV pathogenesis (28). Tcell based responses to HLA antigens and vascular endothelial cell antigens are potential sources of endothelial damage. CD4 lymphocyte-induced upregulation of MHC class II antigens on endothelial cells (subsequent to MHC-I antigen detection by CD8 lymphocytes) elicits a cellular immune response (29). Circulating antibodies against

endothelial cell antigens such as vimentin are associated with CAV (30), providing a possible link between endothelial injury and CAV. Antibody-mediated rejection is gaining acceptance as an important factor in CAV pathogenesis (31). Nonimmunological donor factors, including advanced age, hypertriglyceridemia, hypertension, pre-transplantation coronary artery disease and greater body mass index (32), may modulate CAV progression. Physiological conditions, such as allograft peri-transplant ischemia, donor intracranial bleeding and donor explosive brain death, also are reported to increase the risk of CAV, thought to be largely mediated by the toxic effects of catecholamines. Thus, the temporal sequence of events resulting in CAV begin in the donor prior to engraftment (brain death and catecholamine surge), are propogated by perioperative events of ischemia reperfusion stress and the accompanying 'endothelialitis'. As graft-host interactions (immunological) begin to take precedence and toxic effects of anti-rejection therapy (non immunological effects such as hypertension, hyperlipidemia, renal insufficiency and obesity) are manifest, rapid propagation of CAV ensues.

Fig.1 Cardiac allograft vasculopathy pathogenesis (34)



CAV Risk Factors

Non-immune factors

I/R Injury

I/R injury plays a significant role in endothelial dysfunction and the pathophysiology of CAV.The transplanted organ is vulnerable to I/R injury induced by graft ischemic time, quality of graft preservation during transport, hemodynamic status of the donor, catecholamines used for inotropic support, and reperfusion. The episode of warm ischemia on removal of the heart from the donor, the cold ischemic interval associated with storage and

preservation of the heart, and the period of warm ischemia during engraftment, all this phenomena can induce I/R injury. Compelling evidence supports a molecular and cellular basis for a causal relationship between I/R injury duringrecipients. transplantation and the onset and progression of CAV. I/Rinjury to endothelial cells (ECs) may provide the initialtrigger for atherogenesis by stimulating platelet adhesion,release of growth factors, upregulation of major histocompatibilitycomplex (MHC) class I and II expression, release of donor antigens, expression of adhesion molecules, and proliferation of vascular smooth muscle cells (VSMCs).

Brain Death

A variety of donor-associated risk factors such as brain death can influence the short- and long-term outcomes of transplantation. This form of catastrophic central injury triggers elevated catecholamines, leading to peripheral vasoconstriction and also induces the release of hormones and proinflammatory cytokines and chemokines and adhesion molecules that are detected in the vessels of transplanted organs. Oxidative stress is also involved in brain death associated vascular injury that may contribute to CAV.

Shear Stress

Blood flow–induced shear stress acting on arterial walls plays a critical role in maintaining vascular homeostasis. Endothelial cells act as sensors of shear stress and regulate its levels by adapting the arterial dimensions to blood flow. To allow for variations in arterial geometry, such as bifurcations, shear-stress control is modified at certain eccentrically located sites to maintain low levels. In the presence of atherosclerotic risk factors, low shear stress contributes to endothelial dysfunction and plaque expansion, whereas normal-to-high shear stress is atheroprotective. Initially, lumen narrowing is prevented by vascular remodelling.

However, over an extended period, prolonged unfavorable shear stress conditions augment plaque growth. As atheromas evolve, increasing tensile stress at the shoulder regions renders plaques susceptible to fissuring and thrombosis. Although the role of shear stress in atherosclerosis is well established, its role in CAV is poorly understood.

Immune Factors

Both innate and adaptive immunity play important roles in both atherosclerosis and CAV.. Endothelial injury and activation elicits the release of proinflammatory cytokines, chemokines, and expression of adhesion molecules, which fosters immune cell recruitment and transmigration of immune cells across the endothelial cells (EC) barrier and into the intima..

Infection

Infection has been linked to the onset and progression of atherosclerosis and CAV. Infection in the context of atherosclerosis has been suggested to accelerate progression and activation of unstable plaques, while having no part in the onset of atherosclerosis. Several new studies have demonstrated

specific infections, through activation or inhibiting players of the immune response, have accelerated atherosclerosis. The link between infection and allograft rejection was first suggested in 1970. The same group later linked herpes virus infections (including CMV, herpes simplex, and herpes zoster) to rejection episodes. Subsequent associations were made with influenza and adenoviral infections; however, it is CMV which remains the hypothetical infectious player in the development of CAV. CMV positivity around the time of transplantation is a major predictor of CAV and post-transplant survival.

Hyperlipidemia

Hyperlipidemia is commonly seen in cardiac transplant patients. Many of these patients are hyperlipidemic before transplantation. In addition, the immunosuppressive therapy given to patients, especially calcineurin inhibitors, can result in, or exacerbate preexisting dyslipidemia. Hypercholesterolemia promotes fibrofatty proliferative changes to the intimal hyperplasia seen in most patients with CAV. Hypercholesterolemia, hypertriglyceridemia, HHcy, hypertension, hyperglycemia, obesity, and insulin resistance occur with a high frequency in heart transplant patients. All of these abnormalities are associated with endothelial dysfunction and atherosclerosis in the general population. The link between the metabolic syndrome and the development of atherosclerosis and CAV has been shown to be attributed, at least in part, to reduced NO availability.. In many retrospective studies, hypertriglyceridemia has been identified as a predictor of CAV, indicating that the associated insulin resistance may be important in the pathophysiology of CAV.

Acute Rejection

Acute rejection as a cause or risk factor for CAV has beeninvestigated by several authors. Some groups have reported an association between the severity and frequency of rejection and the severity of CAV; however, others have reported that episodes of acute rejection are not associated with the

development of CAV. One proposed mechanism linking acute rejection to CAV is that the inflammatory process and tissue destruction from rejection result in endothelial damage, which initiates the process of CAV or potentiates the CAV already in progress.

Donor Related Diseases

The incidence of significant donor coronary artery disease (CAD) remains low, at approximately 2%. Donor CAD can serve as a starting point for CAV and may accelerate the disease process. Donor CAD can be important in the prognosis of the transplant patient in that it can progress independently of the CAV process. The impact of native vessel atherosclerosis on CAV remains controversial.

C-Reactive Protein

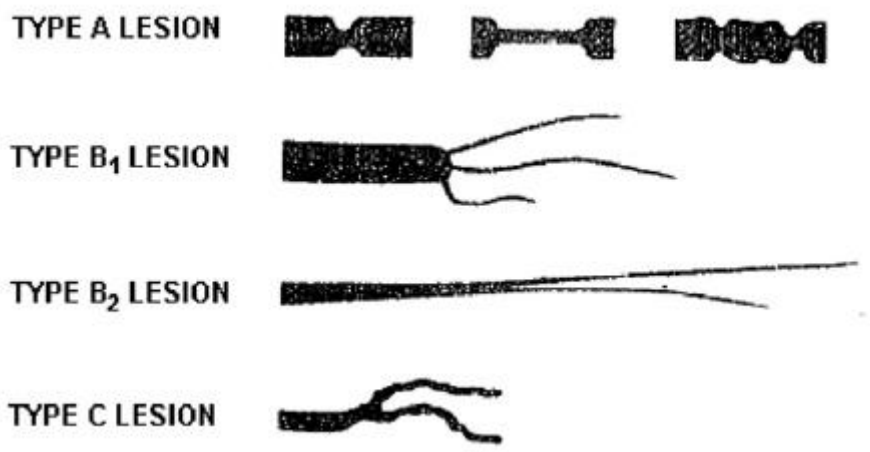
CRP is a protein marker found to be elevated in the blood during inflammation. In vitro experiments have identified several potential proinflammatory roles in cultured ECs, SMCs, and monocytes/macrophages by which CRP may promote atherosclerosis. Exogenous CRP induced the expression of adhesion molecules and decreased endothelial NOS (eNOS). Furthermore, CRP upregulates SMC angiotensin I receptors, thereby increasing reactive oxygen species and proliferation. In addition, monocytes/macrophages exposed to CRP increase release of tissue factor, potentially stimulating cell migration and adhesion to ECs and promoting the uptake of oxidized low-density lipoprotein (LDL).

Hypertension, hyperlipemia, smoking, diabetes mellitus, and other risk factors for atherosclerosis are associated with CAV.

Because CAV involves all the allograft arteries, angioplasty, stenting or bypass grafting are not practical treatment options. Therefore, CAV is the biggest long-term limitation in cardiac allograft

First description of angiographic CAV has been proposed by Gao et al (33) which coded anatomic abnormalities into type A, B1, B2, and C lesions. Type A was discrete or tubular stenosis and multiple stenoses in the proximal, middle, or distal segment branches; type B1 was a proximal vessel maintaining normal diameter with abrupt onset of distal concentric narrowing and obliteration; type B2 was a gradual transition from the normal proximal vessel with tapering, concentric narrowing progressively increasing in severity distally; and type C was a diseased vessel, diffusely irregular that lost small branches with terminations often non-tapered, squared off, and ending abruptly (Figure 2).

Fig. 2. Gao classification of CAV (33)



Anatomic coding might be useful for descriptive purposes, but it did not have prognostic value. For this reason has recently been proposed a new classification (34) based on a multi-

institutional study of 4637 postoperative angiograms at 39 centers from Costanzo and the Cardiac Transplant Research Database (CTRD) (35). Mild CAV is defined as left main (LM) < 50%, or primary vessel with maximum lesion < 70%, or isolated single-branch stenosis < 70%, or any branch stenosis < 70% (including diffuse narrowing). Moderate CAV included LM 50% to 69%, or a single primary vessel < 70%, or isolated branch stenosis <70% in branches of 2 systems. Severe CAV included LM > 70%, or >2 primary vessels >70%, or isolated branch stenosis > 70% in all 3 systems. The term "primary vessels" refers to the proximal or middle 33% of the left anterior descending, left circumflex, and dominant or codominant right coronary artery. "Branch vessels" refer to the diagonal branches, obtuse marginal branches, or the distal 33% of a primary vessel or any part of a non-dominant right coronary artery. The overall likelihood of death or retransplantation (as result of CAV) at 5-year follow-up was 7%. In patients with severe CAV, 50% experienced these end points. Therefore, this CAV classification scheme appears to have prognostic significance.

