UNIVERSITÀ DEGLI STUDI DI PADOVA

Corso di Laurea Magistrale a Ciclo Unico in Medicina e Chirurgia

DIPARTIMENTO DI SCIENZE CARDIO-TORACO-VASCOLARI

E SANITÀ PUBBLICA

Direttore: Prof. Federico Rea

TESI DI LAUREA

VENTRICULAR-ARTERIAL COUPLING IN CARDIAC TRANSPLANTATION: DETERMINING FACTORS AND PROGNOSTIC ROLE

Relatore: Prof. Francesco Tona

Correlatore: Dott. Ricardo Vianello

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ABSTRACT

Background: In transplanted heart patients, adaptation of the new heart to a different afterload to which it has been exposed in the donor's body may bring some consequences on graft function. Usually, ejection fraction (EF) assesses graft performance and when its value is below normal ranges it is known to imply poor prognosis. Nevertheless, even in range of normality some patients have worse outcome than others. In this group of patients, we aimed to study ventricular arterial coupling (VAC) as another tool to assess prognosis because it reflects the interaction between the heart and the arterial system through the ratio arterial elastance (Ea), as an expression of arterial afterload, and end-systolic elastance (Ees) expressing left ventricle contractility. **Aim**: The goal is to create a statistical model using clinical, echocardiographic and VAC derived parameters to determine prognosis in heart transplanted (HT) patients. **Methods**: The study was conducted in HT patients with normal EF, no graft rejection, no cardiac allograft vasculopathy and had survived first year after surgery. Clinical, echocardiographic and pressure-volume derived parameters were gathered 1 year after transplantation and during a 30-year follow-up until death. VAC and other pressure-volume derived parameters were measured with a noninvasive method. Survival predictors were analyzed with univariate and multivariate Cox analyzes. **Results**: HT patients were coupled compared to controls but with higher Ea and Ees. Uncoupled hearts were mainly due to impaired contractility. Male patients and patients with higher end-systolic and end-diastolic volumes were associated with higher mortality, they were also proportionally associated with higher VAC values. Patients with VAC >0.59, Ea >4mmHg, Ees ≤6.75mmHg/ml had a worse long-term prognosis (p= 0.02). Ea and Ees also showed to be independent mortality prognostic factors (p= 0.02 and 0.001 respectively). **Conclusions**: VAC predicts long-term outcomes in transplanted heart patients only as a univariate variable, however, Ea and Ees when studied separately proved to be independent prognostic risk factors. These parameters are particularly useful since they can be easily determined with simple calculations and echocardiographic measurements that are routinely evaluated by cardiologists.

Key words: VAC, cardiac transplant, prognosis, arterial elastance, end-systolic elastance

RIASSUNTO

Premesse: Nei pazienti trapiantati di cuore, l'adattamento del nuovo cuore a un diverso postcarico nel corpo del donatore può portare alcune conseguenze sulla funzione dell'innesto. Di regola, la frazione di eiezione (EF) valuta le prestazioni dell'innesto e quando il suo valore è al di sotto dei valori normali indica una prognosi sfavorevole. Tuttavia, quando rientra in un range di normalità, alcuni pazienti hanno esiti peggiori di altri. In questo gruppo di pazienti, puntiamo a studiare l'accoppiamento ventricolo-arterioso (VAC) come altro parametro per valutare la prognosi in quanto riflette l'interazione tra il cuore e il sistema arterioso attraverso il rapporto elastanza arteriosa (Ea), come espressione del postcarico arterioso, ed elastanza telesistolica (Ees) che esprime la contrattilità del ventricolo sinistro. **Scopo dello studio**: L'obiettivo è creare un modello statistico utilizzando parametri clinici, ecocardiografici e derivati del VAC per determinare la prognosi nei pazienti trapiantati di cuore. **Metodi**: Lo studio è stato condotto in pazienti trapianti di cuore con FE normale, senza rigetto, senza vasculopatia cardiaca da allotrapianto e sopravvissuti il primo anno dopo l'intervento chirurgico. I parametri clinici, ecocardiografici e derivati da pressione-volume sono stati raccolti a partire dall'anno seguente il trapianto e nell'arco di un follow-up di 30 anni fino alla morte. Il VAC e altri parametri derivati sono stati misurati con un metodo non invasivo. I fattori predittivi di sopravvivenza sono stati analizzati con analisi di Cox univariata e multivariata. **Risultati**: i pazienti trapiantati erano accoppiati rispetto ai controlli ma con Ea ed Ees più elevati. I cuori disaccoppiati erano principalmente dovuti a una ridotta contrattilità. Il sesso mascolino e volumi telesistolici e telediastolici elevati erano associati a una mortalità maggiore ed erano anche proporzionalmente associati a valori di VAC più elevati. I pazienti con VAC >0,59, Ea >4mmHg, Ees ≤6,75mmHg/ml avevano una prognosi peggiore a lungo termine (p=0,02). Ea ed Ees hanno anche dimostrato di essere fattori prognostici di mortalità indipendenti (p = 0,02 e 0,001 rispettivamente). **Conclusioni**: Il VAC predice gli esiti a lungo termine nei pazienti cardiopatici trapiantati solo come variabile univariata, tuttavia, Ea ed Ees, quando studiati separatamente, si sono rivelati fattori di rischio prognostici indipendenti. Questi parametri sono particolarmente utili poiché

possono essere facilmente determinati con semplici calcoli e misurazioni ecocardiografiche che vengono valutate di routine dai cardiologi.

Parole Chiavi: VAC, trapianto cardiaco, prognosi, elastanza arteriosa, elastanza ventricolare telesistolica

BACKGROUND

HEART TRANSPLANTATION

Global statistics

Transplant volume statistics:

According to the 35th adult heart transplantation report from the International Society for Heart and Lung Transplantation (ISHLT), since last decade there has been a worldwide increase in heart transplant (HT) volumes. This may be due to various factors such as increased donor availability as a consequence to deaths caused to overdosis after the opioid epidemic in the United States and greater number of cases reported in other countries (1).

An average of 10-19 transplants are performed in most centers, and about 12 perform more than 50 procedures a year (1).

Age of heart recipient and donor:

The average age of recipients is 55 years and has been quite steady throughout the past eras but, it has been evidenced an increase in patients receiving heart transplant at ages over 60 years and a simultaneous reduction in those with ages between 40-59 (1).

The median age for donor varies depending on the area. While North America has the youngest median donor age (28 years in 2016), Europe has the eldest (44 years in 2016) (1).

Main indications for heart transplant:

Cardiac transplantation remains the gold standard in the treatment of advanced heart failure (2). The main primary causes are: non-ischemic dilated cardiomyopathy and

ischemic cardiomyopathy. Although the first is more frequent in other regions, the latter is common in North America (1).

***Comorbidities in recipients**:*

Due to advancement in the medical field, in the last era more patients with comorbidities have been proposed for heart transplantation (3). There is a variability of recipient's comorbidities according to different regions. North America presents higher number of obese and diabetic recipients compared to Europe and other regions. Cigarette smoking in the other hand is more common in European recipients (1).

Finally, since 2007 there has been a rise of patients with mechanical circulatory support (MCS) as a bridge to transplantation. The most common device used is the left ventricular assist device (LVAD) (1). While initially, patients with LVAD had worse outcomes, it is now approaching to outcomes seen in patients without it (3).

Survival outcomes:

Median survival to heart transplant is 10.8 years for adult recipients (transplants performed since 1982 to 2016). Survival after the first year remains above 50% (1).

The latest report from the International Society for Heart and Lung Transplantation (ISHLT) analyzed around 108.034 patients undergone heart transplantation during 1992 to 2018 (3). In the most recent era (2012-2017) 1-year survival has improved despite the increase in medical complexity of transplant recipients. It is generally higher in North America when compared to European and other regions, likely because Europe donors tend to have a more advanced age and high-risk features. On the other hand, one-year survival is not considered as an important performance quality measure in European Standards in comparison with North America which may lead to the performance of higher-risk transplants (3).

On the contrary, the 5-year survival is better in Europe (88.5%) compared to North America (86.9%) and other regions (85.0%) (3).

Italian data on heart transplantation

In Italy, despite the SARS-CoV-2 pandemic in 2020, heart transplant suffered just a minor decrease (-3.2%), from 246 in 2019 to 238 in 2020. As in other countries, the combined transplant (heart with other organs) represents a small percentage (0.8%) of the total of number of transplants (4).

During 2011 to 2020, the centers that had the highest number of operations were (4):

- A.O. of Padova (286)
- ASST Grande Ospedale Metropolitano Niguarda of Milan (271)
- Ospedale Santa Maria della Misericordia of Udine (227)
- Policlinico Sant'Orsola Malpighi of Bologna (223)

Age:

About 50% of the patients that were transplanted in 2020 had ages between 41-60 years, followed by 27.3% in patients between 61-75 years, 15.6% between 18-40 years (Fig. 1) (4). An isolated case of transplant performed on a 76-year-old recipient on the ordinary waiting list was reported: it was an extraordinary event, indicative of the effort to increase the age of heart transplant candidates (4).

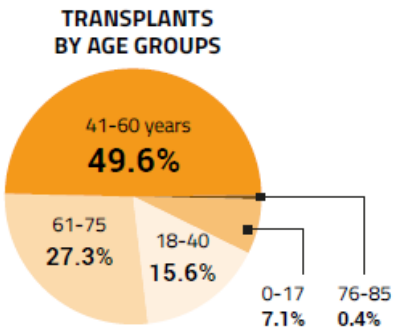

Figure 1: Heart transplant by age group Italy in 2020 (4)

Regions and patients:

The Friuli Venezia Giulia region (61.9%) was confirmed in 2020 to have the largest percentage of transplanted patients living outside the region, followed by Emilia Romagna (33.3%) and Veneto (24.5%) (4).

Waiting lists:

The mortality rate on the list was 3.9% while the average waiting times in 2020 (Fig. 2) were (4), according to the type of list:

- Standard list: 3 years and 7 months approximately
- Pediatric list: 3 years and 3 months approximately
- Urgent list: 8 months approximately

Figure 2: Average waiting times before heart transplantation in 2020 in Italy (4)

Indications for cardiac transplantation

Given the limited number of donor hearts, heart transplantation is generally considered when it is likely to improve quality of life and increase survival (5). In the other hand, a timely referral ensures that the patient will be able to survive on inotropic drugs or mechanical support during the waiting list until the heart is available (6).

Indications in ***Chronic Heart Failure*** (outpatient settings) (5,6):

- Patients on optimal medical therapy who still have limiting symptoms on exertion (NYHA class 3 or 4 or American College of Cardiology stage D patients).
- Patients with frequent readmissions to hospital (≥2 in 12 months) for heart failure exacerbation despite adherence optimal medical therapy.
- Deterioration of renal function attributed to the cardiorenal syndrome
- Limitation (decrease or stop) on the use of potential beneficial medications due to hypotension or renal dysfunction
- Worsening in right ventricular function or rising pulmonary artery pressure due to left heart failure
- Frequent episodes of ventricular arrhythmias despite optimal drug and electrophysiological therapy
- Anemia, weight loss, hyponatremia, or liver dysfunction that are attributable to heart failure.

Indications in ***Acute Heart Failure*** (inpatient settings) (5,6):
This category of patients requires an urgent referral for cardiac transplant.

- Inability to stop intravenous inotropic therapy.
- Need for percutaneous mechanical circulatory support to treat a patient in cardiogenic shock.
- Ventilatory support with use of positive airway pressure for intractable pulmonary oedema.
- Refractory ventricular arrhythmia

Assessment prior to listing for heart transplantation

1) Patient's comorbidities:

- Age:

Patients over 70 years may be considered for cardiac transplantation (7). Nowadays, in selected septuagenarians, 5-year survival is similar to those between 60-69 years. Usually, recipients over 70 years are less acutely ill, have fewer comorbidities and are less likely to have durable LVAD support before transplantation (5).

- Obesity:

Obesity with a BMI >35 Kg/m^2 is associated with a worse outcome after cardiac transplantation. On the other hand, patients with this BMI are less likely to find a suitable donor leading to long waiting times. There for, it is recommended weight loss to reach at least a BMI lower o equal to 35 Kg/m^2 (7).

