Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

Scienze Mediche Specialistiche

Ciclo XXVII

Settore Concorsuale di afferenza: 06/D1

Settore Scientifico disciplinare: MED 11

THE MEDICAL MANAGEMENT AFTER SURGERY FOR ADVANCED HEART FAILURE

Presentata da: Dott. MARCO MASETTI

Coordinatore Dottorato **Relatore**

Prof. Roberto Di Bartolomeo **Dott. Francesco Grigioni**

Correlatori:

Prof. Francesco Moscato
Dott. Luciano Potena
Prof. Andreas Zuckermann

Esame finale anno 2015

INDEX

The new face of heart transplantation: the need of improving outcomes both after transplant and on the waiting list

PART ONE : Major challenges in the long-term complications in heart transplant recipients

Chapter 1. Background on long-term complications and on mTOR inhibitors

1.1 Cardiac allograft vasculopathy (CAV): pathophysiology, the role of CMV and unsolved problems on diagnosis and prevention
1.2 Solid-organ cancers
1.3 mTOR inhibitors: properties for potential use in CAV prevention and in oncological patients

Chapter 2. Cardiac allograft vasculopathy: trying to improve diagnosis

2.1 Static versus dynamic angiographic CAV evaluation: prognostic stratification beyond ISHLT grading
2.2 Interplay of coronary angiography and intravascular ultrasound in predicting long-term outcomes after heart transplantation
2.3 Role of coronary CT in predicting prognosis after heart transplantation

Chapter 3. Cardiac allograft vasculopathy: trying to improve prevention

3.1 Differential effect of everolimus on progression of early and late cardiac allograft vasculopathy in current clinical practice

3.2 Everolimus and valganciclovir prophylaxis: insights from the PROTECT study

Chapter 4. Solid organ cancers after heart transplantation: trying to improve the management

4.1 Factors influencing survival of heart transplanted patients with a solid organ cancer: focusing on the role of immunosuppressive therapy, surgery and oncological status

Chapter 5. Everolimus versus mycophenolate de novo after heart transplantation: does it matter for long-term outcomes?

PART TWO. Left ventricular assist devices (LVAD)

Chapter 1. Background on LVAD

1.1 Technology of the most used LVAD systems
1.2 Physiology of LVAD systems
1.3 The impact of LVAD in the management of the waiting list and on post-transplant outcomes
1.4 The management of LVAD patients: short and long-term complications
1.5 The HVAD system and pump signal analysis

Chapter 2. The combined role of echocardiography and of analysis of pump signals: trying to improve the management

2.1 Continuous monitoring of aortic valve opening in rotary blood pump patients

2.2 Transthoracic echocardiography of outpatients with an intrapericardial left ventricular assist device implanted: a single center experience.

The new face of heart transplantation: the need of improving outcomes both after transplant and on the waiting list

The aim of the Research Project of this Ph D is to improve the medical management after surgery for advanced heart failure, both after left ventricular assist devices (LVAD) implantation, and after heart transplantation in the long-term. Regarding heart transplantation (HTx), the Research Project is focused on diagnostics, classification, prevention and treatment of cardiac allograft vasculopathy (CAV), and on treatment of post-HTx cancers; the results are presented in the first part of this Thesis.
Regarding LVAD, the main focus is on the role of transthoracic echocardiography in the management of patients with a continuous-flow, centrifugal, intrapericardial pump (HVAD, Heartware); this section is reported in the second part of this Thesis.

Heart transplantation (HTx) is actually the best available therapy for end-stage heart failure patients. According to International Society of Heart and Lung Transplantation (ISHLT) Registry[1], taking in account all heart transplants made in the U.S., survival rates are very good, with a reported 1-year survival of 85 %, and of 70 % at 5 years. Survival rates are similar also in Europe.
Besides these beautiful data, the overall success of HTx is impaired by the dramatic drop in the number of donations in last years, particularly in Europe, and by several comorbidities still influencing the late outcome, that has remained unchanged in the last years. The most frequent cause of death long-term after HTx is graft failure, that can be related to cardiac allograft vasculopathy (CAV), rejection (both in the form of cellular-mediated rejection, CMR, or antibody-mediated rejection, AMR, or a mixed form). Post-transplant malignancies are the second more common cause of death, followed by renal insufficiency, and infections. These and other comorbidities may arise as a consequence of long-term immunosuppression. The early causes of death are surgical-related mortality, acute cellular rejection and infections. In the following paragraphs the main aspects of the pathophysiology of these complications as well as their management will be described.
Beside these problems after transplant, the profile of donor hearts has changed in the last ten years, both in Europe and in the U.S., with a decrease in the number of donations, leading to an increase of the waiting time in the HTx list, and to a higher rate of deterioration of the patients. In order to maintain an acceptable survival rate while waiting for a HTx, several medical and surgical options are actually used as “bridge” to HTX, like a widely use of a left ventricular assist device (LVAD), or percutaneous treatments of valvulopathies, especially of mitral regurgitation. Moreover, the quality of donors worsened in the last years, with a higher donor age and longer ischemic time.
As can be easily understood, the overall success of a heart transplant program is related to the careful and strict selection of patients and to the experience of the clinicians in managing both post-transplant complications and the patient on the waiting list, through the use of left ventricular assist devices (LVAD). Thus, there is an actuarial need of improving outcomes both after heart transplantation, through facing long-term comorbidities, and on the waiting list.

PART ONE

MAJOR CHALLENGES IN THE LONG-TERM COMPLICATIONS IN HEART TRANSPLANT RECIPIENTS

Chapter 1.

Background on long-term complications and on mTOR inhibitors

1.1 Cardiac allograft vasculopathy (CAV): pathophysiology, the role of CMV and unsolved problems on diagnosis and prevention

Pathophysiology

Cardiac allograft vasculopathy is main graft-related cause of death after HTx. It is characterized by a diffuse intimal thickening, arising from the small vessels (microvasculopathy) and then spreading to the great vessels, leading to lumen narrowing without focal eccentric stenosis (Figure 1). For this reason, coronary angiography underestimates real CAV incidence, that has been reported as 10% at 1 year and 40-50% at 5 years from HTx, whereas real incidence is higher (80% at 5 years) . Not only progressive increase in intimal area in coronary arteries, but also complex phenomena of vessel remodelling (vessel shrinkage) seem also to contribute to progressive lumen loss, that can lead, especially in the long term, to pruning of distal vessels. Given the involvement of microvessels and the denervation of the transplanted heart, CAV is often asymptomatic. In a frequent scenario, CAV is found in asymptomatic patients; in some cases, asthenia or hypotension are the only symptoms. Most of myocardial infarctions are silent. In other cases, CAV leads to a chronic graft failure, that must be differentiated in clinical diagnosis from chronic rejection, especially AMR.

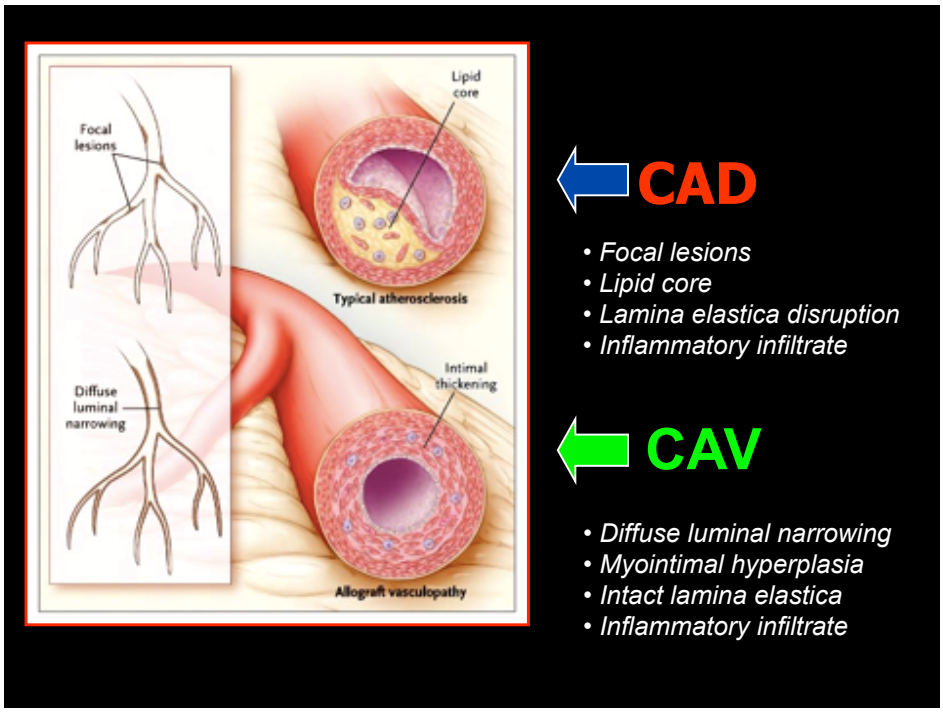


Figure 1. Main morphological differences between “native” coronary artery disease (CAD) and post-transplant vasculopathy (CAV)

Risk factors for CAV are both immunologic and non immunologic, as shown in the below Figure 2. Among the first ones, a key role is played by frequent rejection episodes (especially in the first years) and CMV infection, whereas among the second ones metabolic syndrome, diabetes, hypertriglyceridemia and hypercholesterolemia are the main triggers, especially late in the long term; the role of obesity is less certain. Often an interplay between the various factors coexists, leading to mixed scenarios, especially in the long term, characterized by both intimal thickening (more prevalent in the first years after HTx) and focal stenosis (more frequent in the long term).

All these factors can contribute to inflammation, endothelial damage and intimal hyperplasia, the main morphological features of CAV.

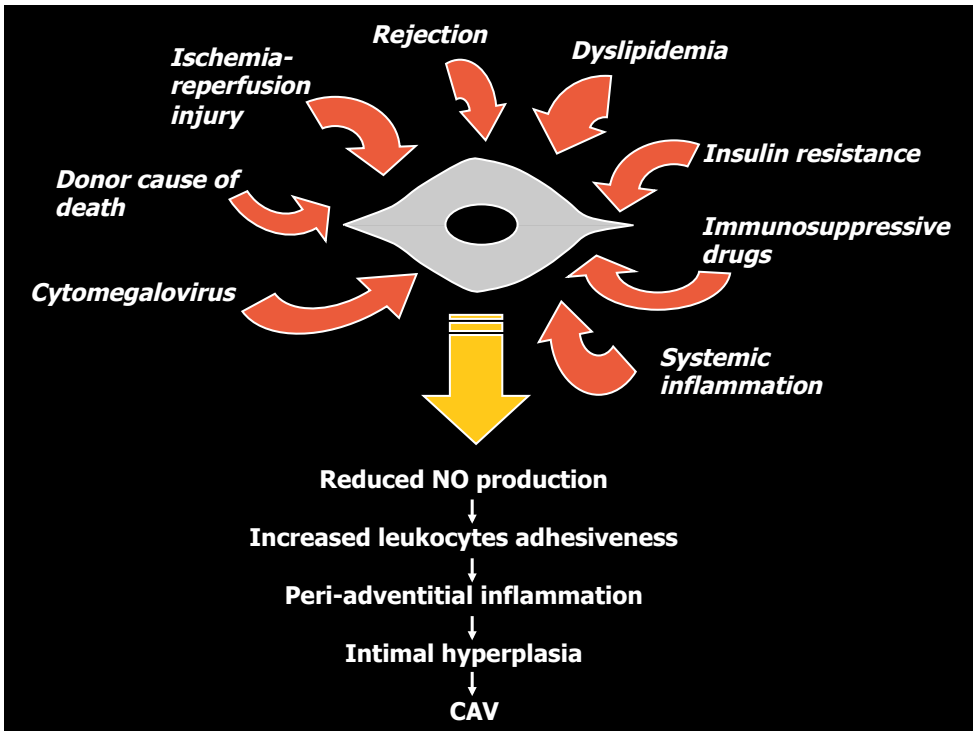


Figure 2. Main pathophysiological mechanisms of CAV

The role of CMV

Given the high prevalence of Cytomegalovirus (CMV) positivity in the general population, CMV reactivation frequently occurs after HTx, favoured by immunosuppression. The risk of virus reactivation or infection is higher in positive recipients (R+), and in negative patients receiving the heart from positive subjects (CMV mismatch, R-/D+). For this reason, the CMV serologic status of the donor and recipient is used to stratify the patient's risk for developing a CMV infection. Prophylaxis is usually started within 24 to 48 hours after HT with valganciclovir.

CMV infection can be completely asymptomatic, especially when viral load is low; when symptoms manifest, are influenza-like, with asthenia, fever, diarrhea (CMV disease); laboratory exams can show leukopenia and anemia. More severe clinical scenarios can manifest with organ involvement: hepatitis, gastroenteritis, pneumonia can occur. Beside the systemic symptoms and effects, CMV infection must be treated because it has an impact on graft function; it has been demonstrated that even subclinical low-grade CMV infection can be a trigger for endothelial damage, and thus for

cardiac allograft vasculopathy (Figure 2). The impact of CMV on the graft appears to be mediated by the action of innate and adaptive immune systems, rather than by a direct viral cytotoxic effect in situ. It has been showed that the more aggressively CMV infection is treated and prophylaxed, the less amount of intimal hyperplasia develops.

By using specific anti-CMV agents such as ganciclovir and valganciclovir, two strategies are recommended for prevention of CMV infection and disease: universal prophylaxis and preemptive therapy. While prophylaxis consists in the universal administration of the antiviral agent to all the patients at risk, in the pre-emptive strategy only patients who develop a "certain" threshold of subclinical infection receive treatment. Their rationales are different: prophylaxis almost abolishes viral replication during the first weeks/months after transplant, when the burden of immunosuppression is higher, thereby delaying the eventual appearance of the infection until a later phase of follow-up, by which time the immunosuppressive burden and risk of rejection should be lower. The pre-emptive strategy permits early low-grade viral replication in the belief that it may stimulate host's own immune response against the virus and will reduce the needing of anti-CMV drugs. Disadvantages are: considerable cost, risk of late CMV disease and ganciclovir resistance for prophylactic therapy; requiring of good logistic organization for preemptive therapy. In addition to specific anti-viral drugs, immunosuppressive agents may also influence occurrence of CMV infection. Inhibitors of the mammalian target of rapamycin (mTOR), tested for prevention of acute rejection in solid organ transplant recipients, appear to have anti-CMV properties and to reduce CAV progression when started early after HTx[6].

Unsolved problems in diagnosis

IVUS is the technique with the best diagnostic sensitivity, allowing the visualization of intimal thickening, the typical feature of CAV; however, due to the economical costs related to IVUS and to the limitation to its performance in case of severe stenosis on left descending artery, actually the widest technique used for diagnosis is coronary angiography. Given the use of iodate contrast agents, kidney function must be taken in account before deciding to perform an angiography; generally, a serum creatinine > 2.5 mg/dl or a GFR > 35 ml/kg/min are contraindications to coronary angiography. The most frequent finding is absence of stenosis at angiography and a diffuse intimal thickening at IVUS (see Fig. 3); however, especially years after HTx, a mixed scenario, with eccentric stenosis, similar to the ones typical of native atherosclerosis, can be found at angiography, especially in patients with metabolic syndrome.

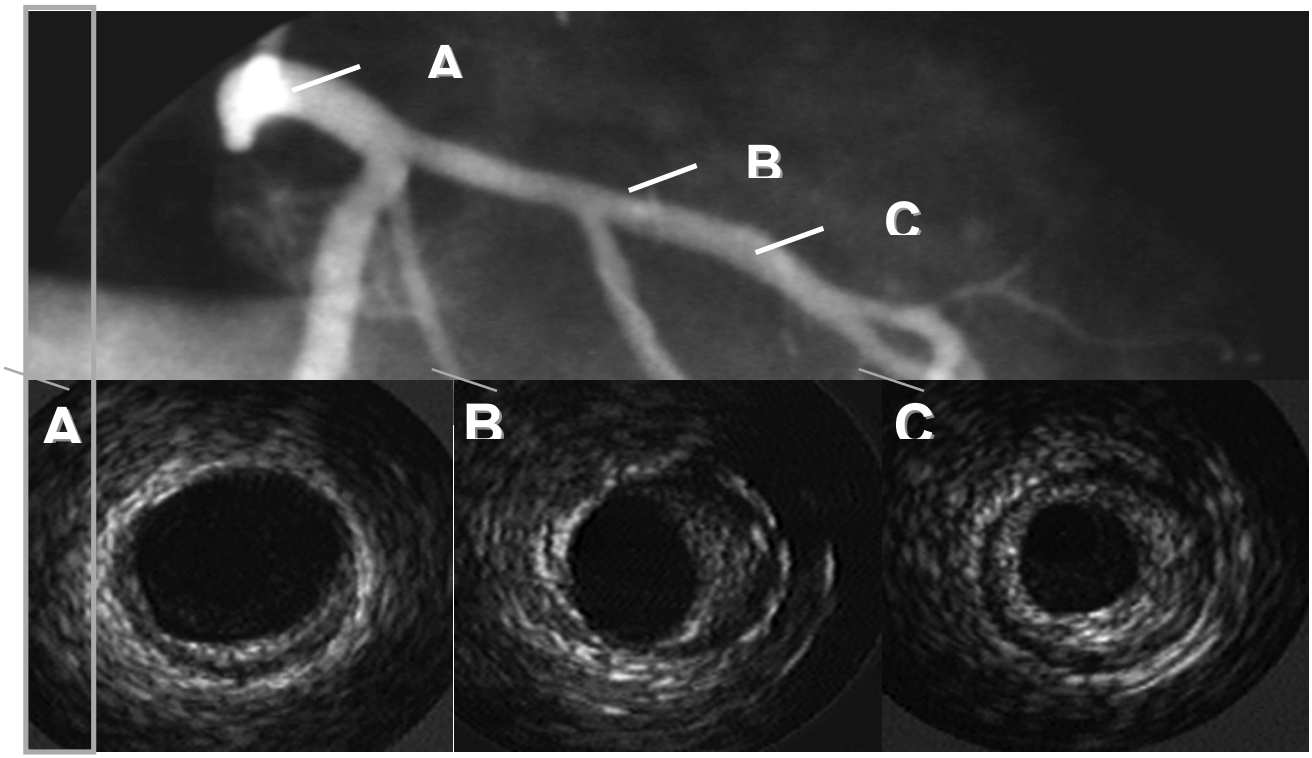


Fig. 3 Typical feature of CAV: normal angiogram with progressive intimal thickening at IVUS , becoming more severe proceeding to distal vessels.

Until some years ago, there was a lack of standardized classification system for CAV. Recently, ISHLT has pointed out a new classification system, taking in account even mild stenosis, in the believe that they could affect post-transplant outcome. The purposed classification system is reported in Table 1 below. However, it is not known if this system can predict cardiovascular prognosis, and it is not known if it can be useful when used both for assess for CAV diagnosis and CAV progression .

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)

Table 1. ISHLT CAV Classification System

The protocol of CAV assessment varies among different Centers and is typically constituted by an angiography one year after HTx and then periodically, with a timeline different among the various

Centers (usually every 2 -5 years).

IVUS is the technique with the highest sensitivity in CAV diagnosis; it allows the identification of changes in maximal intimal thickness (MIT), and the calculations of plaque volume, intimal volume and lumen volumes, as well as of complex remodelling systems in the coronary vessels. Previous studies showed that an increase in MIT $\geq$0.5 mm in the first year after HTx is a marker of subsequent poor cardiovascular outcome. Thus, this cutoff has been used to assess for the efficacy of different prevention and/or therapeutic strategies for CAV in subsequent studies. However, CAV is a continuous process, that develops also beyond the first year from HTx, and it is no known if IVUS-detected changes still retain a prognostic role also after the first year.

Moreover, even if IVUS is more accurate in diagnosing CAV, it is not known if it can add more prognostic information than angiography alone.

Coronary tomography (CT) is a non-invasive screening method for CAD; recently, it has been purposed also fro CAV monitoring, especially in patients with low probability of vasculopathy, performing a subsequent angiography if CT result is uncertain or positive, or in patients who don't want to undergo to coronary angiography. However, it is not known if CT can provide useful prognostic information to assess cardiovascular risk, neither its sensititvity compared to coronary angiography.

Unsolved problems in therapy and prevention

Given the diffuse hyperplasia, percutaneous coronary intervention (PCI) is not possible in the majority of cases; however, in cases in which some focal critical stenosis coexists, similar to the ones of native atherosclerosis, PCI with or without stenting can be made. Globally, the role of PCI in CAV is limited, with high rates of restenosis, even with drug eluting stents, and the real prognostic benefit is uncertain. Moreover, the feasibility of performing an angiography decreases among time, when the probability of finding a stenosis similar to native atherosclerosis is higher, but renal function is worse. The use of coronary by pass is anecdotal and with high rates of mortality in transplanted patients; re-transplantation can be an option in selected cases, but its real feasibility is rare, given the shortage of donations. Medical therapy of CAV is substantially absent. The role of introducing an mTOR inhibitor after an established diagnosis of CAV has been tested, but with uncertain results; anti-platelets agents, if not already started before, are initiated, particularly acetylsalicylic acid, often in association with clopidogrel, the latter suggested in models in vitro to have a slight effect. Statins are also continued

Given the substantial absence of therapy, medical approach to CAV is rather based on prevention strategies. In this context, drugs agent against the various possible trigger of CAV are given (see Fig 5). CMV infection is aggressively prophylaxed and treated, anti-hypertensive agents are given. It has been shown that aggressive prevention of even subclinical CMV infection can reduce IVUS-detected CAV prevention, being universal prophylaxis probably more effective than pre-emptive strategy.

Anti-lipid agents (statins) are a cornerstone in CAV prevention, as it has been shown that they reduce CAV incidence and plaque progression, as assessed by intravascular ultrasound (IVUS) with a class - effect; moreover, they improve prognosis after HTx, with an effect dependent not only by the lipid-lowering effect, but also by anti-inflammatory and anti-rejection properties. For this reason, statins are given to all transplanted patients, regardless of their cholesterol levels; a possible adverse effect can be elevation in creatine kinase (CK), leading to myalgias and even rhabdomyolysis, with acute renal failure. CK elevation is frequent in transplanted patents, given the interaction of some statins with CNI and mTOR inhibitors through P450 cytochrome, often requiring statins temporary suspension or switch from one statin to another one. A target of cholesterol LDL has not yet been well established, but generally a target LDL<100 mg/dl is reasonable. Hypertriglyceridemia is also treated conventionally, and has been suggested to be a strong CAV risk factor. Even if paucity of data exists, acetylsalicylic acid is usually given to prevent CAV. As

written above, in high-risk patients, a more strict angiographic follow-up can be considered.

As explained in Section 1.3 , mTOR inhibitors (PSI: everolimus, sirolimus) have been investigated in CAV prevention, and have been shown to be more effective than mycophenolate mofetil (MMF) in CAV prevention in the first year after HTx

However, it is not known if PSI can have a different prevention effect when combined with different anti-CMV prevention strategies (prophylaxis vs pre-emptive) neither if PSI can be useful in CAV treatment.

1.2 Solid-organ cancers

Malignancies are the first non graft-related cause of death after HTx. The incidence of malignancies in HTx is 3 folds-higher than the general population, and higher than in kidney or liver transplantation, probably because of the higher rates of immunosuppression. According to ISHLT registry, malignancy incidence is 14.2% at 5-years and 27.7% at 10 years, being older age at transplantation the most powerful risk factor. Beside the most frequent malignancies that can be found also in general population, some rare cancers are tipically seen almost only in the transplanted setting like in other cohorts of immunosuppressed patients (i.e. HIV+), like Kaposi's sarcoma. Generally, the clinical course for malignancies after HTx is more aggressive than in the general population, because of immunosuppression, with a subsequent higher mortality rate. Skin cancers are the most frequent malignancies (5-years incidence: 9.4%, 10-years: 19.6%), especially squamocellular cancers (the most frequent ones) and basaliomas, and seem to be related partially to azathioprine. An aggressive dermatological screening, surgical treatment and follow-up is made in these patients, together with the recommendation, especially in the mediterranean countries, to avoid high exposition to UV and sun light. Some patients experience repeated skin cancers, with multiple surgical treatments, and sometimes lymphonodal involvement. Melanoma is also quite frequent, especially in young people.

Among solid cancers, the most frequent are: prostate, colon, lung, kidney, bladder, pancreas, breast, sarcomas, but all organs can be involved[14]. In more than 30% of patients, cancer is diagnosed when already at an advanced stage; overall outcome is poor, with a reported overall survival of <50% 5 years after diagnosis. Aggressive screening protocols are used, but with uncertain results. Once a cancer is found, it must be surgically treated when possible. Chemotherapies and radiotherapy is also possible; during chemotherapeutic treatments, immunosuppressive drugs potentially causing leukopenia are usually reduced or even interrupted. In some selected cases (i.e. kidney, bladder, Kaposi's sarcoma) a switch to an mTOR inhibitor can be considered, especially in those cancers in which these drugs (even if at higher doses than in HTx) are registered as chemotherapeutic agents.

PTLD are also relatively frequent after HTX, especially in young people and women; the most frequent ones are non-Hodgkin lymphomas and sometimes MALT, developing frequently even early after HTx (incidence: 0.5% at 1 year, 1.1% at 5 years, 1.7% at 10 years). These diseases are treated as conventionally, but with a significant poorer outcome (20% survival at 5 years); bone marrow transplantation and even autologous stem cells transplantation are possible when appropriated, with even good results.

Kaposi' sarcoma, a disease exclusive of immunocompromised patients in the developed countries, is not rare after HTx. in these cases, mTOR inhibitors are introduced, given their known effect on this disease, with the potential to achieve a complete remission.

1.3 mTOR inhibitors: properties for potential use in CAV prevention and in oncological patients

The triple combination of steroids, a calcineurin inhibitor (cyclosporine/tacrolimus, CSA/TAC) and an antiproliferative drug (mycophenolate mofetil or everolimus ,MMF/EVE) is actually the most frequent scheme used for long-term immunosupression. Their mechanism of action is depicted in Figure 4.

MMF is a noncompetitive inhibitor of inosine monophosphate dehydrogenase. Proliferating lymphocytes are dependent on this pathway because it is the only pathway for the purine synthesis and DNA replication, whereas other cells use also other salvage pathways for purine synthesis. Therefore, MMF selectively inhibits lymphocyte proliferation in response to allogeneic stimulation without inhibiting other cell lines. MMF is given orally, usually twice a day, and it has been shown in a prospective randomized trial to be superior to AZA on graft survival and in preventing rejection, also when used with reduced CSA doses. It is actually the standard antiproliferative drug. Major side effects are: nausea, vomiting, and diarrhea, which usually are dose-dependent.

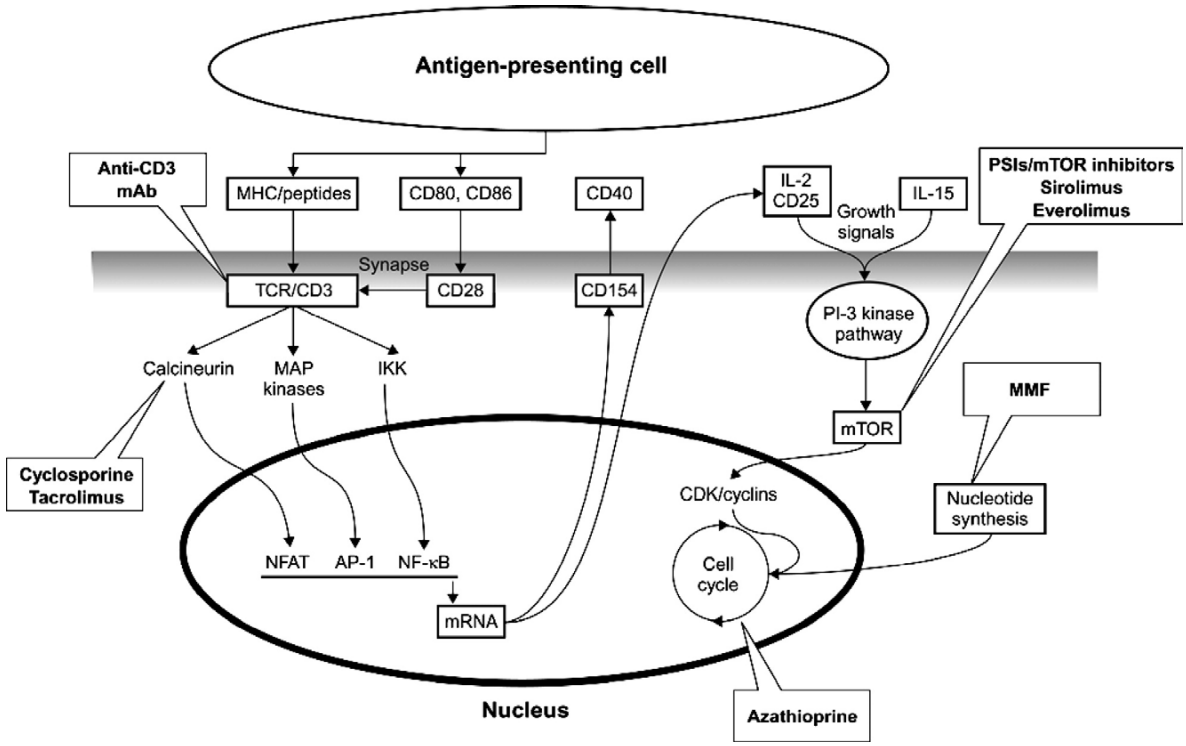


Figure 4. Mechanisms of action of different immunosuppressive drugs

Everolimus (EVE) and its analogue sirolimus (SIR), first isolated in soil samples from Rapa-Nui (Easter Island), is a natural product of the actinomycetes *Streptomyces hygroscopicus*. These drugs bind to the same family of immunophilins as TAC, but rather than blocking calcineurin-dependent T-cell activation, inhibit the target of rapamycin (TOR), a kinase phosphorylating proteins involved in the regulation of the cell cycle, playing a critical role in signals from the growth factor receptors to the cell nucleus for stimulation of growth and proliferation of T and B lymphocyte, smooth muscle cells and endothelial cells.

The frequent involvement of mTOR system in various biological pathways (endothelial cells and smooth muscle cells proliferation, cancerogenesis, healing repair) explains both the adverse effects of this drug and its potential positive effect in various pathological processes. In particular,

mTOR inhibitors have been studied in preventing CAV development (smooth muscle cells proliferation is the basis of intimal thickening, the pathological marker of CAV), in oncology or in transplanted patients developing a tumor. On the same basis, these drugs can lead to a slower wound healing repair process, and to a higher susceptibility to infections, particularly bacterial. The synergic effect with CSA allows a reduction in CSA dose, with the aim to reduce the CNI-related nephrotoxic effect.

Drug concentration in blood is also used for deciding mTOR inhibitors dosing; these drugs are the most complex to use among the various immunosuppressors, because of a narrow therapeutic index and frequent, often dose-related, side effects (about 25% of patients interrupt the treatment). The synergic effect with CSA allows a reduction in CSA dose, with the aim to reduce the CNI-related nephrotoxic effect. The effect of EVE introduction to reduce or at least to delay renal failure is slightly more evident in the earlier stages of kidney disease and in the first years after HTx, especially when pathological proteinuria still has not occurred, and may be is mainly related to the ability of reducing CSA; EVE seems to have a slightly more benefit than MMF in preventing renal insufficiency[10]. The results of randomized and non randomized trials showed that EVE is superior to MMF in preventing rejection and CAV development in the first years after HTx, whereas the real benefit when CAV is already present is less clear and still debated[11].

The prospective randomized trials focusing on comparison between MMF and EVE in de novo use after HTx are reported in Table 2.

Study	Study design	Follow-up	Treatment	N	Primary efficacy endpoint			BPAR	
					Endpoint	%	p value	%	p value
Eisen 2012[a] [3]	Randomized Multicenter Open Label	12 months	EVR 1.5 mg (3–8 ng/mL) Reduced CsA Steroids ± Induction	282	ISHLT grade ≥3A (2R) BPAR, acute rejection associated with hemodynamic compromise, graft loss/retransplant, death, or loss to follow-up	35.1	0.705	22.3[b]	n.s.
			MMF Standard CsA Steroids ± Induction	271		33.6		24.7[b]	
Zuckermann 2011 [4]	Randomized Multicenter Open Label	6 months	EVR (3–8 ng/mL) Reduced CsA ± Induction	99	ISHLT grade ≥3A (2R) BPAR, acute rejection associated with hemodynamic compromise, graft loss/retransplant, death, or loss to follow-up	26.3	n.s.	16.2[b]	n.s.
			EVR Standard CsA ± Induction	100		25.0		21.0[b]	
Lehmkuhl 2009 [5]	Randomized Multicenter Open Label	12 months	EVR (3–8 ng/mL) Reduced CsA Steroids ± Induction	92	ISHLT grade ≥3A (2R) BPAR, acute rejection associated with hemodynamic compromise, graft loss/retransplant, death, or loss to follow-up	32.6	Statistically non-inferior (10% NI)	22.8[b]	0.005 for non-inferiority (10% NI margin)
			MMF Standard CsA Steroids ± Induction	84		41.7		29.8[b]	
Eisen 2003 [30]	Randomized Multicenter Double blind	12 months	EVR 1.5 mg (fixed dose) CsA Steroids ± Induction	209	ISHLT grade ≥3A (2R) BPAR, acute rejection associated with hemodynamic compromise, graft loss/retransplant, death, or loss to follow-up	36.4[c]	0.03 vs Aza	30.6[b,d]	0.001 vs Aza
			EVR 3.0 mg CsA Steroids ± Induction	211		27.0[c]	0.001 vs Aza	21.3[b,d]	0.001 vs Aza
			Aza Standard CsA Steroids ± Induction	214		46.7[c]	–	45.8[b,d]	–

Table 2. Efficacy outcomes of prospective trials on everolimus in de novo heart transplant recipients (from Zuckermann A. Transplantation Reviews 2013; 27: 3: 76-84.)

EVE is the immunosuppressant with the highest anti-CMV activity, regardless of the type of anti-CMV prophylaxis used. Some registries have reported a decreased incidence of de novo cancers in patients continuously treated with EVE, and this drug seems to be at least partially effective in some kinds of cancer developed after HTx.

However, the real clinical benefit of this drug is still matter of debate and must face with problems in tolerability: more than 25% of patients must interrupt the treatment, regardless of the post-HTx phase in which EVE is initiated

The most frequent reasons for interruption in the first months after HTx or after every surgical procedure are problems in wound healing repair and pericardial effusions, often requiring pericardiocentesis, whereas when the drug is started later after transplant, lower limb edema, bacterial infections (especially pneumonia) and dyslipidemia are the most frequent causes of drug interruption. Hyperlipidemia, hypertriglyceridemia, thrombocytopenia, neutropenia, and anemia can occur in every phase after HTx while on mTOR inhibitors therapy. For these reasons, the use of mTOR inhibitors is very individualized and requires an assessment of potential benefits and side effects.

Summary of unclear points and their development in this Research Thesis

CAV diagnosis:

- Is ISHLT classification system for angiographic detected CAV useful for assessing prognosis? (Chapter 2.1)
- Is ISHLT classification system for angiographic detected CAV useful also when used to assess for disease progression? Is even mild CAV progression a marker of worse cardiovascular outcome? (Chapter 2.1)
- Do IVUS detected changes observed after the first year from HTx have a prognostic effect? (Chapter 2.2)
- Does IVUS add any more refined prognostic information compared to angiography? (Chapter 2.2)
- Is coronary tomography (CT) useful in predicting cardiovascular outcome? (Chapter 2.3)
- What is the sensitivity of CT for CAV assessment when compared to angiography? (Chapter 2.3)

CAV prevention/treatment:

- do mTOR inhibitor have a different role in preventing early vs late CAV development? (Chapter 3.1)
- What is the role of the interplay between different anti-CMV prevention strategies and mTOR inhibitors in CAV prevention? (Chapter 3.2)

Post-transplant cancers:

- What are factors influencing survival after post-transplant cancer diagnosis? (Chapter 4.1)
- What is the role of mTOR inhibitors? (Chapter 4.1)

mTOR related clinical benefit:

- Do mTOR inhibitors matter for long-term outcomes? (Chapter 5)

References:

1. Stehlik J, Hosenpud JD, Edwards LB, Hertz MI, Mehra MR. ISHLT International Registry for Heart and Lung Transplantation--into the fourth decade, from strength to strength. J Heart Lung Transplant. 2013; 32(10):941-50.

2. Costanzo MR. The International Society of Heart and Lung Transplantation guidelines for the care of heart transplant recipients J Heart Lung Transplant. 2010; 29:914–956.

3. Potena L, Holweg CT, Chin C, Luikart H, Weisshaar D, Narasimhan B, Fearon WF, Lewis DB, Cooke JP, Mocarski ES, Valantine HA. Acute rejection and cardiac allograft vascular disease is reduced by suppression of subclinical cytomegalovirus infection. Transplantation 2006; 82 (3):398-405.

4. Eisen HJ et al. Everolimus versus mycophenolate mofetil in heart transplantation: a randomized, multicenter trial. American Journal of Transplantation 2013; 13:1203-16.

5. Lindenfeld J, Miller GG, Shakar SF, Zolty R, Lowes BD, Wolfel EE, Mestroni L, Page RL 2nd, Kobashigawa J. Drug therapy in the heart transplant recipient. Part II: immunosuppressive drugs. Circulation. 2004; 110(25):3858-6.

6. Kobashigawa J, Zuckermann A, Macdonald P, Leprince P, Esmailian F, Luu M, Mancini D, Patel J, Razi R, Reichenspurner H, Russell S, Segovia J, Smedira N, Stehlik J, Wagner F; Consensus Conference participants. Report from a consensus conference on antibody-mediated rejection in heart transplantation. J Heart Lung Transplant. 2011; 30:252–69.

7. Kobashigawa JA, Kiyosaki KK, Patel JK, Kittleson MM, Kubak BM, Davis SN, Kawano MA, Ardehali AA. Benefit of immune monitoring in heart transplant patients using ATP production in activated lymphocytes. J Heart Lung Transplant. 2010; 29(5):504-8.

8. Zuckermann A, Wang SS, Epailly E, Barten MJ, Sigurdardottir V, Segovia J, Varnous S, Turazza FM, Potena L, Lehmkuhl HB. Everolimus immunosuppression in de novo heart transplant recipients: what does the evidence tell us now? Transplant Rev (Orlando). 2013; 27(3):76-84.

9. Valantine HA. Cardiac allograft vasculopathy: central role of endothelial injury leading to post-transplant “atheroma”. Transplantation. 2003; 76(6):891-9.

10. Lindenfeld J, Page RL 2nd, Zolty R, Shakar SF, Levi M, Lowes B, Wolfel EE, Miller GG. Drug therapy in the heart transplant recipient. Part III: Common medical problems. Circulation. 2005; 111(1):113-7.

11. Crespo-Leiro MG, Alono-Pulpon L et al. Malignancy after heart transplantation: incidence, prognosis and risk factors. Am J Transpl. 2008; 8:1031-9.

Chapter 2.

Cardiac allograft vasculopathy: trying to improve diagnosis

2.1 Static versus dynamic angiographic CAV evaluation: prognostic stratification beyond ISHLT grading

Introduction:

ISHLT grading of coronary allograft vasculopathy (CAV) provides a static definition of CAV severity that has recently been correlated with adverse outcome. Although the grading system classifies emphasizes even mild stenoses, as they may represent a marker of adverse cardiovascular (CV) outcome, it may not detect the progression of subcritical lesions. Herein we aimed to analyze the prognostic implication of progressing vs. static angiographic lesions within ISHLT classification.

Methods:

All patients receiving heart transplant in 2 large centers (Bologna and Vienna) between 2001-08, surviving >1 year, having undergone 2 coronary angiographies, entered this study.
Angiographic progression (assessed by an operator blinded to the exam report) was defined as any stenosis increase or any new stenosis in any vessel.