Table 3. ISHLT Classification of CAV (34)

ISHLT CAV_0 (Not significant): No detectable angiographic lesion

ISHLT CAV_1 (Mild): Angiographic left main (LM) <50%, or primary vessel with maximum lesion of <70%, or any branch stenosis <70% (including diffuse narrowing) without allograft dysfunction

ISHLT CAV_2 (Moderate): Angiographic LM <50%; a single primary vessel ≥70%, or isolated branch stenosis ≥70% in branches of 2 systems, without allograft dysfunction

ISHLT CAV_3 (Severe): Angiographic LM ≥50%, or two or more primary vessels ≥70% stenosis, or isolated branch stenosis ≥70% in all 3 systems; or ISHLT CAV1 or CAV2 with allograft dysfunction (defined as LVEF ≤45% usually in the presence of regional wall motion abnormalities) or evidence of significant restrictive physiology (which is common but not specific; see text for definitions)

Definitions

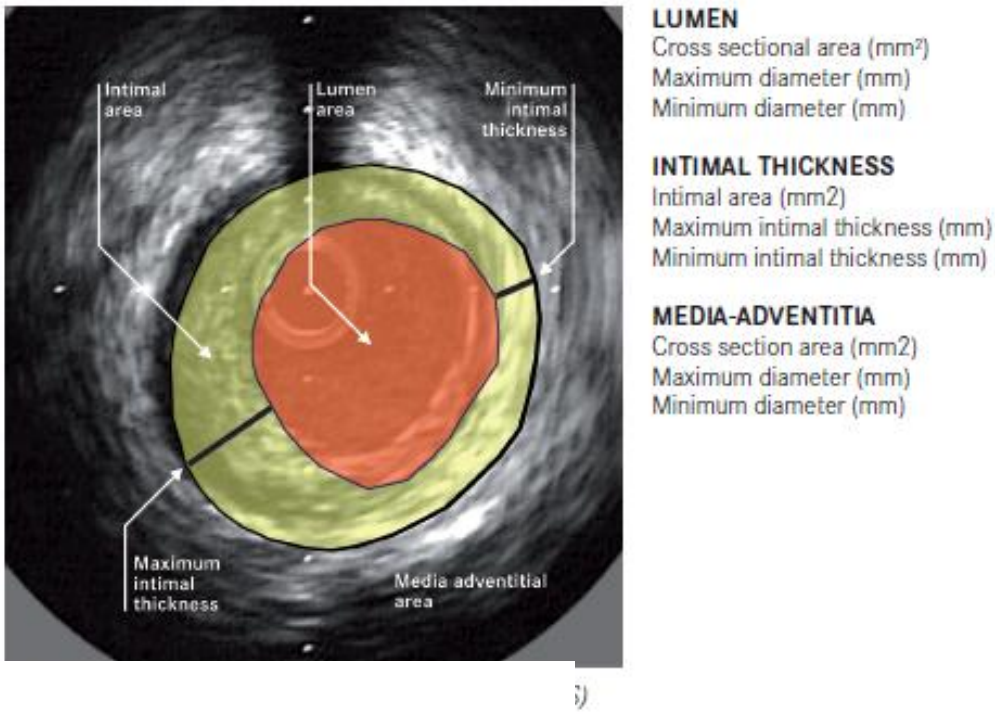
a). A "Primary Vessel" denotes the proximal and Middle 33% of the left anterior descending artery, the left circumflex, the ramus and the dominant or co-dominant right coronary artery with the posterior descending and posterolateral branches.

b). A "Secondary Branch Vessel" includes the distal 33% of the primary vessels or any segment within a large septal perforator, diagonals and obtuse marginal branches or any portion of a non-dominant right coronary artery.

c). Restrictive cardiac allograft physiology is defined as symptomatic heart failure with echocardiographic E to A velocity ratio >2 (>1.5 in children), shortened isovolumetric relaxation time (<60 msec), shortened deceleration time (<150 msec), or restrictive hemodynamic values (Right Atrial Pressure >12mmHg, Pulmonary Capillary Wedge Pressure >25 mmHg, Cardiac Index <2 l/min/m2)

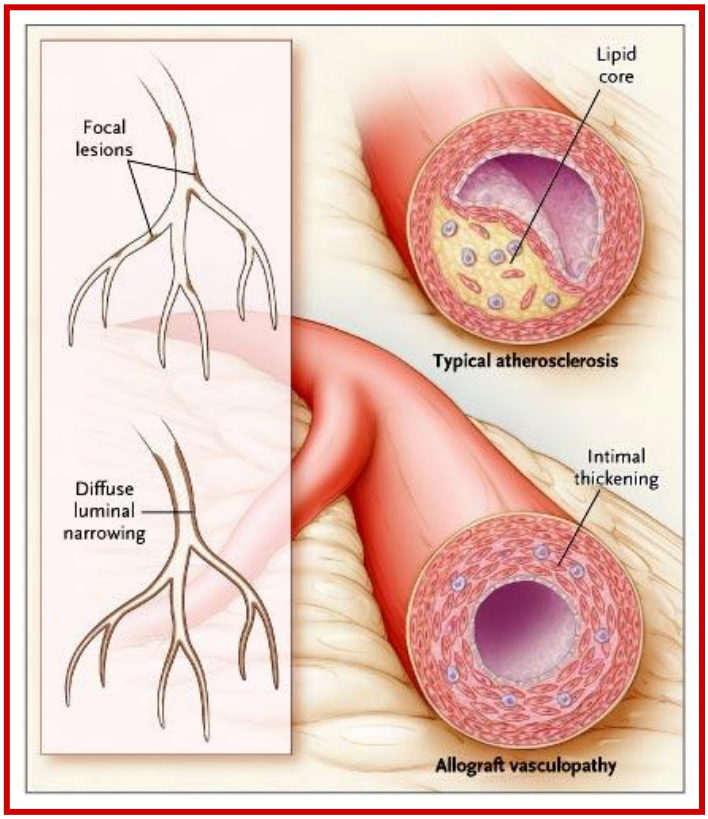
CAV is clinically silent, making early diagnosis and identification of those at greatest risk for progression an important goal. Cardiac angiography is routinely available while intravascular ultrasound (IVUS) is more commonly used in investigational settings. Angiography tends to underestimate the disease process due to the diffuse narrowing characteristic of early CAV, since it evaluates the 'hole' and not the 'wall'. IVUS can accurately characterize the magnitude of intimal thickness as a measure of plaque burden, and allow for an assessment of vascular remodelling Cardiac angiography is less invasive than IVUS but is only moderately sensitive in detecting early CAV. Dobutamine stress echocardiography is less invasive than IVUS or angiography. Although two studies have found its prognostic value to be comparable to these other diagnostic tools (36,37), another has indicated that dobutamine stress echocardiography is poorly correlated with that of IVUS and angiography (38).

Fig.3. IVUS imaging



CAV and atherosclerosis are both characterized by increased cell adhesion molecular expression leukocyte infiltration, similar ambient cytokine profiles, aberrant extracellular matrix (ECM) accumulation, and the early and protracted build up of extracellular and intracellular lipids. Intimal smooth muscle cell (SMC) migration, endothelial dysfunction and abnormal apoptosis are observed in both diseases. Emerging evidence also indicates that stem cells may play a significant role in vascular repair and remodelling in both diseases.

Fig.4 CAV versus Atherosclerosis



The histopathological similarities and differences between atherosclerosis and CAV lie in several key features: fracture of plaques; geometry of luminal narrowing; and the tempo of each disease. Both diseases display fibrofatty plaques, and, in fact, histopathologic analyses show fibrofatty plaques in CAV are indistinguishable from spontaneously occurring atherosclerosis. In CAV, a cellular infiltrate consisting of lymphocytes, macrophages, and

modified SMCs is present, especially in the intima and adventitia. On gross examination they are visible as yellow streaks that follow the direction of blood flow. In fibrous atherosclerotic plaques, lipids are present both in macrophage and SMC foam cells and in the ECM. The intima is thickened because of accumulation of SMCs and ECM proteins. Lipids and macrophages are usually most frequent in the core region, which also contains T lymphocytes and occasional B cells and mast cells. SMCs and ECM are more abundant in the subendothelial region, often forming a fibrous cap covering the lipid and inflammatory cells in the deeper part of the plaque. Although it occurs in both diseases, luminal narrowing is distinctive for each disease. Luminal narrowing in CAV is diffuse, typically concentric intimal thickening of both the major epicardial vessels and the intramyocardial vessels, with comparable severity from proximal to distal in the epicardial coronary tree. In the proximal region of epicardial arteries, the disease begins as concentric fibrous intimal thickening. In contrast, native atherosclerosis is usually a focal, eccentric proliferation of the intima of proximal coronary vessels. There is typically sparing of the intramyocardial vessels. Fatty streaks are seen initially. One of the predominant features of native atherosclerotic vessels as the disease progresses is the deposition of calcium and marked disruption of the elastic lamina. Rarely are veins involved in native atherosclerosis. Another feature is the difference in the manifestations or tempo (rate or pace of progression and severity) of these diseases. In CAV, changes in the intima can be seen as early as 1 or 2 weeks after transplantation. The lesion at this time has mild intimal thickening, early lipid insudation, mild fibrosis, and increases in ECM proteins may be present. In long-term survivors, fibrous and fibrofatty intimal lesions often diffusely involve large and small epicardial and intramural arteries.56 As the intimal disease progresses in severity so does fibrosis of the media. The major epicardial vessels are affected along their entire lengths from the base of the heart to the apex. Both arterial and venous structures can be involved by CAV. In the months following transplantation, intermediate lesions with accumulation of lipid-filled cells in the intima

develop, and atheromatous plaques with wellformed lipid cores of cholesterol clefts and free-lying lipid debris can be seen. Native atherosclerotic plaques display very different tempo as compared with AV. Eccentric intimal thickening has been observed in newborn full-term infants, in whom the thickening occurs in areas of flow turbulence; however, it disappeared after 8 months of age.The plaques appear with impunity at puberty and may continue to progress to complicated lesions.