- Diabetes mellitus (DM):

The patient with DM with end-organ damage (except for non-proliferative rethinphathy) or HbA1c >7.5% or 58mmol/mol despite optimal treatment have a relative contraindication for transplant (7).

- Renal function:

Renal failure is a common finding in patients with advanced HF due to prolonged low CO and/or renal venous congestion. It expressed as an intrinsic renal disease easily diagnosed using renal ultrasound and proteinuria values. In a very early stage, where the patient is euvolemic, an attempt to enhance renal perfusion increasing CO with intravenous inotropic therapy could permit listing the patient for transplantation (5).

- Liver function:

Liver dysfunction (fibrosis or cirrhosis) in the context of a patient with HF could be a consequence of congestive hepatopathy or other coexisting liver disease. Abnormal liver function correlates with poorer outcomes following cardiac transplantation (5).

- Cerebral and peripheral vascular disease:

A clinically evident severe cerebrovascular disease (CVD) may be a contraindication to transplantation. While peripheral vascular disease may be considered a relative contraindication for transplantation when its presence limits rehabilitation and revascularization is not possible (7).

- Pre-existing neoplasm:

Active malignancy, other than localized non-melanoma skin cancer, is a contraindication to transplantation. A relative contraindication is a low-risk recurrence and the absence of metastasis (5).

- Frailty:

A frail elder person is an individual who has a reduction of function in multiple organ systems brought on with minor stressor. The possible symptoms include unintentional weight loss (≥4.5 kg within the past year), muscle loss, fatigue, slow walking speed, and low levels of physical activity (7).

2) Echocardiography:

Provides information on (5):

- Potential etiology of HF
- Assessment of biventricular function
- Presence or absence of associated valvular lesions
- Estimation of pulmonary artery systolic pressure

3) Cardiopulmonary exercise testing:

The cardiopulmonary exercise testing, using a bicycle or a treadmill while simultaneously measuring ventilation and gas exchange, allows to assess the patient's functional capacity. The functional capacity consents to objectively

confirm the patients' perception of exercise limitation and obtain a prognostic evaluation (5).

The elements that are useful to assess poor prognosis are:

- Peak oxygen uptake (peak VO2) <10 ml/Kg/min in patients who achieve maximal exercise (intended as achievement of anaerobic threshold or a respiratory exchange ratio -RER- >1.05) is a strong predictor of poor prognosis. Patients taking beta-blocker or in patients unable to tolerate a beta-blocker, a peak VO2 of <12 ml/Kg/min and <14ml/Kg/min respectively are considered appropriate for cardiac transplant (5).
- Percentage of predicted peak VO2 <50%, in conjunction to peak VO2, can also be used to guide transplant candidacy in young patients (<50 years) and women (5)
- Minute ventilation/carbon dioxide production slope >35 in patients unable to achieve a maximal test is an additional marker of adverse prognosis (5)

It is to note that, the use of cardiac resynchronization therapy (CRT) does not alter the peak VO2 and as so, it is not useful to predict adverse cardiac events (7).

4) Right heart catheterization:

Right heart catheterization (RHC) is mandatory for transplant assessment and is periodically repeated (usually every 3–6 months but must be individualized depending on the situation (7)) in patients on the waiting list. Elevated filling pressures despite optimal treatment are associated with poorer prognosis (5).

It provides useful information on (5):

- Direct measurement of right arterial pressure
- Pulmonary capillary wedge pressure (PCWP)
- Pulmonary artery pressure (PA)
- Mixed venous oxygen saturation
- Cardiac output
- Transpulmonary pressure gradient (TPG): mean PA pressure – mean PCWP

- Resistance in both systemic and pulmonary beds

Pulmonary hypertension is a contraindication for heart transplant, these criteria are (5):

- Pulmonary artery systolic pressure >60mmHg
- Transpulmonary gradient ≥15mmHg, and/or
- Pulmonary vascular resistance >5 wood units

If these criteria are reversible after testing with vasodilators (intravenous nitroglycerin or sodium nitroprusside) or unloading of the ventricle using drugs (diuretics, inotropes, and vasodilators in a 48-period hospitalization) or mechanical devices (like LVAD), then such patients could be eligible for heart transplantation (5,7).

5) Natriuretic peptide levels:

Patients with optimal HF treatment that remain with persistent high NT-proBNP levels are likely to require cardiac transplantation in the following 12 months (5).
In practice, we have found that patients with advanced HF and a suppressed NT-proBNP of <750 pg/mL are highly unlikely to die or require urgent heart transplantation or mechanical circulatory support in the subsequent 2 years (5).

6) Seattle Heart Failure Model (SHFM) and the Heart Failure Survival Score (HFSS):

These are the most widely used risk scores in the advanced HF population and provide an additional prognostic assessment (5).

SHFM and HFSS are comparable and the combination of both improves their predictive ability. SHFM score estimates 1-, 2-, 3- year survival with the use of 20-variabilities easily obtained including clinical, pharmacological, device and

laboratory characteristics. While HFSS uses 7 parameters, including VO2 peak (8,9).

Estimated 1-year survival <80% in the SHFM is regarded as a reasonable cut-off to consider transplant listing, although the SHFM tends to overestimate survival in younger patients with advanced HF. Instead, a HFSS with high to medium risk range is considered the cut point (5,7).

7) Allosensititation:

Allosensitisation to HLA may occur in women who have been pregnant or in patients who have received blood transfusion or transplant. It can negatively impact on the chances of successful donor-recipient matching (5).

Contraindications for cardiac transplantation

- Acute infections. Patients with hepatitis B, hepatitis C or HIV could be considered for transplantation if viral titers are undetectable and have no end-organ damage
- Symptomatic cerebral or peripheral vascular disease
- Poorly controlled DM with end-organ damage
- Current or recent neoplasm (feasibility should be discussed with oncologist)
- Severe lung disease: forced expiratory volume (FEV1) and forced vital capacity (FVC) less than 50% that evidences parenchymal lung disease
- Irreversible pulmonary hypertension
- Renal dysfunction with estimated glomerular filtration rate <30 ml/min/1.73m^2
- Chronic liver disease: e.g., cirrhosis
- Pulmonary embolism in the last 3-6 months

- Non-compliant to medication, ongoing OH or drug abuse, current smoker, inadequate support
- Body mass index ≥35 Kg/m^2 (morbid obesity)
- Disease in various organs, requiring other transplant procedures (e.g renal, hepatic, lung transplant) (5,7)

Complications after cardiac transplant

Early post-transplant problems:

1) Primary graft dysfunction:

Primary graft dysfunction (PGD) is an important problem in the immediate post-transplant period. It is defined as the failure of graft function within the first 24hours after transplantation in absence of a secondary identifiable cause like hyperacute rejection, pulmonary hypertension or known surgical complications such as bleeding or tamponade. Rates go up to 31% and a 44% 1-year survival in severe cases (5). Pathogenesis is not fully elucidated, but risk factors include age of donor and recipient, female donor to male recipient, use of catecholamines at time of death, use of inotropes or mechanical circulatory support at the time of transplantation, ischemic time, among others (2).

2) Rejection (5):

The response of the hosts immune system against the allograft can be cell-mediated or antibody-mediated and can range from a mild rejection (without allograft dysfunction) to severe rejection with hemodynamic compromise. After transplantation, sequentially performed endomyocardial biopsies (10-12 in the first year) are done in order to look for evidence of rejection. The incidence of rejection requiring augmentation of immunosuppression has fallen of almost a half (from 23.5% to 13%) from 2004 to 2014

- Acute cell-mediated rejection (ACR): it is uncommon after the first year in patients on stable immunosuppression. Patients may not have any symptoms and be diagnosed only thorough biopsy. Treatment is usually effective and consists of augmented immunosuppression with corticosteroids.
- Antibody-mediated rejection (AMR): is thought to be caused by complement fixing anti-HLA antibodies which may have been present pre-transplant or develop de novo after transplant. AMR after the first year is associated with graft dysfunction and poorer survival. Survival at 1-year following AMR is not brilliant (50%) despite therapy with plasma exchange, intravenous immunoglobulin, and monoclonal antibodies (e.g Rituximab).

3) Infection (5):

It is obviously favored by the necessary use of immunosuppression therapy and has a higher rate of death during the first-year post-transplant. For this reason, during the first 12 months, prophylaxis is used against opportunistic microbes such as:

- Pneumocystis jirovecii: cotrimoxazole, dapsone, pentamidine
- Cytomegalovirus: ganciclovir or valganciclovir
- Candida: nystatin and fluconazole
- Herpes virus: acyclovir

Vaccination against influenza and pneumococcal infection is recommended.

Late post-transplant problems:

1) Cardiac allograft vasculopathy (CAV):

CAV is a process not fully understood. It produces diffuse and progressive thickening of the intima due to smooth muscle proliferation, accumulation of inflammatory cells and lipid deposition, leading to narrowing and occlusion of coronary arteries of the allograft (5). It is a significant cause of death on late post-transplant, arising to an incidence of nearly 50% after 10 years (2).

Most patients with CAV are asymptomatic and become diagnosed on routine surveillance with angiography (5). When symptomatic, because of denervation of the allograft, these are not like non-transplanted coronary artery disease but instead have dyspnea, gastrointestinal symptoms, heart failure, arrythmias or sudden cardiac death (2).

Revascularization is rarely feasible because of the diffuse nature of the disease. The definite treatment of CAV is retransplantation. Statins and mTORi have shown to delay onset and slow progression of CAV but must be introduced early post-transplant (5).

2) Malignancy (5):

Patients present around 10% risk of de novo solid organ malignancy between 1- and 5-years post-transplant. Many of these tumors are associated to viral infections including HPV (e.g., squamous cell carcinoma of the skin), EBV (e.g post-transplant lymphoproliferative disease) and HHV8 (e.g., Kaposi's sarcoma)

Squamous cell carcinoma of the skin (HPV driven) is the most common malignancy reported in around 18% of 10-year survivors.

Non-lymphoma malignancy is the leading cause of death late after transplantation, accounting for approximately 20% of deaths in those surviving >5 years.

3) Renal disfunction (5):

Within the first year following transplantation almost 9% of recipients have either high creatinine levels or require chronic dialysis or renal transplant. The development of chronic renal dysfunction (GFR: $\leq$29ml/min/m^2) is associated with a fourfold increased risk of death.

Possible causes of this renal impairment are the use of calcineurin inhibitors (CNI) and poorly treated hypertension. CNI can cause a reversible acute toxicity by a mechanism of vasoconstriction of the afferent renal artery, whereas late renal dysfunction is related to tubular damage even when CNI is discontinued.

It is possible to replace CNI with mTORis in favor of a renal advantage, but it has been associated to higher rates of biopsy-proven acute rejection and drug-related adverse events.

4) Hypertension:

Hypertension occurs in about 50-90% of patients Post transplantation. It is due primarily to calcineurin inhibitors (ciclosporin) because of a direct effect and association with renal insufficiency and to altered circadian rhythm in transplanted hearts (no normal blood pressure drops during nocturnal hours and greater 24h hypertensive burden) (10). Hypertension contributes to development of renal dysfunction and CAV (5).

5) Hyperlipidemia:

Possible causes are the pre-existence of elevated lipids, a consequence of immunosuppression therapy (calcineurin inhibitors, prednisone, sirolimus), loop diuretics and renal insufficiency (5,10). Hyperlipidemia plays an important role in peripheral vascular disease (10). Thanks to immune-modulating and lipid-lowering effects, the use of statins has demonstrated to improve survival, reduce severe incidence of rejection and CAV. Current practice is to commence a statin after transplant regardless of lipid levels (5).

6) Diabetes:

Diabetes is present in about 30% post-transplanted patients (10). The use of corticosteroids and CNIs (primarily tacrolimus than ciclosporin) for immunosuppression can contribute to diabetes mellitus (DM) in post-transplanted patients (5,10). A consequence of DM is a greater incidence of hypertension and renal dysfunction (5).