Study endpoints were :

- freedom from cardiovascular death and
- freedom from MACE combined occurrence (CV death, PCI, admissions for heart failure, re-HT) during 5 yrs after the second angiography.

Results:

161 patients (85% males, 16-70 yrs) were included. The 2 angiographies were performed respectively 13 and 61 months (median time) after HTx.
As depicted in Figure 1, CAV occurred in 22.9% of the first angiography (21.1% grade 1; 1.8% grade 2; 0% grade 3), and in 41.5% of the second (34.1% grade 1; 6.2% grade 2; 1.2% grade 3).
As represented in Figure 2 and 3 , ISHLT grading of both angiographies predicted MACE ($p<=0.001$).

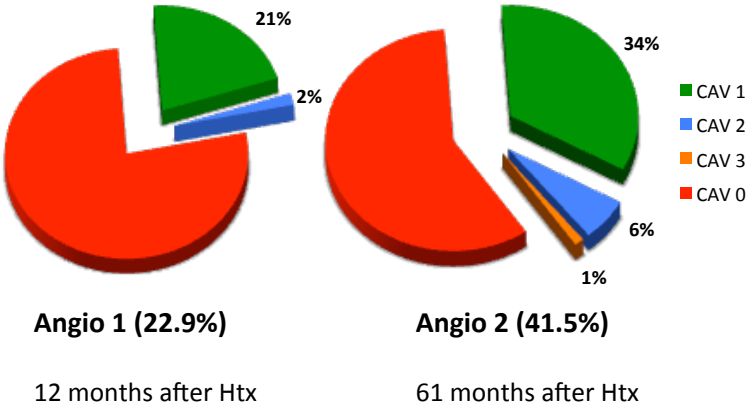


Fig. 1 CAV prevalence

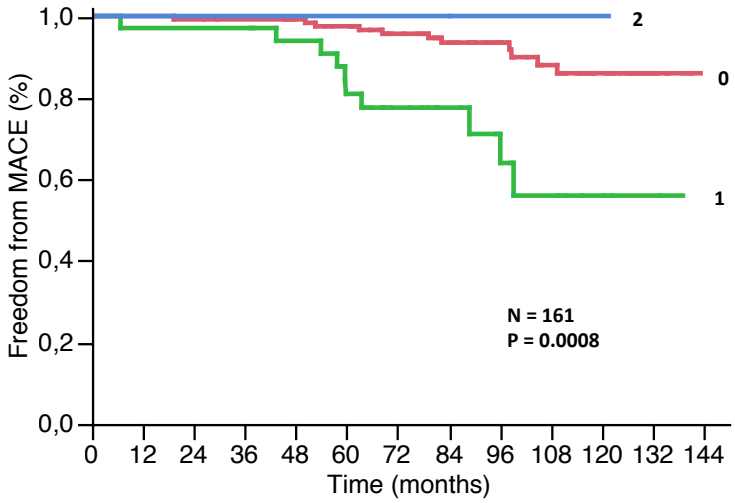


Fig. 2 Effect of ISHLT grading for first angiography on MACE

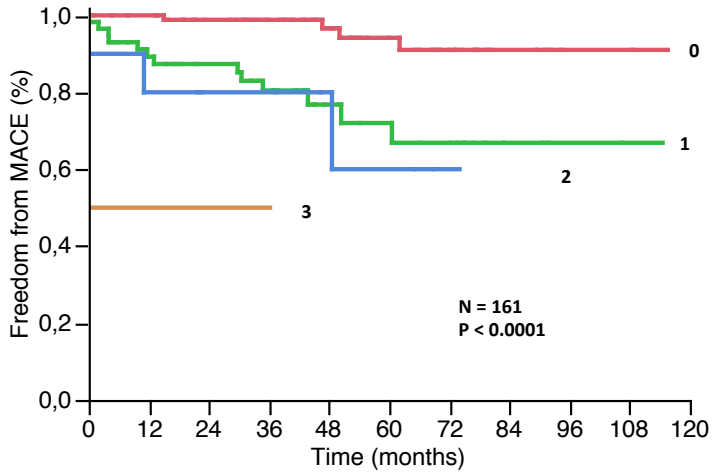


Figure 3. Effect of ISHLT grading for second angiography on MACE

24.8% of patients had an ISHLT-grading progression (75% from 0 to 1, 15% 1 to 2, 5% 0 to 2, 5% 1 to 3), 33.5% of patients had an angiographic progression (Figure 4).

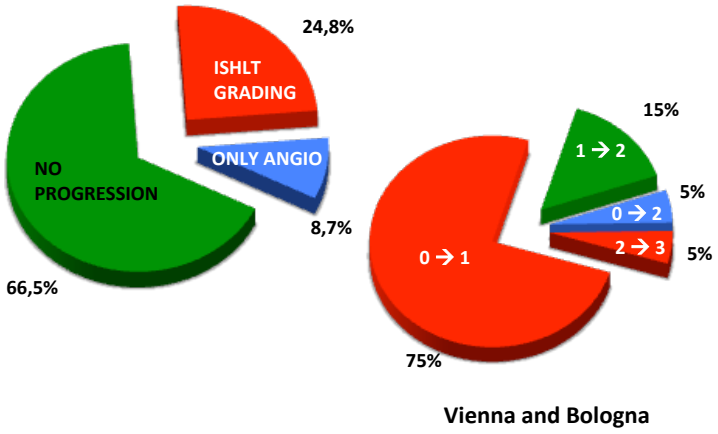


Figure 4. Dynamic CAV assessment: progression in ISHLT grading versus angiographic progression

Grade progression predicted the combined MACE (88.0±4.0% vs. 65.4±10.0%, p<0.01) but not CV death (91.3 ± 3.3% vs. 91.7 ± 4.6% vs. p=0.42), see Fig. 5 and 6.

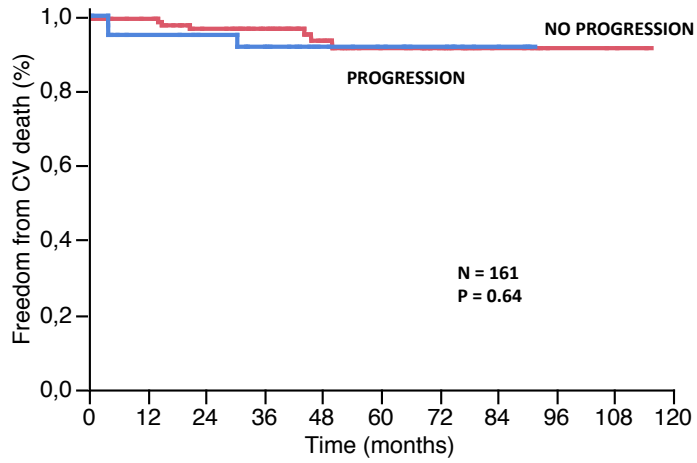


Figure 5. Effect of progression in ISHLT grading on cardiovascular death

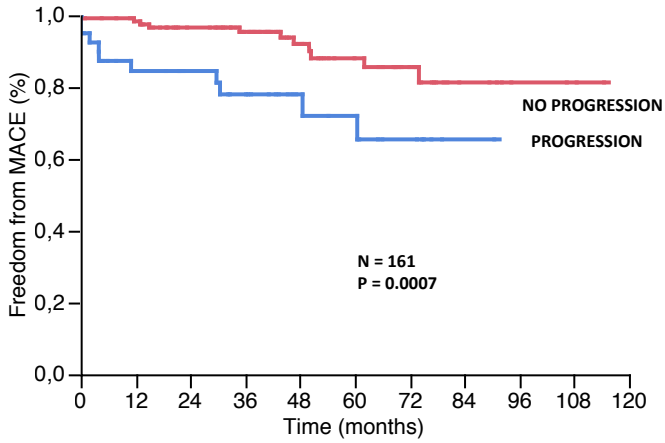


Figure 6. Effect of progression in ISHLT grading on MACE

Any angiographic progression predicted both CV death (95.8±2.6% vs. 82.6±6.3%, p<=0.0108) and MACE (93.9±6.1% vs 59.4±9.2%, p<0.0001), as represented in Figures 7 and 8.

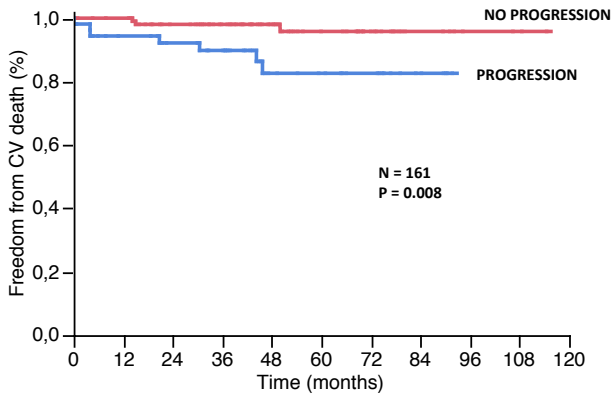


Figure 7. Effect of angiographic progression on cardiovascular death

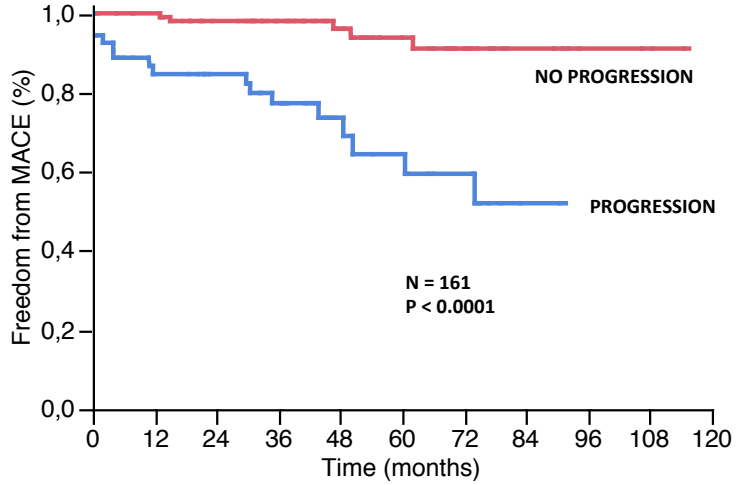


Figure 8. Effect of angiographic progression on MACE

Of note, patients (8.9%) with an angiographic progression within the same ISHLT grade, had a higher incidence of CV death and MACE as compared to the non-progression group (95.8%±2.6% vs. 52.8±19.1%, p<0.001; 93.9%±3.2% vs. 40.3±19.2%, p<=0.0012). These results are represented in Figures 9 and 10.

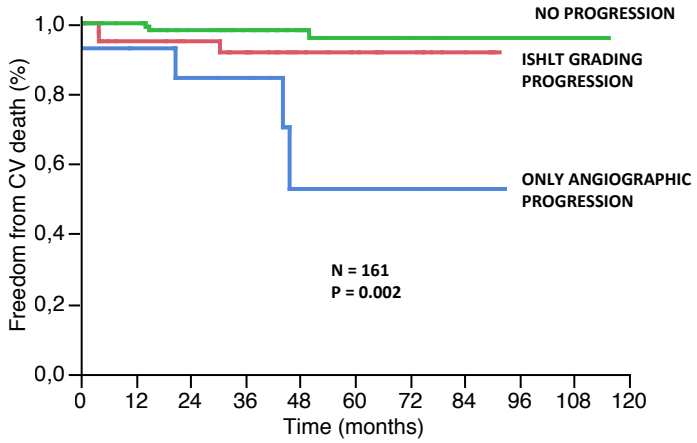


Figure 9. Effect of angiographic progression within the same ISHLT grade on cardiovascular death

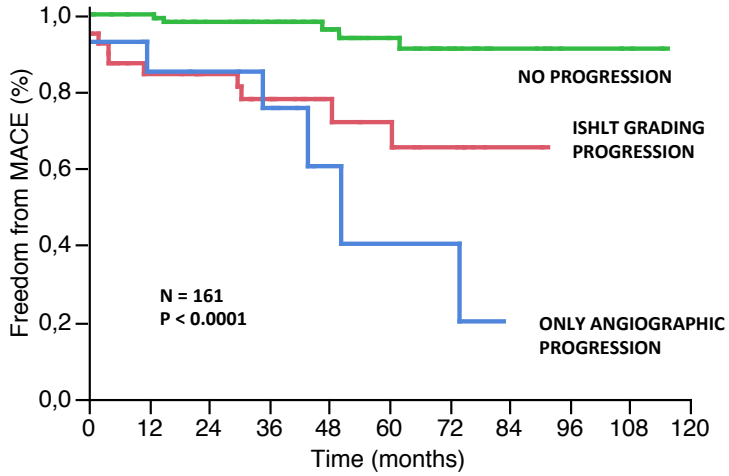


Figure 10. Effect of angiographic progression within the same ISHLT grade on MACE

Entering both static and dynamic features in a multivariate model, static assessment of CAV according to ISHLT grading was able to predict independently MACE. However, ISHLT grading progression appeared to be more sensitive in predicting MACE. (Table 2).

	OR	95% CI	P
"static" ISHLT grading at 2° angio	18.3 (range, non unità)	2.5-107	**0.005**
ISHLT grading progression	1.2	0.4-3.5	0.74
Distance from HTx	3.2	0.1-57.1	0.47

	OR	95% CI	P
"static" ISHLT grading at 2° angio	3.6 (range, non unità)	0.2-32.7	0.32
Angiographic progression	4.8	1.4-20.4	**0.01**
Distance from HTx	2.0	0.06-39.2	0.65

Table 2. Static versus dynamic CAV assessment on MACE prediction at multivariate analysis

Conclusion:

Both static and dynamic ISHLT CAV grading stratifies CV prognosis after HT. However, analysis of progression of mild lesions not captured by changes in ISHLT grading appears to improve angiographic accuracy in stratify prognosis long term after HT, underlining CAV dynamic features and its negative prognostic impact. These data support the inclusion of the concept of "progression" in CAV grading system, identifying mild progressing lesions as potential therapeutic targets to improve prognosis after HTx.

2.2 Interplay of coronary angiography and intravascular ultrasound in predicting long-term outcomes after heart transplantation

accepted for publication on

Journal of Heart and Lung Transplantation 2015

Introduction

Although modern immunosuppressive strategies have led to a marked reduction of acute rejection rates in heart transplant (HT) recipients, cardiac allograft vasculopathy (CAV), the major manifestation of chronic rejection, still remains the leading cause of death in the long-term. (1)

Longitudinal studies based on intravascular ultrasound imaging (IVUS) revealed that early changes in coronary artery geometry, such as increase in maximal intimal thickness (MIT), are clearly related to adverse prognosis, identifying early MIT change as a potential therapeutic target (2-4). Among the several drugs used in the transplant setting, convincing data indicate a drug-related benefit on early CAV development from statins (5, 6) and inhibitors of mammalian target of rapamycin (mTOR) (7, 8). While statins are widely used after HT, and observational registries confirmed their benefit in clinical practice (9, 10), a suboptimal safety profile limited the use of mTOR inhibitors in the early post-HT phase out of the context of randomized clinical trials, and their effect on CAV development in clinical practice is unknown.

Late after the first post-HT year, CAV morphological features and risk factors appear to differ from the early pattern (11-13). However, the effect of pharmacologic interventions on late CAV development has been investigated by a few studies that provided contradictory results (14, 15).

Herein we aimed to explore how therapeutic strategies could affect early and late changes in coronary morphology. In particular, we focused our analysis on the effect of the mTOR inhibitor everolimus on early (year 1) and late (year 1 to 5) changes in coronary morphology after HT, as detected by IVUS, and using patients receiving mycophenolate mofetil (MMF) as a standard comparative cohort.

Methods

Study design

This observational study is a retrospective analysis of data prospectively collected in our institutional database, involving all consecutive HT recipients fulfilling study inclusion criteria. The study design is depicted in figure 1.

Introduction

Cardiac allograft vasculopathy (CAV) remains the major cause of late graft dysfunction and death after heart transplantation (HT)[1] : its prevalence steadily increases during post-transplant follow-up, with at least 50% of patients affected within 10 years after transplant. [1] Nevertheless, according with international registry data, over 50% of long term mortality related to graft failure is not ascribed to CAV: it can be reasonably speculated that a consistent fraction of those deaths may be related to underestimated CAV, underlining the need for improving CAV diagnosis long term after transplant.

Typical CAV features [2, 3] reduce the diagnostic sensitivity of coronary angiography; [4]however, current guidelines indicate angiography, coupled with the assessment of graft function, as the imaging procedure of choice for CAV diagnosis and classification, and to predict long-term prognosis. [5] Despite intravascular ultrasound (IVUS) imaging allows detection of angiographically silent early CAV, not enough data support this technique for routine CAV diagnosis, in particular in the long-term follow-up. [5] While IVUS-detected increase in intimal thickness during the first year post-transplant is a marker for CAV development, and predicts cardiovascular prognosis, whether IVUS performed subsequently to year 1 after transplant bears any additional prognostic information is unknown. [6, 7] Moreover, therapeutic interventions designed to reduce CAV development traditionally focused on the first post-transplant year, using IVUS findings as surrogate endpoint, [8-10] leaving unexplored the effect of therapies and risk factors management on late progression of IVUS and angiographic lesions.

The scopes of this study are: 1) analyze the role of serial coronary imaging in improving the stratification of cardiovascular prognosis late after transplantation, focusing angiography classification on the grades proposed by current ISHLT guidelines [5]; 2) explore the hypothesis that IVUS imaging performed later than the first year of follow-up may provide further information to serial coronary angiography in predicting long term prognosis.

Methods

Study design

This is a single-center observational analysis, aiming to analyze the impact of coronary imaging on the long-term outcome of recipients surviving at least 5 years after transplant. The sole inclusion criteria comprised availability of IVUS performed at 1 (study baseline) and 5 years after transplant, carried out as part of our standard CAV surveillance protocol, since 1998. [11] Severe comorbidities, moderate to severe renal dysfunction, and unwillingness to undergo invasive procedures were the main reasons to withdraw IVUS and angiography performance in our routine clinical practice.

Patient demography, therapies and clinical events were retrieved from a prospectively filed web-based secured database including all transplant recipients ever followed at our Center since 1985, and from the Hospital electronic repository.

This study was approved by local ethical committee and is in compliance with local laws and regulations.

Coronary angiography and IVUS

Coronary angiography was performed with standard technique with at least two planes for right coronary artery and three for left coronary artery examined and digitally recorded for off-line analysis. Angiogram reports were reviewed and classified according with ISHLT guidelines [5] and graded (from CAV 0 to CAV grade 3) blinded to clinical events and IVUS findings. Angiographic progression was evaluated by comparing year 1 and year 5 angiograms and was identified either as ISHLT grade progression, or as any worsening of disease severity within the same grade (e.g. change from mild single vessel disease to mild multiple vessel disease is a progression of CAV extension, although in both cases ISHLT grading remains 1)

IVUS procedure was performed on LAD, after excluding stenoses ≥70%, as detailed previously. [12] Analyzed vascular segments from year 1 and 5 studies were accurately matched, using side-by-side longitudinal reconstruction of the LAD and left main. [13] Our Institutional IVUS Core laboratory performed 2-dimensional and 3-dimensional IVUS analysis by a semi-automated method using Qivus® Clinical Edition software (Medis medical imaging systems BV, Leiden, NL). Changes in maximal intimal thickness (MIT), intimal, lumen and whole vessel volumes were evaluated to describe variability in coronary geometry potentially associated to clinical outcomes. To minimize measurements variability [14] only one individual (V.P.) blinded to the patients' outcome performed all the software assisted measurements reported in this study.

Study endpoints and data analysis

Study outcome was the occurrence of the composite endpoint of fatal and non-fatal major cardiovascular events (MACE) including: myocardial infarction, myocardial revascularization, and admission for acute heart failure (HF). Sudden death, in absence of known potentially fatal non-cardiovascular comorbidities, was considered of cardiovascular (CV) etiology. For descriptive purposes we additionally analyzed separately the occurrence of CV death, but the limited number of this event prevented multivariate analyses. Patients' follow-up was recorded up to 10 years after year-5 coronary imaging (i.e. 15 years after transplantation). Continuous variables are expressed as means $\pm$ standard deviation or as medians (25^{th} to 75^{th} percentile), if skewed distributed. Differences among groups were assessed by ANOVA, Chi square tests as appropriate. Receiver-operating curve (ROC) was used to identify the cut-off in IVUS measurements best predictive of study outcomes. Person-time and incidence rates were calculated and 95% confidence interval was reported. Composite MACE and CV death-free survivals were estimated with Kaplan-Meier method, and differences between groups were assessed with log-rank test. Contribution of clinical and laboratory data to outcomes was identified by Cox's univariate and multivariate regression analysis. The goodness of fit of multivariate Cox regression models including clinical and imaging data was compared by the likelihood ratio test. $P < 0.05$ was considered as significant.

Results

Study population and events

One hundred thirty-one patients, receiving HT between July 1998 and October 2007 were included in the study. A study flow-chart is depicted in the supplementary figure 1. As shown in table 1, between year 1 and 5 a limited number of patients was converted to an mTOR inhibitor and steroid weaning was poor, with still 80% of patients on prednisone at year 5, although average daily dose significantly decreased ($P<0.01$). Statins were widely used, with 91% on medication at year 5, and metabolic parameters, including renal function, remained stable.

Year-5 IVUS was performed between June 2003 and July 2012 and patients were followed for the subsequent 1 to 10 years (689 total patient-years). During the follow-up period, at least one MACE occurred in 21 (16%) patients: 5 admissions for HF, 2 percutaneous coronary revascularization, 2 ST-elevation myocardial infarction, and 12 CV deaths (6 sudden deaths, 6 chronic graft failures). Incidence rate of MACE was 31.8 (95% CI: 20.7 to 48.8) and of CV death was 17.4 (9.9 to 30.6) per 1,000 person-years.

Coronary angiography and prognosis

Figure 1A details progression of angiographic grading between year 1 and 5. Of the 21 patients with angiographic progression, in four cases lesions progressed within the same ISHLT CAV grade. This was the case of mild disease in one vessel (grade 1 CAV), which progressed to mild disease in two to three vessels (still CAV grade 1, but clear angiographic progression). Of note, two of these four patients presented CV death during follow-up. On the other hand, only one of the ten patients with stable CAV 1 had a non-fatal MACE.

As shown in Figure 1B, patients with angiographic progression suffered a significantly greater incidence of MACE as compared with those with stable angiography, either CAV 0 or stable CAV 1 (103 vs. 22 events per 1,000 person-years; incidence rate ratio 4.6: 95% CI: 1.6 to 11.9, P<0.01). Restricting the analysis to CV death only, the ability of angiographic progression in identifying patients at risk only approached statistical significance (44 vs. 13 deaths per 1,000 person-years; incidence rate ratio: 3.3; 95% CI: 0.73 to 12.3; P=0.07).

IVUS measurements and prognosis

Coronary geometry markedly changed between year 1 and year 5, with significant increase in intimal volume and MIT, and in loss of lumen volume. Of note, at year 5, only five (4%) patients showed a normal MIT (i.e. <0.5mm) [15, 16]. Vessel volume did not changed significantly overall (Table 2), but varied widely across study population, with 54% of patients showing positive vascular remodeling.

As shown in Table 3, MIT increase predicted both MACE and cardiovascular death risk. Vessel volume and intimal volume increase, but not lumen loss, were associated with MACE risk only, suggesting a prognostic relevance of vascular wall remodeling.

By building a ROC curve, we identify a MIT change ≥0.35mm as the most accurate cutoff to predict MACE. In patients with MIT change ≥0.35 mm, MACE incidence was 80 per 1,000 patients-year, while in those with MIT change <0.35 mm, MACE occurred in 13 per 1,000 patients/year (incidence rate ratio 6.3; 95% CI: 2.3 to 19.7, P<0.01). Similarly, this cutoff allowed detecting the subgroup of patients with higher incidence of CV death (38 vs. 8 CV deaths per 1,000 person-years; incidence rate ratio 4.5; 95% CI: 1.2 to 20.5; P=0.01). These differences are depicted in the Kaplan-Meier estimates of events shown in Figure 2.

Interplay between IVUS and angiographic progressions in predicting MACE.

Patients with progressing angiographic lesions showed worsening IVUS measurements, in terms of greater increase in MIT (0.39 [0.10-0.95] vs. 0.18 [0.04-0.37] mm; P=0.03) and in intimal volume (77 [10-147] vs. 14 [-19 to 54] mm^3; P<0.01), greater loss in lumen volume (-62[-109 to -6] vs. -13[-64 to 21] mm^3; P=0.03), and higher likelihood of MIT change ≥0.35mm (52 vs. 28%; P=0.03).

To elucidate whether IVUS-detected changes were adding any prognostic information to what could be gained by angiography alone, we stratified the effect of MIT change by angiographic progression. In the small group of 21 high-risk patients with any lesion progression, MIT increase ≥0.35mm did not stratify the estimated incidence of MACE (154 vs. 51 events per 1,000 patients-year respectively; P=0.2). On the other hand, in the subgroup of 110 patients with stable angiographic lesions, MIT increase ≥0.35mm identified those with a significantly greater incidence of MACE (60 vs. 9 events per 1,000 patients-year; incidence rate ratio 6.5; 95% CI: 1.6 to 20.7; P<0.01). Kaplan-Meier analysis shown in Figure 3, highlights that while angiographic progression identified patients with the earliest incidence of MACE, IVUS-detected changes in patients without angiographic progression of the disease discriminates between patients with very low and those with intermediate-late MACE incidence.

In addition to imaging data, clinical and laboratory characteristics at year 5 were analyzed as potential confounders of MACE prediction by IVUS and angiography (Supplementary Table). Using a stepwise

approach, MACE-associated variables with a P value<0.1 were factored in a multivariate model including angiographic progression (Table 4 – Model 1). In this model angiographic progression independently predicts MACE risk, together with age and renal function, while hyperglycemia dropped out of the analysis. After adding MIT change (Model 2), both imaging variables persisted significant, and the Likelihood-ratio test comparing the two multivariate models showed that Chi square of model 2 increased significantly (P<0.01), suggesting that the information added by IVUS to clinical risk factors and angiography evaluation improved prognosis prediction.

Risk factors for angiographic and IVUS progression

In table 5 we analyzed the association of demographic, clinical and laboratory parameters at year 1 after transplant (time of baseline IVUS), with the degrees of CAV progression during the subsequent 4 years. Male gender, pre-transplant ischemic heart disease, fasting hyperglycemia, triglycerides and metabolic syndrome were significantly associated with CAV development, in a proportional fashion to the severity of the disease. Of note, we were unable to find any association with cellular rejection, or with immunosuppressive strategy. Use of anti CMV prophylaxis, on the other hand, was associated with less IVUS-detected progression.

Discussion

By analyzing angiographic and IVUS-detected changes over a 4-year time period, this study provides a first suggestive evidence of the association of late progressing angiographic and IVUS coronary lesions with long-term cardiovascular prognosis, and supports the concept that IVUS imaging may improve prognostic stratification even long term after transplant.

Several observational studies [17-19] clearly show that the severity of angiographic lesions is proportional to the risk of graft loss and MACE. Our results expand these concepts beyond the first years after transplant, focusing on longitudinal lesion progression. In particular, we found that even mildly changing angiographic lesions (i.e. progressing lesions within ISHLT grade 1), are associated with poorer outcome as compared with stable or absent coronary lesions (Figure 1). Although serial evaluation of angiograms may improve

prognostic stratification, a vast majority of patients (76%) presented normal coronary angiography at year 5. On the other hand, at year 5, only 4% of patients showed a MIT below the 0.5 mm threshold, considered as "normal" in the historical IVUS studies, and over 50% showed "severe" intimal thickening. [16, 20] This finding is in line with pivotal IVUS studies showing that at year 1 about 20 to 40% of patients presented with pathological MIT, [6, 7, 15, 16] and that MIT continues to steadily increase after the first year. [21, 22] In this setting, a central question was to ascertain whether in this context of advanced abnormalities [16] IVUS could retain any long-term prognostic information. As previously observed in native atherosclerosis, [23] we found that vessel enlargement in response to plaque increase was predictive of cardiovascular events, regardless of lumen loss (Table X). In addition, MIT change predicted both MACE and CV death and it was possible to derive a cutoff improving the stratification of cardiovascular prognosis obtained by angiography and clinical assessments alone. MIT change ≥0.35mm was found associated with higher incidence of MACE and CV death (Figure 2), identified patients at risk for MACE among those without angiographic progression, (Figure 3) and improved MACE risk prediction (Table 4). Not surprisingly we found a threshold for prognostic MIT change lower than that reported in studies focusing on early follow-up (0.5mm): intimal growth has been reported to be largest during the first year and smaller afterwards [22, 24], and the proportion of patients treated with statins in this cohort is much higher than what reported in earlier studies, (i.e. 30% vs. 90%) further supporting the concept of a strong protective effect of statins on CAV. [6-9, 15, 16, 25]

In addition, the results of this study underscore the importance of the metabolic syndrome milieu (e.g. high fasting glucose, high triglycerides, renal insufficiency) as relevant risk factors for late CAV and for adverse long-term prognosis. [26-28] While the widespread use of statins may explain the lack of association between cholesterol and CAV in this series, [29] low rate of steroid weaning, on the other hand, may at least partially support the relevant contribution of altered glucose metabolism in CAV development (Table 5). Regarding immune-mediated risk factors, we were unable to detect any effect of cellular rejection on CAV or on subsequent prognosis (Table 3), whereas data regarding antibody mediated rejection, or even subclinical detection of circulating donor specific antibodies, were unavailable in the majority of patients and could not be analyzed. Anti CMV prophylaxis was associated with lower IVUS-defined progression,

(Table 5) confirming on the long term our previous findings focusing on first post transplant year. [12] We cannot exclude however that this observation may be biased by an era effect because anti CMV prophylaxis was used only in patients receiving HT after 2005.

Study limitations

This is a single center retrospective analysis, therefore these results should not be considered as definitive but hypothesis generating. However, by using a sample size comparable to that of previous studies we were able to replicate in long-term survivors the same concept of IVUS-detected CAV demonstrated in the first post-transplant year IVUS. [6, 7] In this study, we used grayscale IVUS analysis with volumetric reconstruction of proximal LAD. This approach bears several limitations including that LAD morphology is used as a surrogate for the entire coronary tree and that gray scale analysis does not allow analysis of intimal thickness composition. IVUS with virtual histology and coronary angioscopy, on the other hand, allowed demonstrating that CAV is characterized by heterogeneous plaque morphologies bearing specific prognostic information. [30, 31] Nevertheless, our approach represent a standard, simple and reproducible method that is accepted and validated in early CAV monitoring: our findings support its validity in combination with serial coronary angiography to stratify graft prognosis beyond year 5 after transplantation, despite lacking data on specific plaque composition. Finally, the MIT threshold has been derived from the same cohort of patients which it has been applied on: we cannot exclude that in a validation cohort this threshold may provide different results.

Conclusions

While showing that assessment of late coronary lesion progression provides prognostic information beyond ISHLT CAV classification, we identified late MIT increase as an additional sensitive marker for late adverse CV prognosis. Moreover, this study provide further data supporting pathophysiological similarities between late CAV and native coronary atherosclerosis, [24] including the relevance of metabolic syndrome milieu as a key risk factor for disease progression, and of dilated coronary remodeling as a marker of subsequent cardiovascular events.

Clinical implications

In the effort of achieving the most accurate prognostic stratification in long-term heart transplant recipients, these findings support the strategy of performing routine coronary angiography, with accurate serial match to identify progressing CAV, coupled with IVUS in patients with CV risk factors and normal or non-progressing angiograms. Although we do not provide data supporting specific interventions in patients with progressing CAV, it is reasonable to suggest aggressive management of metabolic abnormalities in the early years after transplant, in light of the prominent association between metabolic syndrome features and CAV progression. Nevertheless, the issue of prevention and treatment of long-term CAV development still remains unanswered: this study may provide the basis to design appropriately sized interventional studies, in which late MIT change may be used as surrogate prognostic endpoint.

Acknowledgements

This work has been supported by a Grant from Banca del Monte Foundation. No commercial entity participated in study funding, design or analysis. None of the Authors has any interest to disclose related to the topics discussed in this manuscript.

Figure legends

Figure 1.

Panel A: Flow chart of CAV grades associated with angiographic progression

Panel B: MACE cumulative incidence according with angiographic classification

Figure 2.

Panel A: MACE cumulative incidence according with IVUS progression

Panel B: Cumulative incidence of cardiovascular death according with IVUS progression

Figure 3

MACE cumulative incidence according with angiographic or IVUS only progression.

Figure legends

Figure 1.

Panel A: Flow chart of CAV grades associated with angiographic progression

Panel B: MACE cumulative incidence according with angiographic classification

Figure 2.

Panel A: MACE cumulative incidence according with IVUS progression

Panel B: Cumulative incidence of cardiovascular death according with IVUS progression

Figure 3

MACE cumulative incidence according with angiographic or IVUS only progression.

Table

Table 1. Patient demography and clinical characteristics at year 1 (study baseline) and 5 after HT

	Study population n=131	
Demography		
Male gender, n (%)	112 (85%)	
Age at transplant, median	56.6 (15-67.5)	
Pre transplant diagnosis, n (%)		
Dilated cardiomyopathy	46 (35%)	
Ischemic	62 (47%)	
Other	23 (18%)	
	Year 1	**Year 5**
Immunosuppressive strategy, n (%)		
Prednisone	128 (98%)	106 (81%)
Average daily dose (mg/kg)	0.12±0.05	0.05±0.03*
Micophenolate mofetil	52 (40%)	56 (43%)
Azatioprine	60 (46%)	39 (30%)
Everolimus	3 (2%)	24 (18%)
Statins	111 (85%)	117 (91%)
Metabolic parameters		
Serum creatinine (mg/dl)	1.53±0.46	1.53±0.43
Serum glucose (mg/dl) §	91 (84-105)	93 (83-108)
Total cholesterol (mg/dl)	196±36	185±37*
HDL cholesterol (mg/dl)	58±15	56±18
LDL cholesterol (mg/dl)	103	94*
Triglycerides	171±77	160±70*
Treated DM, n (%)	32 (24%)	44 (33%)
Metabolic syndrome, n (%)†	50 (38%)	55 (42%)

* P<0.05 for paired analysis between Year 1 and Year 5 values; § Median (25^{th} to 75^{th} percentile); † According with NHLBI-AHA consensus published in Circulation. 2004; 109: 433-438

Table 2. Changes in IVUS-related measurements

	Year 1	Year 5	Change between year 1-5 (median, 25th to 75th percentile)	*P*
MIT (mm)	0.96	1.22	0.2 (0,05 to 0,41)	<0.01
Intimal volume (mm3)	149.9	174.9	18.6 (-11.9 to 61.6)	<0.01
Vessel volume (mm3)	533.9	531.9	8.8 (-44.3 to 57.3)	0.8
Lumen volume (mm3)	383.4	354.3	-18 (-71 to 18)	<0.01

Table

Table 3 Univariate risk estimate for MACE and CV death according with IVUS parameters

	Univariate risk estimate for MACE			Univariate Risk estimate for CV death		
	RR	95% C.I.	P	RR	95% C.I.	P
Year 1 to year 5 changes						
MIT (per mm)	2.93	1.22 – 6.16	0.02	3.72	1.15 – 9.83	0.03
Intimal volume (per 10 mm^3)	1.06	0.99 – 1.11	0.06	1.06	0.99 – 1.13	0.09
Vessel volume (per 10 mm^3)	1.06	1.01 – 1.11	0.04	1.04	0.98 – 1.11	0.19
Lumen volume (per 10 mm^3)	1.02	0.96 – 1.09	0.47	0.99	0.92 – 1.08	0.94

Table

Table 4. Multivariate Cox's models for risk of MACE including imaging and clinical variables

	Model 1			Model 2		
	RR	95% C.I.	P	RR	95% C.I.	P
Angiographic progression	8.92	2.97 – 26.7	<0.01	8.09	2.71 – 24.1	<0.01
Recipient age (per y)	1.08	1.00 – 1.16	0.04	1.08	1.01 – 1.16	0.02
Serum glucose > 125 mg/dl	2.11	0.70 – 6.43	0.18	-	-	-
Serum creatinine (per mg/dl)	7.42	2.67 – 20.6	<0.01	4.52	1.64 – 12.4	<0.01
IVUS progression	-	-	-	4.29	1.61 – 11.4	<0.01
Likelihood Rank Chi ^{2}		33.13			41.11	<0.01*

* P value calculated by the likelihood-ratio test

Table

Table 5. Clinical and laboratory variables at year 1 and subsequent CAV development

Characteristic	CAV progression			*P*
	Angiographic progression n=21	MIT change ≥0.35 only n=31	Stable n=79	
Male gender (%)	20 (95%)	30 (96%)	62 (78%)	<0.01
Pre transplant IHD (%)	15 (71%)	18 (58%)	29 (37%)	<0.01
Recipient age (y)	53±11	56±9	54±10	0.5
Donor age (y)	33±11	32±10	34±12	0.6
Glucose >125 mg/dl (%)	7 (33%)	5 (16%)	6 (8%)	0.01
Treated diabetes (%)	9 (43%)	7 (22%)	16 (20%)	0.10
Total cholesterol (mg/dl)	203±34	201±39	191±35	0.25
LDL cholesterol (mg/dl)	106±29	103±25	101±25	0.36
HDL cholesterol (mg/dl)	57±17	57±14	59±15	0.78
Triglycerides (mg/dl)	208±87	187±70	156±73	<0.01
Serum creatinine (mg/dl)	1.6±0.74	1.5±0.33	1.5±0.4	0.35
Metabolic syndrome* (%)	14 (66%)	12 (39%)	24 (30%)	0.01
Cellular rejection (%)	6 (28%)	9 (29%)	20 (25%)	0.89
CMV prophylaxis (%)	6 (29%)	3 (10%)	24 (30%)	0.05

IHD: ischemic heart disease; DM: diabetes mellitus

* According with NHLBI-AHA consensus published in Circulation. 2004; 109: 433-438