Histopathological Characteristics

Features	CAV	Atherosclerosis
Vessel involvement		
	(1) Epicardial and intramural arteries are involved	(1) Major epicardial muscular arteries are involved
	(2) Veins can also be involved. The only vessels relatively unaffected are those with little or no muscular layer	(2) Largely affects the proximal epicardial coronary arteries. There is usually sparing of the intramyocardial vessels and arteries under muscular bridges
	(3) Diffuse and very extensive vessel involvement	(3) Veins are never involved
	(4) Affects the proximal and distal epicardial vessels, as well as their branches	(4) Three layers, intima, media and adventitia, are involved
	(5) The media can be unaffected or almost completely replaced by fibrous tissue. As the intimal disease progresses in severity so does fibrosis of the media and adventitia.	
	(6) A disease of the intima, media, and adventitia	
Lesion pattern		
	(1) Diffuse, concentric intimal thickening	(1) Focal, eccentric proliferative, and degenerative lesions of the intima of proximal coronary vessels
	(2) Ranges from concentric, diffuse, intimal lesions to advanced fibrofatty plaques with degeneration	(2) Mostly fibrofatty plaques with ultimate necrotic coves and progressively thinned fibrous cap
Initiation, progression, and complication		
	(1) The initial lesions are SMC proliferation in the intima and accumulation of extracellular lipids	(1) Fatty streaks are seen initially
	(2) Accelerated progression of intimal proliferation and luminal stenosis during the early phase of the disease, with foam cell development	(2) Slow progression of lesion development (decades)
	(3) Surface endothelial erosion is not characterized in this setting but may be a rare finding	(3) Surface endothelial erosion is seen
	(4) Fibrous cap thinning and plaque rupture is a rare finding until late in the disease	(4) Thin fibrous cap and plaque rupture are frequently seen in intermediate to advanced lesions

As it has been mentioned above, CAV remains the leading cause of death in cardiac transplant recipients at 5 years post-transplant, accounting for up to 30% of deaths. Below, see the figure showing the influence of CAV on Kaplan-Meyer survival rate In Padua Heart Transplant Centre.

Kaplan-Meier Survival Rate, by Cardiac Alograft Vasculopathy

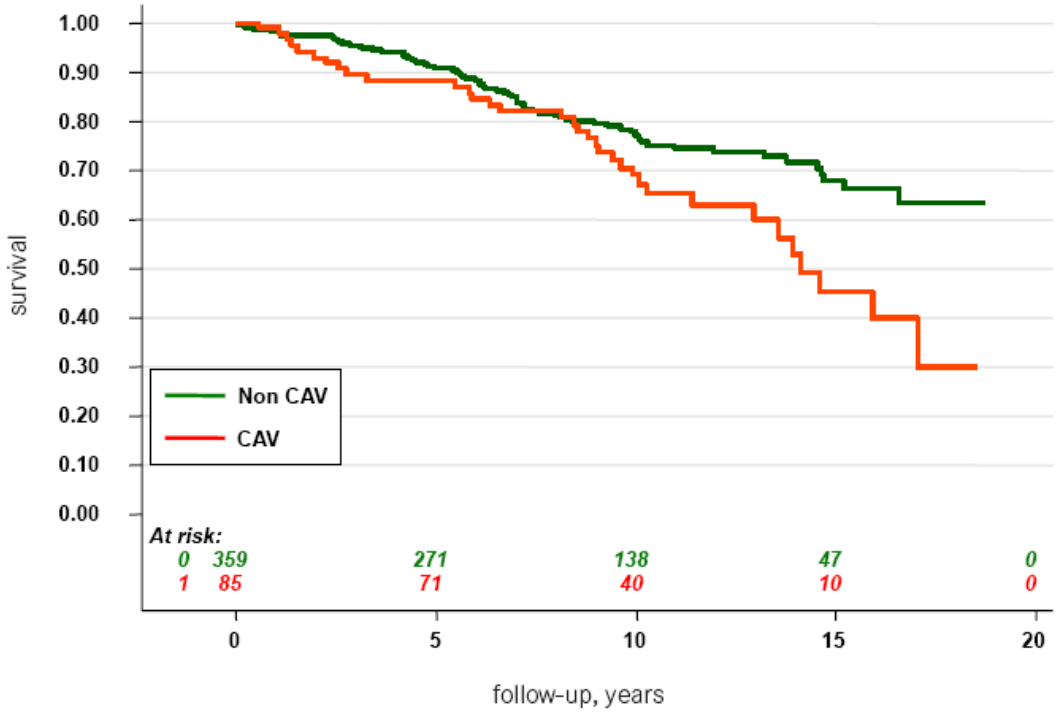


Fig. 2 - HEART TRANSPLANTS
Padua Cardiac Surgery
Kaplan-Meier survival estimate, by graft vasculopathy

ERECTILE DYSFUNCTION

Sexual behaviours and related disorders have been evaluated in patients, on waiting lists for transplantation and those already transplanted, with regard to the quality of life.(39) Sexual activity in patients with end-stage cardiomyopathy is usually reduced in a severe manner, especially in those waiting for transplantation. Questions about sexual activity are also commonly requested by patients and relatives before and after cardiac transplantation, even if

patient collaboration is often inadequate and methodology of examination is obviously limited. The main reported sexual

dysfunction in males is erection, while a rise in libido is also described. Furthermore, still unclear are the role of the pharmacological side effects, the emotional status of the patient, and the affective relationship satisfaction.

Erectile dysfunction (ED) is defined as the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance(40). ED is highly prevalent and by current estimates, 30 million men in the US and 150 million men worldwide are affected (41) and occurs in 19%−64% of men aged 40–80 years, both in developing and industrialized countries. Emotional, physical, and medical factors contribute to ED, and this condition may also be a symptom of various chronic diseases. ED may affect total health, relationships, and overall quality of life (42). Organic causes constitute more than 80% of clinical presentations. Associations include diabetes mellitus, cardiovascular disease, hyperlipidemia, cigarette smoking and obesity, indicating their significance as a public health problem. Furthermore, the disorder is correlated with anxiety, depression, interpersonal relationship difficulties and even violence (43). It is widely known that ED is associated with diseases reported to be related with decreased NO bioavailability such as arterial hypertension, hypercholesterolemia and diabetes. Organic and psychogenic factors may cause alterations in the NO/cGMP pathway and impair smooth muscle relaxation and/or increase smooth muscle contraction, thereby resulting in ED.

Penile erection involves a complex interaction between the central nervous system and local factors. The penis is innervated by autonomic (sympathetic and parasympathetic nerves) and somatic nerve

fibers. Overall, erection is a neurovascular event modulated by psychological and hormonal factors. Upon sexual stimulation, neurotransmitters are released from the cavernous nerve

terminals and also vasoactive relaxing factors from the endothelial cells of the penis, which relax arteries and arterioles supplying the erectile tissue, increasing the penile blood flow. Concomitantly, relaxation

of the trabecular smooth muscle increases the compliance of the sinusoids, resulting in an engorgement of the penis with blood. Therefore, penile erection takes place when both dilation of

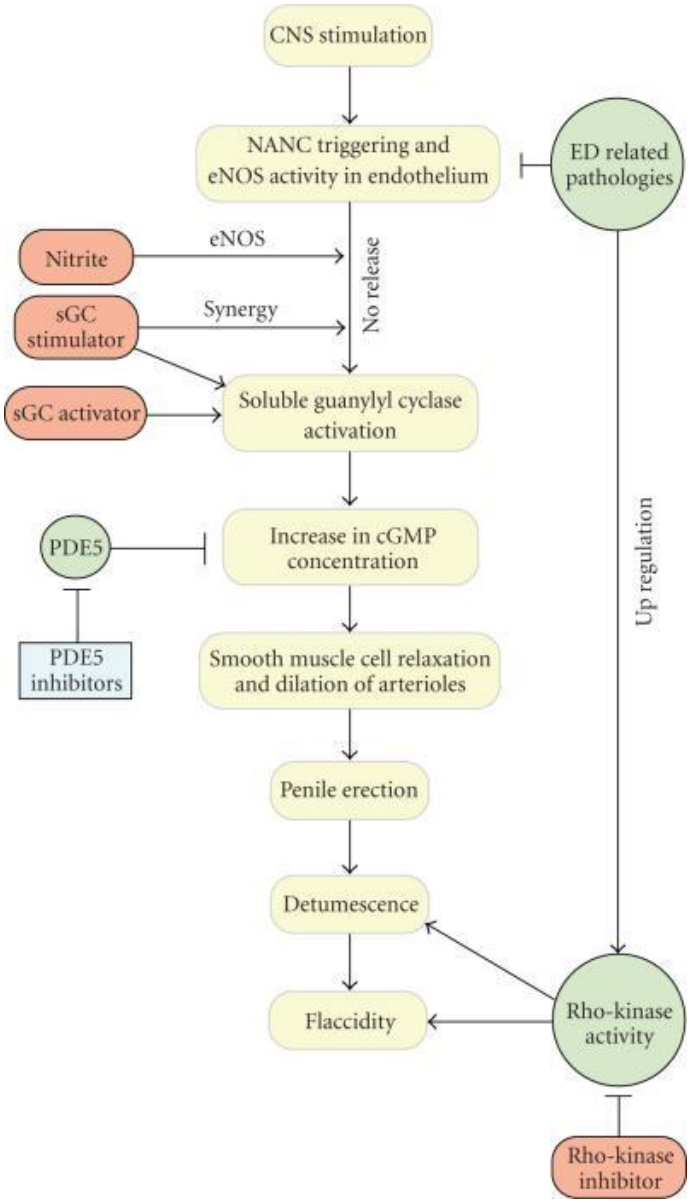
the penile arteries and relaxation of the erectile tissue occur. Because the erectile tissue is surrounded by the tunica albuginea, a tissue that does not distend easily, the increased blood flow to the penis increases not only the penile volume but also intrapenile pressure. This distension causes mechanical compression of the emissary veins, which impedes their ability to drain blood and thereby results in penile rigidity. Detumescence is the result of a cessation of neurotransmitter release, the breakdown of second messengers or sympathetic discharge during ejaculation. Contraction of the trabecular smooth muscle restores the venous outflow, the trapped blood is expelled, and flaccidity returns**.** The nerves and endothelium of sinusoids and vessels in the penis produce and release transmitters and modulators, which interact in their control of the contractile state of the penile smooth muscles. The different structures of the penis are functionally regulated by efferent sympathetic and parasympathetic nerves, and the major neurotransmitters in postganglionic fibers are norepinephrine and acetyl-choline, respectively. Sympathetic input is antierectile, whereas parasympathetic and somatic input are proerectile. Both sympathetic and parasympathetic fibers reach the pelvic or inferior hypogastric plexus where autonomic input to the penis is integrated; the cavernous nerves originate from this plexus, and innervate the helicine arteries and erectile tissue. Intracavernous nerves are encased in fibrous tissue, which prevents their compression during an erection. The dorsal penile nerves, branches of the pudendal nerves, and the ilioinguinal nerve also innervate the penis. These nerves provide sensory input from the glans penis and skin, and penile root (44).

Several investigators provided evidence for functional roles of nonadrenergic noncholinergic (NANC) inhibitory and excitatory nerves, containing transmitters and transmitter/modulator-generating enzymes, such as nitric oxide synthase (NOS) and heme oxygenases (HO). NANC transmitters/modulators may be found in adrenergic and cholinergic nerves(45). Although various polypeptides have been regarded as inhibitory neurotransmitters, the discovery that nitric oxide (NO) functions as a mediator synthesized in and released from the vascular endothelium (46) and as a neurotransmitter in inhibitory nerves innervating the penis represented a breakthrough in the comprehension of the neurophysiological basis of erection.