Death causes in transplanted heart patients

According to the 35th consensus document of the ISHLT, leading causes of death have remained rather stable throughout the years. These main causes are: graft failure (highest in the first 30 days), non-cytomegalovirus infections (within the

first year) and multiple organ failure. Malignancy, cardiac allograft vasculopathy (CAV), and renal failure death increase with time since transplant (1).

***Situation in Padua Hospital (Italy)**:*

A. Angeline and co-workers conducted a study in 2003 that aimed to analyze the causes of death and risk factors of patients that underwent heart transplant in the Hospital of Padua during a 15-year period (from 1985 to 2000). From a pool of 507 transplanted patients, in 96 of them it was performed an autopsy (11).

The causes of death identified were(11):

- Acute graft failure (GF) in 19%,
- Acute rejection (AR) in 14%,
- Infection in 14%,
- Chronic rejection (CR) in 12%,
- Malignancy in 10%,
- Poor preoperative conditions in 7%,
- Intraoperative complications in 5%,
- Pulmonary hypertension and right cardiac failure in 4% and other in 15%.

During the first 6 months, acute graft failure, acute rejection and infections were the most common causes of death, thereafter, chronic rejection and malignancy(11).

Based on a modified ISHLT criteria for heart rejection, they assigned a rejection score (RS). The conclusion at which they arrived were:

- Risk factors for total mortality were (11):
    - Higher rejection score in the total follow-up (TRS)
    - Higher TRS including only severe grades (≥3) (sevTRS)

- Risk factors for cardiovascular death were (11):
    - High steroid dosage at 3 months, 6 months, and 1 year
    - Higher TRS

- Higher rejection score in the first year (RS1yr)
- Higher sevTRS
- Younger age at heart transplant

Higher RS1yr was an independent risk factor for death as well as younger age, a feature known to be associated with high rejection risk (11).

VENTRICULAR-ARTERIAL COUPLING (VAC)

Definition of ventricular-arterial coupling

Ventricular-arterial coupling (VAC) is defined by the interaction between the left ventricular (LV) function and the arterial system (12). It is nowadays recognized as an important determinant of global cardiovascular performance. Therefore, in order to assess LV efficiency, it is important not only the study of the LV properties themselves but also the modulating effect of the arterial system on LV performance and how cardiac energetics are used (13).

Mathematically, VAC is defined as the relation between arterial elastance (Ea) and LV end systolic elastance (Ees). Although these terms will be thoroughly examined further on, Ea represents the arterial afterload while Ees is the load-independent measure of LV contractility (13).

$$VAC = \frac{Ea}{Ees}$$

Both Ea and Ees are expressed in mmHg/ml and the final output Ea/Ees is a ratio without units. Proposed normal values for Ea are 2.2 ± 0.8 mmHg/ml and for Ees 2.3 ± 1.0 mmHg/ml (12).

Effective arterial elastance (Ea)

Effective arterial elastance (Ea) is considered a measure of the net arterial load that is imposed on the left ventricle (LV) (14) in other words, it's the obstacle that the arterial system opposes to the LV ejection (15).

Ea is not a measure of a specific arterial property but an integrative index that incorporates the principal elements of arterial load (14). These elements can be divided in a steady component and in a pulsatile component (Fig. 3), based on the conduit and cushioning functions of the arteries (16).

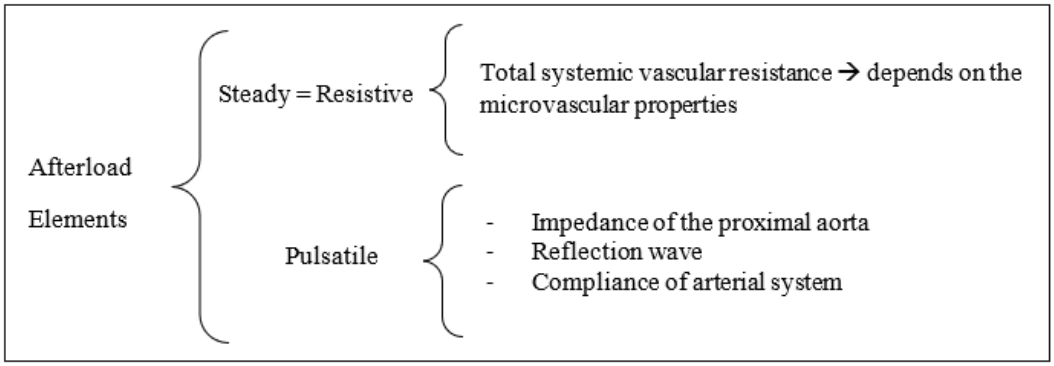

Figure 3: Principal afterload elements

The steady, or also called resistive, component of afterload is represented by the total systemic vascular resistance (SVR) which largely depends on the microvascular properties (12,15).

The pulsatile arterial load is more complex and is affected by various properties of the conduit vessels, such as: impedance of the proximal aorta, magnitude and timing of the reflection wave and the total compliance of the arterial system (15) (Table I).

Table I: Summarized concepts of pulsatile arterial load properties

Elastance (E)	It is defined as the change in pressure (P) resulting from a change in volume (V). It reflects resistance to change the shape when a mechanical load is applied. It can also be understood as the capacity to recoil: $$\boldsymbol{E = \frac{P}{V}}$$
Compliance (C)	It is defined as the change in volume (V) resulting from a change in pressure (P). It reflects the ability to change the shape of the structure when mechanical load is applied, in other words, the ease of expansion: $$C = \frac{V}{P}$$ It is also seen as the reciprocal value of elastance: $$C = \frac{1}{E}$$ Compliance depends mainly on the composition of the arterial wall: smooth muscle cells and connective tissue containing elastin and collagen fibers (16).
Wave reflection	Wave reflation is a physiological property or the aorta in which the pulse wave returns to the LV in diastole, boosting diastolic blood pressure and improving coronary perfusion. When the aorta loses its compliance and becomes stiff (for example, with aging or with hypertension) the reflected

	wave returns to the LV from the peripheral circulation in early/mid systole with and elevated magnitude. This causes systolic blood pressure and central pulse pressure to arise. The consequence is an enhancement of the end-systolic stress and afterload on the LV which subsequently leads to LV hypertrophy (12).
Impedance (Z)	Vascular impedance is thus analogous to vascular resistance, the former expressing pulsatile and the latter "steady-flow" relationships (17).

Ea is determined from the pressure-volume (P-V) loop as the negative slope of the line that connects the ESPVR (End systolic pressure volume relation – corresponding to the upper left-hand corner of the P-V loop) and the end-diastolic volume on the horizontal axis (Fig. 4) (12,18).

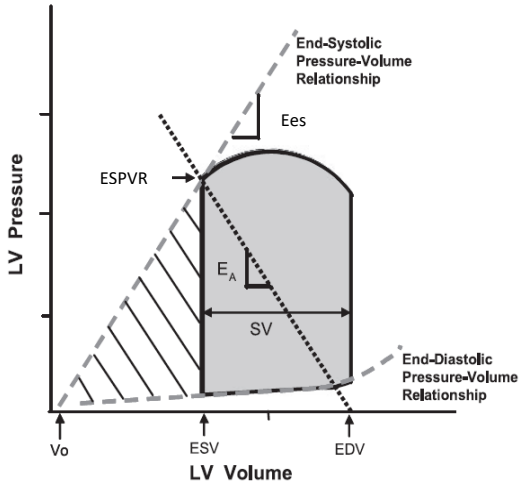

Figure 4: Pressure- Volume (P-V) loop showing effective arterial elastance (Ea) as the negative slope of the line that connects the ESPVR to EDV on the horizontal axis. Ea: effective arterial elastance, Ees: end systolic elastance, ESPVR: end systolic pressure-volume relation, EDV: end systolic volume, ESV: end systolic volume, SV: stroke volume, LV: left ventricle (14)

Assuming that zero stroke volume is associated with zero pressure, then Ea can be approximated by the ratio of the end-systolic pressure (ESP) to stroke volume (SV) (13) (Fig. 5):

$$Ea = \frac{ESP}{SV}$$

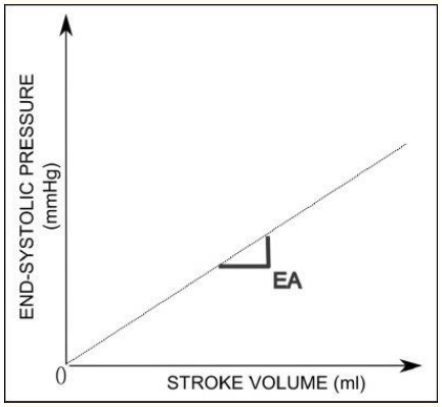

Figure 5: Relation between stroke volume and end-systolic arterial pressure. The slope of this relation represents the arterial elastance (Ea) (13)

On the other hand, End-systolic pressure (ESP) can be calculated as 0.9 times the peak brachial systolic pressure that can be easily estimated using a cuff sphygmomanometer. While systolic volume is determined using doppler techniques at the LV outflow tract (13).

ESP= 0.9 x Systolic blood pressure

Changing ventricular afterload will cause Ea to change proportionally. If afterload increases, Ea increases and ESP will rise while decreasing SV which means that the LV in order to produce a certain stroke volume, must generate higher ventricular pressures. Conversely, if the ventricle is able to generate a high stroke volume with

low pressure, it means that the obstacle of the arterial system is low (15,19) (Fig. 6).

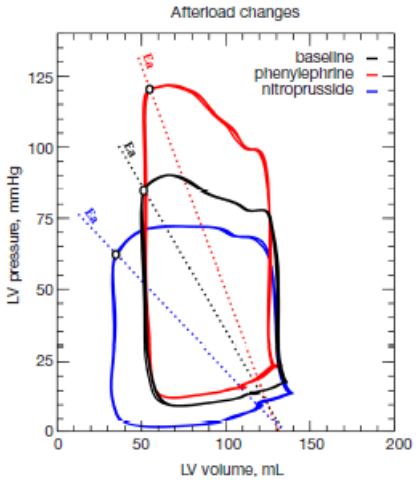

Figure 6: LV pressure volume loops showing the effects of afterload variations on Ea. Afterload was increased with phenylephrine and decreased with sodium nitroprusside. The lines connecting the end-diastolic volume and end-systolic pressure describes the effective arterial elastance at each stage (Ea, dotted colored lines). As afterload increases, so does the value of Ea (19)

Windkessel effect:

The windkessel effect reflects how a reservoir can affect the pulsatile nature of fluid flow. During systole the blood pumped from the LV flows toward the peripheral arteries, but an important amount will be contained in the proximal aorta because of the compliance capacity of this vessel. During diastole the aorta walls return to its original position thanks to its elastic recoil, pumping the containing blood forward (Fig. 7). This means that there will always be blood flowing through the arteries even though the blood isn't being pumped by the LV(20).

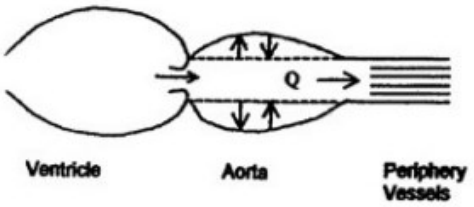

Figure 7: The root of the aorta expands to contain blood during systole thanks to its compliance capacity. During diastole, the elastic recoil allows blood to keep flowing through arteries (21)

The 2-elemnt Windkessel model is the simplest of the windkessel models and it aims to explain what was said before making the analogy to an electrical circuit. In this model the arterial compliance ("stretchiness") is represented as a capacitor (C) with electric charge storage properties that is connected in parallel with a resistor (R) as an expression of the peripheral resistance of the systemic arterial system. These two elements represent the aortic properties and are both connected in parallel with a pump representing the heart (Fig. 8). The flow of blood from the heart is analogous to that of current flowing in the circuit and the blood pressure in the aorta is modeled as a time-varying electric potential (21).