Figure 1
Click here to download high resolution image

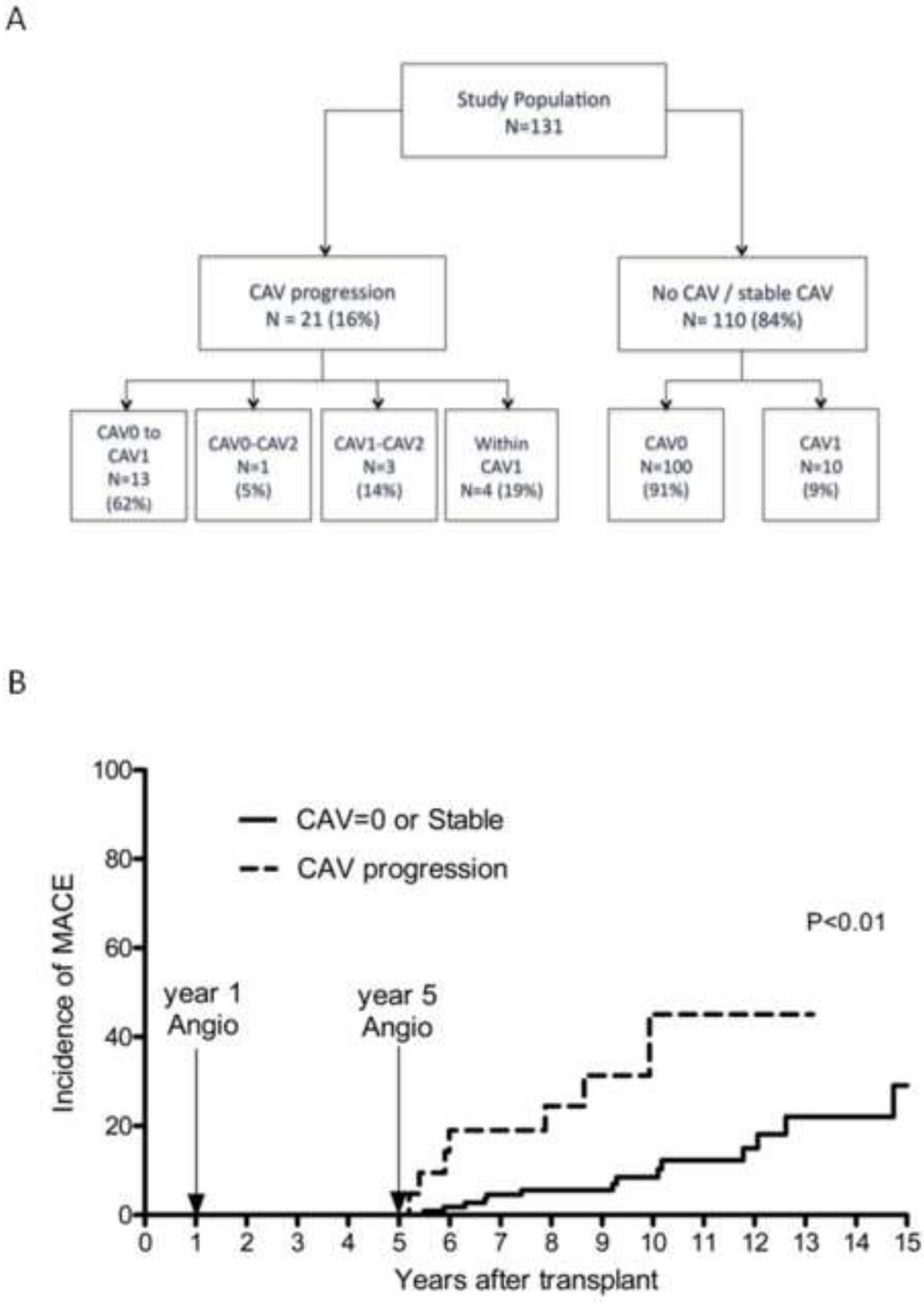
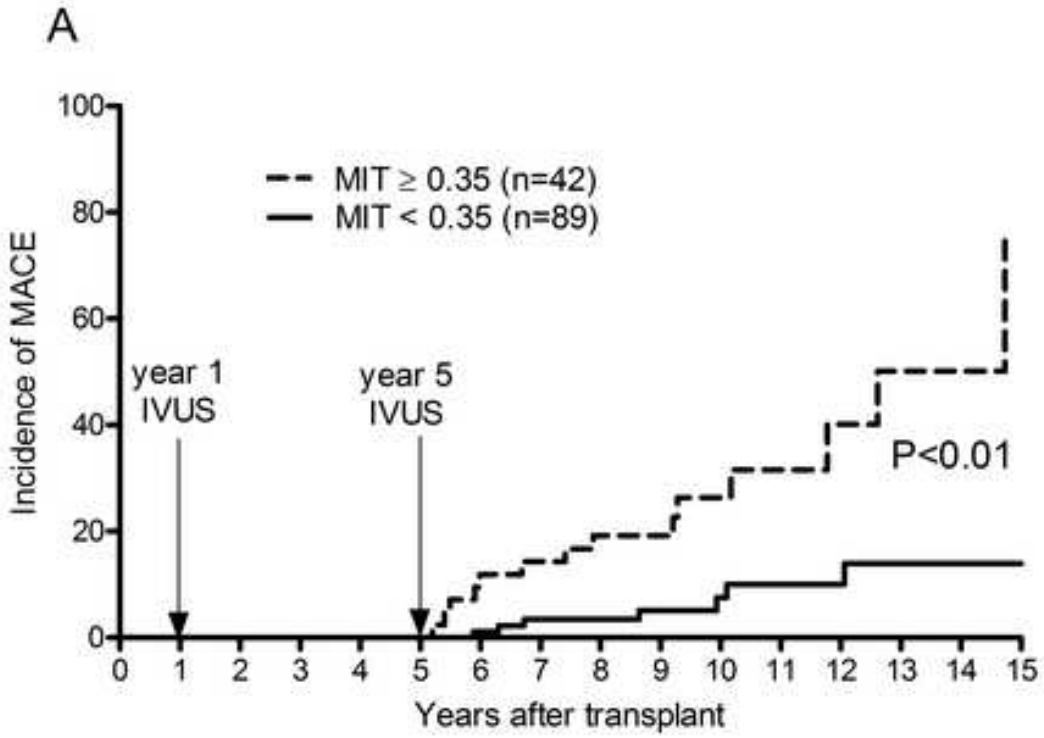


Figure 2
Click here to download high resolution image



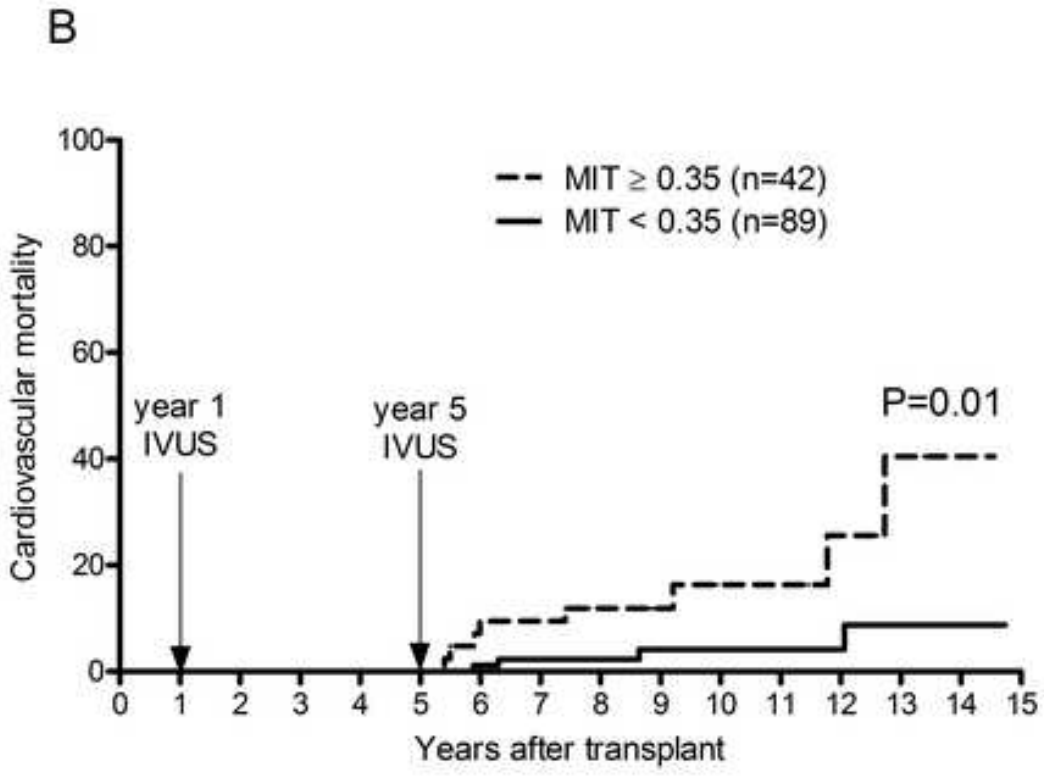
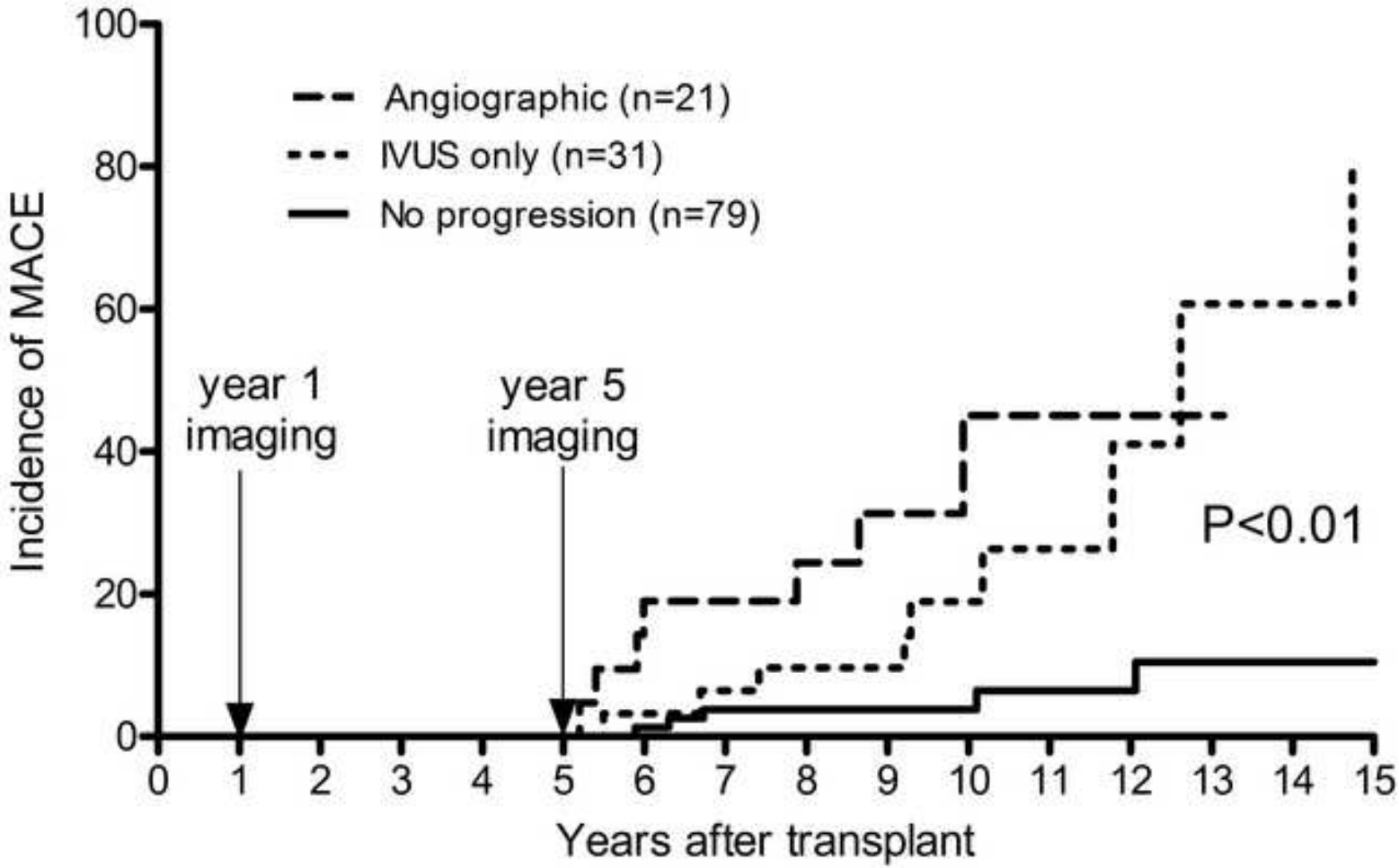


Figure 3
Click here to download high resolution image



Supplementary Table

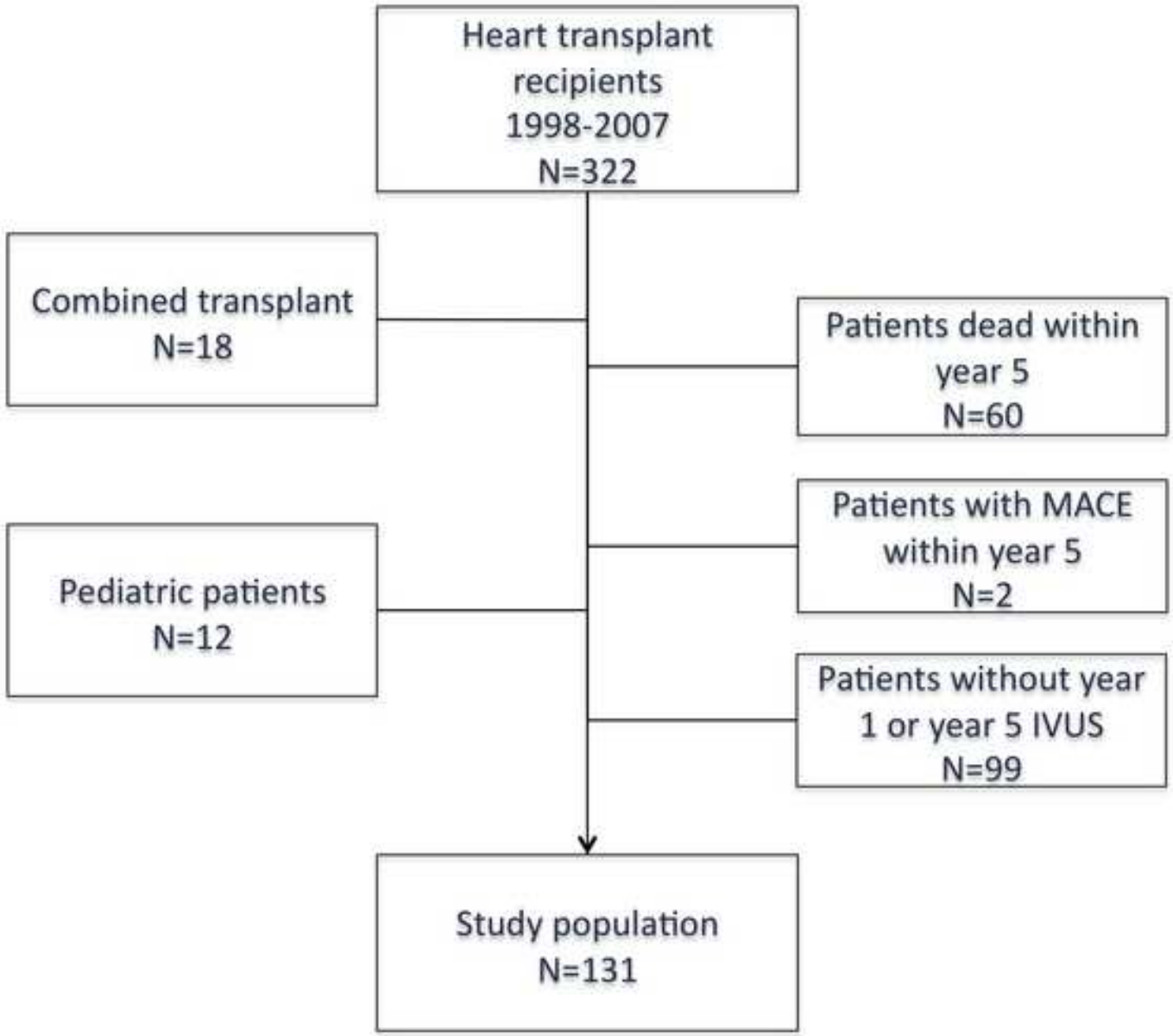
Table S1. Patients characteristics and relationships to the risk of MACE

	RR	95% CI	*p*
Recipient age (per y)	1.06	1.00 – 1.13	0.03
Ischemic etiology	2.25	0.94 – 5.74	0.06
Donor age (per y)	0.98	0.94 – 1.01	0.32
Serum creatinine	4.41	1.87 – 10.3	<0.01
Serum glucose >125mg/dl	2.97	1.06 – 8.30	0.04
Total cholesterol (mg/dl)	0,96 †	0,85-1,08	0.6
HDL cholesterol (mg/dl)	0.81 †	0.61 – 1.04	0.1
LDL cholesterol (mg/dl)	0.99 †	0.84 – 1.14	0.9
Tryglicerides (mg/dl)	1,01 †	0,96-1,07	0.5
Metabolic syndrome*	2.03	0.85 – 4.85	0.1
On everolimus at year 5	0.85	0.13 – 3.13	0.9
On steroids at year 5	0.99	0.36 – 3.5	0.99
Rejection ≥2R during y 1 to 5	1.1	0.39-2.71	0.84

RR: risk ratios; CI: confidence intervals

* According with NHLBI-AHA consensus published in Circulation. 2004; 109: 433-438

Supplementary Figure
Click here to download high resolution image



. 2.3 Role of coronary CT in predicting prognosis after heart transplantation

Introduction:

Cardiac allograft vasculopathy (CAV) is the main long term cause of death after heart transplantation (HTx). The ISHLT guidelines for CAV assessment suggest a role for coronary tomography (CT); however, its prognostic predicting power is unknown, and few data are available about the significance of scoring calcified plaques. Our study's aim was to investigate the role of coronary CT in predicting cardiovascular (CV) prognosis in HTx patients.

Methods:

In this retrospective study of prospective collected data, all patients transplanted in Vienna between 2001-08, with a CT assessment for CAV, were enrolled. A CAV positive CT was labeled as any detected irregularity (stenosis, vessel wall irregularity, calcifications) for better matching with the ISHLT CAV grading system; calcium score (CS) was collected. The primary endpoint was CV death, the secondary MACE (CV death, admission for HF, PCI, re-HTx), both expressed as 5-yrs survival rates

Results:

155 patients entered into the study (81.2% males, 50.3+/-14.0 years at HTx). CT scan was made 44±24 months after HTx; 34.8% of the patients had a CAV positive CT, 25% a positive (>0) CS. Any irregularity in the first CT scan predicted both the primary (98.9+/-0.1% vs. 89.0+/-4.7%, p=0.01) and the secondary endpoint (96.4+/-2.0% vs. 77.2+/_6.1%, p<0.0001), see Fig. 1 and 2.

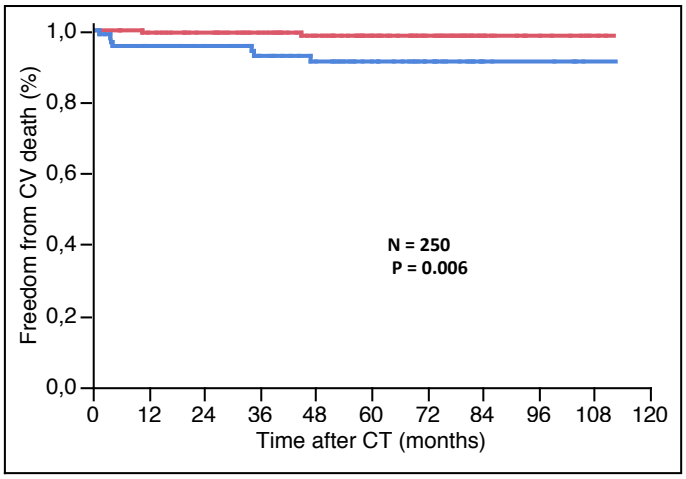
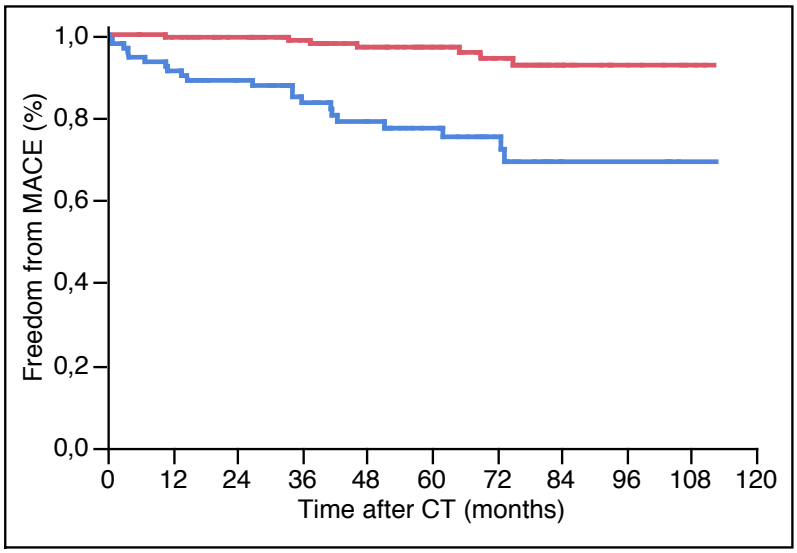


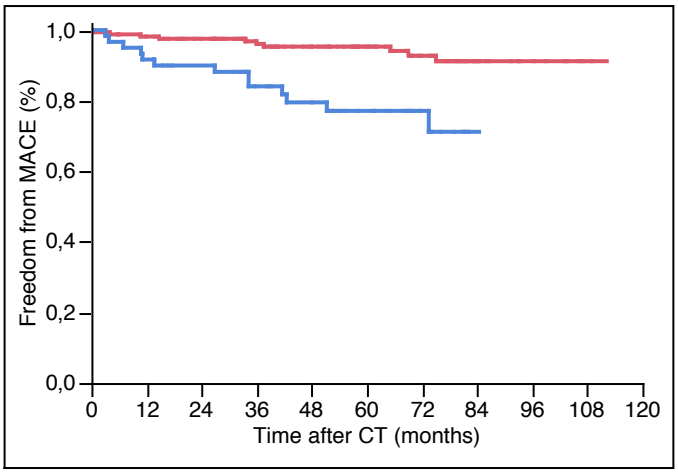
Figure 1. Role of CT scan positive for CAV in predicting CV death



N=250
P < 0.0001

Figure 2. Role of CT scan positive for CAV in predicting MACE

A positive CS predicted MACE (94.0+/-2.3% vs. 78.6+/-7.3%, p=0.01) but not CV death. (Fig. 3 and 4)



N=225
P = 0.0003

Fig. 3 Effect of positive calcium score on MACE

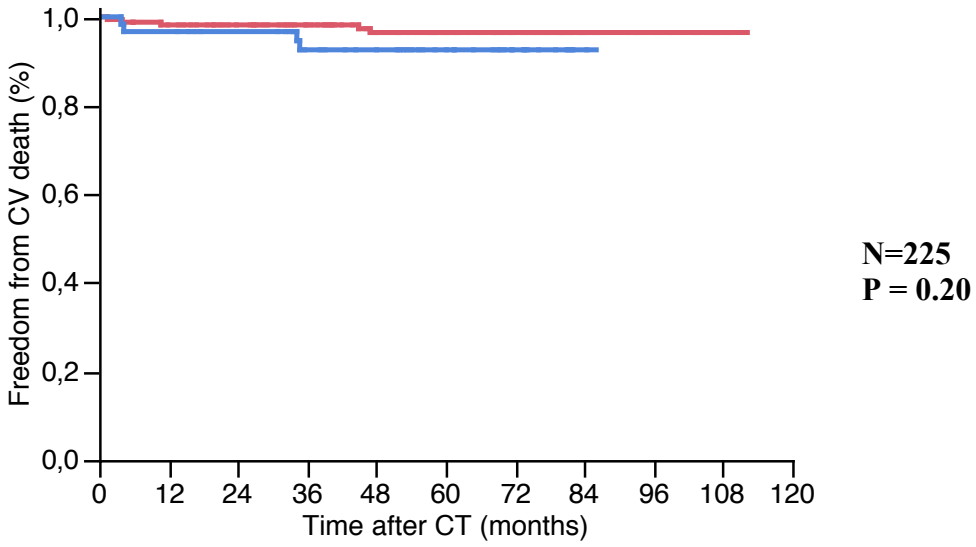


Fig. 4 Effect of positive calcium score on cardiovascular death

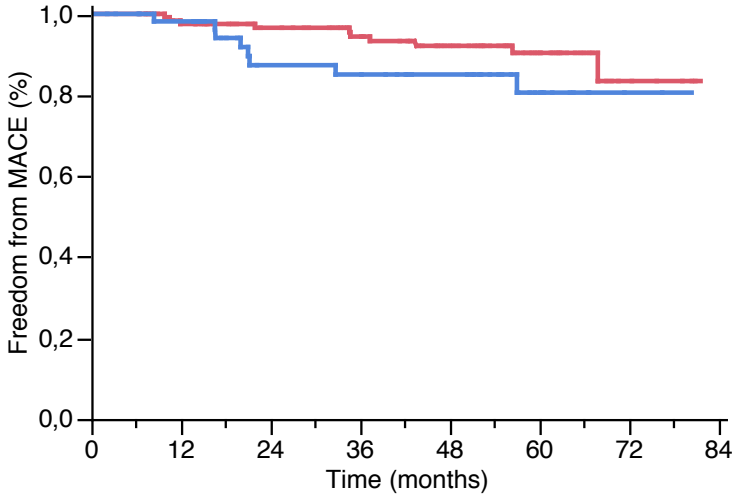
At multivariate analysis, only CT irregularity predicted MACE [OR 2.5 (95% CI 1.1-5.2), p<0.0002]. , Table 1.

	OR	95% CI	P
CT positive for CAV	6.7	1.7-23.4	**0.008**
Positive calcium score	0.93	0.32-3.33	0.90

Table 1. Multivariate analysis on MACE prediction

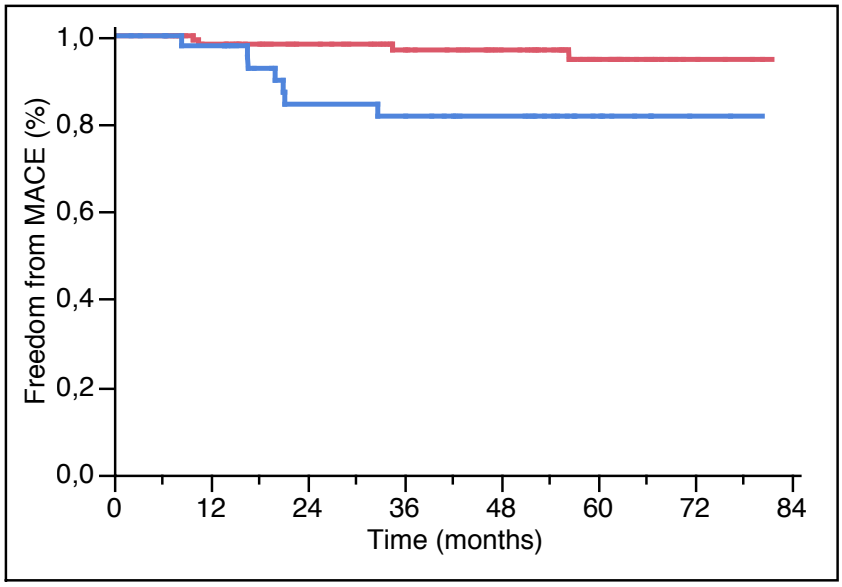
115 patients underwent a second CT, 22±12 months after the first one; 27.8% of them had a CAV progression (increase of a stenosis, new onset stenosis, higher CS), and this predicted outcome with a borderline statistical significance (Fig. 5)

Among the 102 in which CS was calculated, the ones who experienced an increase in CS (28) had a higher rate of MACE (98.6+/-1.3% vs. 82.4+/-9.5%, p=0.03), Fig. 6.



N=197
P=0.09

Figure 5. Effect of CT progression on MACE



N=160
P = 0.006

Fig. 6 Role of calcium score progression on MACE

Among the 39 patients who had a coronary angiography within 6 months from the CT, the ones with CT-detected CAV had a higher rate of angiography detected CAV (88.9% vs. 47.6%,p=0.006); a CT negative for CAV predicted a negative angiography with a 84% sensitivity and a 62% specificity (AUC 0.73,p=0.004).

Conclusion:

CT –detected coronary irregularities can predict CV death and MACE. CS may be used only as a longitudinal parameter to predict CV prognosis in a subset of patients. Even if its sensitivity in CAV assessment is lower than angiography, our study suggests that CT could be used a non-invasive screening tool for predicting prognosis in selected HTx patients.

Chapter 3. Cardiac allograft vasculopathy: trying to improve prevention

3.1 Differential effect of everolimus on progression of early and late cardiac allograft vasculopathy in current clinical practice

American Journal of Transplantation 2013;13(5):1217-26

Introduction

Although modern immunosuppressive strategies have led to a marked reduction of acute rejection rates in heart transplant (HT) recipients, cardiac allograft vasculopathy (CAV), the major manifestation of chronic rejection, still remains the leading cause of death in the long-term. (1)

Longitudinal studies based on intravascular ultrasound imaging (IVUS) revealed that early changes in coronary artery geometry, such as increase in maximal intimal thickness (MIT), are clearly related to adverse prognosis, identifying early MIT change as a potential therapeutic target (2-4). Among the several drugs used in the transplant setting, convincing data indicate a drug-related benefit on early CAV development from statins (5, 6) and inhibitors of mammalian target of rapamycin (mTOR) (7, 8). While statins are widely used after HT, and observational registries confirmed their benefit in clinical practice (9, 10), a suboptimal safety profile limited the use of mTOR inhibitors in the early post-HT phase out of the context of randomized clinical trials, and their effect on CAV development in clinical practice is unknown.

Late after the first post-HT year, CAV morphological features and risk factors appear to differ from the early pattern (11-13). However, the effect of pharmacologic interventions on late CAV development has been investigated by a few studies that provided contradictory results (14, 15).

Herein we aimed to explore how therapeutic strategies could affect early and late changes in coronary morphology. In particular, we focused our analysis on the effect of the mTOR inhibitor everolimus on early (year 1) and late (year 1 to 5) changes in coronary morphology after HT, as detected by IVUS, and using patients receiving mycophenolate mofetil (MMF) as a standard comparative cohort.

Methods

Study design

This observational study is a retrospective analysis of data prospectively collected in our institutional database, involving all consecutive HT recipients fulfilling study inclusion criteria. The study design is depicted in figure 1.

To study early changes in coronary morphology we analyzed a cohort of consecutive adult recipients transplanted between November 2004 and June 2010 (early cohort), who underwent a baseline (within 6 weeks from transplantation) and a 1-year follow-up IVUS study, and with everolimus (marketed in Europe as Certican® by Novartis Pharma, Basel, Switzerland, since 2003) or MMF (marketed as CellCept, by Roche, Basel, Switzerland) added to cyclosporine as maintenance immunosuppression during the first month after transplant.

Late changes in coronary morphology were assessed by analyzing IVUS performed at years 1 and 5 after HT in a cohort of patients transplanted between 1999 and 2006 (late cohort). In this cohort of patients, year 1 was considered as "baseline". We included in the study all patients taking MMF or everolimus for at least 3 of the 4 years of follow-up, with complete year 1 and 5 IVUS data available.

IVUS procedure

In our center, we routinely perform IVUS for CAV progression surveillance at baseline (3 to 6 weeks after transplant), and at years 1 and 5 after transplant, except when contraindicated by renal dysfunction or other severe comorbidities. The IVUS procedure of the left anterior descending artery (LAD) has been detailed previously (16). Briefly, IVUS is performed after documentation of the absence of coronary artery stenosis ≥60% based on angiography, and images were acquired by an automatic pullback (0.5 mm/sec motorized device) of the probe, for offline analysis of the first 30 mm of the LAD. External elastic membrane and lumen cross-sectional borders were identified and used to quantify intimal, vessel and lumen volumes. (17) Our Institutional IVUS Core laboratory performed 2-dimensional and 3-dimensional IVUS analysis by a semi-automated method using Qivus® Clinical Edition software (Medis medical imaging systems bv, Leiden, NL) (18). One individual blinded to the patients' treatment performed all the measurements reported in this study.

Immunosuppression and concomitant therapy

Standard immunosuppressive regimen in our Center consists in induction with thymoglobuline or basiliximab, followed by maintenance with cyclosporine, an antiproliferative agent, and oral steroids. After 2005, in de novo patients MMF (2 to 3 g/day) or everolimus (3 to 8 ng/ml trough levels) were used instead of azathioprine. According to the choice of the in-charge physicians, everolimus could be started a few days

after surgery or introduced after 2 to 6 weeks of MMF treatment. Presence of cytomegalovirus (CMV) serological mismatch, history of pre-transplant neoplasm, or mere preference of the in-charge physician supported the choice of everolimus introduction (intention-to-treat basis).

Late after transplant (late cohort patients) everolimus introduction associated with cyclosporine minimization could be used to substitute azathioprine or MMF combined with standard cyclosporine, as rescue strategy in patients developing renal dysfunction, (19) cancer, (20) or a rapid increase in maximal intimal thickness as detected at year 1 IVUS (14).

Statin therapy was prescribed universally, regardless of cholesterol levels, excluding patients with known or developed intolerance. Statin type and dosage was chosen by the in-charge physician and usually started between months 1 and 2 after transplant. Based on the meta-analysis from Law and co-workers, (21) and on studies from our group, (22-24) we defined “high” and “low” dose statins according with the predicted LDL cholesterol reduction. Table 1 reports only statins type and dosage actually given to the study patients.

Cytomegalovirus (CMV) disease was prevented by either pre-emptive strategy (before 2005), or by universal prophylaxis with valganciclovir, as previously described. (16) All patients were monitored for CMV infection with pp65 antigenemia or whole blood DNA PCR. Patients were treated for CMV infection for antigenemia levels greater than 30 pp65cells/200,000 neutrophils, or 20,000 DNA copies/ml (16).

Data analysis and study endpoints

Early and late cohorts were analyzed separately. The main outcome measure was change in MIT, which was assessed between baseline and year 1 IVUS for the early cohort, and between years 1 and year 5 for the late cohort. Study endpoint, for both cohorts, was a change in MIT >0.5mm, which for study purposes only was defined as “CAV progression”. Additional IVUS parameters used to analyze CAV progression comprised: baseline to follow-up changes in vessel, lumen and intimal volumes, and change rate of intimal index. Intimal index was defined as the rate between intimal and vessel volume, and the change rate as the rate between change in intimal index and baseline intimal index (17). For descriptive purposes we additionally report angiographic CAV classification based on ISHLT nomenclature. (25)

The primary aim of the study was to compare IVUS-defined CAV between patients receiving everolimus with those receiving MMF. In the early cohort, we performed an intention-to-treat (ITT) analysis, based on the drug that patients were taking at the time of baseline IVUS. To account for cross-overs, we additionally performed an on-treatment analysis, including in the EVE group any patients who took at least one dose of the drug during the first year of follow-up.

In the late cohort, shortly after the year 1 time point, the immunosuppressive agent chosen at transplant was changed in several patients, but was then left unchanged until the end of the year 5 follow-up. This allowed us to compare the association of everolimus and MMF with late MIT changes, without the possible confounding effect of late cross-overs. (Figure 2B)

Because of the observational design, to weigh for possible confounders, we adjusted for differences between study groups in laboratory, clinical, and therapeutic variables collected at months 1 and 12 after transplant in the early cohort, and at years 1 and 5 after transplant in the late cohort, by using logistic multivariable analysis. In addition, baseline variables (i.e. month 1 for the early cohort and year 1 for the late cohort) were tested in univariate logistic models where MIT change>0.5 mm (defined as CAV progression) was the outcome variable. Variables associated with CAV progression with a $P \leq 0.1$ at univariate analysis were challenged one at a time in multivariate logistic models against the variable of interest (i.e. everolimus vs. MMF) to identify independent predictors of early and late CAV progression. Among the possible confounders, we devoted particular attention to metabolic variables, statin therapy, and rejection burden. Because the vast majority of our patients routinely receive statin therapy, to analyze the possible role of statins in CAV development we compared patients taking high dose statins vs. those taking low dose or no statin (see supplemental material) (21-24). Rejection burden was analyzed by computing average rejection score, obtained by scoring biopsy grades as follows: grade 0 = 0; grade 1R = 1; grade 2R = 2; grade 3R = 3.

Statistical analysis was performed by JMP® 9.0.1 software. Continuous variables are expressed as mean ± SD if normally distributed or as median (25^{th} to 75^{th} percentile) if skewed, categorical variables as percentages; differences between study groups were assessed by Student's T test, Mann – Whitney or chi-

square test, as appropriate. The odds for CAV progression associated with study variables were estimated by nominal logistic analysis, and a *p* value <0.05 was considered as significant for all comparisons.

Results

Overall, 143 patients entered the study, of which 91 were included in the early cohort and 52 in the late cohort (Figure 2).

In the early cohort, 20 patients were on everolimus and 71 on MMF at baseline (ITT groups). Median (25^{th}-75^{th} percentile) distance from transplant of everolimus start was 5 (3 to 18) days.

Three patients were shifted from MMF to everolimus six to nine months after transplant, because of recurrent CMV infection, Kaposi's sarcoma and CNI-minimizing strategy for renal failure. One patient was shifted from EVE to MMF for an adverse event related to the drug (lower limb edema). Following these cross-overs, the on-treatment groups consisted in 23 patients on everolimus (i.e. anyone who received everolimus for at least 3 months) and 68 on MMF, at year 1

As can be noted from table 1, MMF and everolimus groups were largely comparable at baseline, except for cyclosporine trough levels that were lower in everolimus patients. One year after transplantation, everolimus patients showed higher total and LDL-cholesterol levels (despite a wide and comparable use of high dose of statins in both groups), higher fasting glucose, less steroid dose, and fewer treated CMV infections than the MMF group. Of note, calculated GFR (26) was not significantly different between study groups at year 1. Regarding major adverse events, patients in the everolimus group underwent post-operative pericardial drainage significantly more often than patients in the MMF group (36.8% vs. 13.8%, P=0.02). No difference in severe bacterial infection or other major adverse events was noted.

In the late cohort, 33 patients received MMF and 19 everolimus, for at least 3 of the 4 years of follow-up (Figure 2B and Table 2). Seven patients were on everolimus on a de-novo basis since after surgery, while in the remaining 12 everolimus was introduced as a rescue strategy between year 1 and 2 after transplant for renal failure (n=8), CAV at 1-year IVUS (n=2), recurrent CMV infection despite antiviral treatment (n=1) and recurrent rejection (n=1). Median (25^{th}-75^{th} percentile) duration of everolimus treatment was 41 (37 to 57) months and median distance from transplant of everolimus start was 18 (0.5 to 23) months. At baseline,

the MMF and everolimus groups differed for statin use (P=0.01) and first year rejection score. During the 4 years of follow-up, patients receiving EVE developed a worse metabolic profile compared to MMF, driven by higher total cholesterol (p<0.01), LDL (p=0.01), and triglycerides (p<0.01), despite the similar use of statins at 5 years in both groups (86.3% overall, p=0.39 for EVE vs MMF). Levels of cyclosporine were lower in EVE-treated patients, as expected. Of note, GFR improved from baseline to follow-up in EVE group, while it remained stable in MMF patients (Table 4). Regarding safety, in the EVE group, 4/19 (21%) experienced bacterial infections and two (10%) lower limb edema; however, none of these events caused drug withdrawal.

Predictors of early CAV progression

At year 1, 16 (17%) patients presented mild angiographic CAV (ISHLT grade 1), with only 3 (3%) patients showing angiographic progression from baseline. Progression of IVUS-defined CAV was found in 17 (19%) patients. MMF treatment was associated with higher occurrence of early CAV than everolimus, both by ITT (22.8 vs. 5%; P=0.04) and by on-treatment (24.8 vs. 4.7%; P=0.02) analyses. Accordingly, the MIT change was significantly greater in the MMF than in everolimus group (0.37±0.29 vs. 0.23±0.15 mm, P=0.05, Figure 3 A). Volumetric IVUS analysis revealed that in patients treated with MMF lumen volume decreased (-11%; P<0.01) caused both by a significant increase in intimal volume (+14%; P=0.01) and shrinkage in vessel volume (-5%; P=0.03), while in patients receiving everolimus, vessel volume parameters did not change significantly. Consequently, the median change rate in intimal index was significantly higher in patients receiving MMF (3.2%[-5% to 17%] vs. 11% [0% to 41%]; P=0.02; Figure 3B).

Among all the other variables included in the study, CAV occurrence appeared to be influenced only by the use of high doses of statins. In particular, early CAV tended to develop less frequently among the 31 patients starting aggressive statin therapy at the time of baseline IVUS, than in those with no or low-dose statins (9.7 vs. 25% P=0.06). Of note, high dose statins was also associated with significantly lower MIT increase (0.23±0.19 vs. 0.38±0.29 mm; P=0.01) Multivariable logistic analysis showed that both everolimus treatment (OR[95%CI]=0.14 [0.01-0.78]; P=0.02), and high doses statins (OR[95%CI]= 0.28 [0.06-0.95];

P=0.04) had an independent protective effect on early CAV development, even after adjusting for other possible confounders, such as elevated cholesterol and glucose, and steroid use at year 1.