Synthesis of NO and the consequences of NO binding to soluble guanylyl cyclase is essential for the erectile process. Identification of NO to be a neurotransmitter has been achieved by the use of NOS inhibitors in the corpus cavernosum of the penis (47). NO, an inorganic and labile molecule, is liberated immediately upon synthesis by neuronal NOS (nNOS) from substrate *L*-arginine. To date, it is widely accepted that NO is the main neurotransmitter mediating penile erection, which is released during NANC neurotransmission. Upon its release, NO diffuses locally into adjacent smooth muscle cells of the corpus cavernosum and binds with its physiologic receptor, soluble guanylyl cyclise (48). The enzyme becomes activated following this interaction where upon the enzyme catalyzes the conversion of guanosine triphosphate (GTP) to 3',5'-cyclic guanosine monophosphate (cGMP). This cyclic nucleotide serves as a second-messenger function by activating protein kinase G, alternatively known as cGMP-dependent protein kinase I (cGKI), which in turn exerts actions involving ion channels and contractile regulatory proteins that regulate the contractile state of corporal smooth muscle. The consequence is the decay in cytosolic calcium concentration and relaxation of the smooth muscle, resulting in dilation of arterial vessels and increased blood flow into the sinuses of the corpora cavernosa (49). Thus, at the onset of sexual stimulation, neuronal NO induced by neuronal depolarization and endothelial NO largely generated in response to shear forces brought on by increased blood flow in the penis serve, respectively, as a neurotransmitter

initiating the erectile process and as a paracrine factor sustaining the full physiologic response. On the other hand, phosphodiesterase-5 (PDE5) operates in this signal transduction pathway to restrain erectile effects. This enzyme is predominantly expressed in the corpus cavernosum (50) and functions as a cGMP-specific phosphodiesterase, which catalyzes the hydrolysis of cGMP to GMP. Accordingly, in the penis, the enzyme controls cGMP accumulation caused by NO signaling and consequently limits its relaxant actions.

Fig.5 Pathway for the control of penile erection and detumescence (51)



Stimulation of erection originates in the higher centers of the brain: upregulation of NANC and cholinergic activity and withdrawal of sympathetic activity in the nerves innervating the corpora cavernosa and small arteries of the penis.

This increase in NANC and cholinergic activity results in upregulated NO release from the endothelium and NANC nerve terminals. The NO diffuses into the smooth muscle of the corpora cavernosa and small arteries/arterioles of the penis and binds to the reduced heme iron of soluble guanylate cyclase, activating the enzyme and increasing the formation of cGMP from GTP. cGMP-dependent protein kinase activity opens potassium channels in smooth muscle cells and increases the uptake of calcium into stores. This leads to a decrease in intracellular calcium concentration and smooth muscle cell relaxation. This increases blood flow into the corporal sinuses and the cavernosal sinuses expand trapping blood in the corpora producing a fenile erection

.

Fatigue, endocrine changes and psychogenic disorder combined with arterial disease lead to a high prevalence of erectile dysfunction (ED) in end stage cardiac disease (52).

With the markedly increasing of 5 and 10-year survival rate, ED becomes an important factor with an impact on the quality of life of male recipients (53,54). Furthermore vascular ED is the expression of a systemic vascular disease and in particular of endothelial dysfunction (55). As it has been shown above, emerging evidence indicates that dysfunctional endothelium plays also a significant role in the onset and progression of coronary artery vasculopathy (CAV) in HTx.(56).

Currently, assessment of arterial inflow to the corpora cavernosa is mainly based on ColorDoppler ultrasonography by which it has been shown that the evaluation of cavernous intima-medial thickness (IMT) can be a new parameter able to identify a vascular pathogenesis of ED in a earliers phase when endothelium becomes dysfunctional (57).

Although ED was pointed out with a high prevalence in HTx since 1978 by Barnard et al (58), nowadays few studies have evaluated this symptom in HTx.

ENDOTHELIAL PROGENITOR CELLS (EPC) AND ENDOTHELIAL DYSFUNCTION

Endothelial progenitor cells (EPCs) are circulating bone marrow derived cells which contribute to endothelial cell regeneration and neovascularisation [1]. Although the exact nature of the circulating cells that efficiently promote vascular repair is still uncertain, most

studies to date have focused on circulating cells that are positive for haematopoietic stem cell markers (CD34, CD133) or have the ability to form endothelial-like cells in culture.

Endothelial progenitor cells (EPCs) have endothelial reparative properties by localizing to sites of vascular injury and may contribute to allograft rejection and CAV (59). Normally, the endothelium undergoes dynamic processes of degeneration and regeneration. While EPCs play an important role in this homeostatic mechanism, slight imbalances in the process may cause endothelial dysfunction (59). During vascular injury, circulating EPCs adhere to the vascular wall and replace endothelial cells that have been shed, which promotes healing and prevents plaque formation (60-62). Clinical observational studies (63) demonstrate that the number and migratory activity of circulating EPCs inversely correlate with the number of risk factors for coronary artery disease in which the generation of collateral vessels is reduced. Additionally, both chronic ischemic heart disease and congestive heart failure (CHF) are known to suppress the mobilization of EPCs (64,65) and have an impaired neovascularisation capacity (64). Clinically, the level of circulating EPCs not only correlates with cumulative cardiovascular risk,(66) but it also predicts future cardiovascular events and atherosclerotic disease progression in patients with coronary artery disease (67, 68). In addition, circulating EPCs recently have been observed to increase in the early phase after acute myocardial infarction (AMI) (69). Furthermore, persistent mobilization of EPCs from bone marrow into circulation is associated with favorable left ventricular (LV) remodeling as is evidenced by a reduced LV dilatation and an enhanced contractile recovery after AMI.(70). Numerous studies have demonstrated that increased EPC levels are associated with better cardiovascular outcomes and a reduction of neointimal hyperplasia (65-68).

In transplant physiology, as a result of persistent allograft antigenicity, this EPC homeostatic mechanism may be uncontrolled and pathological. Allograft rejection and CAV may be a result of defective EPC repair mechanisms secondary to allograft recognition (59). A number

of studies have demonstrated a detrimental role of EPCs post-transplant through contribution to allograft rejection and CAV (59, 71, 72) demonstrating EPC seeding at CAV plaque sites, suggesting that circulating EPCs contributed to plaque formation, thus depleting the systemic EPC pool. Endothelial progenitor cells may overwhelm the endothelium causing intimal hyperplasia, giving a plausible explanation for their role in the progression of CAV (59). Also, EPCs may carry the ability to differentiate into both endothelial and smooth muscle cells, making it possible for them to re-endothelialize vessels and cause smooth muscle hyperplasia simultaneously (73,74). This lends more plausibility to the proposed mechanism of CAV, suggesting that EPCs may contribute through preferential differentiation into smooth muscle cells as opposed to endothelial cells.

Until recently, it was believed that progenitor cells contributing to allograft rejection and CAV arose from the allograft itself, namely cells from the local vessel wall. However, recent evidence suggests that recipient-derived cells occupy allograft endothelium (72,75,76). Hillebrands et al. (75) demonstrated the replacement of graft endothelial cells with circulating host-derived cells through the use of a sex-mismatched rat HT model., Hu et al. (76), utilizing a murine model, showed that as allograft age increases, allograft cells are replaced with recipient-derived cells. Furthermore, Simper et al. (72) illustrated the seeding of recipient-derived endothelial progenitors in donor coronary arteries and areas of CAV in human HT patients. Therefore, the idea of a circulating host-derived

progenitor cell that is responsible for allograft rejection and CAV development is well supported and could have numerous clinical implications.

Recent advances in immunosuppressive therapy have allowed for more effective treatment and prevention of allograft rejection. Though many agents have been successful in preventing acute rejection, few have been able to protect against late-stage transplant vasculopathy. Only mammalian target of rapamycin inhibitors (mTORi), such as sirolimus and everolimus, have been shown to attenuate CAV (77, 78). These agents prevent lymphocyte proliferation via

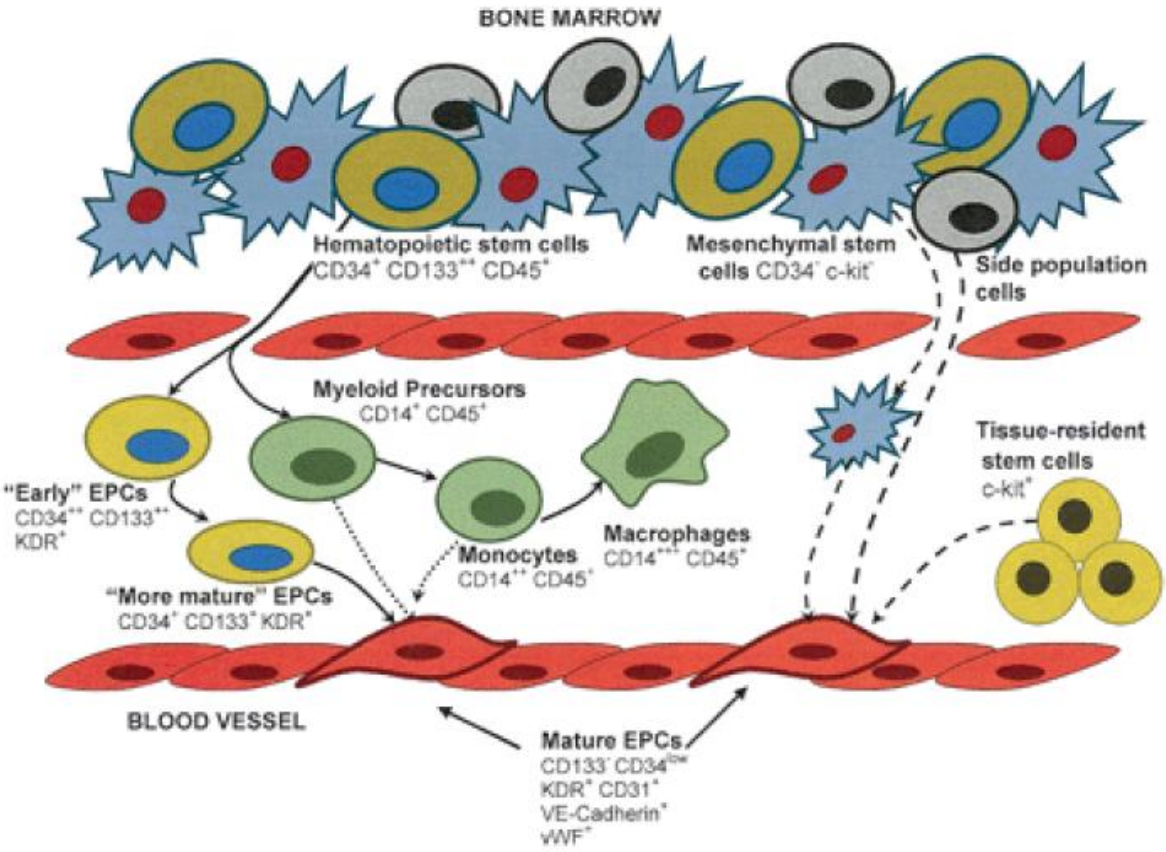
inhibition of the cell-signaling molecule mammalian target of rapamycin (mTOR). Furthermore, it has been shown that sirolimus is cytotoxic to EPCs in vitro; in contrast to cyclosporine and tacrolimus (79).