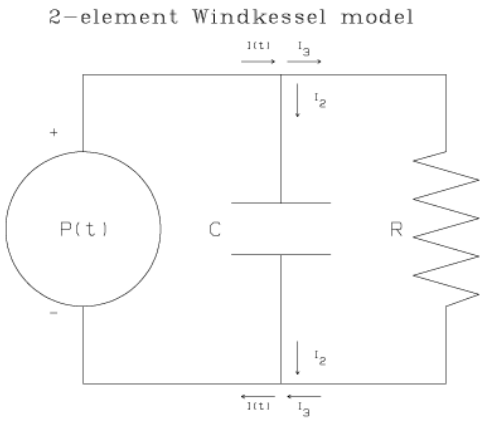

Figure 8: Analog electrical circuit of 2-element Windkessel model. C: capacitor (aortic compliance); R: resistor (peripheral resistance); P (t): Cardiac pump.

The 3-element Windkessel model adds another resistor in series to the already existing parallel combination of resistor-capacitor to account for the resistance to blood flow due to the aortic valve. It simulates the characteristic impedance of the proximal aorta (21) (Fig. 9).

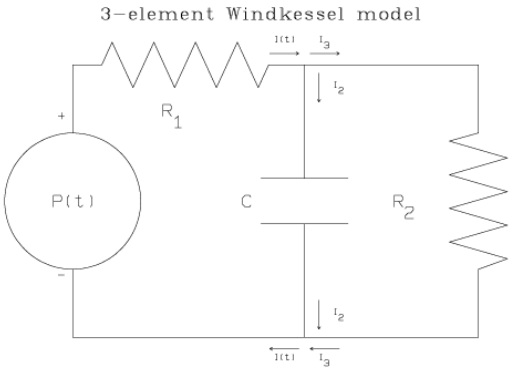

Figure 9: Analog electrical circuit of 3-element Windkessel model. C: capacitor (aortic compliance); R1: resistor 1 (due to aortic valve); R2: resistor 2 (peripheral resistance); P (t): Cardiac pump

Kelly et al. showed that EA measured invasively as ESP/SV closely approximated the arterial load obtained from aortic input impedance and arterial compliance data based on a three-element windkessel model. One limitation of the three-element windkessel model is that it does not include the effects of the reflected pressure waves, which originate from areas of major impedance mismatches or major bifurcations (14).

Left ventricular end-systolic elastance (Ees)

Left ventricle end-systolic elastance (Ees) is defined as the slope of the end systolic pressure volume relationship (ESPVR). The ESPVR is obtained as following: pressure and volume of a LV is plotted while varying the amount of blood in it

(preload), doing so, it is possible to obtain a series of PV loops. The left upper loop corner corresponds to the end systolic pressure (ESP). Connecting all the ESP points obtained by the variation in preload we can draw a straight line, this line represents the ESPVR. The ESPVR has also an intercept with the volume axis called V0, which represents the hypothetical unstressed volume of the ventricle (19) (Fig. 10).

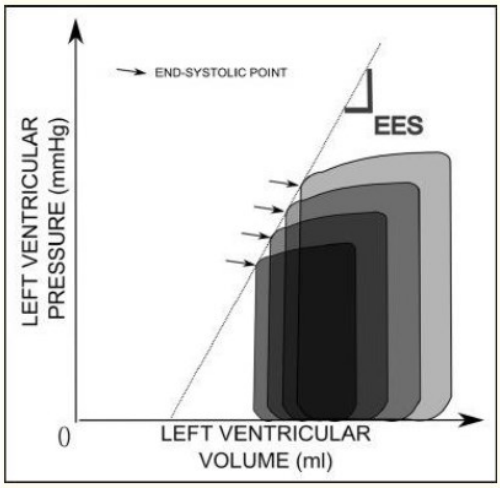

Figure 10: End-systolic pressure point obtained by varying the LV preload. The slope of the line connecting all these ESP points represents the left ventricular end-systolic elastance (Ees) (13)

Sugawa et al were the first to demonstrate that the Ees was a surrogate of the LV contractility using isolated canine heart models (14).

An increase in contractility is reflected as an increase in the slope and a shift in the end-systolic PV relationship to the left, which allows the ventricle to generate more pressure for a given LV volume (14) (Fig. 11).

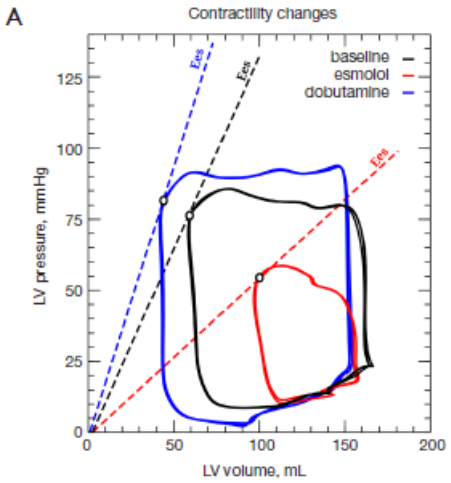

Figure 11: Zero Left ventricle pressure volume loop showing the effects of contractility changes on Ees. Cardiac contractility was increased with dobutamine and decreased with esomolol. The slope of the end-systolic PR relationship defines the end-systolic elastance (Ees) and the contractility performance at each stage (19).

Ees can be calculated as end-systolic pressure (ESP) by end-systolic volume minus V0, where V0 is the x-axis volume intercept of the end-systolic PV relationship. Under physiological loading conditions, these assumptions are only approximations because the calculation of Ees assumes that the end-systolic PV relationship is independent of load, that its slope is linear and that V0 is insensitive to inotropic influences (14).

$$Ees = \frac{ESP}{ESP - V0}$$

Although Ees is considered as a load-independent measure of the left ventricle contractility it can also reflect the geometrical and biochemical changes occurring when LV stiffness is present. Thus, when interpretating Ees caution should be exercised. It is likely that acute changes in Ees (e.g., with inotropic agents or exercise) reflect acute alterations in left ventricular contractility, whereas baseline

values of Ees represent an index that integrates intrinsic left ventricular contractility as well as the modulating effects of the geometric, structural, and functional properties of the LV. Ees should, therefore, be considered an integrated measure of left ventricular chamber performance that can be related to an integrated measure of arterial load (i.e., Ea) (14).

Conceptualizing the left ventricle as a closed volume, Ees represents the necessary intracavitary pressure to increase its volume by one unit (18).

***Time varying LV elastance [E(t)]**:*

The end-systolic elastance concept is intuitive because it is based on a well-defined time point in the cardiac cycle. However, a pressure–volume relationship exists at each instant during the cardiac cycle, giving rise to the concept of the "time-varying" elastance. This model describes the ventricle as a spring that actively increases its stiffness during systole (with a maximum at the end of the systole) and decreases it with the onset of diastole. Therefore, the slopes formed by joining the instantaneous pressure–volume points that occur at similar times during the cardiac cycle in different beats ("isochrones"), increases during systole and becomes steepest toward the end of the systole (red arrow), to then decrease during diastole (green arrow) (19,22) (Fig. 12).

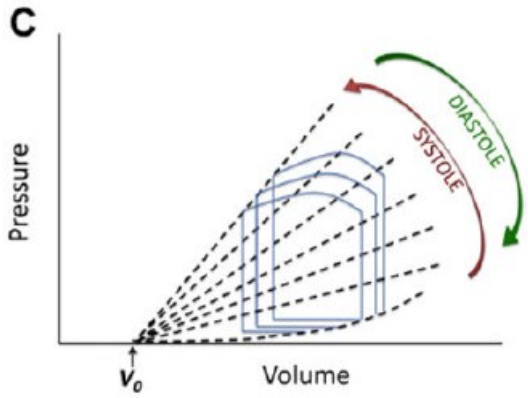

Figure 12: Time varying elastance. The ventricle behaves as a spring increasing its stiffness during systole and decreasing during diastole (22).

Ventricular energetics

Left ventricle PV plot can also provide information about cardiac energetics. For this purpose, it is useful to study the pressure-volume area (PVA) (19) (Fig. 13).

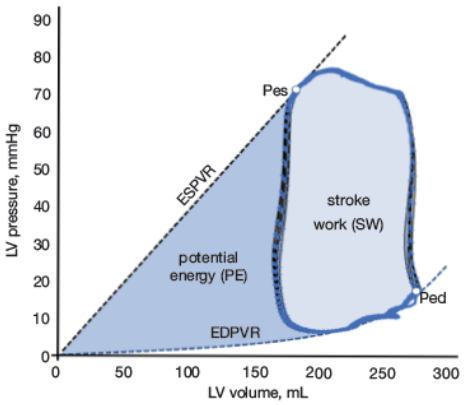

Figure 13: P-V loop representing cardiac energetics. Pressure volume area (PVA) is the result of the stroke work (SW) and potential energy (PE) together and represents the total mechanical energy. ESPVR: end systolic P-V relation; ESDPVR: end diastolic P-V relation (19)

The PVA represents the total mechanical energy generated during systole for a given contractility and loading condition (19,22). It is bounded by:

- The end-systolic P-V relation
- The end-diastolic P-V relation and,
- The systolic trajectory of the pressure-volume loop.

PVA has two components, 1) the LV P-V loop trajectory and 2) the triangled area circumscribed by the following sides (22):

- End-systolic pressure volume relation (ESPVR)
- End-diastolic pressure volume relation (EDPVR)

- Isovolumetric relaxation phase of the PV loop

The first mentioned component defines the ***stroke work (SW)*** and the latter the ***potential energy (PE)***.

SW (the area within the pressure-volume loop trajectory) (light blue in Fig. 13) represents the useful fraction of ventricular energy that is delivered to the arterial system for maintaining forward blood flow and providing adequate transport of oxygen and nutrients to peripheral organs (19).

On the other hand, PE (triangled area in dark blue in Fig. 13) characterizes the ventricular energy that is dissipated as heat during isovolumetric relaxation (19).

PVA has been demonstrated to correlate linearly with myocardial oxygen consumption (MVO_2): higher mechanical energy consumes more myocardial oxygen (22).

The concept of a system's efficiency, understood as the ratio of the energy delivered by a system to the energy supplied to it (23), can also be calculated for the LV. In this case, the **LV efficiency** corresponds to the ratio between the useful ventricular mechanical work (SW) and the O_2 consumed as estimated by PVA (19,22):

$$Ventricular\ efficiency\ \approx \frac{SW}{PVA}$$

VAC and efficiency*:*

When referring to ventricular-arterial coupling energetics we must have two concepts in mind: maximal external work and mechanical efficiency.

Maximal external work is reached when effective arterial elastance and end-systolic elastance are equal (VAC=1) (24).

Conversely, **maximal mechanical efficiency** (defined as the ratio between mechanical work and myocardial oxygen consumption per beat) is maximal when the resistance opposed by the arterial system is half of that of the cardiac output (VAC=0.5) (15,24). In this case, it is said that the heart is working efficiently because it manages to produce the maximum possible stroke work with the minimum energy consumption (15). In a normal healthy heart, VAC is set towards maximal mechanical efficiency, that corresponds to a normal ejection fraction of 60% (15). On the contrary, progressive ventricular dysfunction results in the maximization of external work at the expense of mechanical efficiency (24).

Methods to assess VAC

Although the gold standard method for estimating Ees is the **multibeat invasive method** by intraventricular catheterization (12), the **Echocardiography/doppler single-beat method developed by Chen** and coworkers is, up to date, the gold standard for the determination of Ea/Ees ratio (13).

According to this method, Ees can be calculated noninvasively by the formula (18):

$$\mathbf{Ees} = (\mathrm{DBP} - (\mathrm{End(est)} \times \mathrm{SBP} \times 0.9))/\mathrm{End(est)} \times \mathrm{SV}$$

where DBP and SBP are diastolic and systolic arm-cuff blood pressures, End(est) is the estimated normalized ventricular elastance at the onset of ejection, and SV is Doppler-derived stroke volume. End(est) is described by an apparently very complicated formula:

$$\mathrm{End(est)} = 0.0275 - 0.165 \times \mathrm{EF} + 0.3656 \times (\mathrm{DBP/SBP} \times 0.9) + 0.515 \times \mathrm{End(avg)},$$

where EF is the basal ejection fraction and End(avg) is derived by the following formula:

$$End(avg)= 0.35695 - 7.2266 \times tNd + 74.249 \times tNd2 - 307.39 \times tNd3 + 684.54 \times tNd4 - 856.92 \times tNd5 + 571.95 \times tNd6 - 159.1 \times tNd7$$

where tNd is the ratio of preejection period to total systolic period.