IVUS analysis and predictors of late CAV progression

At year 5, 14 (23%) patients presented angiographic CAV (12 grade 1, and 2 grade 2), with 5 (9.6%) patients showing progression from year 1 to year 5 (2 in everolimus, 3 in MMF groups). IVUS-defined CAV progression was observed in 15 (29%) patients. Intimal volume increased (from 157±87 to 196±97mm^3; P<0.01), causing a significant lumen loss (from 363±118 to 398±110 mm^3; P<0.01), accompanied by lack of vessel wall remodeling (from 556±138 to 560±164mm^3; P=0.8). No significant differences between everolimus and MMF patients were observed in the occurrence of late CAV (31.5% vs. 27.2%, respectively P=0.74), average MIT change (0.34±0.53 vs. 0.27±0.36mm; p=0.57), and average change of all vascular volume parameters (Figure 4; all P values >0.5).

Univariate analysis revealed that high doses of statins at year 1 were associated with a lower occurrence of late CAV (10% vs. 39%; P=0.02). In addition, patients with late CAV also had higher median [25^{th}-75^{th} percentile] triglyceride concentrations at baseline (210[154-245] vs. 153[110-199] mg/dl; P=0.01) than those without CAV. Of note, year 1 triglyceride levels showed a linear correlation with increase in MIT, intimal volume, and lumen loss (Figure 5).

At multivariate analysis, levels of tryglicerides (OR per 10mg/dl increase [95%CI] = 1.11 [1.02 – 1.21]; P=0.01) and high doses of statins (OR[95%CI]= 0.08 [0.01 – 0.47] P<0.01) independently predicted late CAV progression, even after adjusting for everolimus or MMF based strategy.

DISCUSSION

This IVUS-based study analyzes early and late CAV development in two patient cohorts receiving everolimus or MMF in a clinical practice context. We found that everolimus and high dose statins are associated with a low risk for early markers of CAV development. While statins appear to protect against CAV progression

also later after transplant, everolimus treatment does not seem to influence changes in IVUS parameters assessed between years 1 and 5 after transplant.

IVUS-detected increase in MIT during the first year after HT is a marker for early CAV development and has been identified as a surrogate endpoint for long-term survival, being associated with high risk for death and non-fatal cardiovascular events. (2-4) Although MIT progression has been described also after the first year following transplant, its contribution to subsequent prognosis is unknown, and late MIT increase appears to be associated with different remodeling properties of the vessel wall (12). These differences between early and late geometric changes of graft coronaries support the hypothesis that different pathophysiological mechanisms may be implicated in early and late CAV progression. (11, 13). This hypothesis led us to separately analyze early and late morphological changes of graft coronary arteries and compare the effect of everolimus with low-dose cyclosporine to MMF with standard dose cyclosporine in two separate cohorts of patients.

In the early cohort, everolimus was chosen as first choice antiproliferative agent in 20 patients, but was started during a wider period of time than what it was in the major randomized trials (i.e. median (25^{th} – 75^{th} percentile) of everolimus start was 5 (3 to 18) days after HT, instead of 72 hours after surgery). Between months 6 to 9 after transplant, three additional patients were converted from MMF to everolimus, and one discontinued everolimus because of drug-related side effects. Everolimus intake was independently associated with lower early CAV development both in an ITT analysis, and in an on treatment analysis (which includes all patients receiving the drug at any time during the follow-up; Figure 2A), by reducing the likelihood of MIT increase ≥0.5mm, the absolute increase in MIT, and intimal index. This finding extends in a clinical practice context the finding of the randomized trials of everolimus and sirolimus against azathioprine, (7, 8) and of the preliminary analysis of the CRAD 2310 trial, currently available only as part of conference proceedings (27). Our data additionally suggest that the protective effect of the mTOR inhibition may also be achieved by starting the drug slightly later after surgery. This concept may be of particular importance because a delayed start of everolimus may help to maintain efficacy while avoiding some of the side effects typical in the post-operative period (28-30).

At our institution, patients not presenting contraindications, and accepting the procedure, routinely receive IVUS examination at year 5 after transplant. We were thus able to analyze late CAV progression, by defining as an endpoint an MIT change ≥0.5mm between years 1 and 5 after transplant. During the second year after transplant, 20 out of the 52 patients in the late cohort were shifted from the antiproliferative agent they originally received after surgery (Figure 1). We thus compared IVUS change over a 4-year period between groups of patients treated for about three years with the same therapeutic strategy. In particular, median duration of everolimus treatment was 41 (37 to 57) months. We were unable to show any difference in late CAV progression between patients receiving everolimus or MMF, as opposed to what we found in the patients from the early cohort. This finding is in apparent contradiction with the results of a randomized study of sirolimus, in which clinical endpoints were combined with morphological ones (14), but are in line with a recent IVUS-based observational study, comparing patients receiving sirolimus and MMF/AZA on a calcineurin-free regimen, to patients on cyclosporine or tacrolimus and MMF/AZA (31), in which mTOR inhibition had a greater effect in limiting intimal hyperplasia in early converted patients than in patients converted late after transplant. This concept is further supported by the recent NOCTET substudies. (15, 32) In patients randomized to receive everolimus or standard immunosuppression late after HT, NOCTET investigators analyzed MIT changes and plaque composition by using grayscale IVUS and virtual histology technique. While MIT increase was unaffected by everolimus therapy, mTOR inhibition was found to be associated with increased calcified and necrotic plaque component (known to predict adverse coronary outcomes in native atherosclerosis), in particular in patients with more than 5 years of post-transplant follow-up.

Taken together, all these findings support our initial working hypothesis that early and late CAV development are influenced by different pathophysiological mechanisms. Everolimus intake during the first year is likely to reduce coronary intimal hyperplasia by antagonizing the immuno-mediated injury leading to endothelial proliferation (for example by reducing CMV infection, Table 2). On the other hand, metabolic risk factors appear to play a more relevant role in favoring late intimal proliferation compared with what everolimus could antagonize. Of note, late metabolic abnormalities are more likely to be associated with local inflammatory milieu and with plaque composition, as showed the virtual histology study from

NOCTET. (32) Remarkably, we found that triglycerides concentrations at one year were highly correlated with subsequent increase in intimal hyperplasia and loss of lumen volume (Figure 5). In this context, therapy with high dose statins appears to have a protective effect, both on early and late CAV development, confirming and extending the results of a recent randomized study by our group, (23) and supporting the concept that these drugs may interact with immuno- and metabolic-mediated vascular injury in HT recipients. (5, 9)

Our results additionally shed light on the safety profile of everolimus in a clinical practice context, when used in patients with a low-comorbidity profile. While noting that we found no difference in graft loss or death for safety reasons in the study groups, we confirm most of known everolimus side effects, such as a trend towards a worse lipid profile, which however did not appear to influence its efficacy on early coronary intimal hyperplasia. In addition, while we did not record wound-healing or early renal insufficiency issues, it must be noted that patients receiving everolimus in the early cohort had a significantly higher rate of pericardial drainage than those treated with MMF. Overall tolerability was satisfactory, with only one patient in the early group discontinuing everolimus for drug-related side effects, and none who discontinued in the late group, over a 4-year period of follow-up.

Study limitations

The use of IVUS-detected increase in MIT as a surrogate endpoint for long-term graft survival has been validated only in patients receiving IVUS during the first post-transplant year (e.g. our early cohort) and not when occurring late after transplant (e.g. our late cohort). Thus, we are unaware whether our MIT change cutoff of 0.5 mm has any prognostic relevance when measured between years 1 and 5 after transplant. In addition, it must be noted that recent guidelines do not consider IVUS changes sufficient to properly define CAV (25), and that we used the term “CAV progression” to more easily indicate meaningful progression of graft vascular disease. However, in this study we aimed to provide a descriptive analysis of factors influencing coronary morphology and chose established linear and volumetric parameters that clearly relate to CAV pathophysiology. With this approach, we were able to provide evidence supporting the hypothesis that drugs and risk factors have a time-dependent effect on early and late changes in coronary

morphology. In this study we included only patients who were healthy enough to undergo two IVUS examinations. Thus, patients with severe comorbidities or with worsening kidney failure could not be analyzed and we cannot exclude the possibility that their outcome might have provided a different picture to our results. Observational design may represent a further limitation to the study results, and multivariable models may not fully account for uncontrolled potential confounders. In particular, late cohort patients were transplanted during a wide time span in which therapeutic strategy transitioned from azathioprine to MMF or everolimus, quite a few years before early cohort patients, and thus we cannot exclude that other uncontrolled changes in clinical practice could have influenced the different IVUS outcomes in the two study eras. However, our results provide a real-life picture of everolimus timing of use, tolerability, and effects in current clinical practice, besides the protected environment of a multicenter randomized trial, which may often be far from the complex reality of standard heart transplant recipients.

Conclusions

In a clinical practice context, we found that mTOR inhibition by everolimus was associated with a reduced progression of coronary intimal hyperplasia during the first post-transplant year, but not in the subsequent four post-transplant years. High triglycerides, on the other hand, did not appear to influence early CAV, but were highly correlated with late progression of coronary intimal hyperplasia, while high-dose of statins provided beneficial effects on both sides of this spectrum, by limiting early and late CAV progression. By highlighting a differential effect of everolimus and metabolic abnormalities on early and late changes of graft coronary morphology, this observational study confirms the evidence that CAV progression is influenced by risk factors acting at different time points of the CAV time course. Prospective studies are needed to test the hypothesis that time-tailored interventions may grant a major benefit in limiting CAV progression.

Acknowledgements

This study was funded by a grant from Banca del Monte Foundation. The authors are grateful to the Transplant Nurse team for their tireless work in supporting timely follow-up of the patients, and to Susan West for language editing.

Disclosure

The authors of this manuscript have a conflict of interest to disclose as described by the American Journal of Transplantation. L.P. received speaker bureau fees from Novartis Pharma, and A.B. holds an institutional grant from Novartis Pharma.

Table 1. Definition of high and low-dose statins

"High dose" statins (expected 35 to 45% reduction in LDL cholesterol)	Fluvastatin 80mg Rosuvastatin 10mg Atorvastatin 20mg Simvastatin 40mg Ezetimibe 10mg plus Simvastatin 20mg
"Low dose" statins (expected 20 to 35% reduction in LDL)	Fluvastatin 20-40 mg Atorvastatin 10mg Pravastatin 10-40mg Simvastatin 10-20mg Ezetimibe 10mg plus Simvastatin 10mg

See articles (21-24) for reference

Table 2. Baseline characteristics of the early cohort according with ITT therapy

	EVE (n=20)	MMF (n=71)	P
Recipient age (yrs)	50.1 ± 9.2	51.7 ± 11.7	0.57
Recipient sex (males %)	16(80.0)	58(81.7)	0.86
Donor age (yrs)	38.4 ± 13.8	35.0 ± 12.1	0.29
Donor sex (males %)	14 (73.7)	48 (67.6)	0.76
Pre-transplant CAD (n, %)	7 (36.8)	29 (42.6)	0.64
Ischemic time (min)	198.1 ± 57.6	200.7 ± 45.4	0.82
GFR (ml/min)	62.4 ± 20.2	65.7 ± 25.4	0.59
Glycemia (mg/dl)	87.4 ± 33.6	84.5 ± 27.5	0.70
Cholesterol (mg/dl)	223.3 ± 38.8	216.3 ± 60.7	0.62
HDL (mg/dl)	64.3 ± 17.8	58.0 ± 16.0	0.13
LDL (mg/dl)	125.7 ± 37.1	117.9 ± 48.2	0.50
Triglycerides (mg/dl)	173.3 ± 61.4	207.8 ± 105.6	0.16
Body mass index (Kg/m^2)	23.6 ± 3.4	24.0 ± 3.4	0.65
Steroid dose (mg/Kg)	0.16 ± 0.04	0.18 ± 0.05	0.08
Cyclosporine trough (ng/ml)	250.8 ± 85.9	303.3 ± 92.7	0.02
Diabetes (n, %)	8 (40.0)	33 (46.4)	0.73
Aspirin (n, %)	13 (65.0)	38 (53.5)	0.35
Any statin (n, %)	14 (70.0)	51 (71.8)	0.87
High dose statins (n, %)	5 (25.0)	26 (36.6)	0.32
Ejection fraction (%)	65.0 ± 4.5	65.6 ± 6.3	0.70

CAD= coronary artery disease; GFR= calculated glomerular filtration rate

Table 3. Baseline characteristics of the late cohort according with ITT therapy

	EVE (n=19)	MMF (n=33)	P
Recipient age (yrs)	55.1 ± 10.7	55.6 ± 8.8	0.86
Recipient sex (males %)	16 (84.2)	28 (84.8)	0.95
Donor age (yrs)	38.1 ± 12.6	34.9 ± 10.7	0.33
Donor sex (males %)	11 (57.9)	22 (66.7)	0.83
Pre-transplant CAD (n, %)	9 (47.3)	15 (45.4)	0.89
Ischemic time (min)	205.3 ± 57.7	196.3 ± 51.0	0.56
Rejection score during 1st yr	0.68 ± 0.29	0.84 ± 0.27	0.05
GFR (ml/min)	46.0 ± 16.3	53.4 ± 15.9	0.11
Glycemia (mg/dl)	96.4 ± 27.3	99.3 ± 28.7	0.72
Cholesterol (mg/dl)	215.3 ± 48.3	192.9 ± 41.7	0.08
HDL (mg/dl)	60.4 ± 15.8	57.2 ± 14.0	0.45
LDL (mg/dl)	110.4 ± 37.7	97.8 ± 24.1	0.14
Triglycerides (mg/dl)	196.7 ± 62.3	189.1 ± 100.7	0.76
Body mass index (Kg/m^2)	25.7 ± 2.4	26.7 ± 3.5	0.24
Steroid dose (mg/Kg)	0.09 ± 0.06	0.12 ± 0.05	0.08
Cyclosporine trough (ng/ml)	188.6 ± 92.3	224.5 ± 61.4	0.09
Diabetes (n, %)	6 (31.6)	15 (45.4)	0.32
Aspirin (n, %)	8 (42.1)	15 (45.4)	0.81
Any statin (n, %)	14 (73.7)	32 (96.9)	0.01
High dose statins (n, %)	7 (36.8)	12 (36.3)	0.97
Ejection fraction (%)	65.7 ± 4.4	65.1 ± 5.2	0.65

CAD= coronary artery disease; GFR= calculated glomerular filtration rate

Table 4. Early cohort characteristics after one year of follow-up according with ITT therapy

	EVE (n=20)	MMF (n=71)	P
Rejection score	0.72 ± 0.34	0.84 ± 0.32	0.16
GFR (ml/min)	55.0 ± 20.7	53.8 ± 18.5*	0.80
Glycemia (mg/dl)	111.6 ± 51.9*	94.2 ± 22.7*	0.03
Cholesterol (mg/dl)	211.6 ± 34.6	187.5 ± 45.3	0.03
HDL (mg/dl)	56.6 ± 19.5	52.6 ± 15.4	0.33
LDL (mg/dl)	111.4 ± 31.2	96.4 ± 30.8*	0.05
Triglycerides (mg/dl)	183.7 ± 60.2	192.9 ± 88.5	0.66
Body mass index (Kg/m^2)	25.9 ± 3.9*	25.5 ± 3.4*	0.70
Steroid dose (mg/Kg)	0.06 ± 0.04*	0.09 ± 0.05*	0.04
Cyclosporinemia (ng/ml)	78.1 ± 34.7*	219.6 ± 68.7*	<0.01
Diabetes (n, %)	7 (35.0)	22 (30.9)	0.73
Aspirin (n, %)	15 (75.0)	46 (64.8)	0.38
Any statin (n, %)	17 (85.0)	65 (91.5)	0.40
High dose statins(n, %)	12 (60.0)	30 (42.2)	0.15
Ejection Fraction (%)	67.4 ± 5.1	65.4 ± 6.3	0.19
CMV infection (n, %)	1 (5%)	33 (48%)	<0.01

* Significantly different from baseline by paired analysis

Table 5. Late cohort characteristics after four years of follow-up (year 5 after transplant) according with ITT therapy

	EVE (n=19)	MMF (n=33)	P
GFR (ml/min)	55.8 ± 22.4*	54.1 ± 16.8	0.75
Glycemia (mg/dl)	108.3 ± 36.6	108.5 ± 69.3	0.99
Cholesterol (mg/dl)	212.6 ± 27.6	171.0 ± 35.9	<0.01
HDL (mg/dl)	61.2 ± 18.6	52.4 ± 16.6	0.08
LDL (mg/dl)	110.1 ± 33.0	87.3 ± 29.7	0.01
Triglycerides (mg/dl)	199.7 ± 76.8	142.5 ± 64.3*	<0.01
Body mass index (Kg/m^2)	25.9 ± 2.3	27.5 ± 4.3	0.17
Steroid dose (mg/Kg)	0.07 ± 0.05*	0.05 ± 0.03*	0.27
Cyclosporine trough (ng/ml)	91.9 ± 84.8*	158.6 ± 62.4*	0.02
Diabetes (n, %)	6 (33.3)	12 (37.5)	0.76
Aspirin (n, %)	17 (89.4)	27 (81.8)	0.46
Any statin (n, %)	15 (78.9)	29 (87.8)	0.39
High dose statins (n, %)	11 (57.9)	13 (39.3)	0.19
Ejection fraction (%)	66.6 ± 5.0	65.2 ± 5.5	0.36

* Significantly different from baseline by paired analysis

Figure legends

Figure 1.

Study design. ITT, intention to treat; CAV, cardiac allograft vasculopathy; IVUS, intravascular ultrasound; MMF, mycophenolate mofetil.

Figure 2.

Study flow-chart. Panel A: Early cohort; Panel B: Late cohort.

Figure 3.

Change in IVUS parameters between study groups of the early cohort. Panel A: mean and standard deviation of MIT change. Panel B: median and interquartile range of the change rate in intimal index.

Figure 4.

Change in IVUS parameters between study groups in the late cohort. . Panel A: mean and standard deviation of MIT change. Panel B: median and interquartile range of the change rate in intimal index.

Figure 5.

Linear correlations between triglycerides at year 1 and change in maximal intimal thickness (panel A), lumen volume (panel B), and intimal index (panel C) in the late cohort patients.

3.2 Everolimus and valganciclovir prophylaxis: insights from PROTECT study

Purpose:

Cytomegalovirus (CMV) infection may influence the development of Cardiac Allograft Vasculopathy (CAV). In this prospective randomized study we aimed to analyze the interplay of immunosuppressive, anti-CMV strategies and CMV immunity on the risk for CMV infection and CAV during the first year after transplant

Methods:

By a 2x2 randomization process, CMV seropositive heart transplant (HT) recipients were randomized to receive 3 months of valganciclovir prophylaxis (PRO) or a pre- emptive based approach (PRE), and to receive mycophenolate (MMF) or everolimus (EVE) on top of a cyclosporine-based immunosuppressive therapy. All were monitored for CMV infection by whole blood PCR and CMV-immunity reconstitution by elispot assay. At month 1 and 12 after transplant eligible patients underwent intravascular ultrasound (IVUS). Occurrence of CMV infection and change in maximal intimal thickness (MIT) >0.5mm were study endpoints.

Results:

Forty-six patients were randomized: 22 to PRE vs. 24 to PRO, and 24 to EVE vs. 22 to MMF. Six (25%) patients discontinued EVE and 13 (54%) did not completed the 3-months period of PRO for adverse events (mainly effusions in EVE and leucopenia in PRO). Only 23 patients underwent IVUS both at month 1 and month 12. After adjusting for donor serology, PRO and EVE were associated with reduced risk of 70 and 54% respectively for CMV infection in the intention to treat (ITT) analysis ($P<0.05$). However, EVE discontinuation and lack of PRO completion were associated with increased risk for CMV infection. Only 4 (17%) patients developed the MIT endpoint, not allowing any conclusion about treatments efficacy. Nevertheless 100% of the patients who discontinued EVE developed the MIT endpoint. Recovery of CMV immunity at month 1 by Elispot analysis allowed stratifying the risk for CMV infection: patients with lack of immunity were at higher risk of infection, and most likely to benefit from PRO or EVE ($P<0.01$).

Conclusion:

EVE and PRO are protective from CMV infection, but a significant percentage of patients discontinued the treatments for intolerance and appear to be exposed to higher risk of events. Analysis of CMV immunity recovery may provide guidance in customizing therapeutic strategies, by identifying patients in whom aggressive anti-CMV strategies may have a favorable risk/benefit ratio.

Chapter 4.

Solid organ cancers after heart transplantation: trying to improve the management

4.1 Factors influencing survival of heart transplanted patients with a solid organ cancer: focusing on the role of immunosuppressive therapy, surgery and oncological status

Introduction:

Despite advances in oncology and in immunosuppression, solid organ cancers are still the main non graft-related cause of the death in the long-term for heart transplant (HTx) recipients. Whereas some risk factors for the development of post-transplant malignancies have been provided, like global immunosuppressive burden smoking, advanced age, previous malignancy and global. However, after a solid organ cancer has been found in a heart transplanted patient, the challenge for the Transplant Clinician is how to manage these patients, with regard to both immunosuppression and oncological treatment. In the literature, data about factors influencing survival after a cancer has occurred are lacking.
About immunosuppression, in the last years, interest has grown up about mTOR inhibitors (i.e. everolimus or sirolimus), due to their known antiproliferative effect and to the frequent involvement of mTOR pathway disregulation in the pathogenesis of several cancers. Actually, these drugs are approved for chemoterapeutic use in some malignancies (i.e. Kaposi sarcoma, renal cell carcinoma). Basing on these properties, in some consensus conference, mainly based on case reports results, an introduction of an mTOR inhibitor has been suggested in solid organ transplanted patients developing a malignancy. However, it is not known if EVE introduction could influence subsequent outcome.
About the general oncological treatment, it appears obvious that oncological surgery can impact the outcome, but still now no published data have been provided about the feasibility of surgical treatment and about cardiovascular risk and rate of complications for a surgical procedure in this subset of patients.

The aim of this retrospective study is to investigate factors influencing survival after cancer occurrence in HTx patients.

Matherials and methods:

Study design
We retrospectively collected data about cancers occurring in all patients transplanted at our Institution since the beginning of our Transplant Program (October 1988) until July 2011, who survived more than one year after HTx (n=500). Cancers were categorized in four groups: solid organ cancers, post-transplant lymphoproliferative disease (PTLD), non-melanoma skin cancers, Kaposi´s sarcoma. We decided to restrict our analysis to solid organ cancers. We collected oncological data at the moment of diagnosis: histology, organ involved, and stadiation of the tumor, with regards to the presence (M1) or not (M0) of metastases. With the intent to look for factors that could influence post-cancer outcome, we collected data about demographics (age at the moment of cancer occurrence, sex, pre-HTx heart disease, HTx era, induction therapy), oncological

management (surgery, chemotherapy or radiotherapy), immunosuppressive therapy after cancer diagnosis. Regarding this aspect, after recording all changes made in the immunosuppressive regimen after cancer diagnosis, we decided to focus the analysis on the therapy after the diagnosis; patients were divided in two groups for each drug, regardless of the immunosuppression regimen they had before cancer occurrence: steroids, cyclosporine yes/no, antiproliferative agent: azathioprine, AZA yes/no mycophenolate MMF yes/no, everolimus EVE yes/no. In particular, as second step, we focused our attention on the role played by everolimus after cancer diagnosis.: those who received EVE after the diagnosis of malignancy (EVE-group), and those who received an other kind of antiproliferative drug (or any at all) (non-EVE group). Of note, EVE group was comprehensive both of patients in which EVE was started after cancer diagnosis, both of patients who were already on that therapy before. We analyzed the occurrence of cancer-related mortality in the two groups, that included also complications of the malignant status (i.e. pulmonary embolism, sepsis due to severe neutropenia related to anti-cancer therapies)
The primary endpoint was cancer-related mortality. The secondary endpoint was a combined one of cancer-related death and cancer recurrence. The safety profile of EVE after cancer diagnosis was also analyzed, by recording serious adverse events (i.e. severe neutropenia, serious infections, pleural or pericardial effusions, wound problems after cancer surgery), and the cardiovascular and non graft related complications of patients undergone to oncological surgery.

Statistical analysis
The incidence of solid organ cancer in the overall study population was expressed by survival curve (Kaplan-Meyer analysis). Due to the large heterogeneity of cancers observed, an organ- or histological- specific analysis was not possible. Prevalence of metastases at diagnosis was expressed as a percentage. The influence of surgical treatment, chemotherapy, radiotherapy and HTx era on cancer-related survival were analyzed by Cox´analysis ($P<0.05$ considered as significant, OR expressed using CI 5-95%) at univariate and multivariate analysis and expressed with Kaplan-Meyer curve as a 5-yrs survival rate. To analyze the impact of different eras of HTx, we choose to divide the patients before and after 2002 (year in which induction with Thymoglobuline was started in our Institution) EVE was entered in a step-wise model of multivariate analyses including the other variables significant.
Subgroup analysis was performed when appropriate. Differences in categorical variables between EVE and non-EVE group were expressed with t-Student test ($P<0.05$ considered as significant)

Results.

Among 580 patients transplanted in our Center in the period of observation, 500 patients survived more than one year after HTx; 14 developed a PTLD; 7 Kaposi´s sarcoma, 70 non-melanoma skin cancer). 71 experienced a solid organ cancer (10-yrs incidence: 16.8 ± 2.2%).
In Fig. 1 are represented cancers that occurred in the study population: as expected, there was a great heterogeneity. Most common cancers were localized respectively at prostate, colon, lung, kidney, but also less common cancers (i.e. central nervous system and sarcomas) were observed.

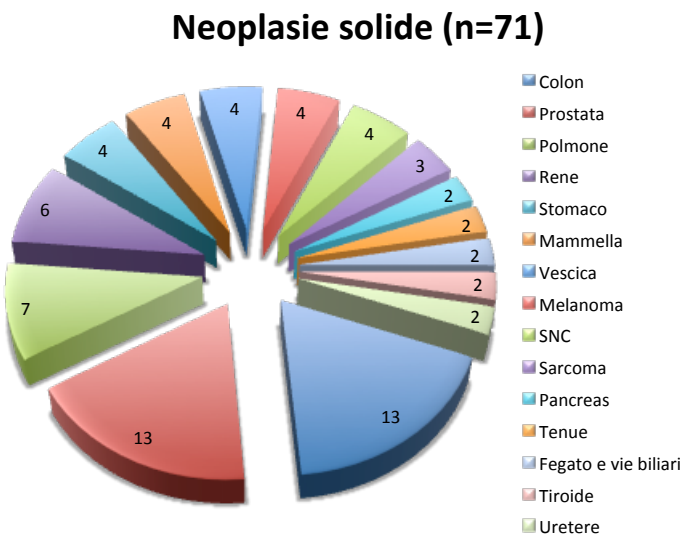


Figure 1. Incidence of cancers

Estimated 5-yrs survival after solid organ cancer diagnosis was overall poor (43.4 ± 6.8%, Fig. 2); in 39.1% of the patients the disease had already reached a metastatic stage at diagnosis.

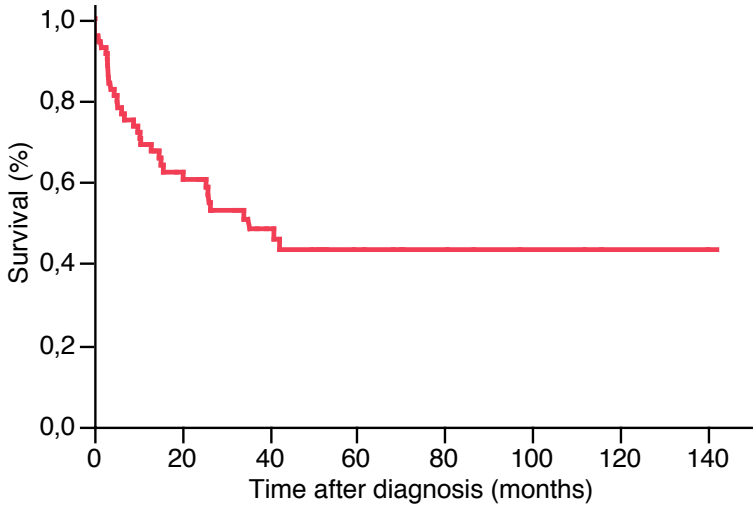


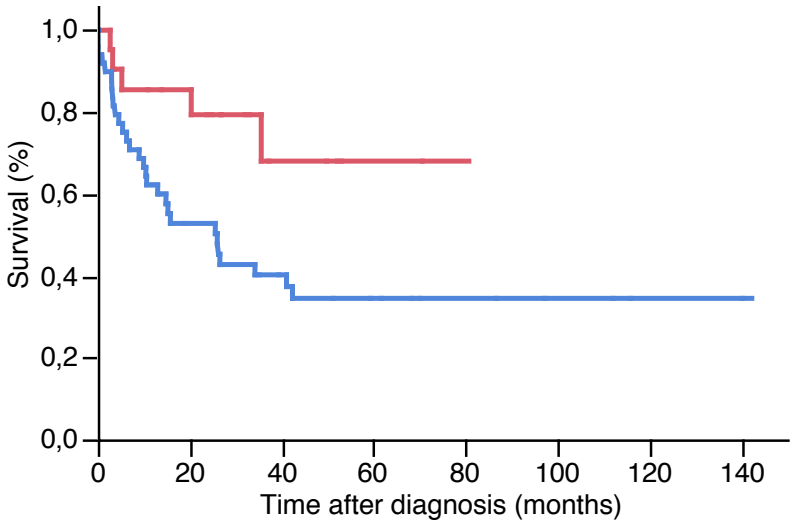
Figure 2. Cancer-related survival

In Table 1 characteristics of the patients at the time of diagnosis are summarized: whereas most of them were on steroid and CNI therapy at the moment of cancer detection, there was eterogeneity on the antiproliferative agent used, thus reflecting different era of HTx.

Sex (%M)	83% (59)
Age at diagnosis (yrs)	63.9 ± 6.9
Transplant after 2002	29.5% (21)
Time after HTx (yrs)	6.7± 4.4
Metastasis at diagnosis (M1)	39.7%
Prednison (%)	88.7% (63)
CNI Cyclo TAC)	94.5% (67), 90.1% (64) 4.2% (3)
AZA (%)	43.6% (31)
MMF (%)	21.1% (15)
EVE (%)	14% (10)

Table 1. Characteristics of the patients at the time of cancer diagnosis

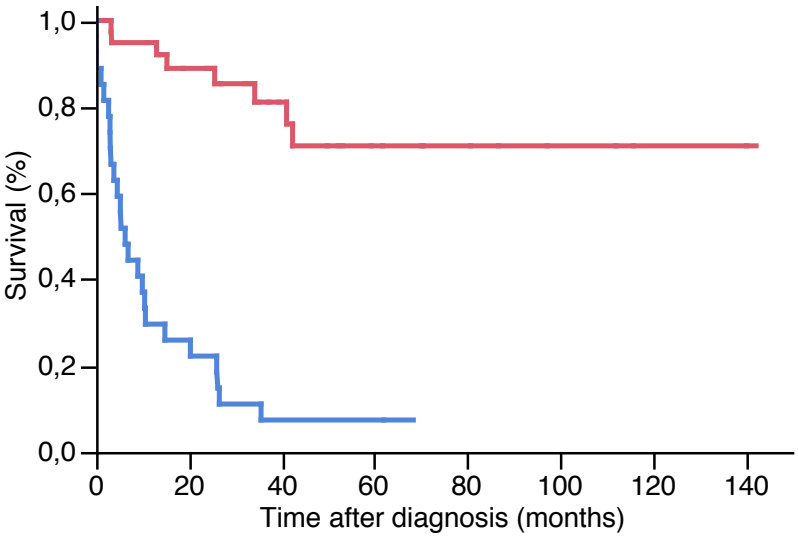
By analyzing the impact of different transplant eras on cancer-related death, we found that, whereas cancer incidence didn´t change significantly (P=0.18), cancer-survival significantly improved after 2002 (5-yrs estimated survival: 68.0 ± 13.2% vs 34.4 ± 7.4 5, P=0.02, Fig. 3a)



P=0.02

Figure 3A. Effect of era on post-cancer survival

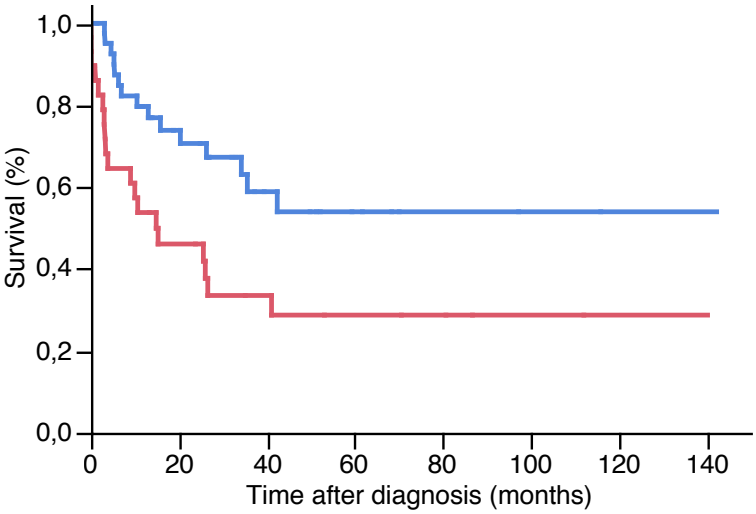
Of note, the percentage of patients with an M1-status at diagnosis was significantly lower (19% vs 47.9%, P=0.02) after vs. before 2002.
At univariate analysis, the presence of metastases at the time of cancer detection (M1 status) significantly affected survival (OR: 9.76 (95% C.I. 4.51-23.52), P<0.0001); median survival of M1-patients was 6 ± 3 months (Fig. 3b)



P<0.0001

Figure 3 B. Role of metastasis (in blue) at diagnosis on cancer-related survival

In 59.7% of the overall population, a surgical treatment of the tumor was offered, and this impacted cancer-related survival (OR: 0.41 (95% C.I. 0.20-0.81), P=0.01, Fig 3c). The possibility of surgically treating the malignancy was overall high and not different before vs after 2002 (P=0.19). Of note, no graft-related complications as well as significant rejections (≥2R) or life-threatening events occurred in the peri and post-operative period. Both chemotherapy and radiotherapy didn´t appear to influence cancer-related survival, as globally considered.



P=0.01

Figure 3 C. Effect of surgery (in blue) on cancer-related death

Role of everolimus on cancer-related death

139 patients who underwent to HTx in the study period were on EVE therapy: 82 of them received the drug as a manteinance therapy, 57 as a de novo therapy. 11/139 patients developed a malignancy during EVE treatment (8 in the manteinance group, 3 in the de novo group), with the following localization: prostate (n=4), colon (n=3), kidney (n=2), bladder (n=1), lung (n=1). In all of them EVE therapy was continued after diagnosis. Among patients (n=60) who developed a solid cancer during another antiproliferative treatment, immunosuppression management was quite different among various era from HTx; 9 of them were switched to an EVE-regimen; overall, EVE was introduced at a median time of 6.3 months after diagnosis, and at a median time of 8.5 months in patients undergone to surgery. Surgical treatment was offered in 70% of patients that were switched to EVE. In patients not shifted to EVE, global immunosuppression was reduced when possible.

Overall, among the study population, 20 patients were on EVE therapy after cancer diagnosis (EVE-group), 11 of them were on EVE as a de novo therapy, 9 as a manteinance therapy after cancer diagnosis.

Cancer-related death was significantly lower in EVE group when compared to non-EVE group (5-yrs survival: 64.8 ± 13.5% vs 36.3 ± 7.6% P=0.01, Fig.4). The prevalence of metastatic disease at diagnosis was not different between the two groups (P=0.14), as well as the feasibility of a surgical treatment (P=0.30).

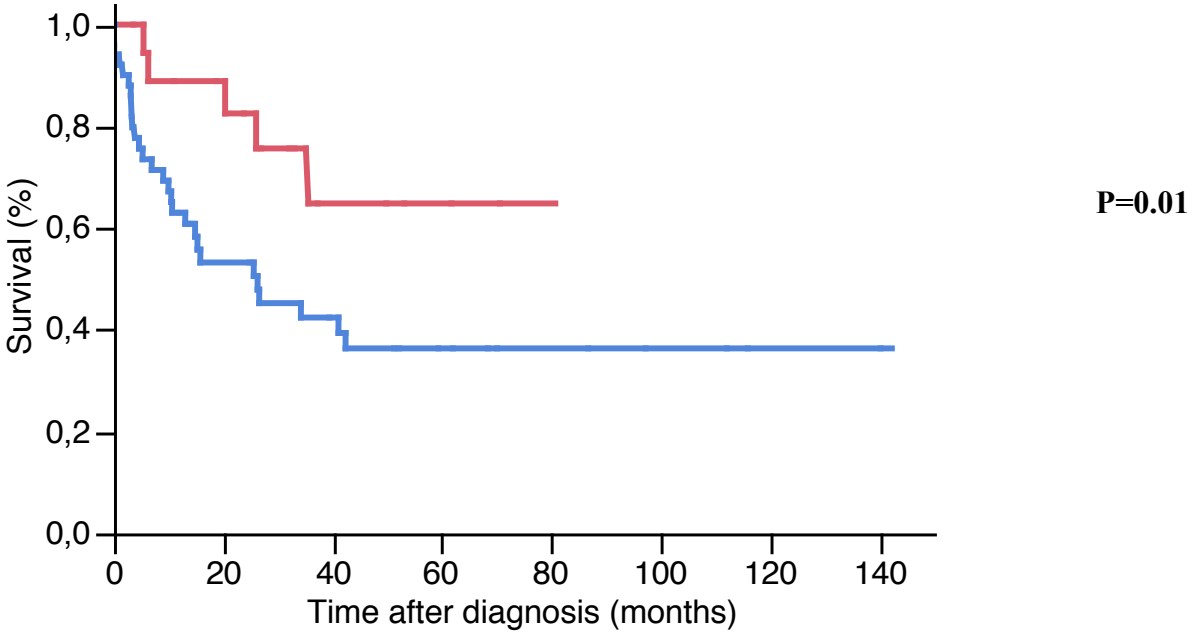


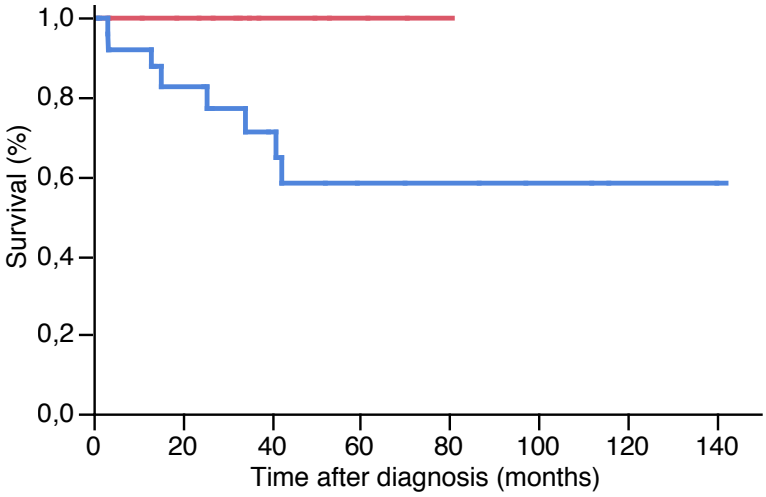
Figure 4. Effect of everolimus (in red) on cancer-related survival

At multivariate analysis, oncological surgery and metastases at diagnosis were found to be indipendent predictor of cancer-related death (respectively OR 0.41 (CI. 95% 0.20-0.83), P=0.01 and 8.92 (C.I. 95% 4.09-21.58), P<0.0001, Table 2), with metastases as the strongest predictor, whereas era of HTx lost of statistical significance. When EVE therapy was entered in a step-wise multivariate analysis model comprehensive of surgery, it showed to influence cancer-related outcome (OR 0.34 (C.I. 95% 0.11-0.81, P=0.01), whereas it reached a borderline statistical significance (P=0.06, OR 0.43, C.I. 95% 0.14-1.04) in a stepwise model with metastases.