Given that mTORi slow CAV progression and have a detrimental effect on EPCs, it is possible to hypothesize that decreasing EPC function may represent a mechanism by which mTORi protect against rejection and potentially CAV.

To date, the true molecular phenotype of EPC is still uncertain, but the majority of the scientific letterature, defined them as CD34+, CD133+ and KDR+ cells (86). Recently a subpopulation of EPC has been described, marked with osteocalcin (OCN), a typical osteo-related protein (87). It has been demonstrated that this population of EPC-OCN+ cells is increased in patients with coronary atherosclerosis, suggesting a pathophysiological role of them in vascular calcification (88) . Foresta et al have demonstrated, according to this hypothesis, that patients affected by erectile dysfunction display a progressive reduction of circulating EPC with the severity of cavernous artery atherosclerosis and a progressive increase of OCN+-EPC (9).

PC, EPC and OCN+-EPC

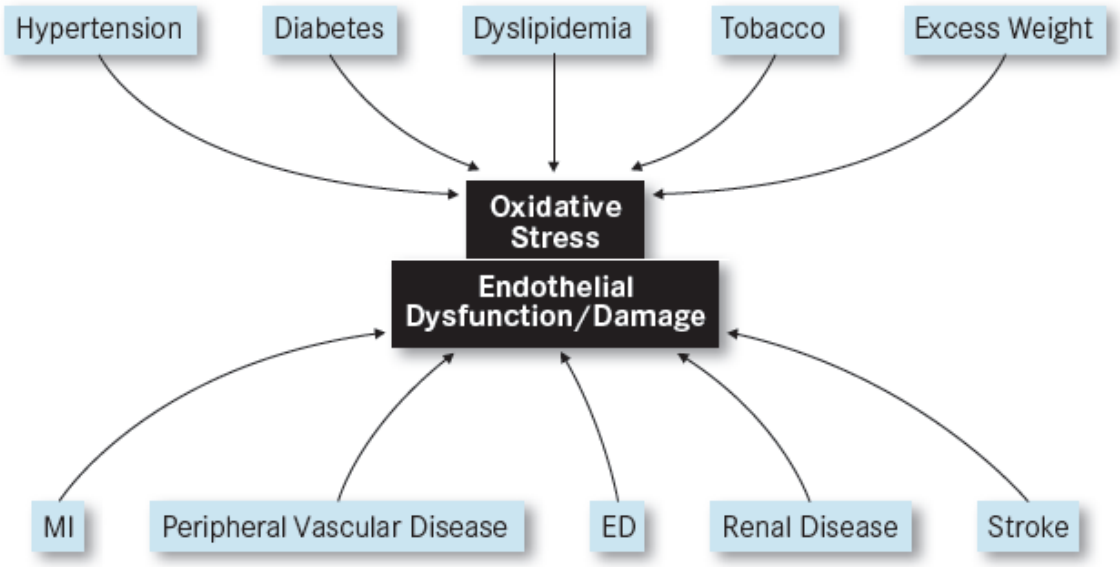
Fig. 6 PC, EPC and OCN-EPC



Endothelial dysfunction may be considered as a systemic disorder and involves different vascular beds.(80, 81) Coronary endothelial dysfunction precedes the development of coronary atherosclerosis (82). Endothelial dysfunction is characterized by a reduction in endogenous nitric oxide (NO) activity, which may be attributed to an elevation in asymmetric dimethylarginine (ADMA) levels (83) ADMA is a novel endogenous competitive inhibitor of NO synthase (NOS) and has been shown to be an independent marker for cardiovascular risk. (84) Thus, the elevation of endogenous ADMA may be associated with the systemic manifestations of endothelial dysfunction

in patients with cardiovascular risk factors. Erectile dysfunction (ED), as we have already mentioned, is a common phenomenon in men with established CAD and is believed to be

another manifestation of atherosclerotic vascular disease. In fact, recent data demonstrate that ED is associated with impaired endothelial-dependent flow-mediated vasodilation in the brachial artery (85) suggesting that ED is associated with peripheral endothelial dysfunction.



AIMS OF THE STUDY

This study has started from the clinical need of facing a problem described in heart transplanted patients. Patients in heart failure often refer sexual disorders and erectile dysfunction, due to the reduced cardiac output. After transplantation both female and male patients progressively "re-born" experiencing feelings that, as they usually say, they had forgotten.

Usually it happens, in the first months after transplantation, that patients recuperate also libido and a normal sexual behaviour but after a variable time they often are frustrated by the comparison of erectile dysfunction.

For this reason, in this study, we decided to investigate on the possible causes of this phenomenon, with the principle purposes of understanding the physio-pathologic mechanism underlying it, ameliorating quality of life of patients.

There are just a few studies, as mentioned above, that evaluate sexual behaviour in transplanted patients; furthermore there are no studies that correlates peripheral vasculopathy (epi-aortic, femoral and penile) with cardiac allograft vasculopathy, that it is still one of the Achille's heel of heart transplant complications.

Purposes of this study are:

1. Evaluation of prevalence of erectile dysfunction after heart transplantation and analysis of related risk factors;

2. Evaluation of the correlation between cardiac allograft vasculopathy, erectile dysfunction and peripheral vasculopaty eventually present

3. Quantification of progenitor cells (PC), endothelial progenitor cells (EPC) and osteocalcin- positive endothelial progenitor cells (OCN-EPC) in male patients after heart transplant;

4. Analysis of the potential correlation between cardiac and penile endothelial dysfunction trough the quantification and characterization of EPC and OCN-EPC;

5. To ameliorating quality of life of male heart transplanted patients by using appropriate pharmacologic treatments and evaluation of therapy on EPC.

MATERIALS AND METHODS

POPULATION

Between march 2009 and june 2010,consecutively 77 male patients undergone to heart transplant in different periods in Padua Heart Transplant Centre have been enrolled in the study. Patients were recruited after informed consent.

Inclusion criteria :

- Age between 18 and 80 years
- Donor cardiac angiography negative for stenosis
- Absence of severe renal insufficiency needing of haemodialysis (creatinine levels < 250 μmol/L)
- Absence of neurologic diseases able to negatively influence social relationships of the patients
- Ejection fraction measured by ultrasound imaging ≥ 45%

Exclusion criteria:

- Age < 18 years and > 80
- BMI > 30

- Hyperprolactinemia
- La Peyronie's disease
- major pelvic surgical interventions
- major psychiatric diseases
- cardiac ejection fraction (FE) <45%.

At the follow-up at Padua Heart Transplant Centre, each patient has been evaluated by accurate medical history, physical examination and ECG; after informed consent all the followed exams have been done:

Instrumental evaluation:

- Left cardiac catheterization, with angiography and ventriculography;
- Coronary flow reserve evaluation by ultrasound with adenosine
- Andrologic examination, with administration of IIEF-5 and penile echocolordoppler ultrasonography;
- Evaluation of peripheral vasculopathy by epi-aortic and femoral echocolordoppler ultrasonography;

Blood test:

- Hematologic test
- Biochemical analysis
- Lipid profile: total cholesterol, high-density lipoprotein cholesterol, triglycerides
- fasting glucose

- hormonal profile (prolactin, total testosterone, PTH, Insuline, osteocalcin, IGF1, SHBG, estradiolo)
- Proteic profile
- Bone metabolic index: 1-25vit D, vit D3, blood and urine P e Ca, deossipiridinolina
- Glycate- Hemoglobin, omocistein

Table 4. Population Variables Analysis

Variable	Average Value or (%)	Range
Age	61.6 ± 10.6	30.9-78.5
Time from heart transplant(HT)	9± 6.2	1.1-23
Transplant cause	36%	
DE before HT	24% (19/77)	
DE after HT	67% (50/77)	

Table 5. Risk Factors:

Variable	%
Smoke	61
Hypertension	85
Diabetes	16
Hypercolesterolemia	77
Familiar Cardiac disease Hystory	6.6

Table 6. Population Parameters Value

Variable	Average Value or (%)	Range	Normal Range
BMI Weight(Kg)/h(m^2)	26,09 ± 3,59	20,52 - 36,33	>25 over weight
EF	63,42% ± 8,75	21 - 88	21 - 88
Tot Colesterol (mg/dL)	198,2 ± 43,51	126 - 403	< 200
LDL (mg/dL)	122,67 ± 39,1	70 - 186	<130
Presence of coronaropathy	35%		
Testosterone (nmol/L)	14,67 ± 5,57	4,75 - 39,14	10-29

Table 7. Percentage of immunosuppressant drugs

Immunuppressant therapy	%
Cyclosporine	100
Azathioprine	19
Everolimus	19
Corticosteroids	42
FK-506	1.3
MMF	42

Table 8. Other therapy percentage

Therapy	%
Beta-blockers	12
Ca-antagonist	28.6
ACE-inhibitors	38
Sartans	23
Furosemide	67
K-Kanrenonoate	8
Doxazosin	38
ASA	66
Allopurinol	62
Statins	59
Ticlopidine	5
Insuline	9
Oral hypoglicemic drugs	7

INTERNATIONAL INDEX OF ERECTILE FUNCTION-5 (IIEF-5)

Erectile dysfunction has been analyzed by administration of the International Index of erectile dysfunction, IIEF-5, which includes five questions of IIEF-15 and scores < 21 were considered as diagnostic for ED .ED is indicated as severe (1-7), moderate (8-11), mild-moderate (12-16), mild (17-21), not present (22-25) (see table 4) .

ULTRASOUND

All subjects were studied with penile (P-CDU), carotid, femoral echocolordoppler ultrasonography.