Similarly, the elastance of the arterial system can be numerically expressed as Ea, using the following formula:

$$\mathbf{Ea} = (SBP \times 0.9)/SV$$

In this way, being Ees and Ea expressed in the same units, the calculation of Ea/Ees ratio is correctly feasible (13).

Because the formulas are relatively complex, they have been implemented in clinical settings as computerized algorithms, or more lately a calculator, which was specifically designed to allow Chen's algorithm to be deployed at the bedside (iElastance_ - Apple iOS App). The operator must enter simple noninvasive parameters that can be collected easily: systolic and diastolic blood pressures, stroke volume, ejection fraction, pre-ejection, and total systolic periods. This has become the most widely used method because of the software and app's ease of-use, and the need for only simple echocardiographic parameters that do not require an updated echo-machine (25). Even though the latter is true, it is recommended using three-dimensional echocardiography or magnetic resonance imaging over two-dimensional echocardiography to measure LV volumes, included in the aforementioned VAC estimation formulas, because more accurate (18).

The overall advantage of this method is that the non-invasive method is applicable for repeated consecutive studies of VAC, e.g., before and after treatment in daily clinical practice (18).

VAC assessment limitations:

Although the assessment of VA coupling with the Ea/Ees framework has proved its usefulness for the understanding of several physiological and pathophysiological processes, it also has a few limitations. Ea in this case is not very accurate because in order to relate Ea/Ees, Ea and Ees must have the same units. This is the reason why Ea has been simplified using the arterial system 3-element windkessel model. This model is a surrogate of the aortic impedance and although it characterizes many of the main features of the arterial impedance, it fails to reproduce the influence of arterial wave reflections and the arterial wave propagation phenomenon (19). Moreover, since Ea gathers all the components of the Windkessel model into one single number, it does not inform about their relative contribution. Therefore, Ea cannot replace the arterial input impedance (which represents the best description of the ventricular afterload) and it should only be used as an integrative measure of the arterial load for assessing VA coupling (19).

The other limitation is related to the slope calculated from the end-systolic PV relation (ESPVR). Experimental studies in isolated heart preparations have shown that the ESPVR is linear, and this assumption is valid in a range of physiological contractile and afterload states in intact cardiovascular systems. But in extreme conditions, such relation tends to be curvilinear doe to length-dependent activation of the myofilaments (19).

Furthermore, Ees does not only vary with changing in contractility but is also influenced by the stiffness of the myocardium secondary to aging or LV hypertrophy doe to arterial hypertension. This suggests that a chronically altered Ees could represent combined effects of contractility and changes of the geometric and structural properties of the ventricle (19).

Finally, in some diseases (e.g., heart failure, hypertension, inflammatory disease, etc), Ea and Ees are simultaneously impaired. The consequence is that the relation Ea/Ees will have an absolute vale of about 1, indicating a normal ratio. Therefore, the importance of evaluating each component separately (18).

Thus, a consensus document emitted by the European Society of Cardiology proposed the simultaneous measurement of arterial and myocardial function markers to provide a more accurate estimation of VAC and its changes in disease or after treatment (18).

Markers of arterial function include, of large vessels: aortic characteristic impedance (Zc), aortic distensibility, beta stiffness index, and large artery stiffness (estimated by aortic pulse wave velocity); of small vessels: central BP and pulse pressure, indices of wave reflection (e.g., augmentation index), brachial-ankle PWV, and total arterial compliance (18). On the other hand, novel markers of myocardial performance are tissue Doppler imaging, two-dimensional and recently three-dimensional speckle tracking which allow the evaluation of myocardial deformation in systole and diastole (18).

TRANSPLANTED HEART HEMODYNAMICS AND VAC

It is reasonable to think that adaptation of a new heart to a previously existent arterial system might not be perfect. There are many factors that influence this interaction and literature can be contradictory. Ventricular-arterial coupling allows somehow to study this cross-talk.

Arterial elastance and heart transplant

One of the main factors that alter arterial elastance in post-heart transplanted patients is arterial hypertension. It is present in about 50-95% of this population and is mostly related to immunosuppressive drugs such as calcineurin inhibitors because of its direct effect on arteries and its association to renal insufficiency (10).

Loss of arterial elasticity due to aging is also significant because most patients receiving heart transplants are of a certain age (7,26). Immunosuppressive drugs may also lead to other conditions like diabetes mellitus (DM), nephropathy and dyslipidemia, important comorbidities that converge on the development of arterial hypertension (10,26). As a matter of fact, De Souza-Neto in their study described DM as the most important factor associated with increased arterial stiffness in transplanted heart patients (26).

Milani et al. refer that even though post-transplant hypertension and vascular age play an important role in decreasing arterial compliance it does not fully explain this phenomenon because arterial stiffness remains high regardless blood pressure control (27).

Arterial stiffening is an independent predictor of cardiovascular events, all-cause mortality and has been demonstrated to be associated to graft vascular disease (GVD, an important death risk factor after 1-year post-transplantation). Hypertension is strongly associated with this loss of compliance and is an independent factor for its development (26). Patients with arterial stiffening manifest with high average diastolic blood pressure and mild nocturnal blood pressure drop (10,26).

Left ventricle responds to this increased afterload with concentric remodeling (27,28), in fact, transplanted patients exhibit twice more concentric remodeling compared to uncomplicated hypertensive patients (27).

On the contrary of what was said before, Xavier-bichart et al. in 1997, found arterial effective elastance (Ea) to remain in normal ranges while Ees was decreased in transplanted patients. The result was a reduced Ea/Ees relation (24).

End-systolic elastance and heart transplant

When it comes to end-systolic elastance, thus contractility, literatures seem very heterogenous. Some studies support the idea that contractility of the LV is conserved after transplant, others suggest otherwise.

Von Sheidt et al., reached to the conclusion that contractility does not decrease after transplantation despite denervation. Therefore, contractility is considered an intrinsic property of the heart, independent of autonomic neural control. Just a limited number of patients (12%) had mildly impaired contractility but was neither associated with structural myocardial or coronary changes, nor with rejection episodes or graft ischemic time. Susception on why of this impairment leads to think that it could be due to damage of the donor heart previous transplantation secondary to toxic effects of catecholamines or inadequate supportive measure after brain death (29).

Similarly, Borow et al., demonstrated that chronically denervated transplanted hearts, without signs of rejection on myocardial biopsy, had normal contractility and contractile reserve (28).

In pro of an impaired contraction is the study conducted by Sagiv et al. They studied the contractility of transplanted heart recipients while doing isometric exercises. Isometric exercise differs from aerobic exercise because contraction of muscles creates obstruction of muscular arteries causing pressure overload, the final effect on the cardiovascular (CV) system is a slight elevation in cardiac output and moderate increase in heart rate. Transplanted heart patient seemed to have impaired contraction because ejection fraction (EF) and left-ventricular pressure/volume ratio were significantly lower previously and during isometric exercise compared with control group (30).

Others sustain that denervation has a negative influence on LV because the release of local norepinephrine from intact sympathetic nerve terminals is required to achieve maximal expected inotropy (31).

Heart rate and heart transplant

Transplanted heart lacks vagal innervation, therefore, heart rates are usually elevated at base and the ability to increase heart rate during exercise is initially limited. Initial increases in cardiac output completely depends on augmented preload and Frank-Starling mechanism. Increases in heart rate during later phases of exercise rely on an increase in circulating catecholamines (31).

A few studies describe a return of normal chronotropic response to exercise in some heart transplanted patients after a period of 1-2 years, suggesting some degree of reinnervation (31).

Cardiac efficiency and heart transplant

Mehra and colleagues' investigation indicated that transplanted hearts had poor contractile efficiency and operated at maximal left ventricular work. As an expression to this ventricular-arterial uncoupling they found increased levels of B-type natriuretic peptide (BNP), independent of alteration in blood pressure (32).

According to the study conducted by Xavier-Bichat et al. transplanted hearts had low work efficiency (Effective work/P-V area) but high energy conversion efficiency (PVA/MVO2) and mechanical efficiency (Effective work/MVO2). This means that the increase in energy conversion efficiency compensated for the decrease in work efficiency allowing a normal mechanical efficiency (24).

OBJECTIVES

A usual assessment performed in post-transplanted heart patients is the left ventricle ejection fraction (EF). EF represents the pump function of the heart and when its value is out of normal parameters it is well known to predict a poorer outcome. The struggle arises in patients with normal EF. In the wide range of normal EF values, patients may have different outcomes, and it is in these patients that we want to study other cardiac properties (such as VAC and its components) that will allow us to create a more sensible mortality predictive model. VAC is an interesting parameter to study in these patients because it helps to explain how well the new heart adapts to the recipient's afterload, considering that the heart has been exposed to particular conditions of pressure and volume during the time spent in the donor's body.

On the other hand, since VAC is a ratio, it loses its ability to discriminate a high VAC due to high arterial afterload or a low ventricular contractility. In this sense, we aim to study Ea and Ees separately to gain further information on the specific influence that these parameters have on ventricular performance.

Considering this background, our research has been directed toward two objectives:

- Create a statistical model to predict mortality using clinical, echocardiographic, ventricular- arterial coupling and its derived parameters as variables, in transplanted heart patients from Padua Hospital in years between 1985 to 2015.

- Identify the determinants that modify ventricular-arterial coupling and its components in these transplanted heart patients.

MATERIALS AND METHODS

This is a retrospective longitudinal study. We selected heart transplanted patients from Padua Hospital during the years 1985-2015 that presented the following characteristics:

- Had survived first year after transplantation
- Had normal ejection fraction (EF)
- Did not present cardiac allograft vasculopathy (CAV) on coronarography at the time of study
- Did not present signs of graft rejection by endomyocardial biopsy

Clinical, echocardiographic, VAC and pressure-volume derived parameters were gathered 1 year after transplantation and compared to a control group of healthy subjects. The same parameters were then measured during a 30-year follow-up until death by cardiovascular causes.

***Clinical parameters**:*

Anamnestic data regarding age, age of transplantation, gender, diabetes, hypertension, hypercholesterolemia, BMI, ischemic heart disease, CKD, presence of left ventricle assistant device, pericardial effusion, and current cardiovascular therapies were gathered.

***Echocardiographic parameters**:*

Left ventricular dimension and wall thickness, end-systolic volume (ESV), end-diastolic volume (EDV), stroke volume (SV), and left ventricular mass index were recorded. LVEF was measured using a modified Simpson's biplane method. Pulsed-wave Doppler parameters included transmitral peak rapid filling and atrial velocity (E and A), deceleration time, and E/A ratio. Using tissue Doppler imaging

measurements, peak systolic (s', an index of global systolic function) and early and late diastolic velocities at the septal mitral annulus (e' and a', respectively) were recorded, and the E/e' ratio were calculated. Systolic and diastolic blood pressures and heart rate were also recorded.

VAC and pressure-volume derived parameters:

To quantify ventricular contractility noninvasively, we calculated Ees as ESP divided by ESV. For arterial elastance, Ea was the ratio of ESP to SV, and VAC was defined as the ratio of Ea to Ees. For these equations, ESV and SV were obtained from echocardiographic results. ESP was defined as 0.9 x systolic blood pressure determined by noninvasive blood pressure measurement at the same time as echocardiographic examination. End-diastolic elastance (Eed) was the ratio of left ventricular end-diastolic pressure (EDP) to EDV. We estimated EDP with a formula using the E/e' ratio (11.96 + 0.596 x E/e'). We estimated mechanical energy including stroke work (SW), potential energy (PE), pressure-volume area (PVA), and left ventricular mechanical efficiency. In Table II shows a summary pressure-volume derived parameters calculation.