	UNIVARIATE		MULTIVARIATE	
	OR	**P**	**OR**	**P**
tastasis at gnosis (M1)	9.76 (4.51-23.52)	<0.0001	**8.92** (4.09-21.58)	**<0.0001**
gery for cancer	0.41 (0.20-0.81)	0.01	**0.41** (0.20-0.83)	**0.01**
E after cancer gnosis	0.36 (0.12-0.86)	0.01	**0.34** (0.11-0.81) 0.43 (0.14-1.04)	**0.01•** **0.06†**
nsplant era (after 2)	0.34 (0.11-0.88)	0.01	0.49 (0.15-1.35)	0.18

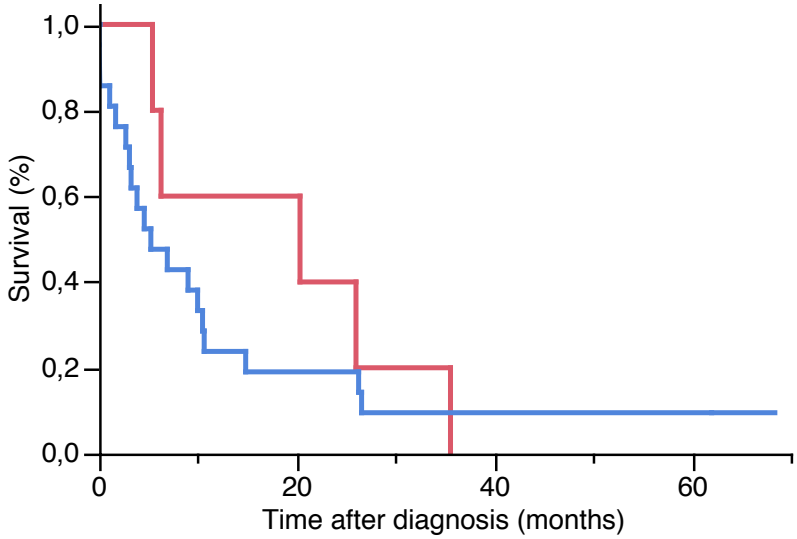
Table 2. Predictors of cancer-related death at univariated and multivariate analysis

By subgroup analysis (Fig. 5 A and B), we noticed that, in the EVE group, no one of the patients who developed a cancer during EVE-therapy (EVE as a de novo therapy) had metastases at diagnosis, and all of them survived until the end of the follow-up period (100%). The prevalence of a metastatic disease at diagnosis was similar between patients among the EVE group who were switched to EVE and non-EVE group patients (55.5% vs 43.7 %). 5-yrs survival was 34.5 ± 18.3 % vs 36.3 ± 7.6 % respectively for EVE switched vs. non-EVE group patients.



P=0.04

Fig. 5 A. Subgroups analysis: effect of EVE (in red) on survival in M0 patients



P=0.18

Fig. 5 B Subgroups analysis: effect of EVE (in red) on survival in M1 patients

Globally, therapy with EVE after cancer diagnosis appeared to influence the outcome in patients in M0 status (P=0.04), whereas it didn´t appear to influence the outcome of patients with metastases (P=0.18)

Discussion:

Whereas efficacy of EVE in the treatment of some types of cancers is known, the efficacy of its introduction after a cancer occurrence in solid organ transplantation has been poorly investigated and shown to be effective in some special cases, such as Kaposi´s sarcoma in renal transplanted patients. However, data about the role of EVE on subsequent outcome in HTx are lacking.
In our institution, immunosuppression widely varied among last years: in the 90´s , AZA was the only antiproliferative agent used, subsequently replaced by MMF since 2003. EVE was used in our institution since 2007, both as a de novo and a rescue therapy. In our Center, EVE as a de novo therapy has been used in patients enrolled in multicentric or monocentric prospective randomized studies of comparison with MMF or AZA or, more recently, for clinician's choice (i.e. patients with serological mismatch for CMV); even it was not the aim of the present study, overall incidence of cancer in patients on EVE de novo was low. Even if the main indication for the introduction of EVE as a manteinance therapy in our Center is renal failure in the setting of a CNI-reducing strategy, in 9 patients this drug was introduced after cancer. In the last years, after the development of a solid organ malignancy, the main strategy in our Center in patients who developed a solid organ cancer was to reduce overall immunosuppression and when possible to introduce EVE, in absence of controindication (i.e. severe proteinuria or leucopenia).
In this study, we observed a lower incidence of cancer-related death in patients on EVE after cancer diagnosis; of note, the prevalence of the two strongest predictors of survival (metastatic disease at diagnosis and the feasibility of a surgical approach) was not different in EVE vs non-EVE group. However, at step-wise multivariate analysis , the effect of EVE was retained only in a model comprehensive of surgery, but not in the one with metastasis, even if with a borderline statistical significance. It must be noted that patients without metastasis at diagnosis had a benefical effect on survival by EVE therapy, whereas those with an M1 status did not. Moreover, in the EVE group, cancer-related death of patients in which EVE was introduced after the diagnosis of cancer was similar to the one that patients in the non-EVE group had, whereas no one of patients who developed a cancer during EVE-therapy died. It must be noted that no one of patients in the EVE-de novo group had metastases whereas M1-status prevalence in the patients that were subsequently switched to EVE was similar to the one of patients in the non-EVE group. However, it is also possible that EVE could have played an addictive role also in the EVE-de novo group, or

especially in the subgroup of patients that didn't undergo to surgery. This hypothesis is difficult to prove but also to exclude, due to the different pathobiology of the different types of tumors in all groups of the study, that is, undoubtly, an important study limitation. However, it gives a rationale to the introduction of EVE after a cancer diagnosis; of note, EVE was introduced regardless the presence o not of metastases (p= EVE vs non EVE).
Surgery is an effective treatment of cancer, and it can improve survival when it's possible to be offered in general oncological patients. The decision to offer a surgical treatment for cancer has to be balanced on one hand with the risk of cancer recurrence, and on the other with the peri- and post-operative risk and complications, of which the cardiovascular one are the most threatening. In HTx patients, these two topics are matter of an increased concern, because of the believed increased risk of cancer recurrence after surgery, due to overall immunosuppression, and because of the fear of graft-related (i.e. rejection, CAV) and non-graft related (i.e. infections) complications. In our study, surgery was performed in a great majority of patients, so very aggressive, appeared to add a benefit on cancer-related risk of death and to be safe. We didn't experience any-graft related serious complication and only few, not life-threatening episodes of infection (XX). Of note, the feasibility of surgery was apparently not influenced by the presence of a metastatic status at the moment of cancer diagnosis.
It has been shown that EVE may delay wound healing; for this reason, we waited a median time of 8.5 months before to introduce it in the cohort of operated patients (70% of the overall cohort of patients switched to EVE after cancer diagnosis). The introduction of EVE after surgery appeared to be safe: we didn't observe any impaired wound healing neither significative adverse events (i.e. pleural or abdominal effusions, pneumonia or bacterial infections). The drug was well tolerated also in the overall EVE group.

Study limits
The sample size of this study was limited, but however regardable for a single-center study testing a long-term complication. Apart from the observational nature, the most important limit of our work is undoubtly the heterogeneity of the population due to the different cancers observed. It could be that the effect of EVE on cancer occurrence and prognosis was different in different subsets of tumors in our population. EVE was introduced in a cohort of people not affected by the same tumor and not all people affected by a certain kind of tumor were switched to EVE therapy; however, we can't exclude that this drug was introduced on a subset of patients with a cancer biology favourable to respond to mTOR inhibition, thus introducing a biological-based bias in the evaluation of its effect. On the other hand, it has also to be said that the unselective mTOR inhibition played by EVE could have also mitigated the potential anti-proliferative effect of mTOR inhibition, paradoxically favouring the progression of cancer in certain hystobiological subtypes.
These limitations are however difficult to contrast: ideally, a prospective randomized trial about the introduction of EVE after cancer should be made in a subset of patients affected by the same cancer, and should require a multicentric design due to the small number of cancer events, even in a long-experienced and large transplant program.
However, an indagine about the biological effect of EVE was not the purpose of this study. Our aim was to obtain grossolani data that could provide a rationale for the clinicians to the use and introduction of EVE after a cancer has occurred.

Conclusions:

The efficacy of the introduction of an mTOR inhibitor after a cancer diagnosis occurring in a heart transplant patient has been poorly investigated, both on the safety and on the efficacy site. In our observational study including 500 HTx patients, the presence of metastasis at diagnosis and an highly-aggressive surgical treatment were the strongest outcome predictors. Surgical treatment of cancer in HTx patients, when possible, has a favourable efficacy-safety profile, without life-threatening complications.
EVE introduction was safe, and appeared to influence cancer-related survival even when compared to surgery, whereas its benefic effect was shown only in patients without metastasis at diagnosis. Despite the heterogeneity of its population, this study provides a strong rationale for the introduction of EVE after cancer for the clinicians taking care of long-term heart transplant recipients.

Chapter 5.
Everolimus versus mycophenolate de novo after heart transplantation: does it matter for long-term outcomes?

Background:

Several large prospective randomized trials (RTs) compare EVE and MMF de novo after HTx; however, long term comparison on clinically meaningful endpoints is lacking.

Methods:

In this study we included all patients enrolled in our Center in RTs about EVE de novo. We analyzed 5-years (yrs) incidence of fatal and non fatal major cardiovascular events (MACE), all-cause mortality, rejection, CMV infection, cancers, GFR (by MDRD). Given the frequent cross-overs between the two drugs, we performed both an Intention to treat (ITT) and an On-Treatment (OT) analysis, defined retrospectively as the drug taken for most of the time.

Results:
93 patients (80% males, 53±11 yrs, HTx 2005-14) were enrolled: 57 randomized to EVE, 36 to MMF. Study population characteristics are depicted in Table 1.

Parameter	Everolimus N=57	Micofenolato N=36	P
Gender (M,%)	84,2%	72,2%	0,16
Age at HTX (years)	52,7 ± 11,02	53,8 ± 11,31	0,62
Donor age (years)	41,7 ± 12,48	38,8 ± 12,19	0,27
Donor gender (M,%)	80,7 %	75 %	
VFG base (mL/min)	68,63 ± 30,42	66,49 ± 36,06	0,75
Pre-HTx Cardiopathy (N)	CAD 22 DCM 22 HCM 4 RCM 3 Valvular 2 Others 4	CAD 13 DCM 9 HCM 4 RCM 4 Valvular 3 Others 3	0,5

Table 1. Study population characteristics

29 underwent at least one cross over, 10 for clinical reasons (cancers, renal failure, rejection), 19 for drug intolerance. Tolerability was lower in the EVE arm (p=0.05), see Figure 1 mostly due to

pericardial effusions, but comparable to MMF after 3 month from HTx (p=0.42). Causes of drug interruption due to intolerance are depicted in Figure 2.

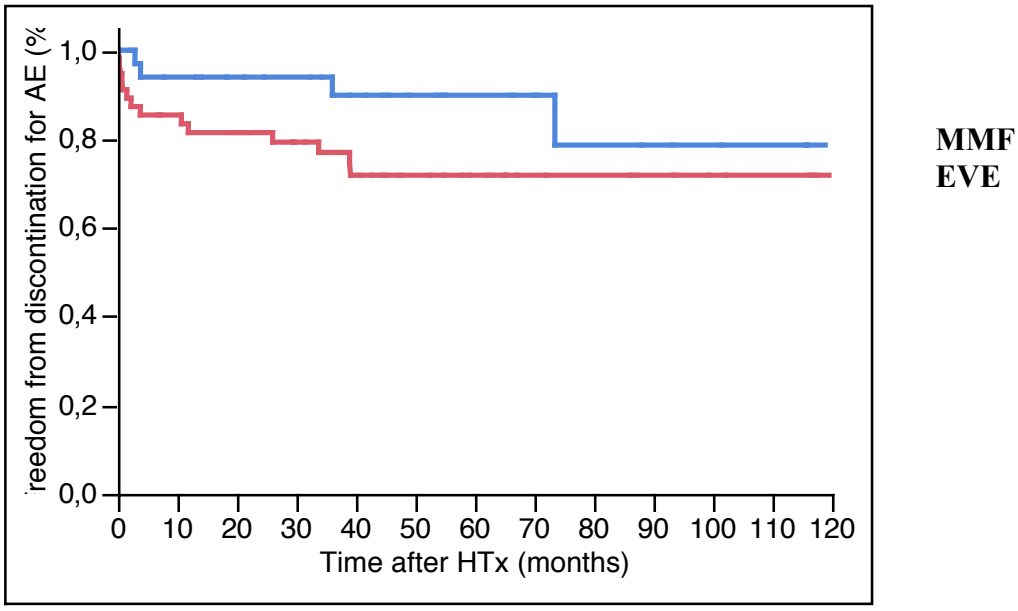


Figure 1. Study drug interruption due to tolerance problems

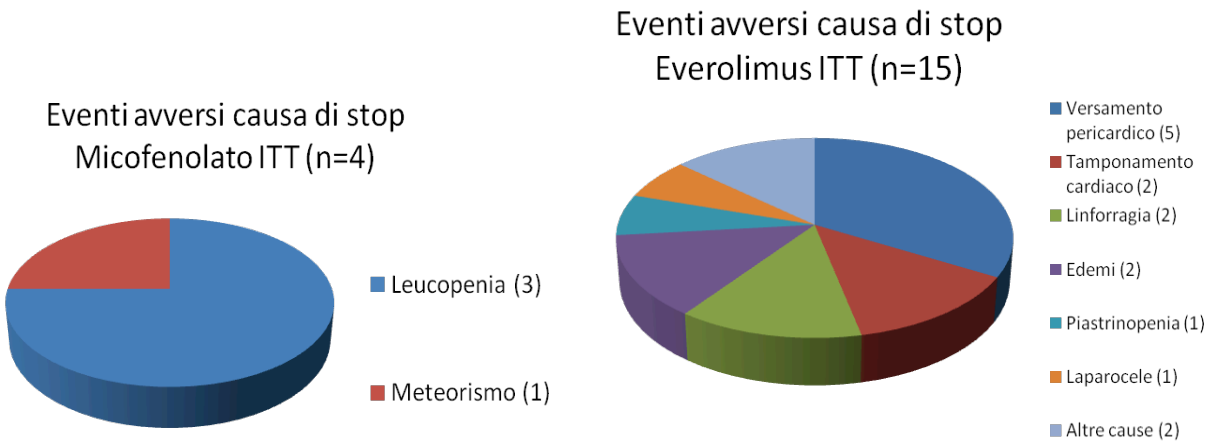


Figure 2. Causes of drug interruption

At ITT analysis, we found no differences about MACE (Fig. 3), overall mortality (Fig. 4), rejection, CMV infection, cancers, (Figures 5 A, B, C), renal function (p all not significant).

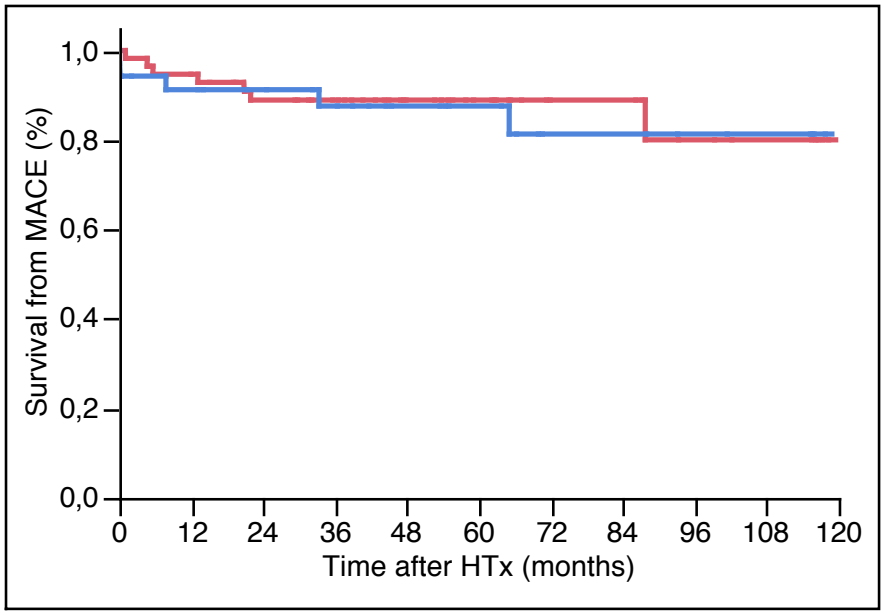


Figure 3. Difference about MACE (ITT analysis)

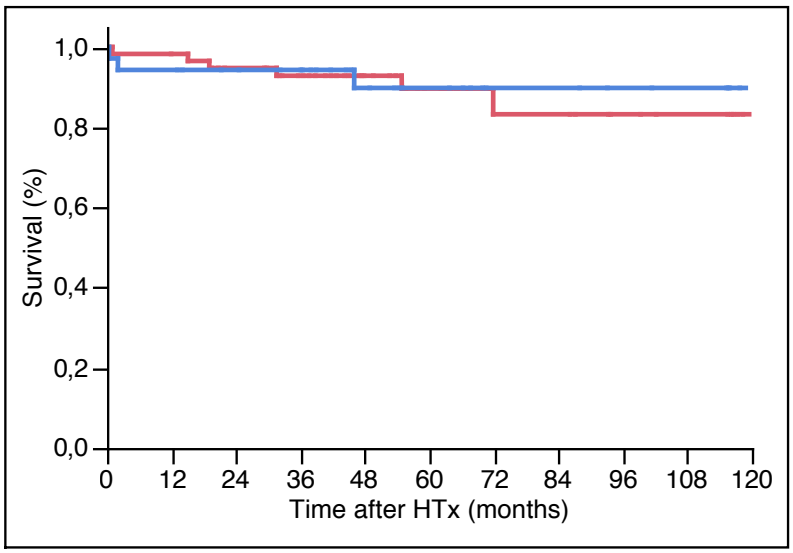
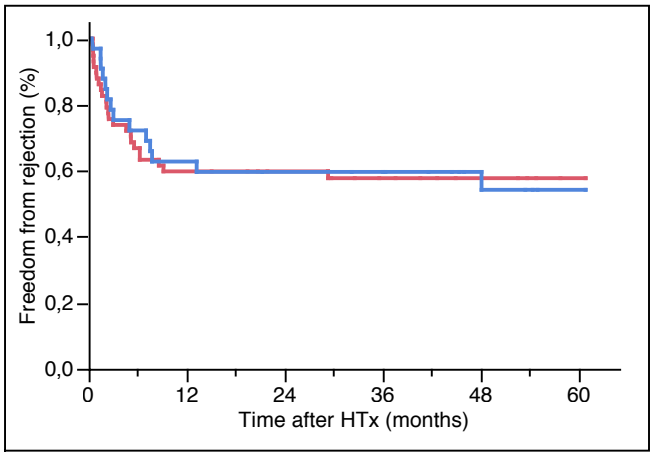


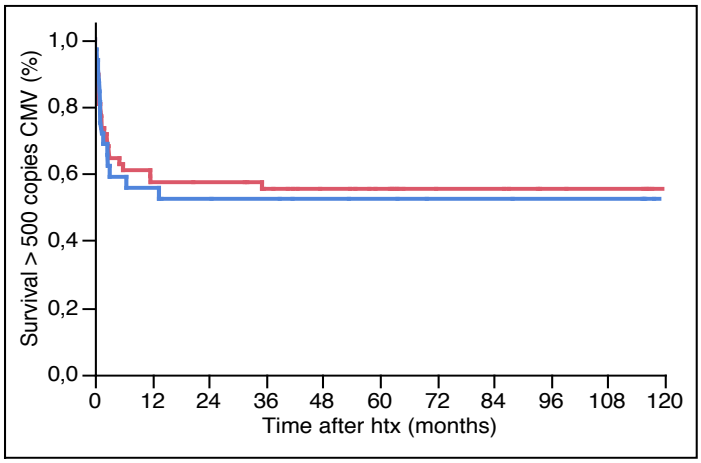
Figure 4. Overall survival (ITT analysis)



EVE
MMF

P=0.75

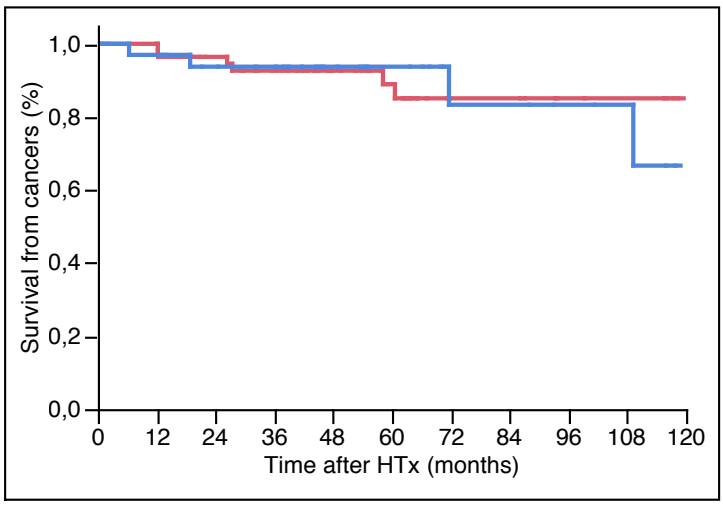
Figure 5 A. Rejection (ITT analysis)



EVE
MMF

P=0.86

Figure 5B. CMV infection (ITT analysis)



EVE
MMF

P=0.86

Figure 5C. Difference on cancer incidence (ITT analysis)

At OT analysis EVE group (42/93) had a lower incidence of MACE (2.6±2.5% vs 19.0±5.8%, p=0.01), (see Fig. 6) and CMV infection at one year (28.8±7.0% vs 44.0±7.3%,p=0.02), (Fig. 7), with similar mortality (p=0.53) and renal function (p=0.45) and a promising lower estimated incidence of cancers at 10 yrs (9.5±5.4% vs 38.7±16.2%, p=0.33).

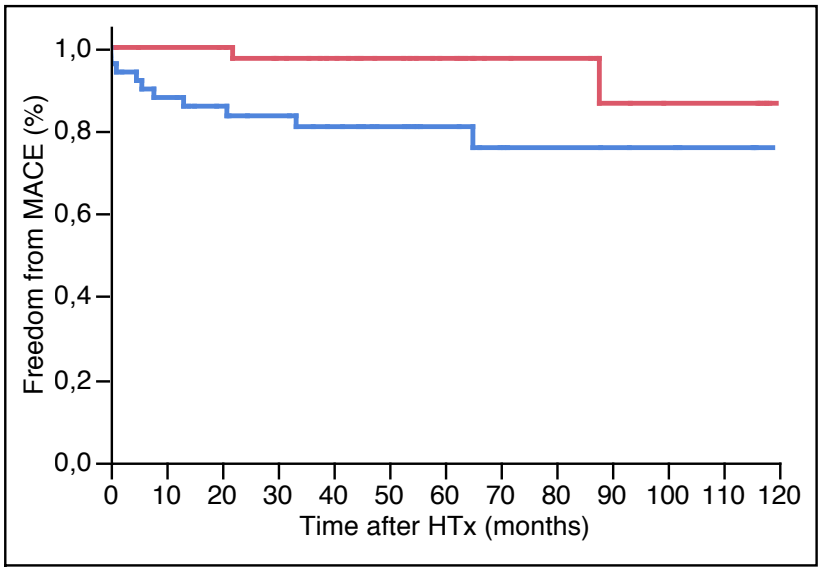


Fig. 6 Difference on MACE incidence (on treatment analysis)

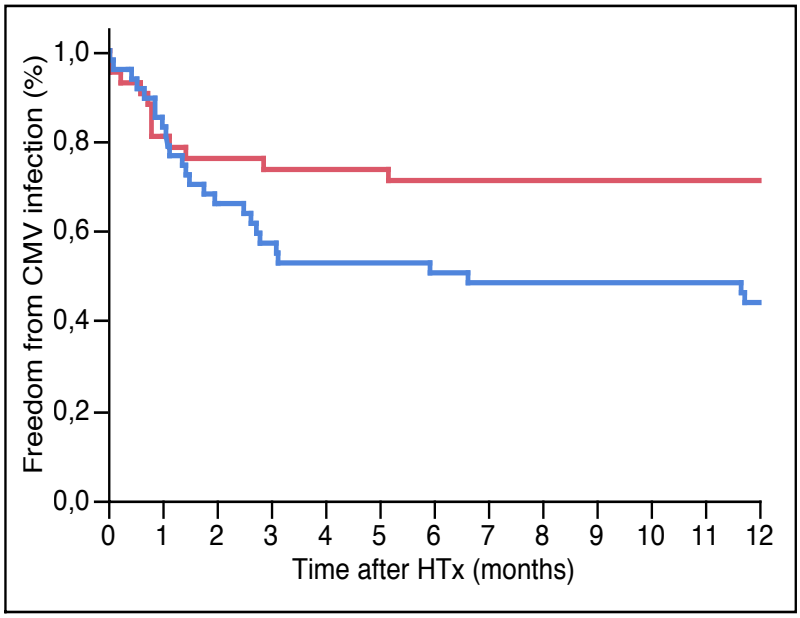


Fig. 7 Difference on CMV infection at one year (on treatment analysis)

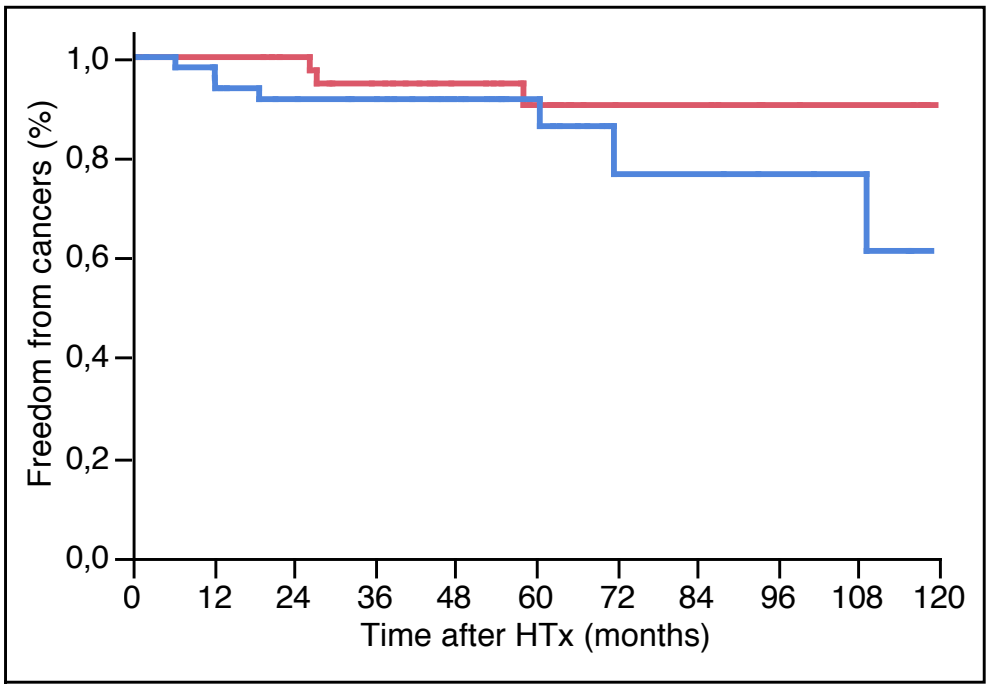


Figure 8. Difference on cancers' incidence (on treatment analysis)

Conclusion:

This is a single-center post-hoc analysis of long-term outcomes in patients initially randomized in trials with different endpoints. While confirming problems in maintaining EVE therapy in the early post-HTx period, we found suggestive evidence of potential long-term benefits of EVE in reducing MACE, CMV infection and cancers. Although these data are only hypothesis generating, they suggest a delayed introduction of EVE to favour its tolerability, aiming to take better advantage of its possible long-term benefits.

PART TWO

LEFT VENTRICULAR ASSIST DEVICES (LVAD)

Chapter 1.

Background on LVAD

Among durable devices for mechanical circulatory support (MCS), the ones supporting left ventricle (left ventricular assist devices, LVAD) are the widest used in clinical practice, according to data from INTERMACS, the Registry of ISHLT for MCS. LVAD represent 95% of all MCS, being right ventricular assist devices (RVAD) and biventricular assist devices (BIVAD) respectively 4% and 1%.

1.1 Technology of the most used LVAD systems

The first pumps available on the market provided pulsatile-flow through specific valves; however, despite a more physiologic flow, it was demonstrated that they were associated with a worse outcome than the second-generation pumps, that provide a continuous flow (CF).
The widest used CF-LVAD is Heartmate II device, from Thoratec. It is an axial pump, usually positioned extrapericardial.
The other largely implanted pump is HVAD, from Heartware; it's a pump with a centrifugal design, implanted intrapericardially, bearingless, and providing a continuous flow; it has a magnetic levitation system. This pump has been approved by FDA for the use "bridge to transplant" in 2012, actually, it is used also as destination therapy (Fig.1)

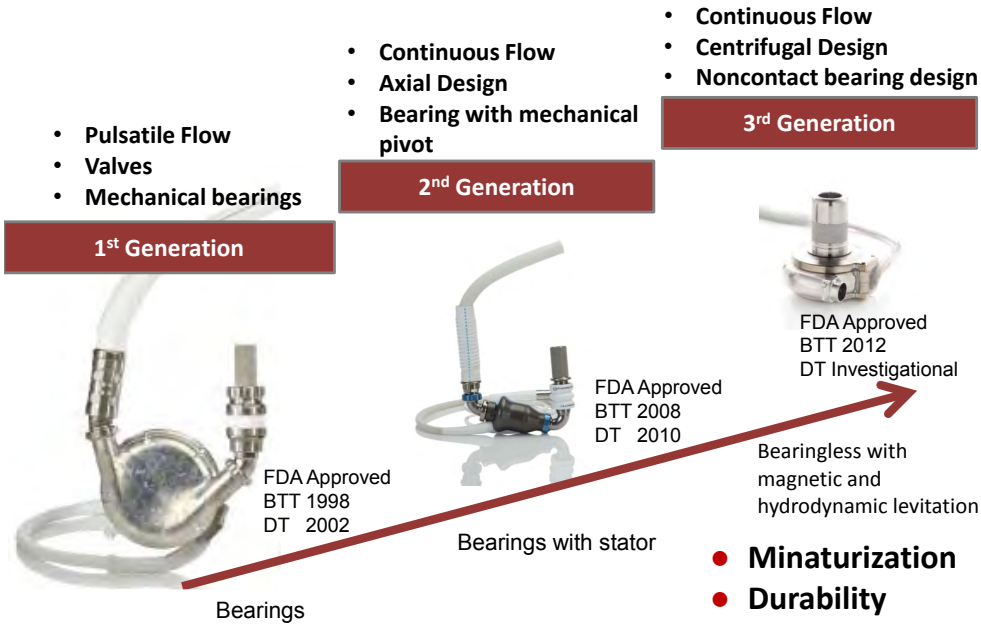


Figure 1. Evolution of LVADs' technology

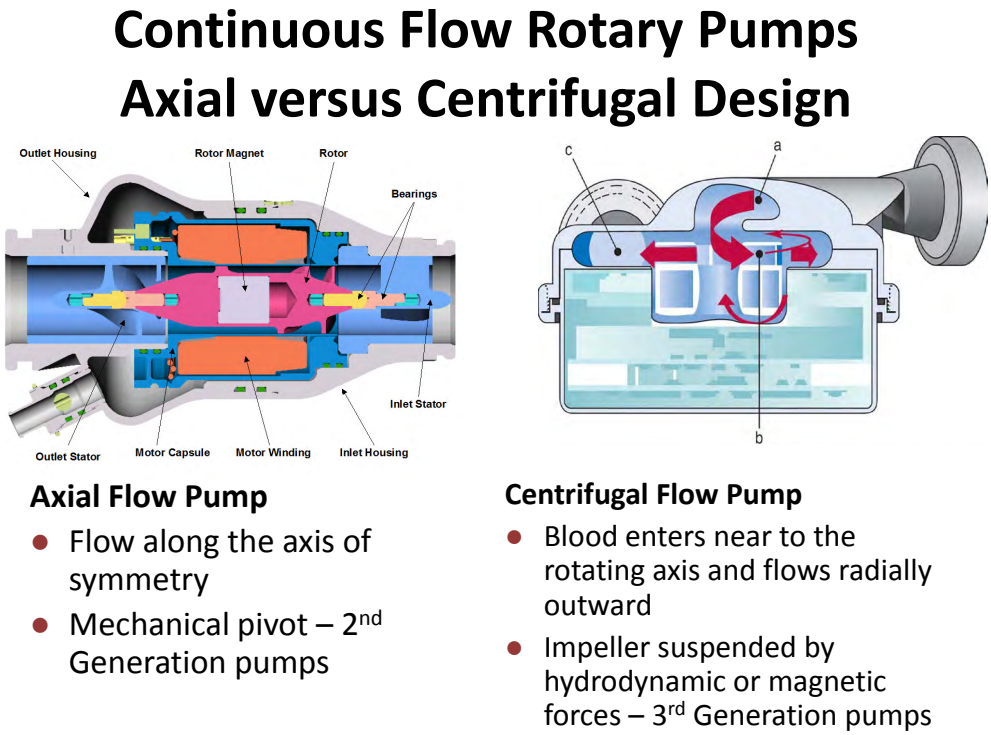


Figure 2. Differences between axial and centrifugal design

Differently from axial flow pump, in a centrifugal flow pump , the blood enters near to pump rotating axis and flows radially outward. In the HVAD centrifugal pump, the impeller is suspended by hydrodynamic or magnetic forces.

1.2 Physiology of LVAD systems

Even if they provide flow continuously, CF LVAD do not carry the same output through cardiac cycle or in different physiologic conditions, and they are influenced in some degree by pulsatility of the heart, that is related to systolic performance of left ventricle.

In particular, pump flow depends on :

- Type of the pump (axial vs centrifugal)
- pump speed
- residual left ventricular function
- the pressure gradient between pump head (left ventricular pressure) and after the pump (aortic pressure), that is :

$$\Delta P = \text{aortic pressure- left ventricular pressure} = P_{AO} - P_{LV}$$

Pump speed is regulated externally. Thus, left ventricular function and the gradient between aortic pressure and left ventricular pressure are crucial in determining LVAD flow. Aortic pressure depends on overall fluid status and from systemic medications normally used for heart failure's treatment, like ACE inhibitors or beta blockers. Left ventricular filling pressure is determined by left atrial pressure, that depends on left ventricle relaxation properties (diastolic function) and, in absence of mitral stenosis and/or significant pulmonary regurgitation, on right ventricular output. Thus, in these conditions, right ventricle determines the preload of the pump, whereas systemic aortic pressure is the afterload of the pump. From this assumption, one can understand that pump flow is different among the different phases of cardiac cycle and among different settings of pump speed. Flow curves have been built among different types of pumps, extrapolating the

haemodynamic features from animal models of isolated hearts. A different shape of flow curve corresponds to a different pump speed

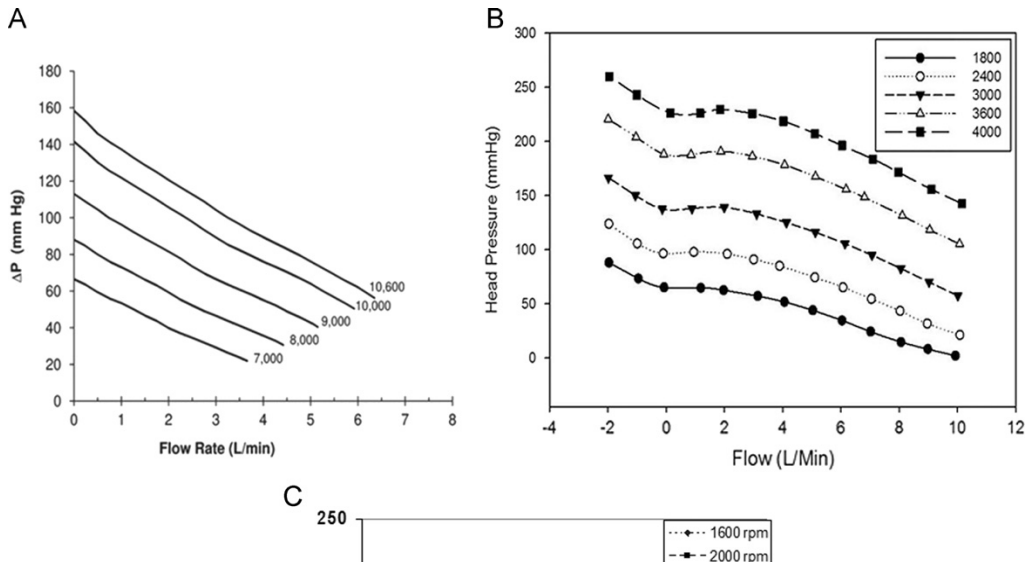


Figure 3. Pressure-flow curves: at left, axial pump, at right, centrifugal pump.

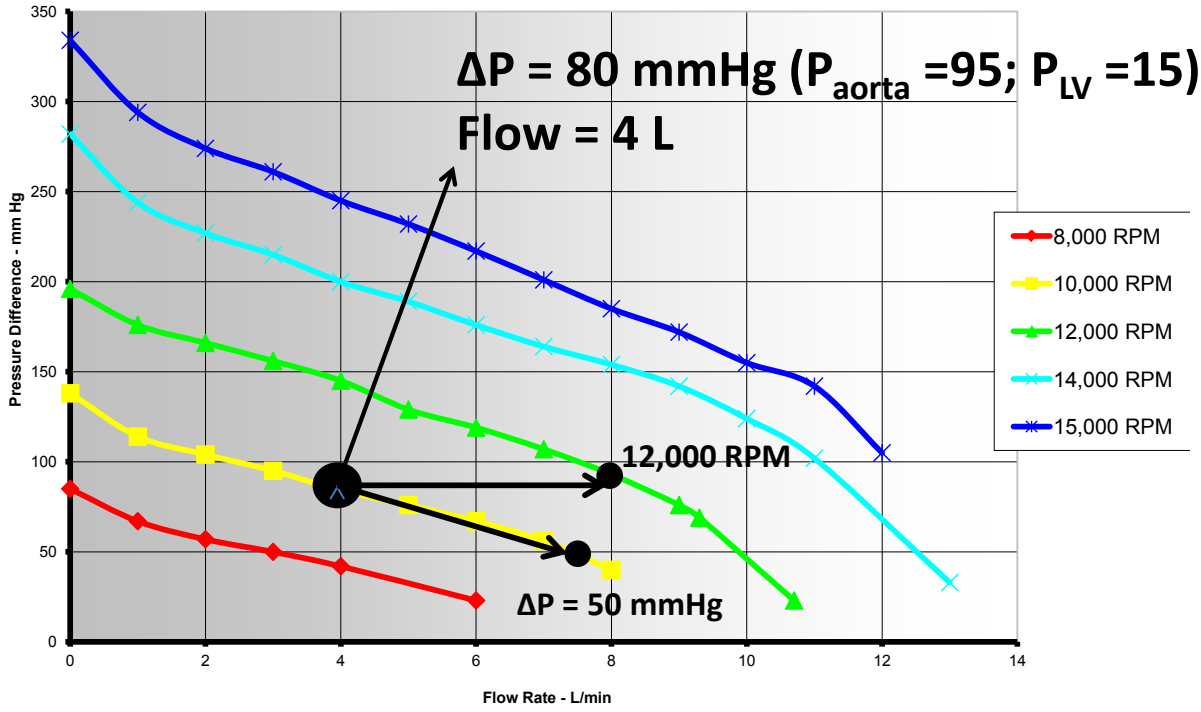


Figure 4. Effect of different pump speeds on pressure flow curves

As depicted in Figure 4, at the same level of pressure difference across the pump, an increase in pump speed leads to an increase in pump flow.