Penile-color Doppler ultrasound (P-CDU) was performed with a high resolution Echo-Color-Doppler (iU22, Philips, Netherlands) after intracavernous injection of 10 mg alprostadil as previously described (7). Cavernous intima media thickness (IMT) was measured choosing the best rectilinear portion at low magnification. Afterwards, the selected portion was studied at high magnification (24 X zoom), regulating the partial and total B-mode gain to reduce the noise at minimum level. The degrees of cavernous artery stenosis were quantified by a double measurement of the lumen, respectively, in the thinner tract of the stenosis and in the closer site not involved in the lesion. Arterial wall measurement was repeated three times: mean values were considered for statistical analysis. A cavernous plaque was defined as an IMT > 0.4 mm or a two-fold thickening compared to the closest tracts. Cavernous artery alterations, according to previous published data (57), were sub-divided into:

- normal cavernous IMT (IMT < 0.3 mm);
- increased cavernous IMT (0.3 ≤ IMT < 0.4);

- cavernous plaque (IMT ≥ 0.4)

US study of carotid and femoral districts, including bilateral repeated measurements of common carotid and femoral artery IMT, were performed as previously described (90). According to literature, a value of carotid m-IMT < 0.9 mm was considered normal, whilst a 0.9–1.2 mm range of m-IMT was defined as increased thickness and m-IMT ≥ 1.3 mm was defined as plaque (91).

Every arterial wall measurement (cavernous, carotid and femoral artery) was repeated three times :mean values were considered for statistical analysis. The degree of stenotic tracts of the cavernous, carotid, femoral artery were quantified by a double measurement of the lumen, respectively in the exact tract of the stenosis and in the neighbour site not involved in the stenosis.

CARDIAC ANGIOGRAPHY

This procedure is performed in a catheterization laboratory by an expert interventional cardiologist. After cannulation of peripherial artery (femoral or radial), coronary ostia are selectively cannulated by a small diameter catheter under X- ray guidance. Contrast media is injected through the catheter to selectively visualize the main coronaries and their branches by different standardized projections. CAV was diagnosed anytime a coronary disease with a degree of ISHLT CAV ≥ 1 was found (34)

ECHOCARDIORAPHY

An echocardiogram was obtained in all patients within 48 h of coronary angiography. From the parasternal long-axis view, M-mode measurements were performed to determine the end-diastolic thickness of the interventricular septum and the left ventricular posterior wall. Left ventricular hypertrophy was defined as a septal plus posterior wall thickness ≥24 mm (92). Left ventricular ejection fraction was measured using Simpson's method. CFR was evaluated using CE-TTE before and after adenosine infusion, as described (93)

Contrast-enhanced transthoracic Doppler echocardiography

Echocardiography was performed for coronary flow evaluation using CETTE before and after adenosine infusion, with an ultrasound system (Sequoia C256, Acuson, Mountain View, CA) connected to a broad-band transducer with second harmonic capability (3V2c). Briefly, CFR was measured in the distal portion of the LAD, first obtaining a modified foreshortened two-chamber view or, if a distal LAD flow recording was not feasible, using a low parasternal short-axis view of the base of the heart (93). Administration of the contrast agent (Levovist, Schering AG, Berlin, Germany) was performed both before and during adenosine intravenous administration (94).

CORONARY FLOW VELOCITY RESERE ASSESSMENT

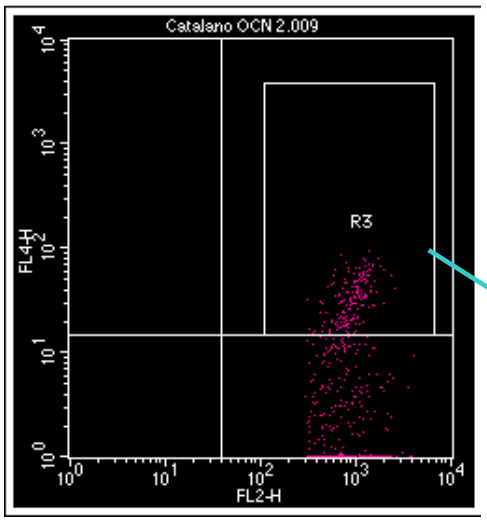
All patients had Doppler recordings of the LAD with adenosine infusion at a rate of 0.14 mg/kg/min for 5 min (94). Cardiac drugs were not interrupted before testing, although all

methylxantine-containing substances or medications were withheld 48 h before the study. CFR in the LAD was calculated, as the ratio of hyperemic to basal diastolic flow velocity, by an experienced echocardiographer, blind to angiographic and clinical data. For each variable in the CFR calculation, the highest three cycles were averaged (94)

DETERMINATION OF CIRCULATING EPC

Circulating levels EPC and OCN+-EPC were evaluated by flow cytometry. All incubations with antibodies were carried out for 15 min, at room temperature in the dark, followed by extensive washing with phosphate buffered saline. 450 μL of whole blood sample were incubated with biotin-coniugated monoclonal antibody anti-human KDR (Sigma-Aldrich, Milan, Italy) and goat anti-human OCN antibody (V-19, Santa Cruz Biotechnology, Santa Cruz, California). After further incubation with phycoerythrin-conjugated monoclonal antibody anti-human CD133 (Milteny Biotec, Bergisch Galdbac, Germany), streptavidin-fluorescein isothiocyanate (Sigma-Aldrich), and phycoerythrin-cyanine7-labeled donkey anti-goat immunoglobulins (Santa Cruz Biotechnology). Red cell lysis was then performed by treatment with Pharmlyse reagent (Becton Dickinson) for ten minute at room temperature in the dark. Samples were finally washed, resuspended in PBS and analyzed with a FACSCalibur flow cytometer (Becton Dickinson). CD34+ mononuclear cells were gated in a proper dot plot and then evaluated for the expression of CD133 and KDR. EPC were considered as the cell population simultaneously positive for all 3 markers. Finally, OCN+-EPC were defined as the OCN-positive fractions of EPC: Data are expressed as cells per millilitre of peripheral blood (cells/mL

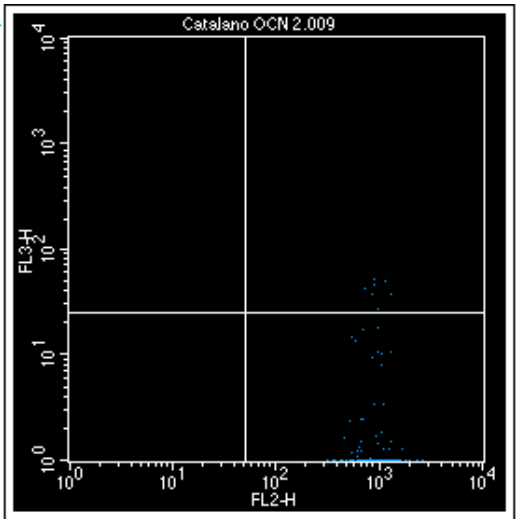
PC and OCN+-PC



Gate: G3
Gated Events: 503
Total Events: 657150
X Parameter: FL2-H (Log)
Y Parameter: FL4-H (Log)
Quad Location: 39, 15

Quad	Events	% Gated	% Total
UL	0	0.00	0.00
UR	167	33.20	0.03
LL	0	0.00	0.00
LR	336	66.80	0.05

CD34^{+}/CD133^{+}

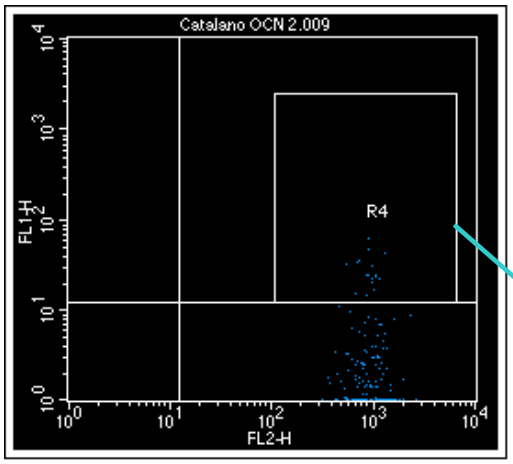


Gate: G4
Gated Events: 169
Total Events: 657150
X Parameter: FL2-H (Log)
Y Parameter: FL3-H (Log)
Quad Location: 51, 25

Quad	Events	% Gated	% Total
UL	0	0.00	0.00
UR	7	4.14	0.00
LL	0	0.00	0.00
LR	162	95.86	0.02

CD34^{+}/CD133^{+}/OCN^{+}

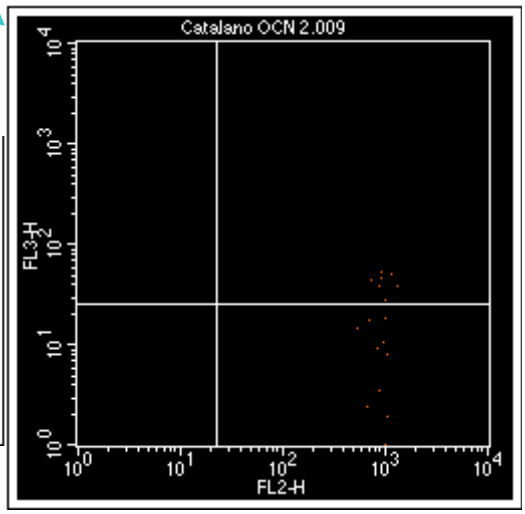
EPC and OCN+-EPC



Gate: G4
Gated Events: 169
Total Events: 657150
X Parameter: FL2-H (Log)
Y Parameter: FL1-H (Log)
Quad Location: 12, 12

Quad	Events	% Gated	% Total
UL	0	0.00	0.00
UR	17	10.06	0.00
LL	0	0.00	0.00
LR	152	89.94	0.02

CD34$^+$/CD133$^+$/KDR$^+$



Gate: G5
Gated Events: 17
Total Events: 657150
X Parameter: FL2-H (Log)
Y Parameter: FL3-H (Log)
Quad Location: 22, 25

Quad	Events	% Gated	% Total
UL	0	0.00	0.00
UR	7	41.18	0.00
LL	0	0.00	0.00
LR	10	58.82	0.00

CD34$^+$/CD133$^+$/KDR$^+$/OCN

STATISTICAL ANALYSIS

Quantitative variables were expressed as mean ± standard deviation and categorical variables as percentage. Comparison between groups was performed by Student's *t* test or Wilcoxon test for quantitative data and with chi-square test or Fisher test for categorical data. P values <0.05 were considered statistically significant. All statistical analyses were performed using the SAS package, rel. 9.02 (Cary, SAS Institute

Table 9.