Table II: Pressure-volume derived parameters and its calculation

PV derived parameters	Calculation
End-systolic pressure (ESP)	= systolic blood pressure x 0.9
Arterial elastance (Ea)	= ESP/stroke volume (SV)
End-systolic LV elastance (Ees)	= ESP/end-systolic volume (ESV)
End-diastolic LV elastance (Eed)	= EDP/end-diastolic volume (EDV)
Ventricular-arterial coupling (VAC)	= Ea/Ees
Stroke work (SW)	= ESP x SV
Potential energy (PE)	= ESP x ESV/2
Pressure-volume area (PVA)	= PE + SW
LV work efficiency	= 100 x SW/PVA

Coronary angiography:

Coronary angiography was used to evaluate the presence, onset, and development of cardiac allograft vasculopathy (CAV). It is part of the routine assessments performed in post-transplanted patients and is done close after surgery, at one year, at 2 years, at 3 years and then every 2 years. Coronary angiographies were analyzed by a hemodynamic cardiologist. The CAV was defined and classified according to the standard of International Society for Heart and Lung Transplantation (ISHLT) criteria. Maximum stenosis at the common trunk primary vessels and secondary branches were assessed. Hemodynamic evaluation must be correlated to graft function assessed by imaging.

Endomyocardial Biopsy and Rejection Score*:*

Monitoring of acute rejection was performed by endomyocardial biopsies according to established protocols (once a week in the first month, once every two weeks until the third month, once a month until the first year; in the presence of a grade 2 rejection, the biopsy was performed again after 10-15 days). No biopsies were performed after the first year, except in cases of suspicion of a clinical rejection. A rejection score was assigned based on a modified version of that of ISHLT: 1A = 1, 1B = 2, 2 = 3, 3A = 4, 3B = 5, 4 = 6. It was then calculated, for each patient, the following parameters: rejection score at 1 year (RS 1year), severe rejection score at 1 year (sev RS), total rejection score (TRS) and total severe (grade ≥3A) rejection score (Sev TRS). The score was normalized by dividing the sum of the scores by the number of biopsies performed in the single patient.

Statistical analysis*:*

Numeric variables with normal distribution were expressed as mean ± standard deviation (SD), numeric variables with non-normal distribution were expressed as median ± interquartile, and categorical variables were expressed as percentage frequencies. Comparison of means was performed by Student's t test for

independent samples, comparison of medians was performed by Mann Whitney or Wilcoxon test, and frequency comparison was performed by Chi-square test or Fisher's exact test. The Levene test was used to determine the equality of the variances of the comparison groups. Correlations between variables were analyzed by Pearson's correlation index or Spearman's rank correlation index, depending on whether the distribution was normal or non-normal, respectively. The determinants of dichotomous parameters were identified by univariate and multivariate logistic regression analyzes. The determinants of parameters intended as continuous values were identified by univariate and multivariate linear regression analyzes. Survival predictors were analyzed with univariate and multivariate Cox analyzes. The multivariate analysis was performed in a *backward* manner and the variables found to be significant in the univariate analysis were included in the model. For the multivariate analysis, the analysis of ROC curves was used to evaluate the effectiveness of the prediction.

The results were considered statistically significant for a p under 0.05. Statistical analysis was conducted with SPSS software version 24.0 (Chicago, SPSS, Inc., Chicago, Illinois).

RESULTS

Our population consisted of 345 transplanted heart patients with normal ejection fraction and no signs of graft rejection at the time of study. Echocardiographic measurements were made 1-year after transplantation and then subsequent follow-ups were carried out until death. Our control group involved 100 healthy adults.

Echocardiographic parameters between healthy controls and heart transplanted patients:

Left ventricular diastolic and systolic dimension, wall thickness, and mass index were higher in patients than in controls ($p < 0.0001$). ESV, EDV, SV, and EF were also significantly higher in patients than in controls ($p < 0.0001$). All measurements from transmitral flow and tissue Doppler imaging were significantly higher in patients than in controls ($p < 0.0001$). See Table III

Table III: Echocardiographic parameters of controls and transplanted patients 1-year after surgery:

	Controls (n=100)	**HT patients (n=345)**	***p* value**
LVEDD (mm)	48 (43-50)	25 (24-27)	**<0.0001**
LVESD (mm)	31 (29-34)	14 (13-16)	**<0.0001**
LVPWT (mm)	8.3 (8-9.2)	11.1 (1.1-13.1)	**<0.0001**
IVSWT (mm)	8.4 (8.1-9.3)	11 (10-12.1)	**<0.0001**
LV mass index (g/m^2)	81 (74-86)	95 (79-117)	**<0.0001**
EDV (ml)	108 (87-114)	86 (73-104)	**<0.0001**
ESV (ml)	40 (32-50)	32 (25-40)	**<0.0001**
SV (ml)	61 (52-80)	55 (45-64)	**<0.0001**
EF (%)	60 (57-61)	62 (58-67)	**<0.0001**
Peak E velocity (cm/s)	62 (54-68)	79 (56-90)	**<0.0001**

Peak A velocity (cm/s)	58 (51-58)	48 (39-60)	**<0.0001**
DT (ms)	201 (191-220)	165 (140-198)	**<0.0001**
e' (cm/s)	7.2 (7-8.6)	11 (8-13)	**<0.0001**
a' (cm/s)	8.6 (8.2-9.4)	7 (6-8)	**<0.0001**
s' (cm/s)	7.5 (7.1-8)	8.8 (7.8-9.4)	**<0.0001**
E/A	1.12 (0.9-1.22)	1.44 (1.13-2.02)	**<0.0001**
E/e'	6.4 (6-7.2)	7.4 (5.8-10.4)	**<0.0001**

Data are presented as median (25^{th}–75^{th} percentiles). LVEDD: Left ventricular end diastolic diameter. LVESD: Left ventricular end systolic diameter. LVPWT: Left ventricular posterior wall thickness. IVSWT: Interventricular septal wall thickness. LV mass index: Left ventricle mass index. EDV: End diastolic volume. ESD: End systolic volume. SV: Stroke volume. EF: Ejection fraction. DT: Deceleration time. e': Early diastolic mitral annular tissue velocity. a': Late diastolic annular tissue velocity. s': Systolic tissue velocity. E/A: Early to late diastolic transmitral flow velocity. E/e': LV pressure filling.

***Pressure-volume curve derived parameters between healthy controls and transplanted heart patients**:*

Both arterial elastance (Ea) and LV end-systolic elastance (Ees) were significantly higher ($p < 0.0001$) in transplanted heart patients compared to healthy control group, as seen in Table IV. Since VAC is a ratio, the simultaneous increase in Ea and Ees lead to no difference in VAC ($p = 0.7$). When studying mechanical energy exerted by the left ventricle, stroke work (SW), potential energy (PE) and pressure-volume area (PVA) were all increased ($p < 0.0001$) while efficiency showed no difference ($p = 0.4$). End-diastolic elastance (Eed) was also found increased ($p < 0.0001$).

Table IV: Pressure-volume-derived parameters in controls and transplanted patients 1-year after surgery:

	Controls (n=100)	**HT patients (n=345)**	***p* value**
E_a (mmHg/ml)	1.65 (1.31-2.07)	3.89 (3.24-4.61)	**<0.0001**
E_{es} (mmHg/ml)	2.47 (1.86-3.23)	6.01 (4.86-7.74)	**<0.0001**
VA coupling	0.66 (0.42-0.73)	0.64 (0.54-0.71)	0.7
SW (mmHg.ml)	6336 (5616-8118)	3676 (3039-4452)	**<0.0001**
PE (mmHg.ml)	2070 (1620-2376)	1172 (930-1421)	**<0.0001**
PVA (mmHg. ml)	8352 (7371-9936)	4807 (3997-6036)	**<0.0001**
Efficiency (%)	75 (73-82)	75 (73-78)	0.4
E_{ed} (mmHg/ml)	0.14 (0.13-0.19)	0.33 (0.26-0.40)	**<0.0001**

Data are presented as median (25^{th}–75^{th} percentiles). Ea: Effective arterial elastance. Ees: End-systolic elastance. VA: Ventricular-arterial. SW: Stroke work. PE: Potential energy. PVA: Pressure volume area. Eed: End-diastolic elastance.

***Clinical variables as mortality predictors**:*

We studied the following clinical parameters seen in Table V in transplanted heart patients that had survived or died after at least the first-year post-transplantation. Almost 86% of non-survivors were men (p= 0.008). Patients receiving male hearts and higher donors age were also significant in non-survivors population (p= 0.03). Total rejection score (p=0.02), rejection score in the first year (p<0.001), severe

rejection score (p<0.001) and sever rejection score in the first year (p<0.001) were also significant. The other parameters were non-significant between those who survived or died.

Table V: Clinical parameters in heart transplanted patients who survived or died 1 year after transplantation:

	Survivors (n=286)	**Non-survivors (n=59)**	***p* value**
Time from HT (years)	1 (0.5-2)	1 (0.5-2)	0.2
Male recipient gender, n (%)	83 (70.3)	96 (85.7)	**0.008**
Age at HT (years)	50 ± 16	48 ± 17	0.3
Ischemic time (min)	18 ± 59	18 ± 57	0.9
Recipient BMI (Kg/m^2)	24 ± 4	24 ± 4	0.9
Diabetes, n (%)	24 (20.3)	24 (21.4)	0.5
Hypertension, n (%)	75 (63.5)	66 (60)	0.4
Hyercholesterolemia, n (%)	36 (30.5)	39(34.8)	0.8
Obesity, n (%)	14 (11.8)	12 (10.7)	0.9
Male donor gender, n (%)	66 (55.9)	78 (69.6)	**0.03**
Donor age (years)	33 ± 14	35 ± 16	**0.03**
Donor BMI (Kg/m^2)	24 ± 3	24 ± 3	0.8
GFR (ml/min/1.73m^2)	43 (35-50)	41 (20-61)	0.2
Total rejection score	0.60 (0.28-0.82)	0.73 (0.70-.)	**0.02**
Rejection score 1st year	0.67 (0.37-0.92)	0.77 (0.72-.)	**<0.001**
Severe rejection score	0.00 (0.00-0.32)	0.40 (0.26-.)	**<0.001**
Severe rejection score 1st year	0.00 (0.00-0.33)	0.44 (0.30-.)	**<0.001**

Data are presented as n (%), mean ± standard deviation or median (25th–75th percentiles). HT: Heart transplant. BMI: Body mass index. GFR: Glomerular filtration rate.

When confronting these clinical data with VAC divided by tertiles, we observe that transplanted male patients (p= 0.008), male donated hearts (p= 0.03) and donor age (p= 0.03) presented higher VAC values (present in highest VAC tertile with VAC >0.66), as seen in Table VI.

Table VI: Clinical parameters by tertiles of ventricle-arterial coupling 1-year after transplantation:

	Lowest tertile (≤0.52) (n=115)	**Middle tertile (0.52-0.66) (n=118)**	**Highest tertile (>0.66) (n=112)**	***p* value**
Time from HT (years)	2 (1-3)	1 (0.5-2)	1 (0.5-2)	0.2
Male recipient gender, n (%)	81 (70.4)	83 (70.3)	96 (85.7)	**0.008**
Age at HT (years)	50 ± 16	50 ± 16	48 ± 17	0.3
Ischemic time (min)	183 ± 54	18 ± 59	184 ± 57	0.9
Recipient BMI (Kg/m^2)	24 ± 4	24 ± 4	24 ± 4	0.9
Diabetes, n (%)	18 (15.6)	24 (20.3)	24 (21.4)	0.5
Hypertension, n (%)	77 (70)	75 (63.5)	66 (60)	0.4
Hyercholesterolemia, n (%)	38 (33)	36 (30.5)	39(34.8)	0.8
Obesity, n (%)	12 (10.4)	14 (11.8)	12 (10.7)	0.9
Male donor gender, n (%)	62 (53.9)	66 (55.9)	78 (69.6)	**0.03**
Donor age (years)	38 ± 14	33 ± 14	35 ± 16	**0.03**
Donor BMI (Kg/m^2)	24 ± 3	24 ± 3	24 ± 3	0.8
GFR (ml/min/1.73m^2)	73 (48-81)	43 (35-50)	41 (20-61)	0.2

Data are presented as n (%), mean ± standard deviation or median (25^{th}–75^{th} percentiles). HT: Heart transplant. BMI: Body mass index. GFR: Glomerular filtration rate.