However, as represented in Figure 3, there is a substancial difference between axial and

centrifugal curves. Centrifugal pumps have a flat head curve, where they operate over a very wide range of flows for a very small change in ΔP across the pump. The example in Figure 5 shows that for one cardiac cycle in which ΔP swings from 40 to 80 mm Hg, centrifugal pumps have a very large swing in flow (0 to 10 liters/min), acting almost like a pulsatile pump; this creates inherent high pump flow pulsatility in response to changing LV pressures. In contrast, a typical axial-flow pump has a steep head curve where there is a linearly related increase and decrease in flow with decreasing and increasing pump ΔP; in this example, the 40- to 80- mm Hg swing across the pump conduits produces less flow pulsatility, ranging from 3 to 7 liters/min during a cardiac cycle. Thus, centrifugal pumps (like HVAD) are more sensitive to ΔP variations and therefore to afterload changes.

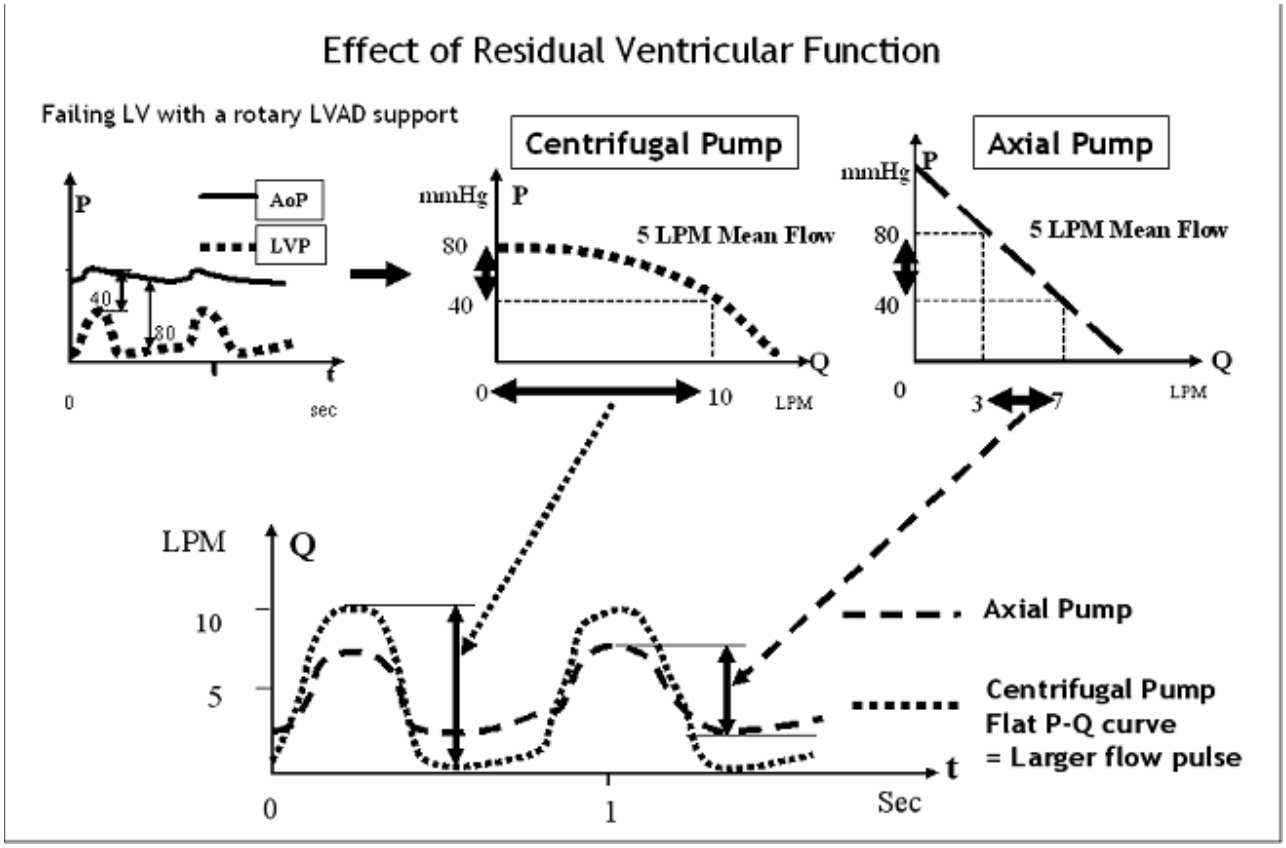


Figure 5. Different behavior of centrifugal and axial pumps in respect to different ΔP (afterload) values

Given the sensitivity of pump flow to ΔP, significant changes occur during cardiac cycle. During systole, when LV pressure is higher, ΔP decreases, leading to an increase in pump flow; during diastole, when aortic pressure increases, the increase in ΔP leads to a decrease in pump flow (Fig. 6). Residual left ventricular systolic function influences also pump flow, by influencing the capacity of left ventricle of generating higher pressures in the systolic phase.

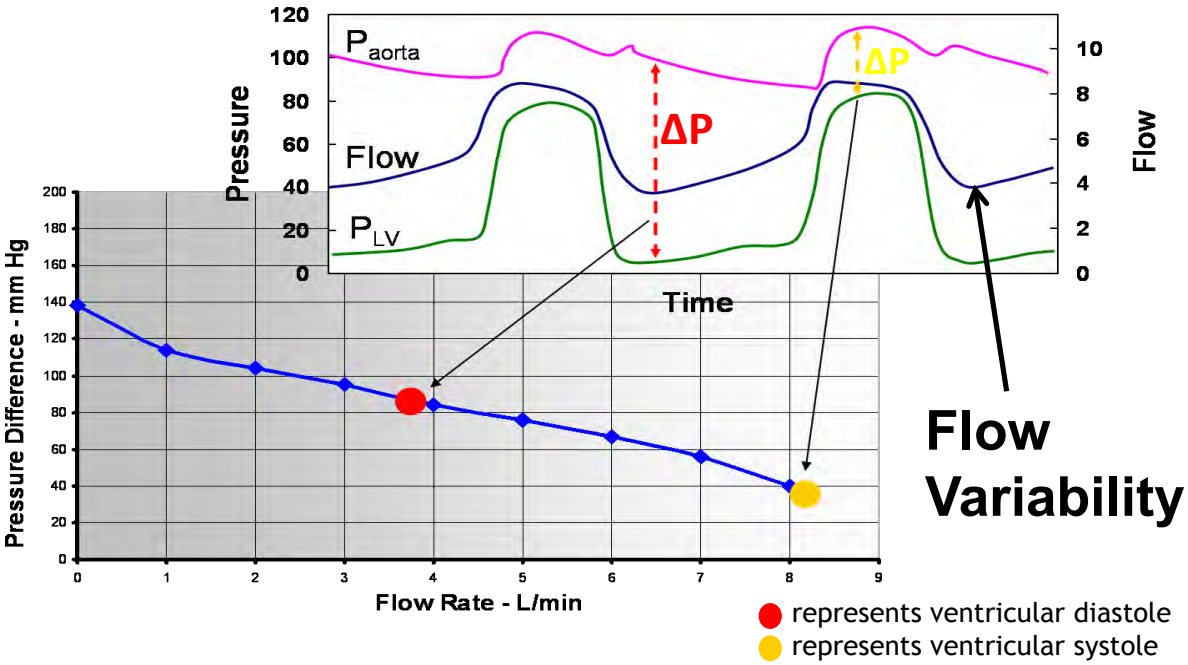


Figure 6. Pump flow during the different phases of cardiac cycle.

Pre-load sensitivity, as it relates to mechanical circulatory assist devices, mimics the relationship between LV filling pressures and ventricular stroke volume defined by the Frank-Starling curves. As right ventricular output and volemia are the preload determinant, right ventricular failure or dysidratation can lead to decrease in LVAD flow, thus of the overall cardiac output.

Data from Salamonsen et al on afterload sensitivity of CF rotary pumps support the general understanding that these pumps have higher after-load sensitivity than the human heart. This high after-load sensitivity creates the need to control systemic vascular resistance (SVR) in these patients to guarantee sustained outputs. Typically, the targeted mean systemic arterial pressures for CF pump patients is 70 to 90 mm Hg, with pressures exceeding 90 mm Hg to be avoided. If SVR were to increase (ΔP increase), this would produce an instantaneous drop in pump causing a lower flow condition. In contrast, the steeper head curve of axial pumps responds to an increase in SVR by increasing the pressure generated across the pump ports, limiting the decrease in flow by increasing outlet pressure. At low-flow conditions, this retains the capability to enforce high blood pressures with adequate LV volume. However, it also results in high inlet suction that in a low LV volume state can potentiate arrhythmias, create suction events, or lead to hemolysis. Thus, as depicted in Figure 5, the dependence of axial pumps and centrifugal pumps from preload and afterload is different, being centrifugal pumps more sensitive to afterload changes.

Flow pulsatilty, that is related to the difference between the systolic and diastolic amount of flow, is inversely related to the degree of LV unloading by the pump and directly proportional to the strength of LV contraction and as such can be used as a measure of the LV function under VAD support. Any significant decrease in pump flow pulsatility without a change in pump speed should be investigated clinically for causes of decreasing LV pressures during LVAD support. This typically includes decreasing LV contractility or low LV volume states caused by right heart failure or dehydration. Reasons for the flow PI to increase, without a change in pump speed, include an increase in LV contractility via inotropes, myocardial recovery, and exercise or increased pre-load Starling effects.

Low flow occurring in the pump or a condition of excessive unloading is dangerous, as can lead to

contact between inflow cannula and interventricular septum, and ventricular tachycardias, thus leading to further low flow and to a loop of low flow, hypotension and ventricular arrhythmias.

In the circulation assisted by LVAD, flow is directed from left ventricle (LV) into ascending aorta through LVAD, and no flow is observed through LV outflow tract (LVOT), thus remaining aortic valve closed during the entire cardiac cycle (Fig. 7A). However, if LV is not completely assisted by the LVAD, a certain amount of blood will reach ascending aorta through LVOT, thus allowing opening of aortic valve (AV) (Fig. 7 B).

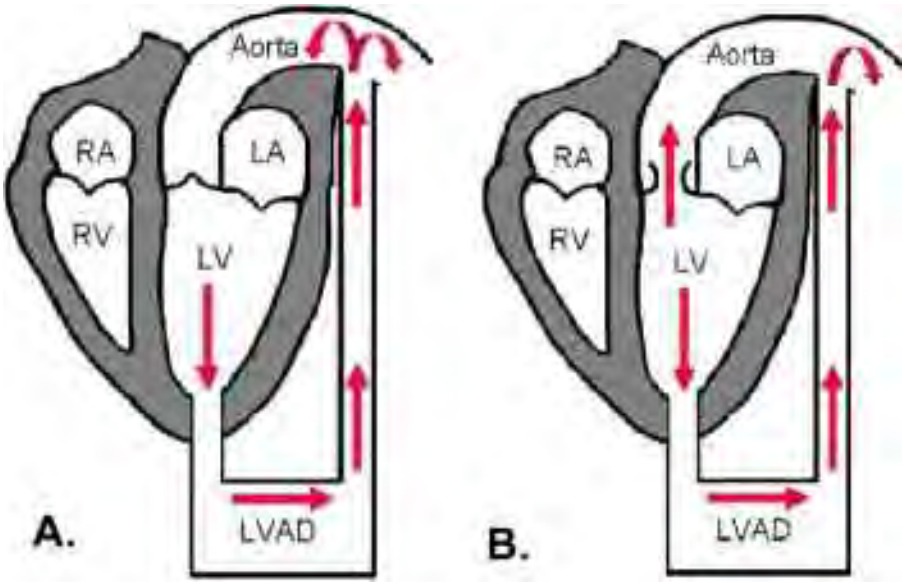


Fig.7 A. Full support condition. B. Partial support condition

Right ventricle receives the overall output carried by the LVAD and by LVOT. Therefore:

RV output= systemic output = LVAD output + output through LVOT

In condition of full support, output through LVOT is = 0, becoming RV output= LVAD output

In condition of partial support, RV output = LVAD output + output through LVOT

Of course, these two conditions are the two extremes of a wide spectrum of haemodynamic situations, in whom different degrees of support are possible. thus, it has been suggested to consider AV opening as a marker of LV support by the LVAD, However, this sentence is not completely true, as, even if various studies showed the relationship between the extent of pump support and the opening time and area of the AV, other factors influence LV unloading conditions and AV opening.

Factors influencing AV opening in a LVAD patients are:

- preload and afterload pressures (right ventricular function, liquid intake and systemic aortic pressure)
- LV unloading support by LVAD
- LV residual function
- Pump speed

A situation in whom LV is not completely assisted by the LVAD can occur in case of a low pump support (low speed settings) or of a strong LV function, such in case of myocardial recovery.in this case, the ventricle is still able to generate pressures greater than the aortic pressure (AoP), and blood is ejected in parallel to the RBP via the AV. A condition of complete support can happen in case of a very weak ventricular function; in this case, the AV remains closed throughout the whole heart cycle, and the blood flow occurs entirely through the RBP. However, the AV opening is certainly affected by afterload and preload pressures: an decrease in preload, like right ventricular failure or a status of hypovolemia, can reduce LV filling pressures, thus reducing LVAD flow and changing aortic valve status from open to closed.

However, at fixed afterload and preload conditions, AV opening status the evaluation of the AV opening provides information about the native heart function and the interaction with the LVAD. It has been reported that a fixed closure of AV can lead to aortic leaflets' fusion, aortic insufficiency, and formations of thrombi in the aortic root (see Chapter 1.4).

The assessment of the opening state of the AV is currently performed with echocardiography. Echocardiography has a key role in the optimization of pump settings and in the management of these patients, because it has the potential to help to recognize some of LVAD-related complications and to differentiate between the different possible causes of fluctuations in pump flow, like right ventricular failure or aortic insufficiency. Moreover, it has been reported that it can provide information about the unloading status of LV. These aspects are illustrated later.

In clinical as well as experimental animal data, it has been observed that the state of the AV (open or closed) is related to the shape of the systolic portion of the pump flow signal. In case of partial support (AV opens), the pump flow signal shows a flat plateau in the systolic portion of each heartbeat; in case of full support (AV always closed), the systolic peak is sharper (Fig. 8). This observation has a basic hemodynamic explanation: during full support, the almost sinusoidal shape of the left ventricular pressure (LVP) signal mainly affects the systolic shape of the pressure head waveform across the pump. Consequently, this results in an almost sinusoidal pump flow signal. On the contrary, if the AV opens, the pressure difference between the AoP and the LVP during ejection becomes slightly less than zero and does not change substantially as long as the AV remains open, which leads to the flat plateau in the pump flow signal. As shown from Granegger et al, the state of the AV can be automatically determined by quantifying the different shapes of the pump flow waveform during systole, as shown in Figure 8.

Figure 9 shows pressure patterns (LVP and AoP) and pump flow waveforms during speed changes in an animal experiment. In Fig. 9, the changes in the systolic portion of the pump flow waveform as well as the shape change in the histograms at the different speeds can be clearly observed. The flat plateau of the pump flow wave- form vanishes when the AV remains closed during the whole heart cycle. Similarly, the peak in the right side of the histograms decreases with increasing pump speed and vanishes when the AV is closed. The peak in the systolic portion of the histogram plot is thus equivalent to the flat plateau in the time course, whereas the absence of a

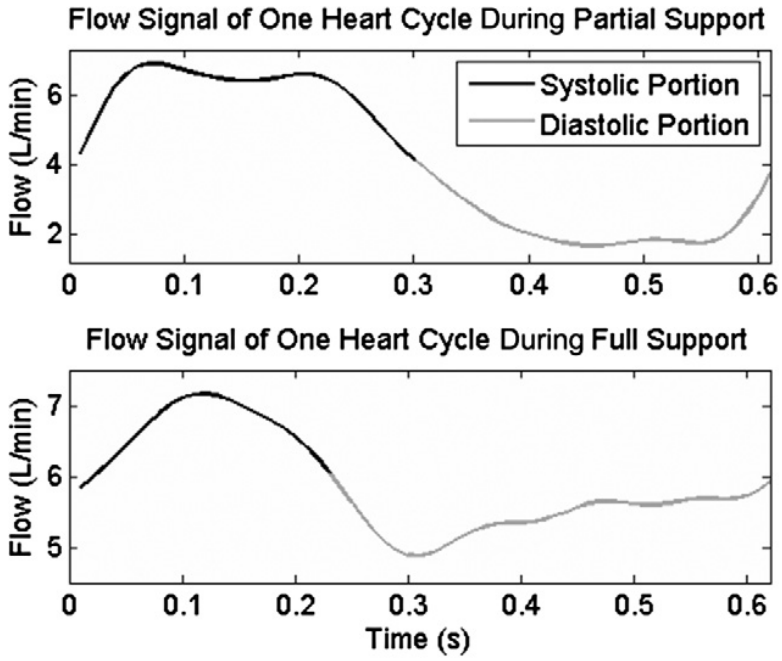


Fig. 8 (from Granegger M et al, Artificial Organs 2013)

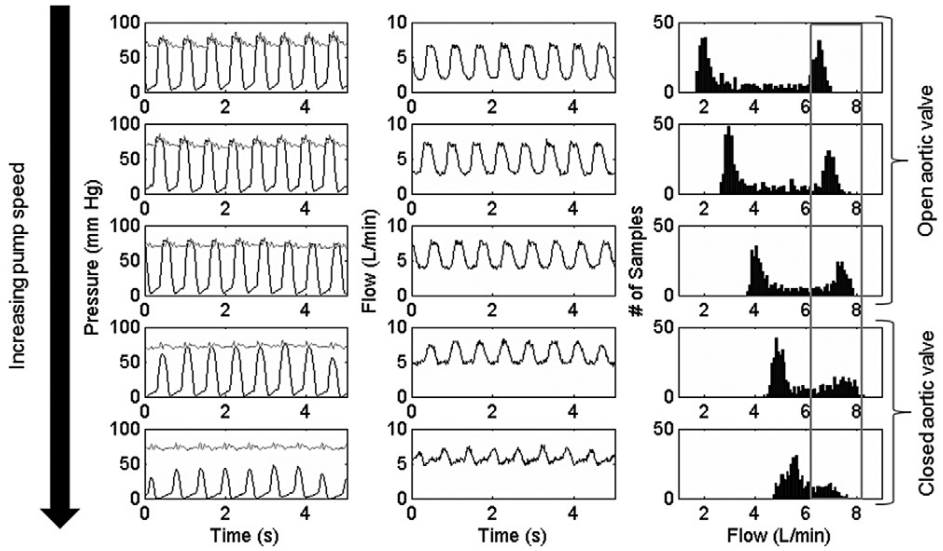


Fig. 9 (from Granegger M et al, Artificial Organs 2013)

1.3 The impact of LVAD in the management of the waiting list and on post-transplant outcomes

Indications for implantation
Patients being considered for an LVAD are normally within NYHA III and IV classes. However, as shown in the literature, these two classes contain a wide spectrum of patients with different characteristics. More in detail, the Clinicians actually refer to 7 INTERMACS classes (Table)

INTERMACS Profile Level	Status		Time frame
1	Critical cardiogenic shock	„crash and burn“	hours
2	Progressive decline	„sliding fast on inotropes“	Days to weeks
3	Stable but inotrope dependent	„stable on inotropes“	weeks
4	Recurrent advanced HF	„fequent flyer“	Weeks to few months
5	Exertion intolerant	„housebound“	Weeks to months
6	Exertion limited	„walking wounded“	months
7	Advanced NYHA class III		

Table 1. INTERMACS profiles within NYHA III and IV classes

The main indications for LVAD implantation are classically 4:
Bridge to transplant, bridge to candidacy, bridge to recovery, destination therapy.

- bridge to transplant (BTT): patients listed or suited to be listed for heart transplantation, having an high risk of clinical deterioration while on the waiting list;
- bridge to candidacy (BTC): patients having a temporary and potentially reversible contraindication to HTx, such as high pulmonary vascular resistances, solid organ cancer within the previous 5 years, smoking status, mild obesity;
- bridge to recovery (BTR): patients in a life-threatening condition, such as post-cardiotomy cardiogenic shock, acute myocarditis, in whom an LVAD is a potential life-saving procedure leading to an improvement in left ventricular function;
- destination therapy: patients with a fixed, irreversible contraindication for HTx (older age (>70 yrs), COPD or renal function contraindicating transplantation, severe obesity), having a life expectancy < 2 years and no other comorbidities potentially limiting survival in the mid term.

However, this classification is not fixed, as a sustancial proportion of patients switch from a group to another (cit Teuteberg); for example, patients initially considered for heart transplantation and implanted with a BTT or BTC intention, can subsequently stay permanently with an LVAD, thus becoming DT patients, because of the development of a long term complication, or persistence of a previous temporary contraindication to heart transplantation, or because of a mild clinical improvement, so that HTx is no more necessary. Conversely, patients implanted with a DT intention can be considered for heart transplantation, thus becoming a BTT group, if improvement of a believed fixed contraindication for heart transplantation occurs; in some patients, indeed, the decrease of left ventricle filling pressures and the increase in cardiac output carried by the LVAD can lead to an improvement in pulmonary vascular resistances and in renal function, so that HTx become a more suitable option.
According to INTERMACS data, about XX % of patients switch from BTT/BTC to DT and XX% from DT to BTT; at the end, as reported in Fig. 1, after 2 years from implantation, only 55% of patients in the BTT group remain effectively listed, in 20% of patients HTx is a probable option, whereas 20% of patients are in the DT group. In DT group, about 5% of patients after 2 years are listed for HTx. In the “transplanted –likely groups” (BTT, BTC, BTR) are effectively listed, and XX% of the DT .

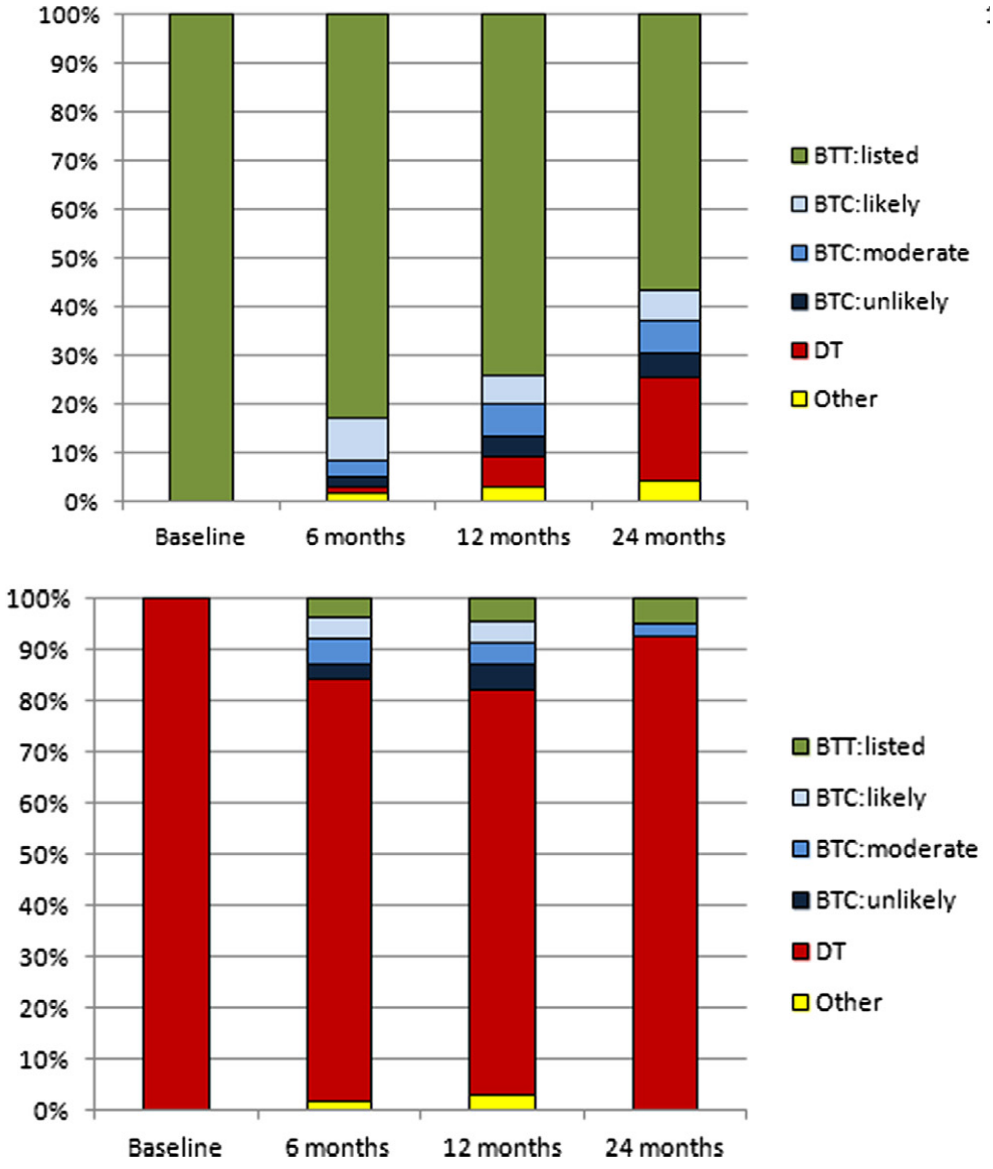


Fig. 10 Changes in being considered for HTx within BTT class (A) and DT class (B).

1.4 The management of LVAD patients: short and long-term complications

Main complications arising after LVAD implantation are:

- driveline and/or pump pocket infections
- haemorragic or non haemorragic stroke
- pump thrombus
- aortic insufficiency
- right ventricular failure
- gastrointestinal bleeding
- suction and arrhythmias

Time course of these complications is different, being respectively bleeding, RV failure and infections more frequent in the early phase , and cardiac problems and bleeding and thrombosis in the later one.

The management of driveline infection is based on antibiogram-driven antibiotics; in extremely selected cases, pump pocket exchange can be necessary. Driveline infection is a significant risk factor for stroke, and can be a cause of change in waiting list status for patients with an LVAD as BTT.

Haemorragic stroke and/or systemic bleeding is a frequent condition; patients with LVAD require a combined anticoagulant and antiplatelet therapy, that exposes the patient to a higher risk of bleeding. Moreover, colon angiodysplasias are believed to be more frequent in patients with a new generation pump, probably because of the continuous flow. Bleeding risk is high during overall support condition but is higher in the first post operative phase. On the other hand, driveline infections, problems in anticoagulation management and/or temporary anticoagulation reduction because of bleeding, are potential causes for ischemic stroke in these patients. Pump thrombosis is a dramatic event, that leads to a rapid increase in pump power (because of increased resistance forces) and to an erroneous higher estimated pump flow. Treatment of pump thrombosis is based on intravenous thrombolytic agents.

Aortic insufficiency is a frequent long-term complication in LVAD patients; it has been reported that a permanently closed AV can have damage in aortic leaflets, leading to leaflets' fusion, and to aortic regurgitation (AR). AR is deleterious for LVAD: if a moderate to severe AR occur, a consistent part of the blood carried by the LVAD returns in the LV, thus leading to a decrease in systemic output and to an increase in LV filling pressures. In the long term, this can worsen LV function, haemodynamic parameters and even RV function. For this reasons, aortic valve repair or substitution with a biological prosthesis is made preoperatively in patients with a more than mild AR. Of note, AR can be continuous through the entire cardiac cycle, thus being systodiastolic. Recently, it has been suggested an intermittent opening of AV to try to reduce AV damage, but the target of AV opening has not yet been established. High systemic blood pressure is avoided, as it can increase AR.

Right ventricular failure is a frequent long term complication, but can occur also intraoperatively. After pump starting during the operation, a sudden increase in LV output occur, therefore causing an increase in right ventricular (RV) preload and output. If preoperative RV function is moderately reduced, RV could not be able to tolerate the sudden increase in volume, and this could lead to intraoperative RV failure, causing low-flow in the LVAD. This condition can happen also chronically because of the chronic RV volume overload carried by the LVAD. However, other factors not completely clear could contribute to its pathogenesis. RV failure is usually treated with levosimendan and with slight reduction in pump speed.

Suction can occur in all condition of too high support: this can happen either when pump speed is too high either when hypovolemia is present. Also arrhythmias, like atrial fibrillation or ventricular tachycardia, by reducing LV filling status, can lead to a relative too high condition of support by the pump, thus causing suction. When suction occurs, a contact between inflow cannula and interventricular septum or papillary muscles can occur, causing further arrhythmias, potentially life threatening, and leading to a loop of hypovolemia-arrythmias, that must be corrected by increase in fluid intake, antyarrithmic agents and reduction in pump speed. Typically, in these condition, interventricular septum is “sucked” towards LV cavity and has a more “left-oriented position”. Therefore, position of LV is considered another marker of LV unloading status and of interaction between the heart and the pump.

1.5 The HVAD system and pump signal analysis

HVAD system is constituted by HVAD pump, driveline, two external batteries, driveline, and a controller (Fig. 11)

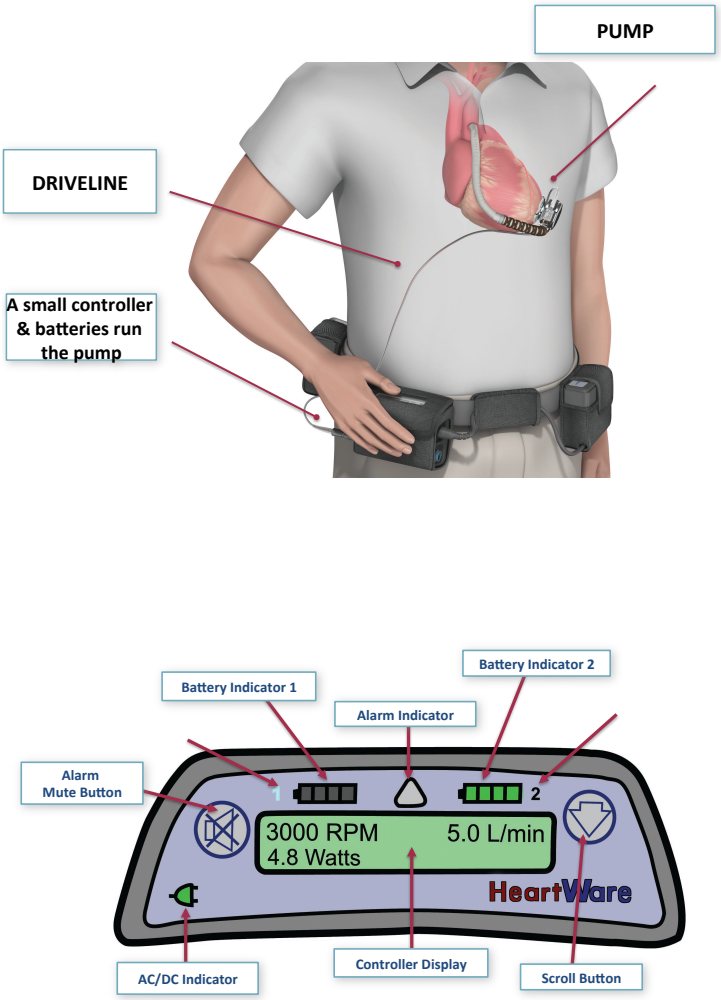


Fig. 11 HVAD system (Heartware)

On HVAD monitor the flowing information are displayed (Fig. 12):

- pump flow (estimated by haematocrit values)
- pump power

- pump speed

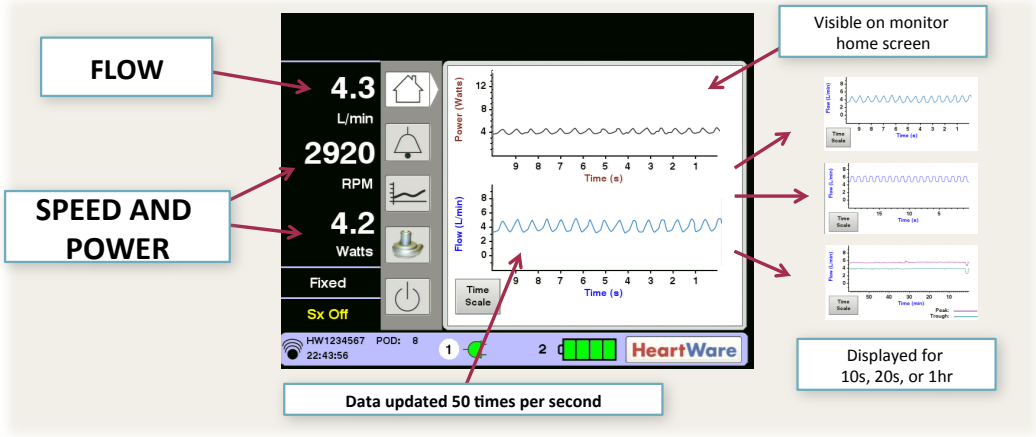


Fig. 12 HVAD monitor

As shown by Moscato et al (Fig. 13), different information can be derived by the analysis of pump flow curves.
Pulsatility index informs about LV contractility, the shape of the systolic portion pf pump flow about AV opening status, the diastolic part of the pump curve about suction events.

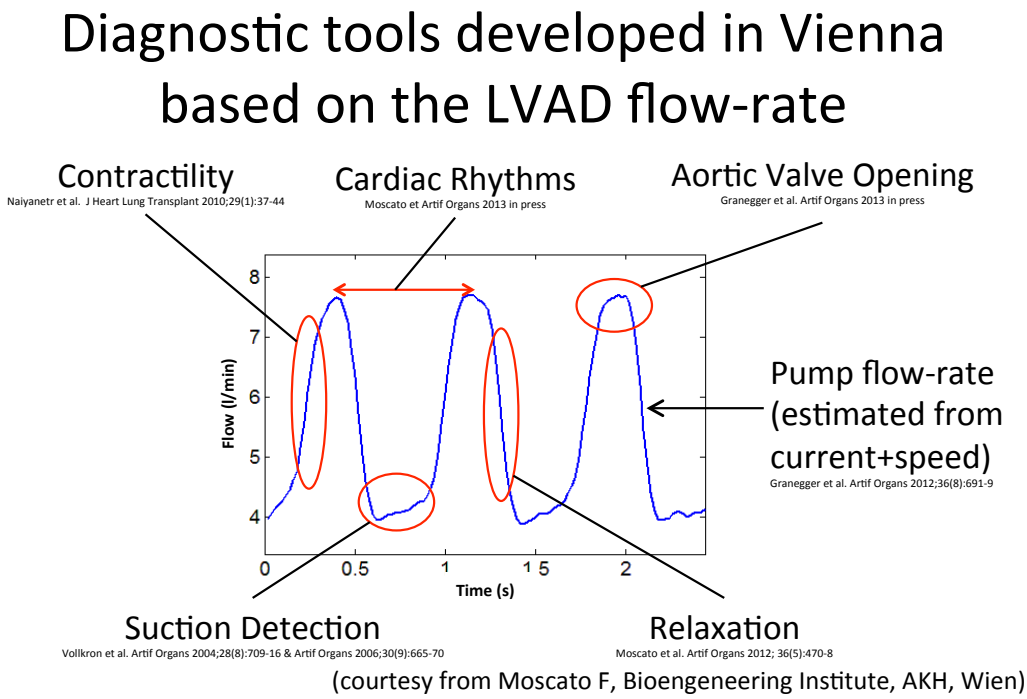


Fig. 13 Information derived from pump flow signal

Time course variations in pump flow or power also give important information about volemic status, grade of support and potential life-threatening situations. HVAD monitor has different grades of alarms. The most important alarms are: low flow alarm, high watt alarms and batteries charge status (see Fig. 14-16)

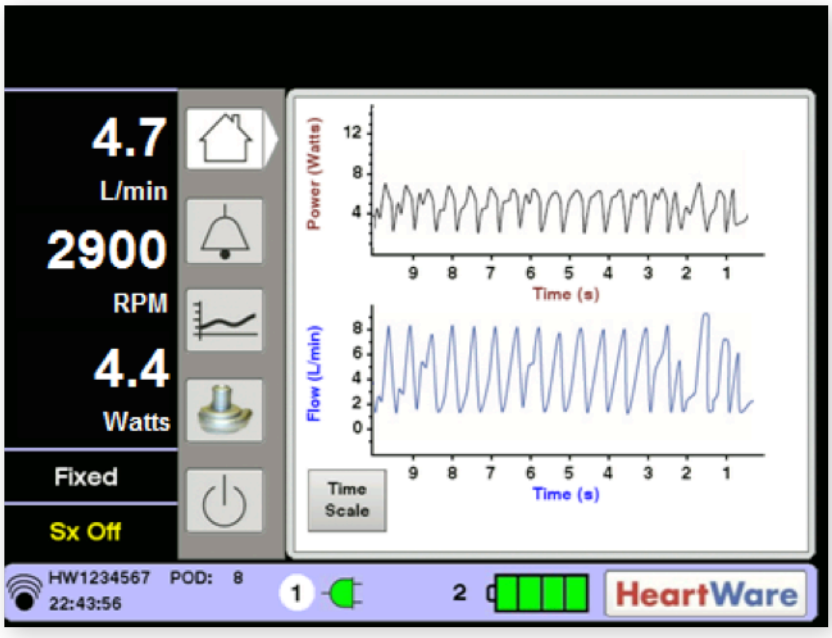


Fig. 14 Suction

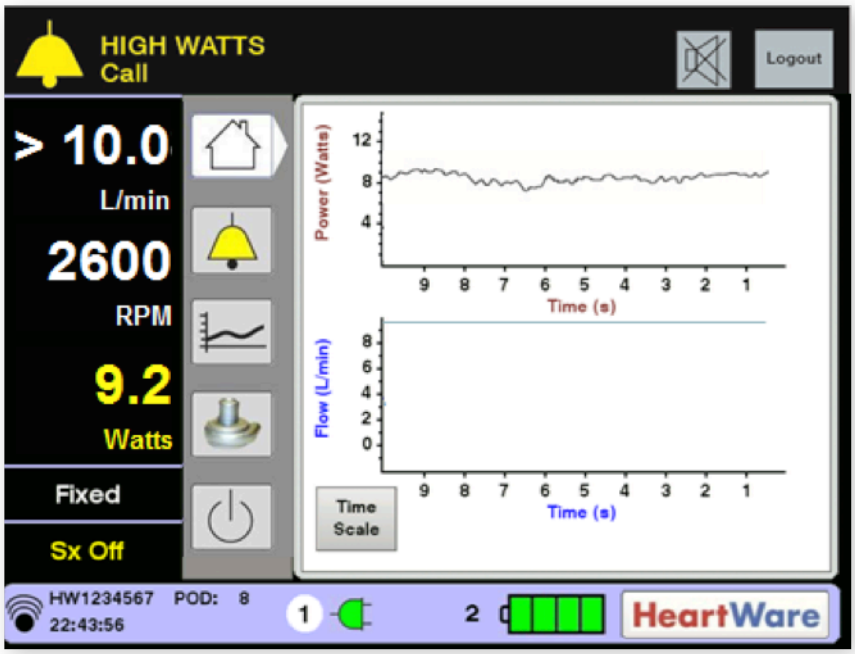


Fig. 14 High watts

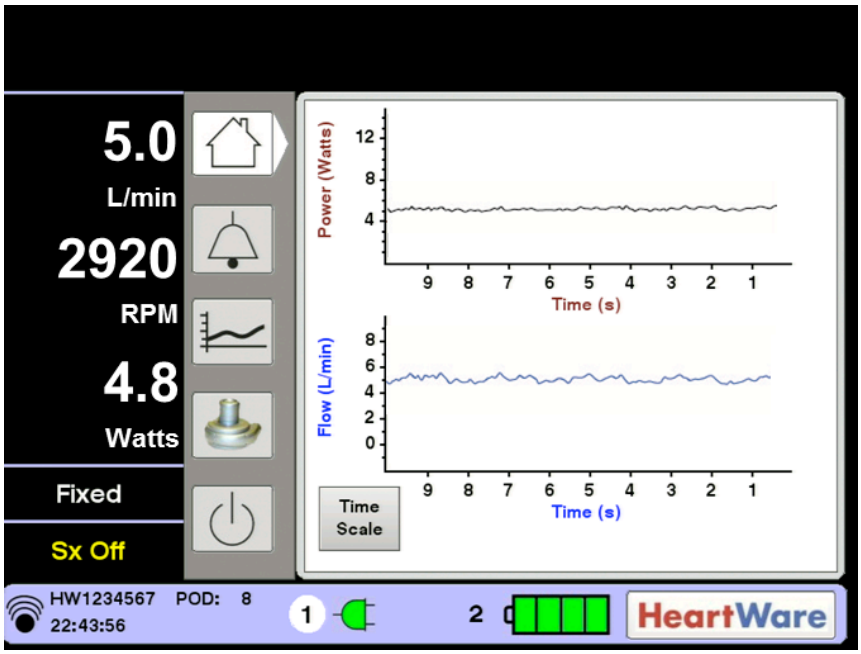


Fig. 15 Hypovolemia and excessive pump speed

HVAD monitor allows to achieve a lot of information, as already written.
Transthoracic echocardiography (TTE) is the other method that allows to recognize most of LVAD related complications and to distinguish between the different causes of alarms, as reported in Table 2. LV diameters as assessed by M-Mode, the position of interventricular septum and aortic valve opening status are parameters indicative of the unloading status of left ventricle provided by the pump. Pump flow can be also measured by transthoracic echocardiography (TTE), through visualization of inflow and outflow cannula and Doppler analysis.