IIEF-5

1. How do you rate your confidence that you could get and keep an erection?	Score
- Very low	1
- Low	2
- Moderate	3
- High	4
- Very High	5

2. When you had erections with sexual stimulation, how often were your erections hard enough for penetration?	
- I am currently not sexually active	0
- Never or almost never	1
- A few times (less than half of the attempts)	2
- Sometimes (approximately half of the attempts)	3
- Most times (more than half of the attempts)	4
- Always or almost always	5

3. During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner?	
- I am currently not sexually active	0
- Never or almost never	1
- A few times (less than half of the attempts)	2
- Sometimes (approximately half of the attempts)	3
- Most times (more than half of the attempts)	4
- Always or almost always	5

4. During sexual intercourse, how difficult was it to maintain your erection to completion of intercourse?	
- I am currently not sexually active	0
- Extremely difficult	1
- Very difficult	2
- Difficult	3
- Slightly difficult	4
- Not difficult	5

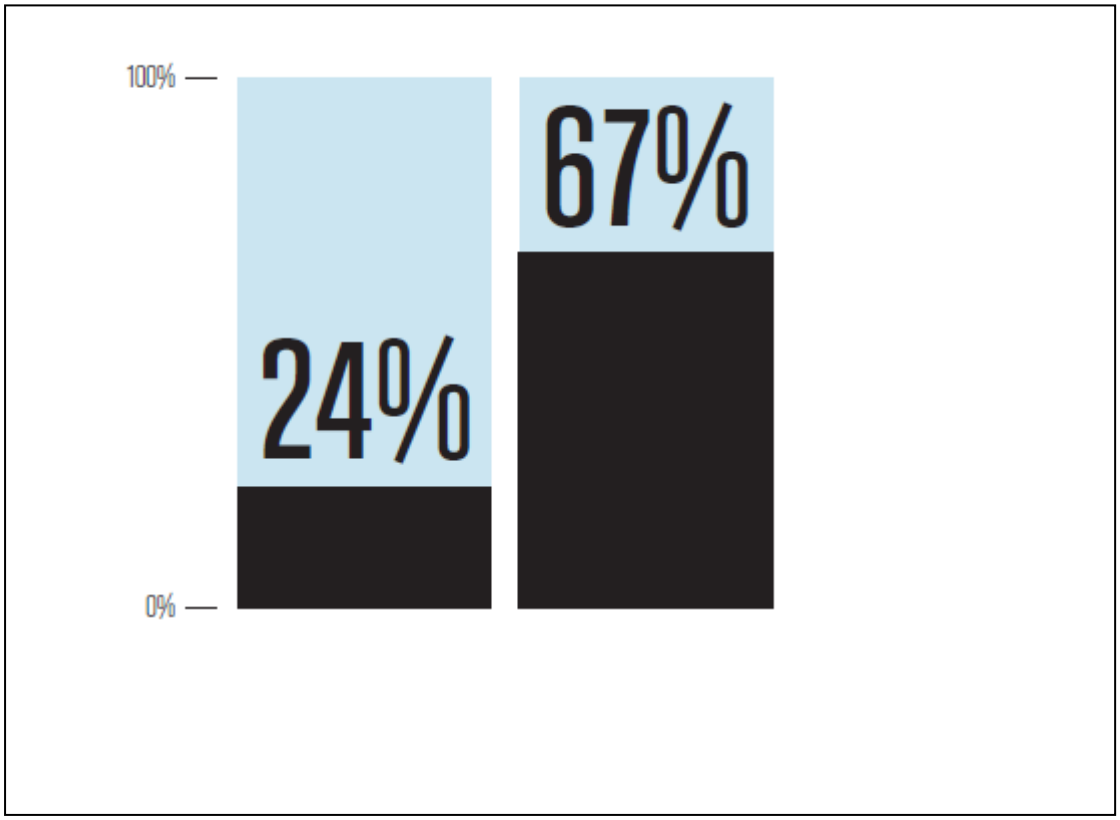
5. When you attempted sexual intercourse, how often was it satisfactory for you?	
- I am currently not sexually active	0
- Never or almost never	1
- A few times (less than half of the attempts)	2
- Sometimes (approximately half of the attempts)	3
- Most times (more than half of the attempts)	4
- Always or almost always	5

RESULTS

Cardiac catheterization has shown a prevalence of coronaropathy in the population in study of 35% (28 patients), classified, after revision of the images of the cardiac angiography, as common atherosclerosis in 15.6% (12 patients) and as cardiac allograft vasculopathy in 11.7% (9 pts).

The first interesting result concerns the incidence of erectile dysfunction in the heart transplanted population, which increases more than 3 folds compared to the "normal" population, with a value of 67% (50 patients).

Fig. ED before and after HT



To analyze results the population in study has been divided, regarding the presence or absence of erectile dysfunction, the pre-transplant diagnosis of post-ischemic disease and

presence and absence of cardiac allograft vasculopathy. In each group medical history, clinical and instrumental results of patients have been analyzed.

Table 10. Variables values in patients with or without DE

Variable	DE	No DE	Normal Range
Tot Colest (mg/dL)	204.6+47.2<	185.31.7	<200
Glycemia (nmol/L)	5.8+1.49+16	5.2+0.92	3.3-6
HDL (mg/dL)	54.3+16	53.30+12.1	<39
LDL(mg/dL)	127.8+42.36	111.52+28.56	<130
Triglycerides (mg/dL)	123.59+50	106.03+32.01	<200
Tot T (nmol/L)	14.4+5.08	15.3+6.63	10-29
Free T	0.29+0.09	0.32+0.08	

A vascular origin of the ED in the transplanted patients has been postulated and, for this reason, penile and peripheral vascular data in DE/no DE groups have been compared.

From the table below, it emerges that patients with ED show vascular abnormalities in the cavernous analysis, as IMT (intimal-media tickening) which is augmented and as PSV which is conversely decreased. Furthermore, similar results have been obtained in the carotid district showing increased plaques in the DE group.

These data, all together, highlight that there is a multi-district vascular alteration in the ED group

Table 11. Vascular districts analysis in DE/no DE group

	DE	No DE	p
IMT cav (mm)	0,3 ± 0,08	0,2 ± 0,06	<0,05
PSV art cav (cm/s)	48,42 ± 20,25	66,21 ± 21,39	<0,002
IMT TSA (>0,9mm)	16,67% (10)	4% (1)	<0,005
Epiaort. Plaque	60,87% (28)	26,09% (6)	0,01
Cav. plaque	26,7% (12)	5,26% (1)	<0,05

To further investigate the origin of ED in each group the PCs, EPCs, and the OCN+-EPCS have been quantified. Even if the only statistical significant data concerns the progenitor cells (PCs), it is evident a trend which shows an altered balance between the EPC and OCN+-EPCs, with a stable quantity of EPCs but an increased number of OCN-EPCs in ED patients.

Table 12. PC, EPC and OCN+-EPC in DE/no DE groups

	DE	No DE	p
PC (cell/ml)	929,7 ± 559,71	1200,13 ± 477,19	<0,04
EPC (cell/ml)	64,59 ± 92,47	62,46 ± 47,16	0,9
EPC-OCN+ (cell/ml)	23,6 ± 19,32	15,3 ± 22,74	0,11

Finally, the correlation between DE and cardiac vasculopathy, intended as either atherosclerotic either *allograft* vasculopathy has been analyzed.

In the table below, see the results:

	NO CORONAROPATIA	CORONAROPATIA	p
No DE	75 % (18)	25% (6)	< 0,05
DE	54,17 % (26)	45,83 % (22)	< 0,05

To understand if the correlation showed between coronaropathy and ED was specific of CAV incidence of CAV in patients with and without ED has been investigated:

Table14.

	No CAV	CAV	p
No DE	91,3% (21)	8,7% (2)	< 0,05
DE	74,47% (39)	25,53% (12)	< 0,05

In the table below it is reported the concerning the nature pre-transplant pathology, differentiating between two big categories of post-ischemic disease and not-ischemic disease in which there are primitive dilatative cardiomypathy, post valvular diseases, infections with predominance of myocarditis and other causes:

Table 15.

	Post-ischemic	**Not-ischemic**	**p**
NO DE	80% (20)	20% (5)	0.03
DE	54% (28)	45.1% (23)	0.03

Interestingly, there are not significant differences comparing the pre-transplant diagnose, the levels of PC, EPC and OCN-EPC, nor with the presence of peripheral vasculopathy.

Below, table 16 shows the correlation between coronaropthy and ED. The significant result shows an increased ED in patients with cardiac vasculopathy, confirming the data above:

Table 16

	NO-ED	**ED**	**p**
No Coronaropathy	40.9% (18)	59.1% (26)	<0.005
Coronaropathy	21.43% (6)	78.571% (22)	<0.005

Finally levels of PCs, EPCs and OCN+-EPCs have been evaluated in patients with or without cardiac vasculopathy (both atherosclerotic or as manifestation of CAV). Number

of OCN+-EPCs are significantly increased, whilst number of PCs and EPCs were not significantly different. If it is true, as indicated in the literature, that the OCN+-EPCs are expression of a calcification potential and endothelial dysfunction, the result we obtained is very important, confirming that endothelial dysfunction is the common factor shared by peripheral and cardiac vasculopathy, in transplanted patients.

As shown in the table below (table 17) in patients with cardiac vasculopathy, there are also penile alterations; last but not least, also the coronaric flow reserve, a marker of the cardiac micro-circulation, is significantly reduced in patients with coronaropathy.

Finally, also testosterone appears to be decreased in this group of patients.

Table 17.

	Coronaropatia assente	Coronaropatia presente	p
PC (cell/ml)	1082,8 ± 554,93	971,67 ± 524,79	0,43
EPC (cell/ml)	59,2 ± 43,92	79,38 ± 121,73	0,33
EPC-OCN+ (cell/ml)	14,32 ± 18,43	29,79 ± 23,87	< 0,003
IMT a cav dx (mm)	0,25 ± 0,08	0,3 ± 0,09	< 0,02
IMT a cav sin(mm)	0,25 ± 0,08	0,33 ± 0,17	< 0,02
CFR	3,05 ± 1	2,44 ± 0,74	< 0,02
T totale (nmol/L)	15,8 ± 6,06	12,79 ± 4,16	< 0,03
T libero (nmol/L)	0,31 ± 0,09	0,27 ± 0,08	< 0,05

Lastly a correlation between immune-suppressant therapy and levels of EPCs has been analyzed, but data were not significant. Similarly, immuno-suppressant therepy seems to be not influent on testosterone levels, differently from what we expected. Testosterone resulted only in patients under everolimus. These data are probably influenced by the low number of patients divided based on immunosuppressant regimen, so further investigations need to be conduced.

Table 18.

	T (average) **nmol/L**	**p**
NO-Everolimus	15.38±5.62	<0.02
YES-Everolimus	11.43±4.74	

DISCUSSION AND CONCLUSIONS

Cardiovascular diseases are well known to share same risk factors than erectile dysfunction, as hypertension, hypercolestherolemia, smoke habit, diabetes and obesity. Surprisingly, the correlation between erectile dysfunction and heart transplantion has not been studied and it is still unclear.