***Echocardiographic variables as mortality predictors**:*

Echocardiographic data from cardiac transplanted patients who survived 1-year post surgery and those who didn't survive show that the later presented higher EDV (49ml vs. 54ml.), ESV (18ml vs. 23ml) (p=0.0001) and interventricular septal wall thickness (1.15mm vs. 1.40mm) (p=0.04). Other parameters showed no statistical significance (see Table VII).

Table VII: Echocardiographic parameters in heart transplanted patients who survived or died 1 year after transplantation:

	Survivors (n=286)	**Non-survivors (n=59)**	***p* value**
LVPWT (mm)	1.05 (0.90-1.20)	1.30 (1.30-.)	0.06
IVSWT (mm)	1.15 (1.00-1.30)	1.40 (1.20-.)	**0.04**
EDV (ml)	49 (42-55)	54 (45-64)	**<0.0001**
ESV (ml)	18 (16-19)	23 (19-28)	**<0.0001**
EF (%)	61 (58-64.25)	68 (50- .)	0.9
E/A	1.58 (1.09-2.40)	2.10 (1.60-.)	0.9
E/e'	7.14 (6.04-10.65)	7.50 (4.63-.)	0.6

Data are presented as n (%), mean ± standard deviation or median (25^{th}–75^{th} percentiles). LVEDD: Left ventricular end diastolic diameter. LVESD: Left ventricular end systolic diameter. LVPWT: Left ventricular posterior wall thickness. IVSWT: Interventricular septal wall thickness. LV mass index: Left ventricle mass index. EDV: End diastolic volume. ESD: End systolic volume. SV: Stroke volume. EF: Ejection fraction. DT: Deceleration time. e': Early diastolic mitral annular tissue velocity. a': Late diastolic annular tissue velocity. s': Systolic tissue velocity. E/A: Early to late diastolic transmitral flow velocity. E/e': LV pressure filling.

When doing a comparison of these echocardiographic parameters with VAC divided per tertiles, we see that, patients with higher EDV and ESV where those

who had higher values of VAC (highest tertile, VAC= >0.66) (p=<0.0001). See Table VIII.

Table VIII: Echocardiographic parameters by tertiles of ventricle-arterial coupling 1-year after transplantation:

	Lowest tertile (≤0.52) (n=115)	**Middle tertile (0.52-0.66) (n=118)**	**Highest tertile (>0.66) (n=112)**	***p* value**
EDV (ml)	44 (35-58)	49 (42-55) ^	54 (45-64)	**<0.0001**
ESV (ml)	15 (11-20)	18 (16-19)*	23 (19-28) †	**<0.0001**

Data are presented as median (25^{th}–75^{th} percentiles). LVEDD: Left ventricular end diastolic diameter. LVESD: Left ventricular end systolic diameter. LVPWT: Left ventricular posterior wall thickness. IVSWT: Interventricular septal wall thickness. LV mass index: Left ventricle mass index. EDV: End diastolic volume. ESD: End systolic volume. SV: Stroke volume. EF: Ejection fraction. DT: Deceleration time. e': Early diastolic mitral annular tissue velocity. a': Late diastolic annular tissue velocity. s': Systolic tissue velocity. E/A: Early to late diastolic transmitral flow velocity. E/e': LV pressure filling.

* p <0.0001 vs. the lowest tertile of ventriculo-arterial coupling.

^ p <0.001 vs. the lowest tertile of ventriculo-arterial coupling

†p <0.0001 vs. middle tertile of ventriculo-arterial coupling

VAC, Ea, Ees and ventricular mechanic energetics in transplanted patients:

We compared VAC components and LV energetics using VAC as a dichotomic variable according to its median value as, coupled when VAC was ≤0.59 or uncoupled when >0.59. From this analysis we can deduce that VA uncoupling, in heart transplanted patients after 1 year from surgery, is predominantly caused by a

lower Ees (p<0.0001) while Ea had no statistical significance (p= 0.1). VA coupling was significantly higher (p <0.0001), see Table IX.

Patients with VA uncoupling presented higher SW and PE, and lower efficiency (p< 0.0001). PVA showed no significant difference (p= 0.6).

Table IX: Pressure-volume-derived parameters by ventriculo-arterial coupling 1-year after transplantation:

	VA coupling (≤0.59) (n=171)	**VA uncoupling (>0.59) (n=174)**	***p* value**
E_a (mmHg/ml)	2.03 (1.75-2.41)	2.18 (1.82-2.76)	0.1
E_{es} (mmHg/ml)	4.21 (3.54-5.03)	3.14 (2.41-3.92)	**<0.0001**
VA coupling	0.50 (0.44-0.55)	0.70 (0.64-0.76)	**<0.0001**
SW (mmHg.ml)	6806 (5564-7817)	6815 (5083-8301)	**0.007**
PE (mmHg.ml)	1638 (1274-1993)	2379 (1742-3034)	**<0.0001**
PVA (mmHg.ml)	8314 (6968-9577)	9040 (6918-10933)	0.6
Efficiency (%)	80 (78-81)	74 (72-75)	**<0.0001**
E_{ed} (mmHg/ml)	0.19 (0.17-0.21)	0.18 (0.13-0.21)	0.3

Data are presented median (25^{th}–75^{th} percentiles). Ea: Effective arterial elastance. Ees: End-systolic elastance. VA: Ventricular arterial. SW: Stroke work. PE: Potential energy. PVA: Pressure-volume area. Eed: End-diastolic elastance.

In this graph we plotted the relationship between Ea and Ees, the points where each intercept corresponds to VAC. We can appreciate how uncoupled hearts (red dots) gather towards the left indicating higher Ea with lower Ees (Fig. 14).

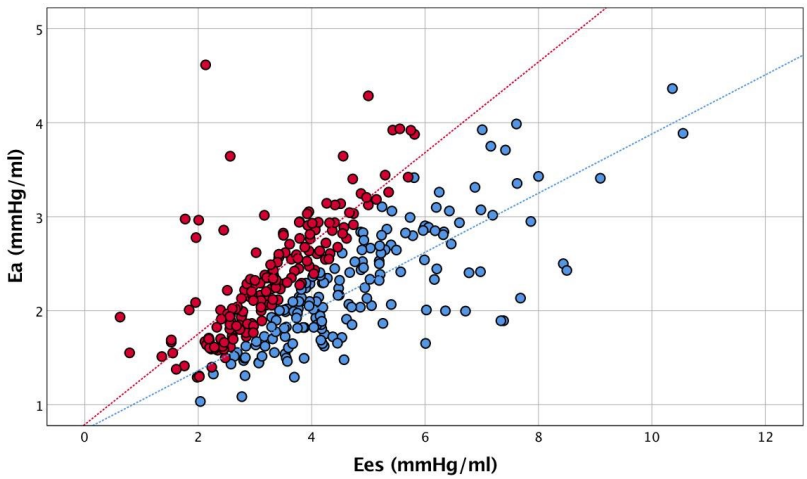

Figure 14: Relationship between afterload (Ea) and end-systolic elastance (Ees) and coupling

***Survival analysis in a 30-year follow-up**:*

The patients form the study were evaluated in a 30-year follow-up and survival was taken in account after the first year from transplantation. The data obtained was survival at 5 years of 96.6%, 10 years 93.4%, 15 years 87.1%, 20 years 74.1%, 25 years 59% and finally 30 years 38%.

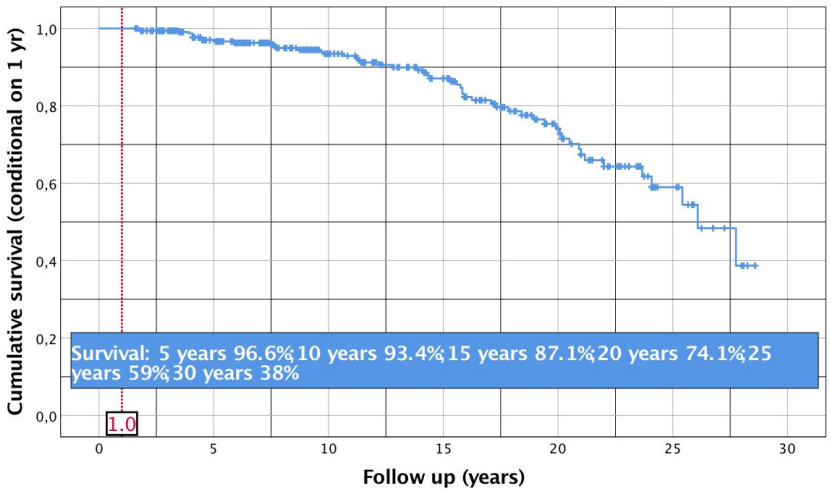

Figure 15: Cumulative survival (conditional on 1 year) in a 30-year follow-up

Survival according to VAC and its components:

Following these patients through a 30-year laps we can appreciate how transplanted hearts with VA coupling above the median (>0.59) showed lower survival (p=0.02) (Fig. 16) and a hazard ratio of 1.6 at the univariate analysis.

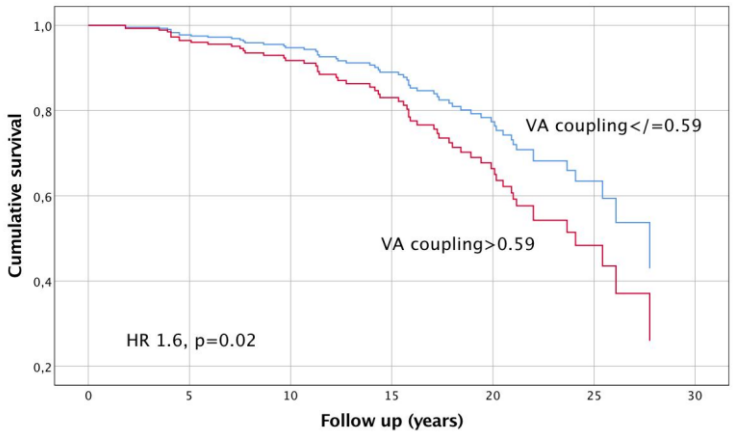

Figure 16: Cumulative survival throughout a 30-year follow-up according to VAC median

The univariate analysis of Ea and survival for a 30-year lapse, showed a lower survival for those transplanted heart patients with Ea above the median value (4mmHg/ml), p= 0.02 and HR=1.8 (Fig. 17).

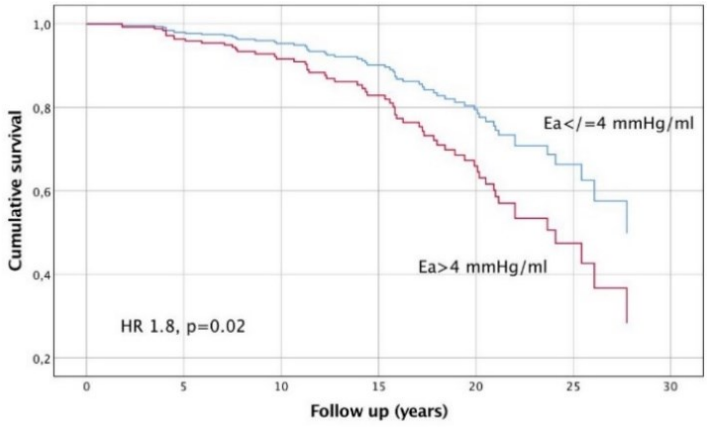

Figure 17: Cumulative survival throughout a 30-year follow-up according to Ea median

The univariate analysis of Ees and survival for a 30-year lapse, showed a lower survival for those transplanted heart patients with Ees under the median value (6.75mmHg/ml), p= 0.02 and HR=1.8 (Fig. 18).

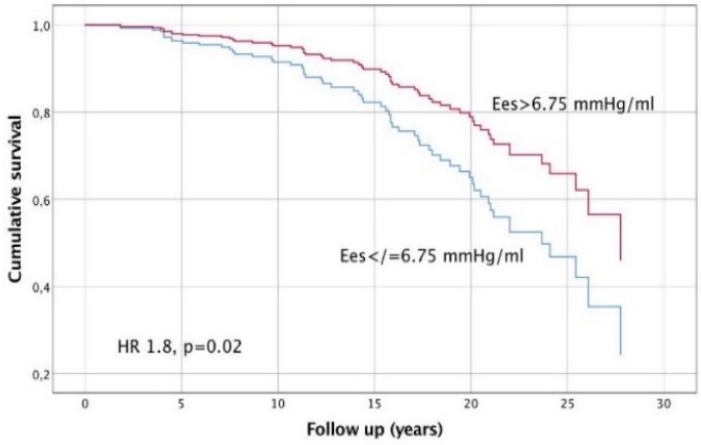

Figure 18: Cumulative survival throughout a 30-year follow-up according to Ees median

In the multivariate analysis, adjusted to other variables, VAC showed no statistical significance (p=0.06) as a long-term survival predictor (Fig. 19=.