However, almost the totality of the echocardiographic data about LVAD are derived from Heartmate II device, whereas there is paucity of data in the literature about the feasibility of a TTE exam with HVAD

Pericardial effusion with or without cardiac tamponade
RV failure (increased RV size, decreased RV systolic function, increased right atrial pressure, and increased tricuspid regurgitation)
Inadequate LV filling (small LV dimensions)
LVAD-induced ventricular ectopy or tachycardia (underfilled LV and mechanical impact with septum)
LVAD-related continuous aortic insufficiency (aortic regurgitation throughout cardiac diastole and systole)
Intracardiac thrombus (including right and left atrial, LV apical, and aortic root thrombus)
Pulsatile pump inflow valve regurgitation (apical inflow cannula turbulent flow detected by color Doppler during LVAD ejection, dilated LV, frequent opening of the AV, and reduced outflow graft flow <1.8 m/s)
Pulsatile pump apical inflow obstruction (intermittent interruption of usual laminar LVAD diastolic inflow using pulsed-wave Doppler with inflow velocities >2.5 m/s and color flow aliasing at the cannula orifice)
Continuous pump apical inflow abnormality due to inflow cannula obstruction, malposition, or hyperdynamic apical LV function (color Doppler high-velocity aliased flow at the cannula orifice with a peak Doppler velocity ≥ 2 m/s)
Cannula kinking or complete thrombosis (loss of Doppler signal in all echo views and loss of RV outflow tract stroke volume with speed change)
Hypertensive emergency, continuous flow pump (minimal AV opening, dilated LV, worsening MR, and peak outflow cannula velocity >2 m/s)
Impeller cessation, continuous flow pump (dilated LV, acute reversal of apical inflow flow direction using spectral or color Doppler, worsening MR, and decreased RV outflow tract stroke volume)

Table 2. Information derived from transthoracic echocardiography

Giving these considerations, it appears useful to integrate data derived from pump curves analysis and from TTE. However, it is not known if aortic valve opening status assessed by pump curves' analysis is consistent with TTE, and what is the real feasibility of TTE in HVAD system, considering its intrapericardial position.

Summary of unclear points and their development in this Research Thesis

- Is aortic valve opening status assessed by pump curves' analysis consistent with TTE? (Chapter 2.1)
- what is the real feasibility of TTE in HVAD system? (Chapter 2.2)

References:

1. Moazami N, Fukamachi K, Kobayashi M, Smedira N, Hoercher K , Massiello A, Lee S, Horvath D, Starling RC. Axial and centrifugal continuous-flow rotary pumps: a translation from pump mechanics to clinical practice. J Heart Lung Transpl 2013; 32: 1-11.

2. Shah NR, Cevik C, Hernandez A, Gregoric ID, Frazier OH, Stainback RF. Transthoracic echocardiography of the HeartWare left ventricular assist device. J Card Fail. 2012; 18(9):745-8

3. Roman P, Walker A, Rajagopal K, Mazzeffi M. Transesophageal echocardiographic measurements of HVAD flows J Cardiothorac Vasc Anesth. 2014 Sep 25

4. Slaughter MS, Rogers JG, Milano CA, Russell SD, Conte JV, Feldman D, Sun B, Tatooles AJ, Delgado RM, III, Long JW, Wozniak TC, Ghumman W, Farrar DJ, Frazier OH. Advanced heart failure treated with continuous-flow left ventricular assist device. *N Engl J Med* 2009;**361**(23):2241-2251.

5. Kirklin JK, Naftel DC, Kormos RL, Stevenson LW, Pagani FD, Miller MA, Ulisney KL, Baldwin JT, Young JB. Third INTERMACS Annual Report: the evolution of destination therapy in the United States. *J Heart Lung Transplant* 2011;**30**(2):115-123.

6. John R, Mantz K, Eckman P, Rose A, May-Newman K. Aortic valve pathophysiology during left ventricular assist device support. *J Heart Lung Transplant* 2010;**29**(12):1321-1329.

7. Morgan JA, Brewer RJ, Nemeh HW, Henry SE, Neha N, Williams CT, Lanfear DE, Tita C, Paone G. Management of aortic valve insufficiency in patients supported by long-term continuous flow left ventricular assist devices. *Ann Thorac Surg* 2012;**94**(5):1710-1712.

8. Crestanello JA, Orsinelli DA, Firstenberg MS, Sai-Sudhakar C. Aortic valve thrombosis after implantation of temporary left ventricular assist device. *Interact Cardiovasc Thorac Surg* 2009;**8**(6):661-662.

9. May-Newman K, Enriquez-Almaguer L, Posuwattanakul P, Dembitsky W. Biomechanics of the aortic valve in the continuous flow VAD-assisted heart. *ASAIO J* 2010;**56**(4):301-308.

10. Tuzun E, Rutten M, Dat M, van d, V, Kadipasaoglu C, de MB. Continuous-flow cardiac assistance: effects on aortic valve function in a mock loop. *J Surg Res* 2011;**171**(2):443-447.

11. Granegger M, Schima H, Zimpfer D, Moscato F. Assessment of Aortic Valve Opening During Rotary Blood Pump Support Using Pump Signals. *Artif Organs* 2013.

12. Granegger M, Moscato F, Casas F, Wieselthaler G, Schima H. Development of a pump flow estimator for rotary blood pumps to enhance monitoring of ventricular function. *Artif Organs* 2012;**36**(8):691-699.

13. Moscato F, Granegger M, Naiyanetr P, Wieselthaler G, Schima H. Evaluation of left ventricular relaxation in rotary blood pump recipients using the pump flow waveform: a simulation study. *Artif Organs* 2012;**36**(5):470-478.

14. Moscato F, Granegger M, Edelmayer M, Zimpfer D, Schima H. Continuous Monitoring of Cardiac Rhythms in Left Ventricular Assist Device Patients. *Artif Organs* 2013.

Chapter 2.

The combined role of echocardiography and of analysis of pump signals: trying to improve the management

2.1 Continuous monitoring of aortic valve opening in rotary blood pump patients

Introduction:

In patients with end-stage heart failure, rotary blood pumps (RBPs) for left ventricular support have become the therapy of choice for bridging the patient to heart transplantation. More recently RBPs even emerged as an alternative to heart transplantation (1-4). However, especially during prolonged left ventricular support, adverse events such as aortic valve (AV) commissural fusion, aortic insufficiency and thrombus formation in the aortic root, which may cause neurologic events, were observed (5-8). These complications are most probably related to the diminished or absent opening of the AV in patients with a RBP: In case of high pump support or a weak ventricular function, blood flow occurs entirely through the RBP (9, 10) and the AV remains closed during the entire heart cycle. Consequently, a continuous back-pressure load is exerted on the AV, which does therefore not experience the physiologic cyclic load changes. To prevent these adverse events it might be of importance to allow - at least intermittently - the opening of the AV by appropriate periodic pump speed adjustments. To determine the duration and magnitude of these speed adjustments a continuous and simple monitoring of the AV opening would be required. Additionally, a continuous monitoring of the AV state would provide important information about the native heart function and the condition of the cardiovascular condition: a change of the AV state from closed to open at a given level of pump support could indicate an improvement of heart function and could be a first indicator of cardiac recovery.

Currently, the clinical assessment of the AV condition is performed with echocardiography, which is conducted by experienced personnel and it is time-consuming and cumbersome. Therefore, it cannot be performed frequently especially because of the increasing number of patients with a RBP implanted and the limited hospital resources. In this framework, a method that allows monitoring of the AV state continuously from available pump data, without any required additional intervention, would represent a great benefit for the treatment of this patient population. In previous work a method was developed to discriminate between an open and closed AV using pump flow data from numerical and animal models, respectively (11). This algorithm is based on the fact that the state of the AV (open or closed) is related to the shape of the systolic portion of the pump flow signal: In case of partial support (AV opens) the pump flow signal shows a flat plateau in the systolic portion of each heartbeat, in case of full support (AV always closed) the systolic peak is sharper.

The aim of the current study was to apply and validate the previously developed algorithm to detect AV opening in RBP recipients using the estimated pump flow signal from a centrifugal pump (HeartWare Ventricular Assist Device (HVAD), HeartWare, Inc., Miami Lakes, FL).

Materials and Methods:

A single-center, prospective clinical study was approved by the Review Board of the Medical University of Vienna. Fifteen hemodynamically stable patients with an implanted HVAD were enrolled into this study. Before each examination potential thrombus formations in the left ventricle and the aortic root were excluded by echocardiography. Pump speed was then reduced stepwise

reduced from baseline speed by 200, 400, 600, 800, 1000, 1200, 800 and 400 rpm but not below a minimum speed of 1800 rpm) in 20 second intervals. After each speed reduction speed was returned to baseline for a minimal interval of 60 seconds (Figure 1). Using M-mode echocardiography the AV state was assessed and a video was continuously acquired via the output of the ultrasound device (iE33 xMATRIX Ultrasound, Philips Medical Systems, Andover, MA, USA) for subsequent beat-to-beat offline analysis. Pump data (speed and current) were collected simultaneously by continuously recording the RS-232 datastream which is available from the HVAD pump controller.

Classification of the AV state based on pump data

Basically, the same algorithm for pump flow analysis as presented in (11) was used. This method is based on three features (skewness, kurtosis and crest factor) calculated from the systolic portion of the pump flow signal. In the following text the major differences to the algorithm described in (11) will be highlighted:

- We made use of the previously reported flow estimator (12), which is able to estimate the pump flow signal based on the speed and current signal with a frequency content of up to 15 Hz. This was necessary because the frequency content of the signal is of major importance to reflect the plateau in the systolic portion of the pump flow signal in partial support.
- To detect accurately the systolic portion of the estimated pump flow signal and minimize the effect of noise, the signal was band-pass filtered (0.8 - 10 Hz). The frequency content was limited to 10 Hz, since this is sufficient to represent the plateau in the flow signal in the partial support condition and, at the same time, limits the amount of noise in the pump signal. However, in some cases the estimated pump flow signal was still affected by disturbances. For the beat-wise analysis a moving average filter for each of the three features (skewness, kurtosis and crest factor) over 10 beats was applied before classification to reduce the effects of noise.
- After preliminary experience with clinical data, the detection of the systolic portion of the flow signal had to be slightly modified: If the pump flow pulsatility (Q_{p2p}) value exceeded 4 L/min and the heart rate was equal or below 70 bpm, $1/4^{th}$ of the Q_{p2p} value was subtracted from the high-pass filtered flow signal before determining the zero-crossing and consequently the rising and falling edges that delimited the systolic portion of the flow.

The three features of the systolic portion of the pump flow signal were used for training and testing of two different classification algorithms: a) a linear classifier that attempts to express the dependent variable, in our case the state of the AV, as a linear combination of the three features (13); b) a quadratic classifier that attempts to express the dependent variable as a quadratic combination of the three features (13).

Classification of the AV state based on echocardiography

Each beat in the M-mode echocardiography was classified according to the AV state by one cardiologist and two biomedical engineers. Three levels of certainty were introduced:

1. If the state of the AV could clearly be identified based on M-mode echocardiography (AV opening time could be measured or AV leaflets did not move during systole) the beat was classified into a beat with the AV open or closed with 100% certainty.
2. If the state of the AV could not clearly be identified based on M-mode echocardiography, the three evaluators/referees/experts ### classified the beat as AV open or closed basing their decision on additional information (e.g. AV leaflet motion in B-Mode echocardiography).
3. If the state of the AV could not clearly be identified based on M-mode echocardiography and additional information was not reliable, the beat was rejected and excluded from further analysis.

Beats which were classified into the first two categories were used for the validation of the the developed method.

Data analysis
Three different procedures for data analysis were performed to validate the developed algorithm:

1. *Validation of the algorithm with all beats of the entire patient population*

   To validate the ability of the algorithm to discriminate beats with an open and closed AV with the entire dataset, the whole sample of classified beats of all patients was divided randomly into 5 subsamples. Of the 5 subsamples, 4 subsamples were used as training dataset and one as test dataset for the evaluation of the classifiers. This cross-validation process was then repeated 5 times, with each of the 5 subsamples used once as the validation data. Performance statistics were calculated as an average from the obtained 5 cross-validation results.

2. *Patient specific validation of the algorithm*
   a. Beat-wise analysis: To predict the reliability of the classification process for each single patient, a cross-validation was performed that used all classified beats from 14 patients as training dataset and the beats of one patient as validation dataset. This process was repeated 15 times, with each patient data being the test dataset once.
   b. Speed-wise analysis: To quantify the ability of the classification algorithm to determine the AV state at a given speed setting, the classifiers were also trained with average feature values calculated over each speed setting. The reference AV state during each speed setting was considered to be the most frequent AV state occurrence classified by echocardiography. Also here a cross-validation was performed that used all speed settings from 14 patients as training dataset and the speed settings of one patient as validation dataset. This process was repeated 15 times, with each patient data being the test dataset once.

The performance statistics of the classification were expressed as specificity and sensitivity. Specificity measured the ability to correctly identify a beat with a closed AV state. Similarly, sensitivity measured the ability to correctly identify a beat with an open AV. Negative (NPV) and positive predictive values (PPV) were used to represent the likelihood that an AV state determined as closed (open) was actually closed (open). The correct classification rate measured the proportion of correctly classified samples among all classified samples.

Results:

The demographics as well as hemodynamic and key echocardiographic parameters of the 15 patients are summarized in Table 1.

Table 1 Demographics as well as hemodanymic and echocardiographic parameters at baseline pump speed of the 15 patients enrolled in the study.

Age (years)	56.7 ± 9.8 (21-75)
Etiology (DCMP/ICMP)	6/9
BMI (kg/m^2)	25.5 ± 4.7 (15.8-37.7)
Male gender (%)	80
Time on device (days)	231 ± 221 (19-610)
Baseline pump speed (rpm)	2721 ± 212 (2400-3100)
Heart rate (bpm)	86 ± 13 (67-113)
Mean pump flow (L/min)	4.7 ± 0.8 (3.0-6.0)
Pump flow pulsatility (L/min)	3.5 ± 1.0 (2.0-6.0)
LV end-diastolic diameter (mm)	60 ± 14 (35-87)
LV end-systolic diameter (mm)	51 ± 15 (21-76)
Ejection fraction (%)	26 ± 7 (15-43)

Patients with AV open at baseline speed (%)	27
Patients with AV regurgitation (% grade 0/1/2/3)	53/40/7/0

DCMP: Dilated Cardiomyopathy, ICMP: Ischemic Cardiomyopathy, BMI: Body Mass Index, LV: Left Ventricle, AV: Aortic Valve.

Figure 1 shows the pump speed signal during a typical experiment. An example of the synchronized echocardiographic and pump data used for classification purposes is presented in Figure 2. On the left side of Figure 2 the AV is clearly closed (full support) whereas at the right side after second 206 the AV opens (partial support). Note the difference in shape of the systolic portion of the signal: during full support the shape is sharper than in the partial support condition, where the plateau can be observed (11).

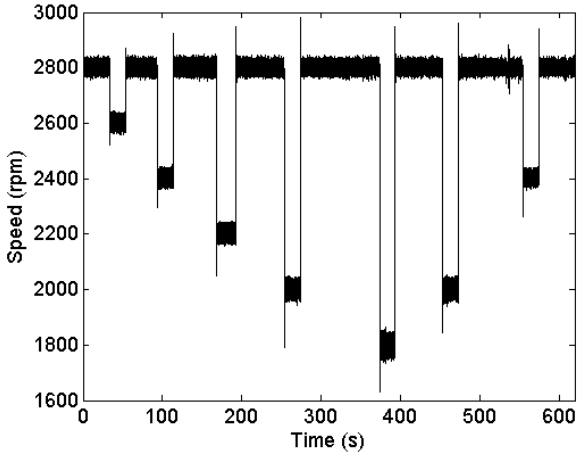


Figure 1 Time course of the pump speed signal during a typical speed step experiment. The pump speed was stepwise reduced from 2700 rpm to the lower speed limit of 1800 rpm.

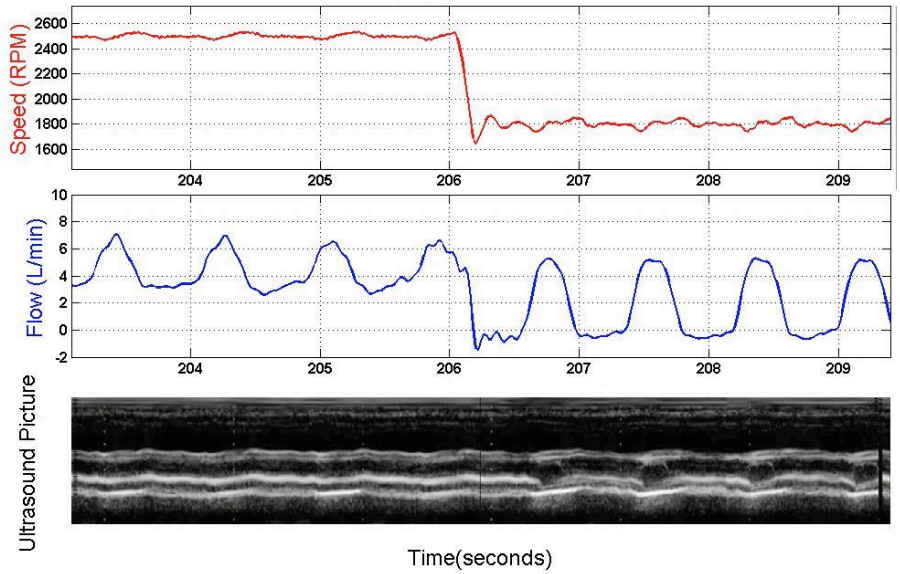


Figure 2 Pump speed (upper panel) flow (middle panel) and synchronized M-mode picture (lower panel) during a speed step experiment from 2500 rpm to 1800 rpm. In the right half of the figure the AV opens.

Validation of the algorithm with all beats of the entire patient population

From the 15 patients 9757 beats were recorded. 7384 beats could be classified as beats with an AV open or closed and were used for further analysis. In 3497 beats the AV was open; in 3887 beats the

AV remained closed during the entire cardiac cycle. In Figure 3, the three single-beat features extracted from the pump data are plotted for all 15 patients in a three dimensional graph and the classification based on the M-mode echocardiography is represented by dots in black and gray. The linear discrimination plane determined by the classification procedure is also indicated. The specificity/NPV and sensitivity/PPV values achieved in this cross-validation process are presented in Table 2.

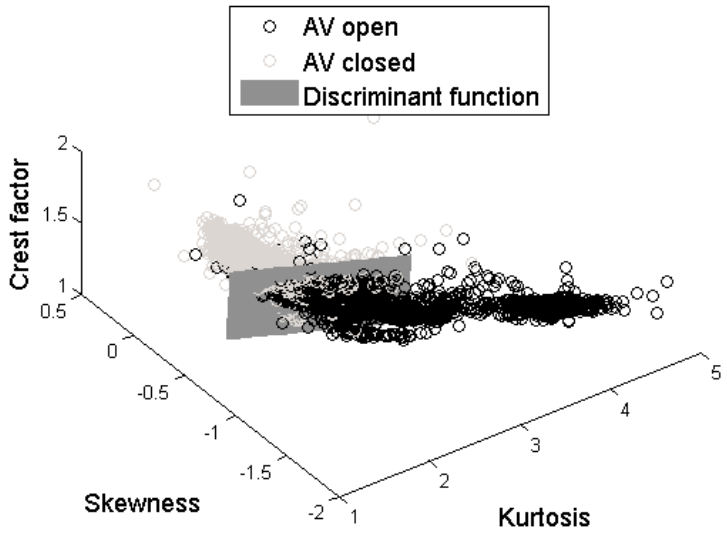


Figure 3 All beats (n=7384) with an open (black) and closed (gray) AV of 15 patients and the identified linear discrimination plane.
Table 2 Performance of the two classifiers to distinguish between an open and closed aortic valve tested with clinical data. NPV=Negative Predictive Value, PPV=Positive Predictive Value.

Linear classifier	91.2/90.3	91.0/91.8	91.1
Quadratic classifier	95.7/85.3	81.6/94.5	89

Patient specific validation of the algorithm

a. Beat-wise analysis

The correct classification rates of the cross-validation, using the beats of 14 patients as training dataset and the beats of one patient as test dataset, are presented in Table 3. The mean correct classification rate was 86.9±10.1% and it was greater than 82% in 12 of 15 patients using the linear classifier. It must be noted that the results of the statistical analysis are strongly influenced by the AV condition at baseline speed: Due to the performed speed step protocol (speed decrease for 20 seconds followed by 60 seconds of baseline speed) the proportion of beats recorded at baseline speed is obviously much higher than in any other speed setting; therefore, if the baseline speed setting is in a AV borderline condition between open and closed this may lead to many misclassified beats, this has a strong influence on the correct classification rate (indicating the ratio between misclassified and correctly classified beats) even if the beats recorded in all other speed settings were classified correctly (Patient 1, 14 and 15). Therefore, in the next step, a speed-wise analysis was performed. In this analysis the proportion of beats at different speed settings does not have an influence on the results.

Table 3 Correct classification rates for each patient for the linear and quadratic classifier.

Patient 1	71.4	73.9
Patient 2	84.4	86.5
Patient 3	99.5	99.3
Patient 4	99.9	99.9
Patient 5	82.8	91.2
Patient 6	94.4	94.1
Patient 7	89.6	92.0
Patient 8	92.1	92.8
Patient 9	88.9	91.0
Patient 10	91.6	82.4
Patient 11	83.4	78.9
Patient 12	96.3	94.1
Patient 13	87.7	87.8
Patient 14	63.7	52.8
Patient 15	77.9	76.9
ALL (Mean±SD)	86.9±10.1	86.2±12.1

b. Speed-wise analysis

The correct classification rates of the cross-validation, using the speed settings of 14 patients as training dataset and the speed settings of one patient as test dataset, are presented in Table 4. With the linear classifier in two patients (Patients 1 and 2) only one speed setting was misclassified, in the other patients all other 76 speed settings were classified correctly, resulting in a mean correct classification rate of 97.4±7.2% of performed speed steps.

Table 4 Number of performed speed steps and percentage of correctly classified speed steps with the linear and quadratic classifier. Additionally, the status of the AV at baseline speed is presented.

Patient 1	7	85.7	100
Patient 2	4	75	75

Patient 3	7	100	100
Patient 4	9	100	100
Patient 5	3	100	100
Patient 6	5	100	100
Patient 7	5	100	100
Patient 8	5	100	80
Patient 9	3	100	100
Patient 10	3	100	100
Patient 11	5	100	100
Patient 12	4	100	100
Patient 13	7	100	85.7
Patient 14	6	100	100
Patient 15	5	100	100
ALL (Mean±SD)	5.2±1.7	97.4±7.2	96.1±8.4

Discussion:

In this study a previously developed method (11) was validated in the real clinical setting. It allowed the discrimination between beats with an open and closed AV employing an estimated pump flow signal with high reliability. In a first validation step, using the entire dataset, the basic ability of the algorithm to discriminate between beats with an open and closed AV was proven. In the beat-wise analysis both linear and quadratic discrimination analysis showed a correct classification rate of approx. 90% of all beats, which is similar to the previously reported performance in animal experiments (11). Beside the validation of the algorithm using the entire dataset, also a patient specific analysis was performed. The result of such an analysis predicts the performance and reliability of the developed algorithm when it is applied to new patients, with completely unknown AV state. Whereas the beat-wise analysis indicated a slightly worse result (correct classification rate 86.8 ± 10%) compared to the analysis with the entire dataset, in a speed-wise analysis only 2 out of 78 speed steps (Patient 1 and 2) were misclassified in the 15 patients. However, in this analysis the information about the frequency of beats with an open/closed AV is lost; even borderline cases of the AV state would be classified as open/closed, losing the information of the ratio between open and closed beats. This might be of importance especially in case of arrhythmia where each beat is characterized by a different contraction force. Nevertheless, an analysis over a time-window of e.g. 20 seconds could indicate accurately whether the AV is currently more frequently open or closed within this interval.

The developed algorithm determines the presence of a plateau in the pump flow signal, which is more prominent during the ejection phase. In (11) we found in data derived from a numerical hemodynamic model that this plateau could only be detected reliably in case a volume of more than 3 ml was ejected via the AV. In this study, from the M-mode echocardiography the ejected volume could not be determined, therefore the AV leaftlet motion and the actual ejected volume could not be correlated. This might also explain one of the misclassified speed steps in the speed-wise analysis (Patient 2): The AV was classified as closed by the algorithm even if the AV leaflets were opening. However, the volume ejected may had not been relevant because the dynamics of the AV leaflet opening was slow and not even complete in most beats. However, since we could not measure the volume of ejected blood, this remains speculative.

The examinations were performed on the laying patient, not reflecting the daily life activities of out-of-hospital activities; however, different pathologies as well as a wide range of ventricular function, heart rate, pump settings, flow pulsatility, waveform and arrhythmia were observed in the 15 patients (Table 1). This indicates the robustness of the algorithm regarding this diversity but a validation during physical activity (e.g. during exercise stress tests) seems important.

To the authors' knowledge there is only one study that addresses the assessment of the AV opening by using pump data only without any additional intervention. In Bishop et al. (14) this

method was clinically evaluated. Their results with 6 patients indicated the ability to discriminate between an open and closed AV in 4 out of 6 patients. The two remaining patients suffered from mild aortic insufficiency, which might have affected the results in these cases. In the presented study with 15 patients various degrees of aortic regurgitation (grade 0 to 2) were observed, however, this did not impair the discrimination abilities of the presented algorithm.

The patient specific validation of the alogrithm provided very good results demonstrating that only one single discrimination plane for all patients was required and no patient specific training was necessary for correct discrimination. This allows a simple implementation of this algorithm into a controller of a RBP system which would be valid for any patient without any additional intervention required. Once implemented into the controller of a currently clinically used RBP system this method would allow continuous monitoring of the AV opening with a high accuracy. The physician could be provided with the long-term trend of the AV opening and could adapt the treatment and the pump speed setting accordingly. Frequent AV opening could be especially of importance in bridge-to-recovery candidates, where it is obviously beneficial that an aortic stenosis/insufficiency is prevented. In this study in 4 out of 15 patients the AV was permanently open at baseline pump speed (Table 1), in all other patients a decrease in pump speed was required to force the AV to open. In patients with a weak cardiac function it might be disadvantageous and even dangerous to decrease the pump speed permanently; therefore, in these patients an intermittent pump speed reduction to allow AV opening could be used. To determine for each patient the amount and duration of speed reduction a continuous monitoring of the AV status would be necessary to achieve a certain ratio between beats with an open and closed AV. It must be however noted that because of the lack of continuous monitoring of the AV status the optimal ratio between an open and closed AV to prevent adverse events as aortic leaflet fusion, insufficiency and thrombus formation in the aortic root is not known yet.

In conclusion, this study demonstrates that the AV opening can be reliably detected using the estimated pump flow signal in RBP patients. Combined with other non-invasive diagnostic methods based on intrinsic pump signals as suction detection (15), determination of contractility and relaxation properties (16, 17), heart rate and its variability (18), the method of continuous monitoring of the AV state could be integrated into an automated patient (tele-) monitoring system. This would not only provide important information for the treatment of the patient but detect occurrence of adverse events at an early stage.

2.2 Transthoracic echocardiography of outpatients with an intrapericardial left ventricular assist device implanted: a single center experience.

Introduction:

With the increasing number of implants {Kirklin, 2014 #973}}[1], the clinical interest about the role of transthoracic echocardiography (TTE) in the management of patients with a left ventricular assist device (LVAD) in the outclinic setting is growing.
The two actually most frequently implanted pumps (Heartmate II, Thoratec Inc., Pleasanton, CA USA, and HVAD, Heartware Inc., Framingham, MA, USA) while providing both a continuous flow, have substancial differences {Slaughter, 2010 #1199}: axial and extrapericardial the first, centrifugal and intrapericardial the second. Both pumps have a monitoring system, providing information about pump speed, pump estimated flow, power consumption and pulsatility index; in the HVAD monitor these parameters are shown both in real time and in their fluctuations over time, as well as different kind of alarms, thus allowing to achieve useful information[3,4] for pump setting regulations in the outclinic management. To help to discriminate among different causes of variations in pump data and alarms, together with physical exam and laboratory exams, transthoracic echocardiography plays a key role, helping the Clinician in the patients' management, in optimization of pump settings and in the assessment of some mid or long-term complications after LVAD implantation (i.e. aortic regurgitation (AR) and right ventricular (RV) failure).
However, almost the totality of the patients included in the clinical series and observational prospective studies focusing on the role of TTE had been implanted with a Heartmate II pump[5,6,7,8]. Echocardiographic data about the other largely implanted pump, HVAD, are less often documented; one possible explanation could be the theoretical difficulty on performing TTE due to its intrapericardial placement.
In the unique, small clinical series (19 patients) focused on TTE on HVAD[9], only three kind of information are provided: a) the feasibility of visualizing inflow and outflow cannula in most of the patients; b) the impossibility to have a Doppler signal inside the inflow cannula, whereas it's possible to be achieved in the outflow cannula; c) the artifacts precluding mitral valve color Doppler examination when inlet cannula is included in the screen. Thus, in the current literature there is lack of data and reports about the feasibility of performing a comprehensive standard TTE examination and measurements in HVAD patients, including Doppler measurements (i.e. PAP estimation, mitral inflow pattern, Tissue Doppler) when inlet cannula is not included in the screen. Moreover, it has not been reported if non-standard echographic views are needed in HVAD patients and there are no data about the possibility of evaluating the flow provided by the pump or by the overall circulating system through a TTE exam; there is only one clinical case in which pump flow through outflow cannula was evaluated by transesophageal echocardiography (TEE).
The aim of this clinical series is to provide echocardiographic insights about the feasibility of performing standard and pump-specific measurements in patients with HVAD when evaluated in a routine, outpatient setting. Herein, we report echocardiographic data of patients with HVAD enrolled in a prospective study made in our Clinic validating the ability of a mathematical algorithm in predicting aortic valve opening based on pump flow signal; we mainly focus on the feasibility of standard TTE measurements, as well as on specific non-standard TTE approaches.

Materials and methods:

Study population
17 outclinic patients (14 males, 3 females), implanted at Vienna General Hospital (AKH) between 2011 and 2013 and routinely followed in the outclinic ambulance, were evaluated during 2013, and

TTE were performed. The almost totality (n=16) of patients had been enrolled in a study protocol evaluating the ability of a mathematical algorithm in predicting aortic valve opening (as assessed by TTE) based on pump flow signal at different HVAD speed settings during the exam and approved by the local Ethic Review Board; the informed consent to the study protocol was collected before TTE exam. All of them were clinically stable patients. Only in one patient, TTE was made by clinical reasons (assess for left ventricular recovery); in this case, pump speeds were not changed. Demographic characteristics are shown in table 1; surgical implantation technique was mini-invasive in all cases (mini-left thoracotomy and right subclavear incision), except that for one patient

Echocardiographic measurements

TTE was made only once in 15 patients; in 2 patients, TTE was repeated respectively 3 and 2 times as follow-up; in the first case the TEE was repeated to monitor pump speed reduction after the first exam due to left-shifted position of interventricular septum, and in the second case the two exams were motivated by medical therapy optimization after implantation. Thus, overall, 20 TTE were performed. All TTE were made by the same operator, a Cardiologist trained in echocardiography using the ultrasound device iE33 xMATRIX Ultrasound (Philips Medical Systems, Andover, MA, USA). During the exams, all standard views and measurements normally performed in clinical practice and described by the American Society of Echocardiography (ASE) were tried to be achieved. In addition, when an echographic information was not easily achieved by standard approaches, additional non-standard views were explored, having the aim, in most of cases, to avoid the artifacts related to the pump (especially the ones due to the inflow cannula), or to obtain the visualization of inflow and outflow cannula. Images were optimized by modifying the gain, brightness, compression, and time-gain compensation settings. Before starting pump speed changes, the presence of thrombus in aortic root or left ventricle (LV) was checked. A cardiac technician team expert in LVAD management was present during all the examinations.

A standard M-mode examination from parasternal long-axis (PLAX) view as recommended was performed. Atria dimensions were measured in apical 4-chambers (4C) view. Aortic valve opening status was assessed by M-Mode in parasternal long-axis view putting the sample cursor at the level of aortic leaflets and recording the images for more than 5 seconds. LV filling pattern was assessed as recommended, and systolic function was assessed by calculating ejection fraction from LV end-diastolic and end-systolic volumes (Sympson's method), when possible, or in alternative by integrating visual estimation with Teichholz's method. Determination of valvular regurgitation with color Doppler was attempted first with quantitative methods and than with semiquantitative methods (vena contracta for aortic and mitral regurgitation, ratio regurgitant jet/left atrium area) made both qualitatively according to the guidelines of ASE (from grade 1 to 4) in all views both.

Regarding RV assessment, in every patient all the RV views were done sistematically: apical (trying to obtain a true, non-foreshortened view) and the parasternal view for RV inflow, PLAX, parasternal short-axis (PSAX), and subcostal views, trying to calculate the following parameters, following ASE guidelines' reccomendations: end-diastolic and end-systolic areas and RV diameters in the 4C view; RV shortening fraction, two-dimensions lateral tricuspid annular motion (TAPSE), RV index of myocardial performance (RIMP), S' wave at Tissue Doppler (TDI). Then, RV function was assessed and qualitatively graded using a four-point grading system (normal, mild, moderate, or severly reduced), when at least 2 of the 4 standard predefined RV dysfunction criteria (low TAPSE, low RVFAC, low tricuspid s', or high right ventricular index of myocardial performance, using the American Society of Echocardiography cutoffs) were met. Pulmonary artery pressure was estimated using the modified Bernoulli formula: [4 (peak systolic tricuspid regurgitation [TR] velocity at end-expiration)^2] + right atrial pressure estimated by the inferior vena cava diameter as well as its response to inspiration, as previously described. Total RV output, (corresponding to the sum of HVAD output and the flow ejected by LV) was calculated, as recommended, using the formula (RVOT/2) ^{2} x π x RV flow VTI. RVOT was assessed in the PSAX view at the level of great arteries, RV VTI (velocity-time integral) by pulsed-wave Doppler at the same level. The interference of the pump on PW-Doppler signal in the LV outflow tract didn't allow to calculate LV

output. In patients where the aortic valve was closed, as the RV output is supposed to be virtually equal to the HVAD output, we compared the RV output with the pump flow estimated by HVAD controller, taking in account also eventual valvular regurgitations.

Inflow cannula was assessed in parasternal long-axis view and apical 4C and 2C views; sometimes, off-axis images (i.e. para-longitudinal views) were required.
Outflow cannula was assessed, to look for the anastomosis with ascending aorta, as previously described by others, in right parasternal views (turning the patient to the right side), proceeding from the lower to higher intercostal spaces, or by high-left parasternal view; flow velocity in the outflow cannula was assessed 1 cm proximal to aortic anastomosis, as previously described in Heartmate II-Echo studies. The direction of the flow was also assessed by color-Doppler, and had different directions, depending on transducer's position.
Pericardial effusion was investigated in all views.

Other data collected
During the execution of TTE, data from the pump controller were simultaneously collected: flow (estimated by considering the hematocrit value of the day of TTE), power, speed. Blood pressure, heart rate were also collected

Study endpoints
The primary endpoint of this report is the feasibility of performing a measurement normally included in a TTE exam; this was defined as the possibility of measuring it and it was verified in every single TTE; its values are expressed as percentage among the total number of TTE.
We described also the difficulties encountered in TTE performance across the different measurements and the need for specific non standard views.
The quality of a single measurable echocardiographic parameter was defined using a scale: good (grade 2) if the endocardium border was clearly visible in B-mode or if there weren't significant disturbances on Doppler signal; acceptable (grade 1) if myocardium was clearly visible but endocardium contour was not completely visible in all wall segments or if significant disturbances were present on Doppler signal but allowing data measurement; poor (grade 0) if endocardium border was not clearly assessable in most of wall segments or if a Doppler measurement was not calculable because of significant interferences.

Results:

Feasibility and quality
The overall exam quality was good in 80% cases. Feasibility was different among various echographic parameters, and it is reported in Table 1. In all TTE exams, to calculate some of the echocardiographic data, it was necessary to obtain non-standard views; the quality of the data achieved is reported in the same Table, evaluated as explained in the Methods' section. Herein, we report insights about the specific need of non standard views.

Parameter	Feasibility	Quality 0	Quality 1	Quality 2
M-Mode of left ventricle	100%	0%	0%	100%
SAX view	100%	0%	0%	100%
Left ventricular ejection fraction by Teichholz's method by Simpson's method	100% 20%	0% 80%	0% 0%	100% 20%
Left ventricle filling pattern	100%	0%	50%	50%

Aortic valve opening status	100%	0%	0%	100%
Aortic regurgitation	100%	0%	0%	100%
Mitral regurgitation (by color Doppler)	100%	0%	0%	100%
Tricuspidal regurgitation (by color Doppler)	100%	0%	0%	100%
Systolic pulmonary artery pressure	100%	0%	50%	50%
Right ventricular areas	100%	0%	30%	70%
TAPSE	85%	15%	35%	50%
Right ventricular myocardial performance index (MPI)	85%	15%	40%	60%
Right ventricular function	100%			
TDI of right ventricle	85%	15%	35%	50%
PW/CW Doppler of RVOT and RV output calculation	85%	15%	0%	85%
Inflow cannula visualization PW Doppler	100% 0%	0%	0%	100%
Outflow cannula visualization PW Doppler (when visualized)	95% 100%	5% 0%	0% 0%	95% 100%
Left ventricular output	0%	100%	0%	0%
PW/CW Doppler of aortic valve	0%	100%	0%	0%

Table 1. Feasibility of different echocardiographic parameters

Assessment of parameters of left ventricular unloading.
Aortic valve opening status (open or closed) was assessable in all patients by B-mode in PLAX view; however, given the small openings of the valve observed in some patients and their possible underestimation by B-mode, aortic valve status was verified by M-Mode from the PLAX, placing the cursor at aortic leaflets' level (Fig. 1). This allowed to clearly assess valve status (open or closed) in all TTE, even if not in all beats (75% overall). Even if aortic valve opening area was not calculated, the degree of excursion of the leaflets was, as expected, different among the patients, being influenced by mean aortic pressure, pump speed and left ventricular function, thus reflecting different support conditions. To assess for support and grade of unloading given by the pump, the position of interventricular and interatrial septum were also easily investigated, and than integrated with the analysis of pump curves obtainable from HVAD monitor to estimate the degree of pump support. In all patients, when pump speed was modified during TTE performance, the change in aortic valve opening status (from closed to open or vice versa) and/or in leaflets' excursion was assessed by M-Mode in PLAX view, and corresponding to change in pump flow and curves morphology in HVAD monitor.