It is evident that erectile dysfunction is drastically increased after heart transplantation, arriving to percentage of 67 % in the populationin study; this phenomenon cannot be explained only with the evidence that some risk factors are more present in the transplanted patients; there is an high prevalence of actual or, more often, formal smockers, hypertensive patients and renal abnormalities but, in contrast, other risk factor, such as obesity, diabetes and hypercolestherolemia are not different than in normal population.

Certainly, transplanted patients are strongly medicated, with immunosuppressant regimen which is not poor of side effects. All patients enrolled in the study, are under cyclosporine. The 42% of patients are under corticosteroid treatment at low dosage and often re-introduced after years from transplant. The politic of Padua Heart Transplant Centre, in fact, concerning cortiosteroids is the suspension within the first year of transplant; metilprednisolone is usually re-introduced to ensure the possibility for patients to reduce Calcineurin inhibitors (CNI sparing protocol) avoiding or reducing their side effects.

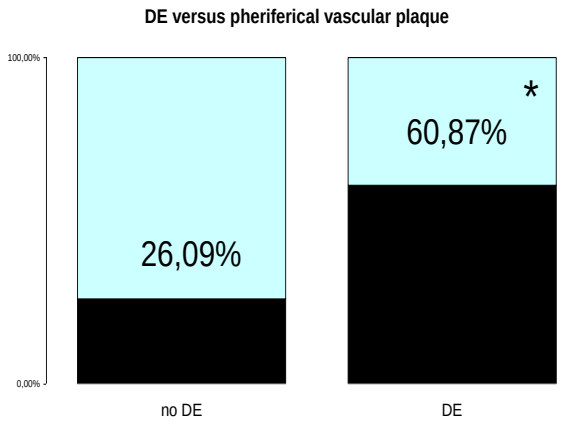
Azathiorine, that in the past, was always used together with cyclosporine and corticosteroids in the “triple regimen”, is now less used, because of the introduction of new immunosuppressant drugs, as mycophenolate and everolimus. However, patients are not only under immunosuppressive medication; all

hypertensive drugs are used also in this category of patients, based on the individual characteristics.

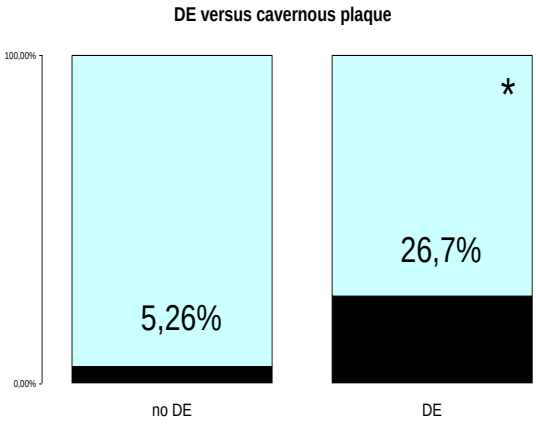
Analyzing our results in the group of patients, the one with erectile dysfunction and the one without, it appears that there are not significantly differences between hematologic blood test, lipidic, proteic and hormonal profiles, even if patients affected by erectile dysfunction present higher levels of cholesterol, HDL, triglycerides and glucose and lower levels of testosterone.

On the opposite side, in the case of erectile dysfunction patients, there is a significant higher incidence of vasculopathy, intended as cardiac (both forms: atherosclerotic and as a consequence of CAV) and peripheral vasculopathy, as shown in the figures below.

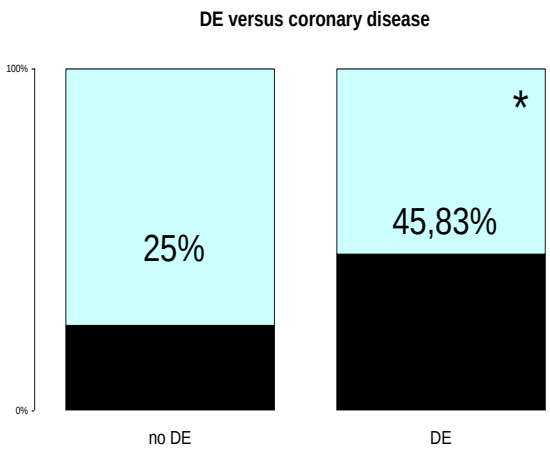
A)



B)



C)

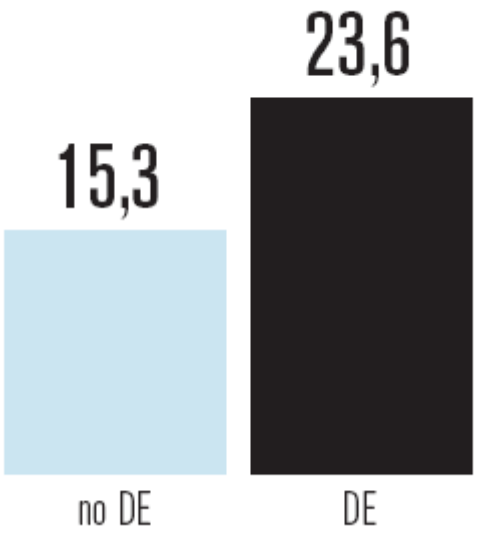


From this graphics, it is evident that patients with ED suffer of higher incidence of femoral or carotid plaques (60.87% versus 26%), higher incidence of penile vaculopathy (26.7% versus 5.26%) and also they present major coronary diseases (45.83% versus 25%).

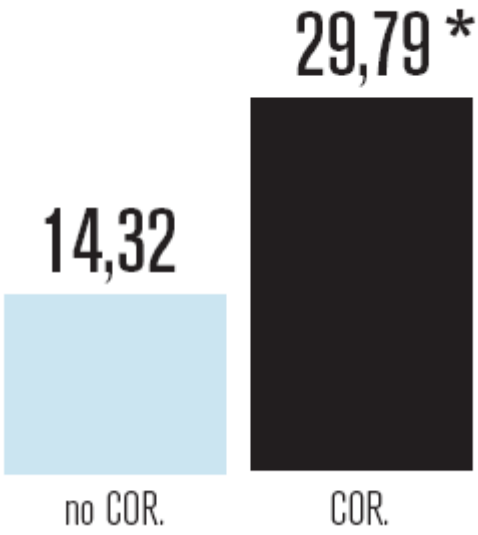
We can conclude, from these results, that erectile dysfunction in the transplanted patients is a marker of a multi-district vascular pathology, which concerns both micro- and macro-circulation.

Starting from the illustrated results it has been hypothesized that the multi.-disrtict vasculopathy might be due to endothelial dysfunction; for this reason, levels of endothelial progenitor cells, which are deputized to endothelial repair, have been further evaluated. The fact that PCs and EPCs are not significantly decreased in paients with erectile dysfunction, is only apparently anomalous; it is in fact the balance within EPCs and OCN+-EPCs that is modified, in favour of the OCN-EPCs, which result significantly increased. This subpopulation of EPCs, have a totally different role compared to the EPCs, accordingly to literature and they seems to be responsible, at least in part, of the vascular pathogenesis, showing a major calcifying potential.

DE versus OCN+



Cardiac vasculopathy versus OCN+



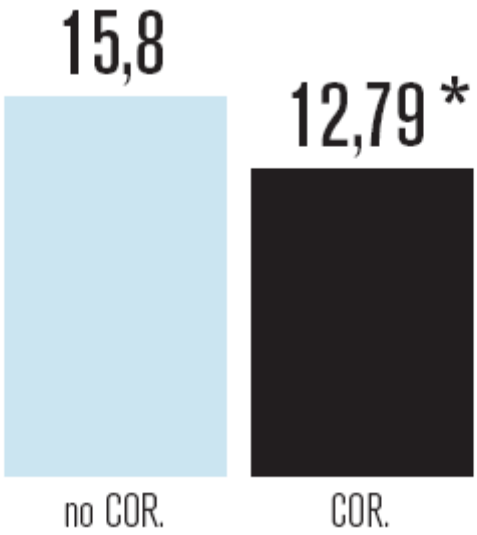
From these data, it is plausibly to conclude that endothelial dysfunction plays a predominant role in the vasculopathy in transplanted patients, and that it is a shared mechanism of micro- and macro- circulation alterations.

This is confirmed by the reduction, in patients with cardiac vasculopathy, of the coronary flow reserve (CFR), which is widely accepted as an index of micro-coronaric circulation. Patients with cardiac vasculopathy, demonstrated by cardiac angiography, present also a reduced CFR < 2.5, while patients without cardiac vasculopahy show levels of CFR < 3.

In literature it has been described that the micro-circulation vasculopathy is due to a proliferation of smooth muscle cells in the small vases and in capillaries, determining lumen stenosis, that could sometimes underestimates by angiography, especially at initial phases (95,96).

In the population variables analysis, only testosterone levels have shown a significant difference between patients, regarding blood test. It has been found to be reduced which is reduced in patients with cardiac vasculopathy as shown in the fig. below.

Coronaropathy versus Total Testosterone



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This data reflects what has been described in literature, regarding the reduction of total testosterone in cardiac diseases; it seems possible an influence of testosterone on the endothelial progenitor cells, switching toward OCN+-EPCs as described in atherosclerotic plaques formation.

In this study there are no differences due to immunuppressant therapy on the vasculopathy and on the levels of EPCs. To our opinion, this is explainable, at least partially, with the modest numerosity of patients when subdivided by different immunuppresant regimens. It is, indeed, possible to hypothesize a role of the new immunuppressant drugs, as everolimus and mycophenolate, which have an anti-proliferative action, on delaying the comparison on vasculopathy, even if their action can also be direct toward EPCs and testosterone production.

The modest numerosity of the different groups of patients based on immunuppressant regimens is one of the main limitation of this study; for this reason further enrolment need to

be done; future perspectives are represented by in vitro experiments to seed EPCs in different coltures with the presence of the different immunuppressant drugs to better understand the effect of each medication on the endothelial progenitor cells proliferation.

It seem also extremely important to test weather the use of conventional drugs used for the erectile dysfunction, as PDE5 inhibitors or substitutive testosterone administration, might ameliorate the quality of life of patients, reducing symptoms related to erectile dysfunction, and increasing EPCs proliferation switching the balance toward EPCs (and not OCN+-EPC).

We can conclude that erectile dysfunction, in heart transplanted patients, is a praecox symptom that needs to be fast investigated, to improving quality of life of this population. I Furthermore, it might represent an index of vascular pathology, anticipating the cardiac involvement. Its diagnosis can orientate physicians in the best therapeutic option and in the decision making process in the follow up, to preventing peripheral or cardiac vasculopathy.

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