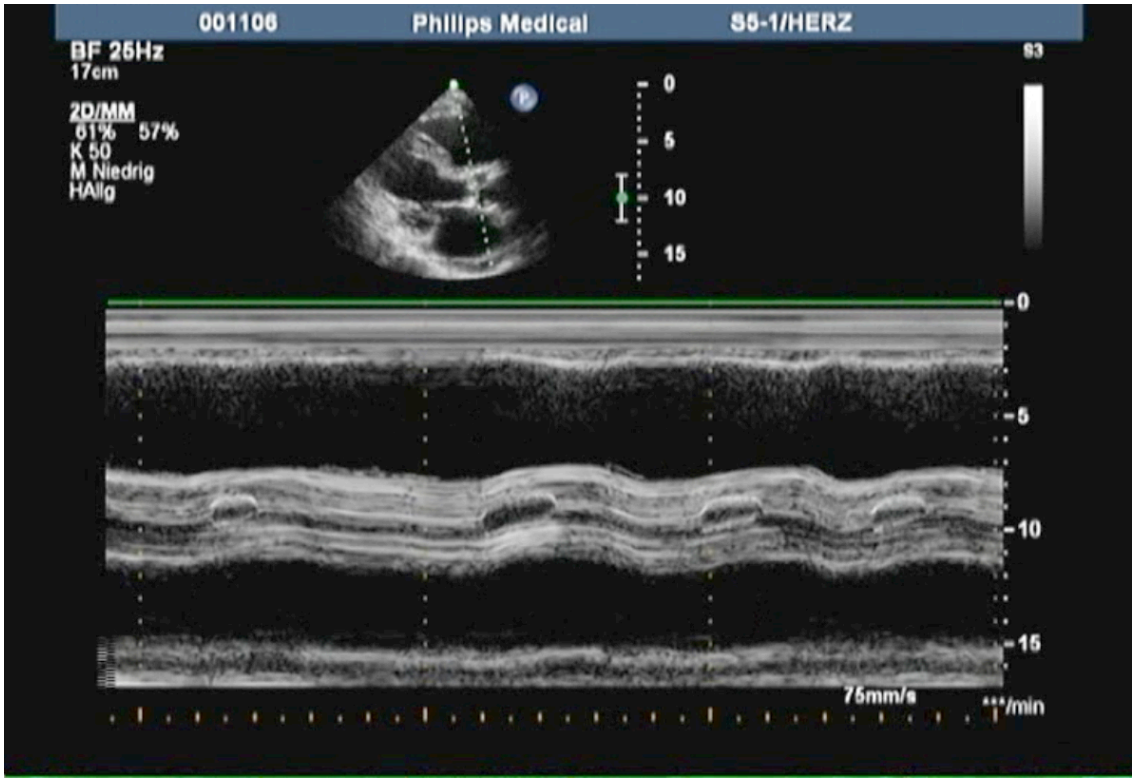


Fig. 1 M-mode analysis of aortic valve: a) assessment of aortic valve opening status by M-Mode in PLAX, b) continuous aortic regurgitation

Doppler examination.
A comprehensive Doppler assessment (both PW and CW-Doppler) was possible for mitral, tricuspid and pulmonary valves in the great majority of cases (Table 1), thus allowing the achievement of useful hemodynamic information, such as LV filling pattern and estimated pulmonary artery pressure.
In standard 4C view, a significant “window effect” on color-Doppler signal didn’t allow PW or CW Doppler measurements (Fig. 2A).

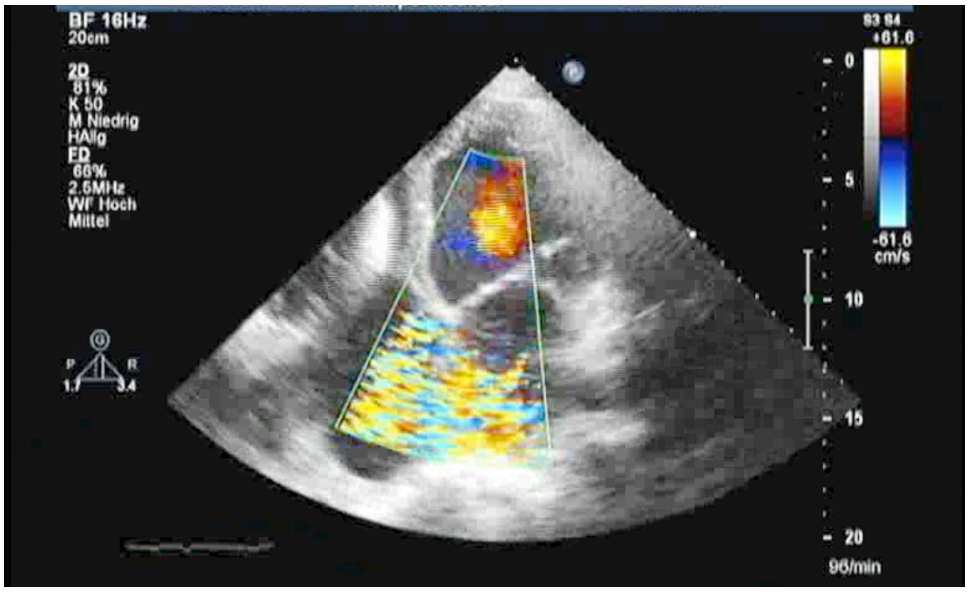


Fig. 2 A Standard apical 4 chamber view

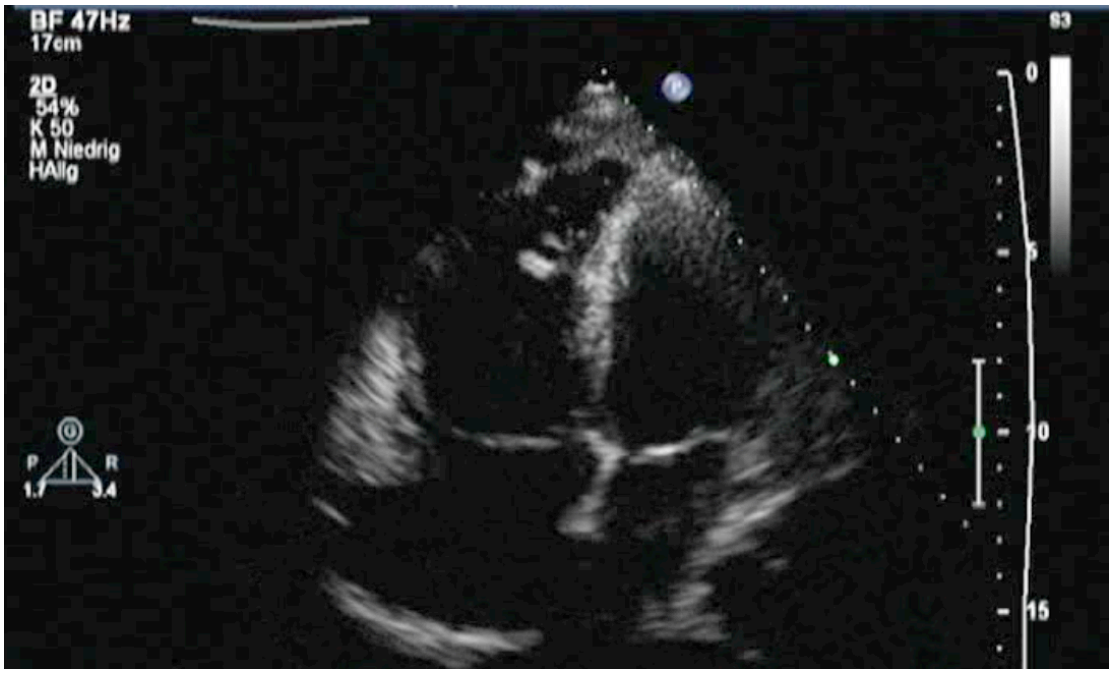


Fig. 2 B Modified 4 chamber-view

PW-Doppler of mitral valve for evaluation of left ventricle filling pattern was obtained by a "modified" 4C view (Fig. 2B), by placing the probe at 1-2 higher intercostal spaces and directed more medially than the standard apical approach (1-2 cm from left mid-clavicular line), thus allowing filling pattern assessment in 100% of cases (Fig. 3A).

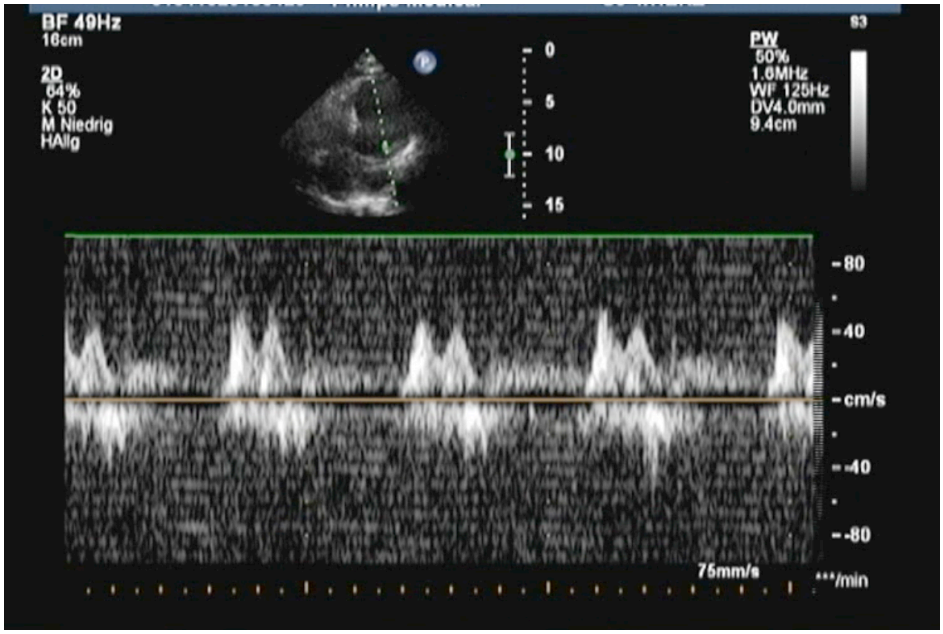


Fig. 3 Doppler analysis feasibility: a) filling pattern of left ventricle, b) pulmonary pressure assessment c) RV outflow tract Doppler assessment (for RV output calculation)

Figure 3 A. Filling pattern of left ventricle

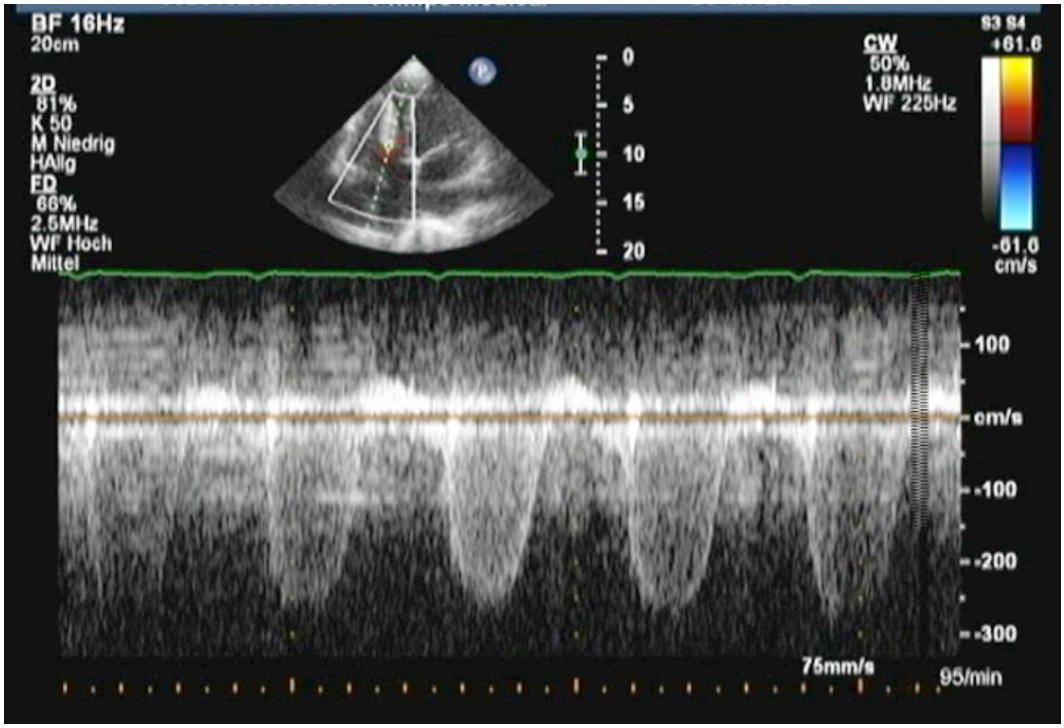


Figure 3 B. Assessment of systolic pulmonary artery pressure

By the same approach, CW Doppler exam of tricuspid valve was possible in 100% of cases, thus allowing calculation of estimated systolic pulmonary pressure.

PW and CW Doppler evaluation of aortic valve was not possible in standard fifth-chamber view from apical approach. Tilting the probe more upwards from the modified 4-C approach previously described, only in a few cases color Doppler evaluation was possible.

Doppler evaluation of pulmonary valve was possible in 85% of cases by the standard PSAX view; this allowed to calculate RV acceleration time and right ventricular output (Fig. 3C) . We compared, for descriptive purposes, RV output and the pump flow as estimated by the HVAD monitor; results are reported in Table 2. As previously described, in patients with a closed aortic valve, without significant valvular regurgitations, RV output is theoretically equal to the LVAD output: the two values were comparable with a maximal error of 20%. The greatest differences between the two values were more often observed in patients with a concomitant significant valvular regurgitation. In the same table, we reported HVAD flow and the RV output estimated by Echo in patients with an open aortic valve for descriptive purposes.

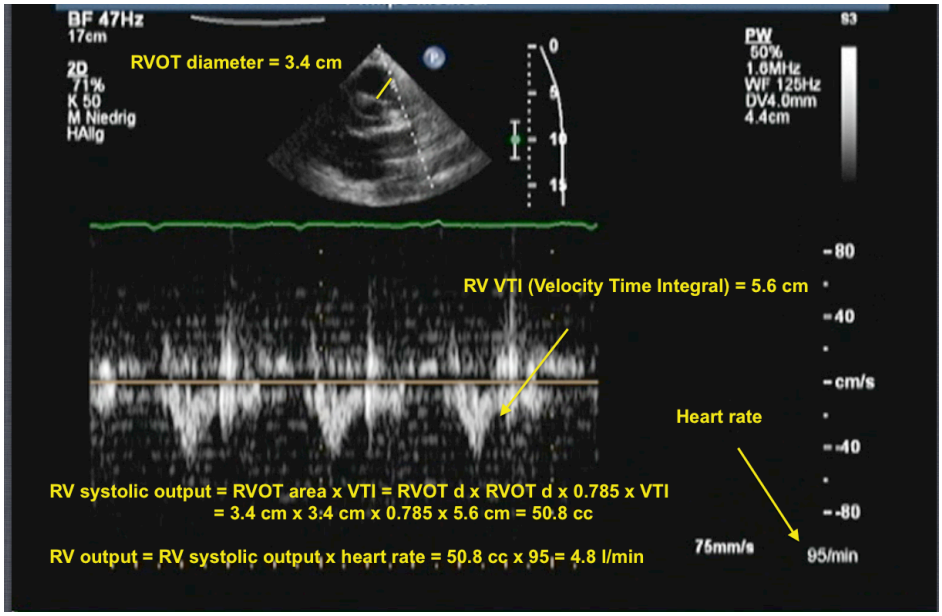


Figure 3C. Calculation of right ventricular output through right ventricular outflow tract

Patient #	LVAD flow (l/min)	RV output (l/min)	Aortic valve status	AR grade	MR grade	Pump speed	Heart rate	Blood pressure (mmHg)	Pump power (Watts)
1	4.6	4.8	Closed	1.5	0	2680	71	70	3.9
2	5.4	5.7	Closed	0	0	2600	95	80	3.8
3	4.6	4.1	Closed	0	0	2640	62	75	3.7
4	5.0	3.9	Closed	0	0	2500	72	70	3.4
5	5.2	5.1	Closed	1.5	2	3000	75	75	5.2
6	5.3	5.2	Closed	0.5	1	2700	61	80	4.2
7	6.3	5.2	Closed	1	0	3000	97	90	6.0
8	5.3	4.8	Closed	2	1	2800	95	88	4.5
9		5.0	Mostly open	0	0	NA	85	NA	NA
10	4.8	6.6	50% open	0.5	1.5	2800	73	78	4.2

Table 2. Comparison between controller-estimated LVAD-output and right ventricular output by Echo

Assessment of valvulopathies.
Assessment of valvular regurgitations by quantitative methods was never possible. Assessment of valvular regurgitation by semiquantitative methods was possible in all cases. Mitral regurgitation (MR) was easily visualized by parasternal approaches; however, to avoid interference with inflow cannula, it was necessary placing the probe 1-2 intercostal spaces lower than the standard PLAX view. At the same way, MR was easier assessed in the "modified" 4C view rather than the standard one.
Aortic regurgitation (AR) assessment through vena contracta measurement was done in 100% of cases by standard PLAX view; by modified 4C view in 50% of cases, AR was assessed. As CW and PW Doppler for aortic valve were never possible, quantitative determination of AR was not

done. Aortic regurgitation was in most of cases continuous during cardiac cycle (systo-diastolic); this could be assessed by color M-mode, and it has been already reported in echocardiographic studies involving HM-II (Figures 4A and B). In 4 exams, a flow in red at color Doppler was visualized in the medium tract of left ventricle; PW analysis allowed to distinguish if it was due to aortic regurgitation or to flow inside inflow cannula.
Quantification of tricuspidal regurgitation (TR) was made by modified 4C view in 100% of cases; when TR was more than mild, it was easily visualized also by projection for right ventricle inflow from parasternal view and form short-axis.

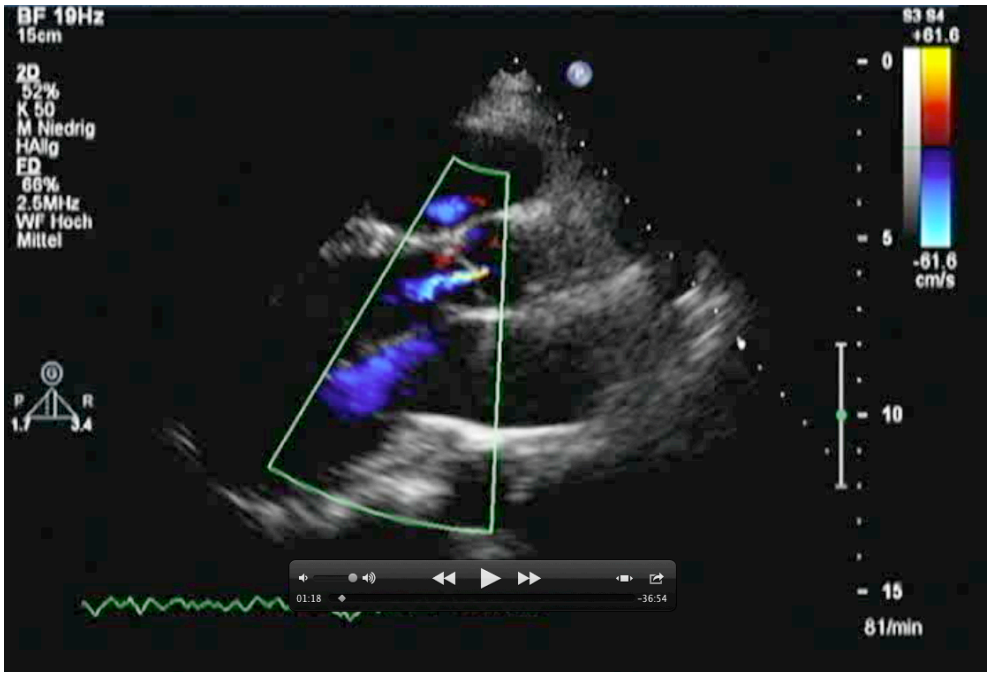


Fig. 4 A. Aortic regurgitation by PLAX view

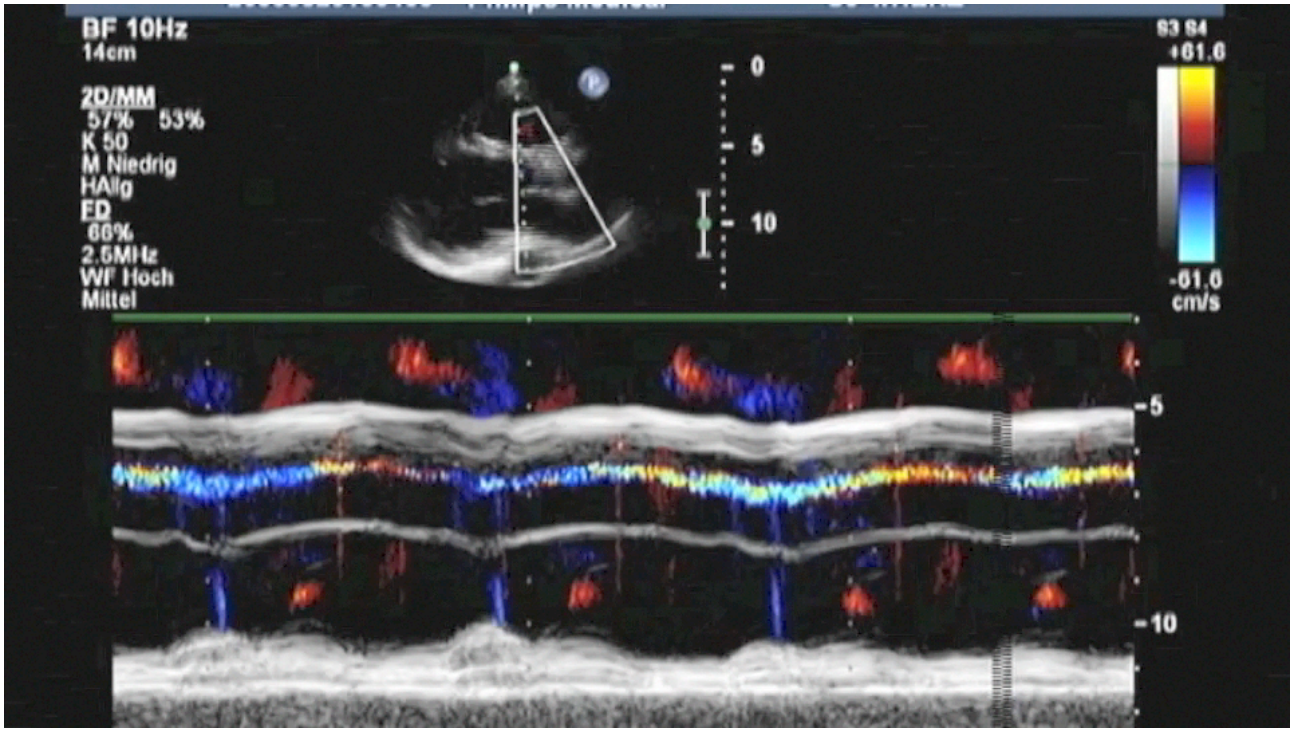


Fig. 4 B. Color M Mode of aortic regurgitation

Assessment of left ventricular function

Left ventricular diameters were assessed by standard PLAX in all cases. Clear visualization of apical endocardium in standard 4C view was not possible in most cases (80%) , whereas it was clearly identifiable in modified 4C view in all cases, even if, in this projection, left ventricular volume are probably underestimated. Given the not clear visualization of LV in most of cases, LV ejection fraction (LVEF) was calculated by Simpson's method only in 20% of cases, while in the other cases,it was calculated first by Teichholz's method and then visually estimated.

Assessment of right ventricular function

All traditional parameters of right ventricular function were calculated from modified 4 C view. Their feasibility was high, as reported in Table 2. S wave at Tissue Doppler were easily assessed in most cases by 4-chamber view, as well as MPI. The mean value of each parameter is reported in Table 3.

Characterization of overall RV function was possible in 100% of cases; the degree of RV systolic impairment was evaluating taking into account all the multiple parameters of RV function possible to be measured, as described in the methods section.

Visualization of inflow and outflow cannulas

Inflow cannula was visualized in all patients in PLAX view and in 4-C apical view. A tract of outflow cannula was visualized in most patients (95%) anteriorly to right ventricle in the PLAX view or anteriorly and laterally to the right ventricle in the 4-C apical view. The anastomosis of outflow cannula to ascending aorta was visualized in 80% cases by using or an high-left parasternal view (2-3rd intercostal space) or by a right parasternal view, obtained positioning the probe between the 2nd to the 4th right intercostal space, and turning the patient to right. In all cases in which outflow cannula was visualized, it was possible to measure flow velocity by pulsed-wave Doppler. Normally, we measured the velocity about 1 cm proximally to the anastomosis between outflow cannula and ascending aorta. The velocity spectrum appeared as a negative flow (see Fig.5); mean velocity was 135 cm/sec, similar to what declared by Heartware .

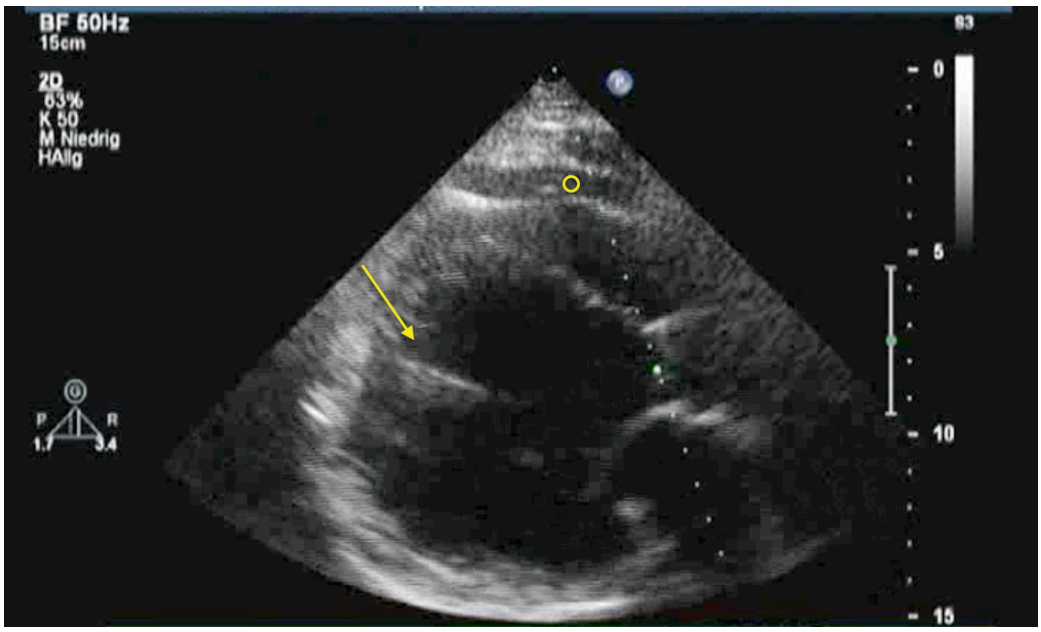


Figure 5A. Visualization of inflow and outflow cannulas by PLAX. Inflow cannula is indicated with the circle, outflow cannula with an arrow

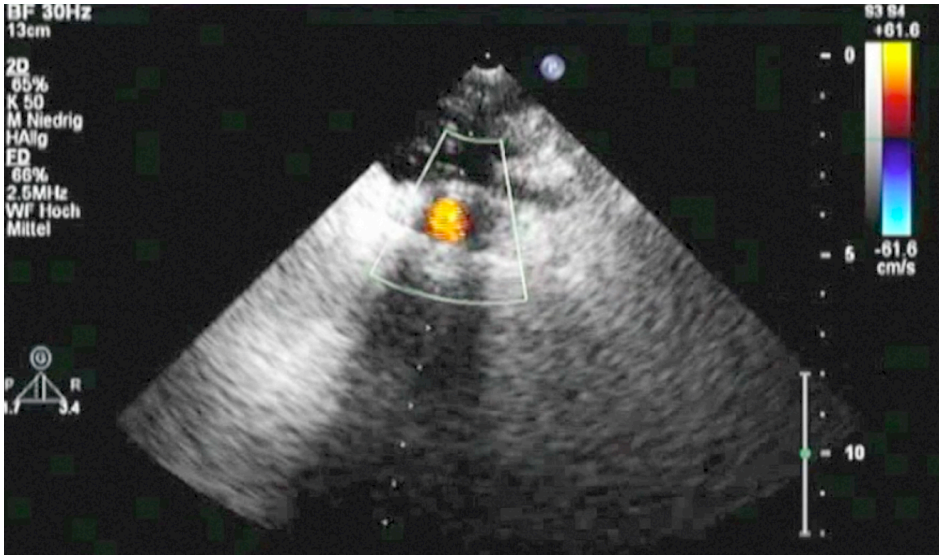


Figure 5B. Outflow cannula visualization by right parasternal view

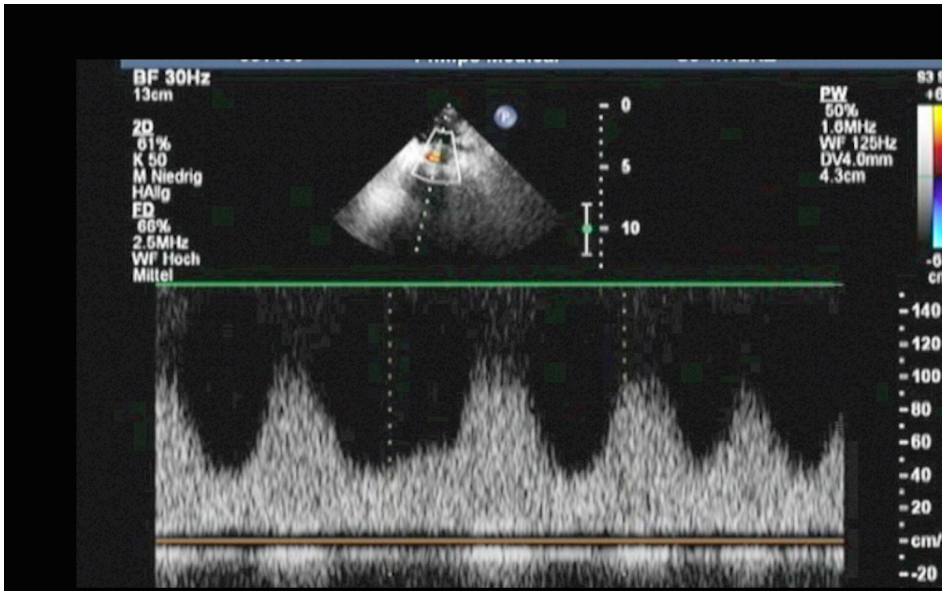


Figure 5C. Doppler analysis of outflow cannula

Echocardiographic data observed
For descriptive purposes, we reported in Table 3 the results of echocardiographic measurements performed.
As reported, right ventricular function was impaired in most cases, and aortic regurgitation was often present.

Left ventricular M-Mode measurements	
End-diastolic diameter (LVEDD, mm)	58.8 ± 13.5
End-systolic diameter (LVESD, mm)	49.0 ± 14.9
Septum diastolic thickness (IVSd, mm)	9.6 ± 1.4
Septum systolic thickness (IVSs, mm)	11.5 ± 2.4
Posterior wall diastolic thickness (LVPWd, mm)	9.4 ± 2.8
Posterior wall systolic thickness (LVPWs, mm)	13.1 ± 3.2
Aortic root (mm)	33.8 ±
Left ventricular ejection fraction (EF,%)	26.1 ± 6.7
Left ventricle filling pattern	
Normal , n (%)	5 (26.3%)
Impaired relaxation , n (%)	3 (15.6%)
Pseudonormal, n (%)	1 (5.2%)
Restrictive, n (%)	10 (52.6%)
Aortic valve status	
Open (%)	4 (20.8%)
Mostly open (%)	1 (5.2%)
Mostly closed (%)	1 (5.2%)
Closed (%)	13 (67.6%)
Aortic regurgitation	
None (%)	12
Mild (%)	5
Moderate (%)	2
Severe (%)	0
Mitral regurgitation	
None (%)	12
Mild (%)	6
Moderate (%)	1
Severe (%)	0
Tricuspidal regurgitation	
None (%)	9
Mild (%)	6
Moderate (%)	4
Severe (%)	0
Systolic pulmonary artery pressure (mmHg)	35 ± 10
Right ventricular end-diastolic area (cm2)	
Increased (%)	11
Normal (%)	8
TAPSE (mm)	13.3 ± 1.8
Right ventricular myocardial performance index (MPI)	
Right ventricular function	
Severly reduced , n (%)	0
Mildly reduced , n (%)	2
Slightly reduced, n (%)	7
Normal, n (%)	10
Pericardial effusion	
No, n (%)	14
Mild, n (%)	5

Table 3. Echocardiographic parameters of the study population

Discussion:

This single center small clinical series provides for the first time, to the best of our knowledge, insights about the feasibility of a comprehensive transthoracic echocardiographic exam of HVAD, a wide implanted intrapericardial pump. Beside the specific echocardiographic data achieved in our population, the main information provided by our report is that, even if non-standard views are often required to achieve the data normally evaluated in a standard echocardiographic exam, thus making overall difficult and perhaps longer its performance, a comprehensive TTE is generally possible in patients with an HVAD implanted.

As already reported in literature, TTE can be helpful in dissecting among the different possible causes of complications in LVAD patients, like right ventricular failure or suction. Given the considerable information achievable from HVAD monitor system, through analysis of pump flow curves and variations over time of flow, power and alarms, the integration of this information with TTE data may help in patients' management. We could verify that within this cohort of patients, in those cases in whom TTE was performed more than one time because of clinical reasons. In one case of low flow alarms, with evidence of suction events, TTE confirmed the diagnosis of excessive left ventricular unloading, through the visualization of a left-sided interventricular septum and dilated right ventricle with a only slightly reduced systolic function, thus excluding a problem of RV failure. After a reduction in pump speed and an increase in liquid intake from the patient, a subsequent TTE revealed a position of interventricular septum more directed to right, with a decrease in RV dimensions and an increase in RV output; accordingly, the pump controller showed an increase in estimated flow. In another case, TTE helped in confirming the decision of wean a patient from HVAD: monitor has shown an increase in pulsatility and a supposed opening of aortic valve in all beats, echocardiography confirmed a permanently open aortic valve and a LV with normal diameter and ejection fraction >45%, with trivial mitral regurgitation. Moreover, in most of cases the calculation of RV systolic pressure was possible (and well related with RHC?)

In our series, TTE in HVAD achieved the calculation of the standard haemodynamic parameters assessing the grade of the efficacy of LV unloading and the effect of HVAD on pulmonary pressures, like left ventricular filling pattern and pulmonary systolic pressure.

Right ventricle is a crucial player, as recently underlined, in determining the clinical outcome of LVAD patients. Clinicians know that RV assessment by TTE is challenging, and of course remains so also in this setting of patients. In a recent study (cit.), given the objective difficulty of assessing RV function by TTE, it has been suggested to evaluate multiple parameters, like TAPSE, RV areas, TEI index. Our study suggests that most of parameters for assessment of RV function can be calculated in HVAD patients, thus giving a strong effort in using this technique for specific longitudinal studies of RV function along time. Particularly, we stressed the importance of S wave, as assessed by TDI, of TAPSE, and of TEI index. In previous larger studies about HM-II patients, S wave was found to be one important prognostic marker of RV failure post-LVAD implantation, and TEI index may be also affected by the grade of unloading provided by the LVAD. However, their prognostic relevance in patients with a centrifugal pump must yet be demonstrated.

The good relationship between pump-estimated flow and the RV flow calculated by TTE (when the aortic valve is closed) also in a centrifugal pump is an important acquisition and may be of interest, especially when an evaluation of loading status is needed and a pump controller is not immediately available; moreover, one may speculate that it could be useful to calculate RV output even when part of the flow is provided through the LV: in this case, the difference between RV output and the flow estimated by the controller may give information about the output trough the LV and, indirectly, about the residual LV contractility reserve. However, one must also take in account also valvulopaties (i.e. aortic regurgitation).

This study is, to best of our knowledge, the first one assessing the issue of the practice feasibility of a complete TTE exam in HVAD. In the literature, most of the available data and studies about TTE in patients with an LVAD involve HM-II device. A paper specifically reported a single center experience on TTE in HVAD patients, but it provided only information about the visualization of the cannulas, and involved 19 patients. Our study provides, with a similar size sample, more detailed and comprehensive information. The lack of reports in literature about TTE feasibility in HVAD is difficult to explain. Probably, it could be explained with a general assumption that theoretically one

should not expect to observe significant differences are supposed between two continuous-flow pumps with respect to echo. However, the intrapericardial implant of HVAD is supposed to generate more acoustic problems, and this could explain the scares enrollment of HVAD patients into ramp studies. Even if our aim is not to speculate and discuss about TTE differences between the two pumps, we observed two main differences with respect to published Echo paper on HM-II: the impossibility of achieving a Doppler signal of the inflow cannula and of the aortic valve, thus not allowing calculation of left ventricular output, the difficulty in achieving a standard apical 4-C view, thus rending necessary a paralongitudinal view. On the other hand, several theoretic similarities were also observed: Doppler analysis of outflow cannula, feasibility of achieving hemodynamic information. On the other hand, one may speculate a different importance of some TTE parameters with respect to the two pumps: an assessment of LV (and/or RV) output by TTE appears to be mandatory for HM-II patients, as flow estimation by the controller is less accurate , whereas appears to be less important with HVAD, where controller provides a more accurate flow estimation.

The artifacts due to the intrapericardial placement of the pump require non-standard echocardiographic views, to which the sonographer should become familiar, considering the increasing number of ambulatory patients with this device.

Study limitations

The major study limitations are: limited number of patients, single center study, case-reporting design. Moreover, a potentially bias is the patient selection (specifica i criteri di selezione per la scelta dei pz nel lavare; guarda anche il lavoro di marcus. However, the aim of our study was simply to give a clinical-oriented, practice description of TTE exam in an outpatient setting. Even if the number of patients enrolled is small, it is superior to the number of HVAD patients enrolled in single echocardiographic studies, and similar to the one reported in another recent echocardiographic series about HVAD.

Conclusions:

This clinical series provides for the first time data about the feasibility of a complete TTE exam in HVAD patients, one of the commonly implanted pumps, in a real-world outpatient setting. According to our experience, non standard views are required to assess some of the parameters of a stan Specific problems and possible tricks are presented. Even if studies enrolling a larger sample size are required, we believe that TTE examination of HVAD patients requires pump-specific expedients, but it is possible and allows complete and useful hemodynamic information in the great majority of cases, thus allowing the echo- guided optimization of the pump settings, and the study of the complex interaction between the pump, the patient and the heart.