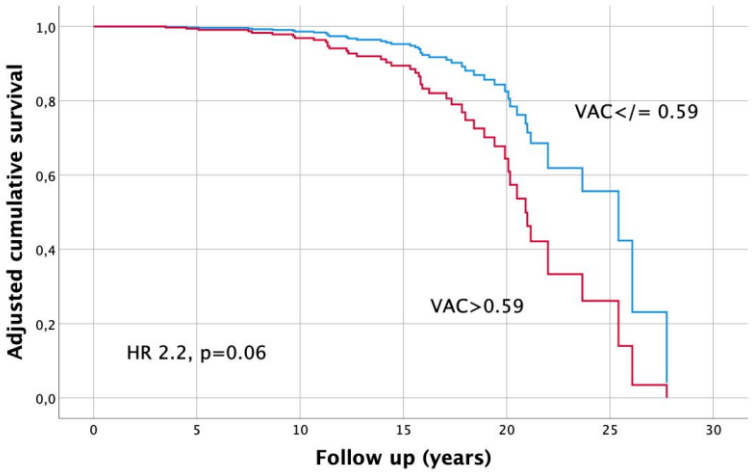

Figure 19: *Adjusted cumulative survival throughout a 30-year follow-up according to VAC*

Instead, the multivariate analysis of Ea and Ees showed that, Ea above the median (4mmHg/ml) (Fig. 20) and Ees under the median (6.75mmHg/ml) (Fig. 21) demonstrated to be independent death prognostic factors in transplanted heart patients (p= 0.02) (Ea HR= 2.01, Ees HR= 2.72).

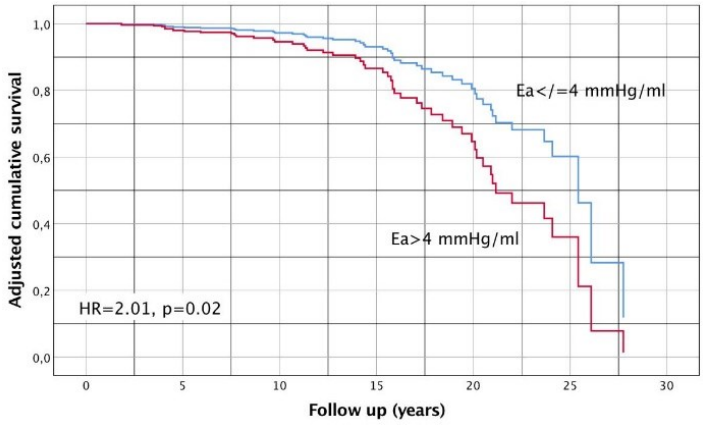

Figure 20: Adjusted cumulative survival throughout a 30-year follow-up according to Ea

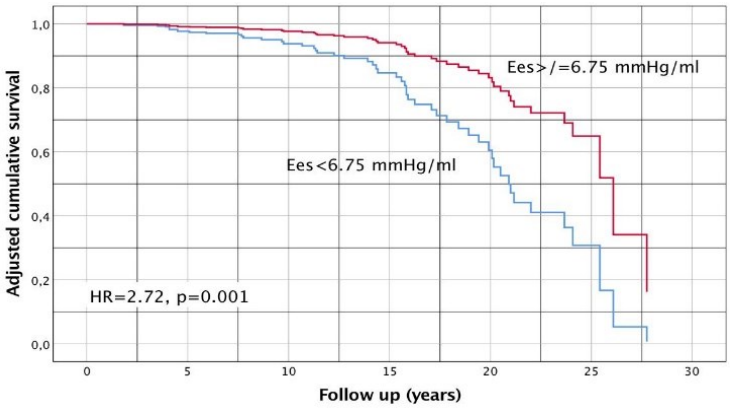

Figure 21: Adjusted cumulative survival throughout a 30-year follow-up according to Ees

***Survival and cardiac mechanical energetics**:*

Variation in SW, PE, PVA, and efficacy are not related with long term survival (Tables 22 to 25).

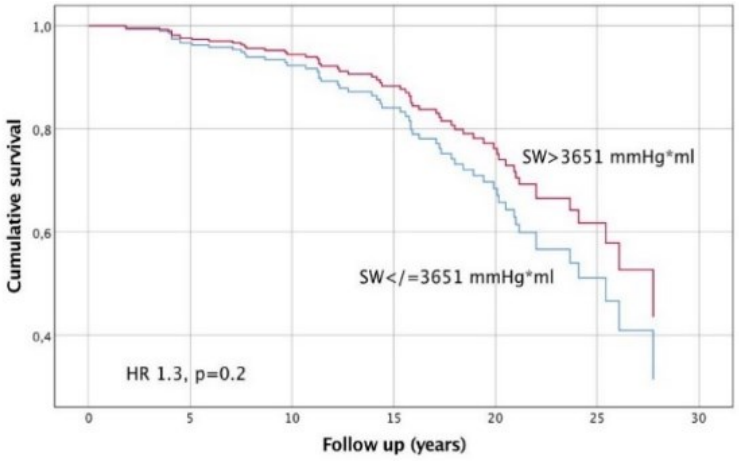

Figure 22: Cumulative survival throughout a 30-year follow-up according to stroke work

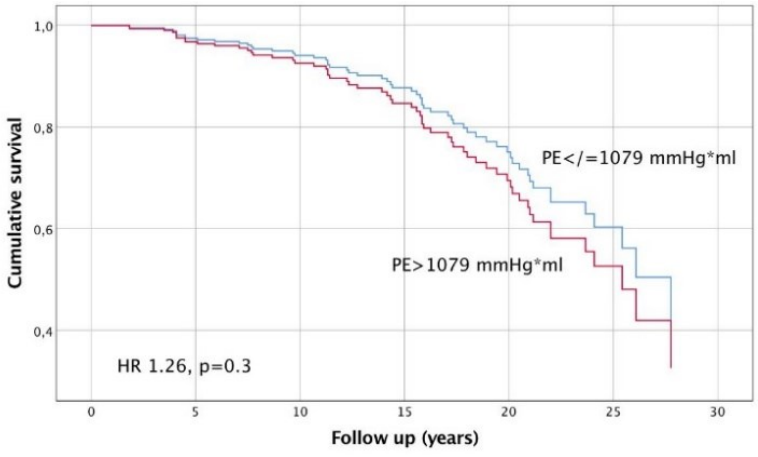

Figure 23: Cumulative survival throughout a 30-year follow-up according to potential energy

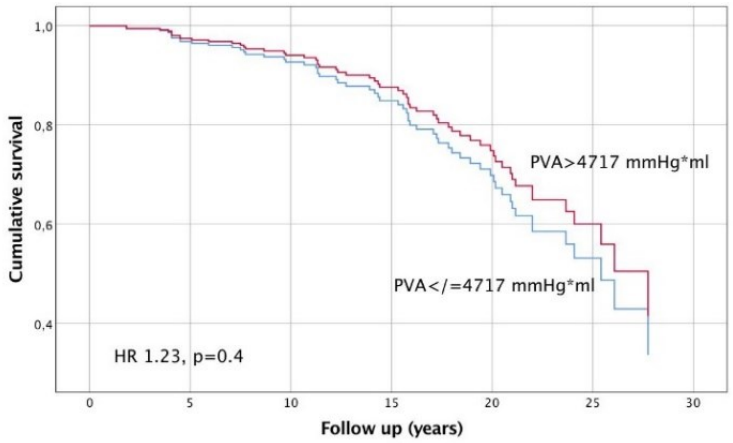

Figure 24: Cumulative survival throughout a 30-year follow-up according to pressure-volume area

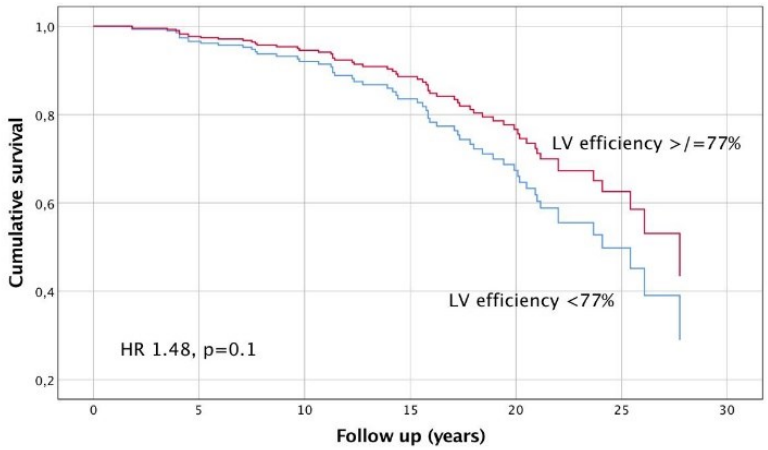

Figure 25: Cumulative survival throughout a 30-year follow-up according to LV efficiency

***Survival and LVEF**:*

Patients under and above LVEF median (62%) showed no difference in survival in our population (p= 0.1) (Fig. 26). It is to note that these patients were in normal LVEF ranges because they were selected with those characteristics for our investigation.

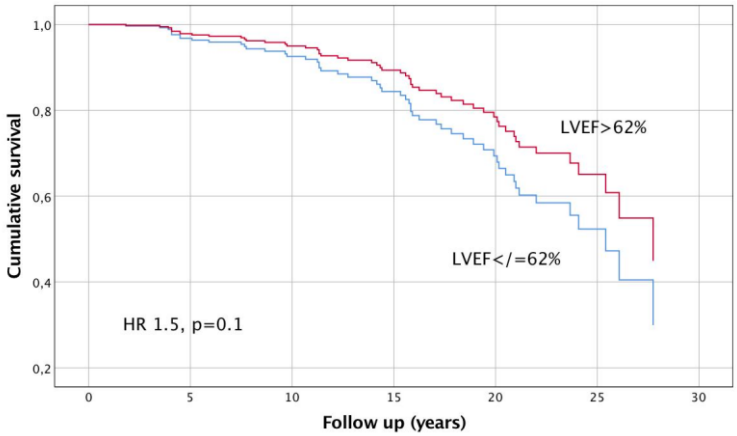

Figure 26: Cumulative survival throughout a 30-year follow-up according to LVEF

***Survival and gender**:*

Male transplanted patients presented a lower long-term survival (p= 0.04) and a HR= 2.07, as seen in Table 27. When studying in detail gender graft mismatch between recipients/donor and survival (Table 28), we see that woman receiving a male heart do no present significant difference compared to when receiving a female heart (p= 0.8), on the contrary, men receiving a female heart have lower survival than when receiving a heart from the same gender (p <0.01).

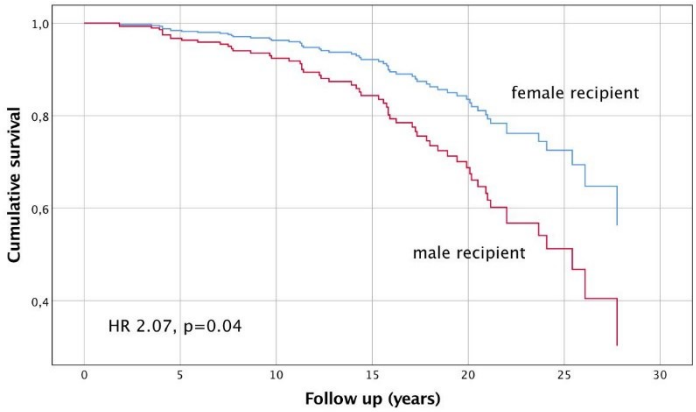

Figure 27: Cumulative survival throughout a 30-year follow-up according to gender recipient

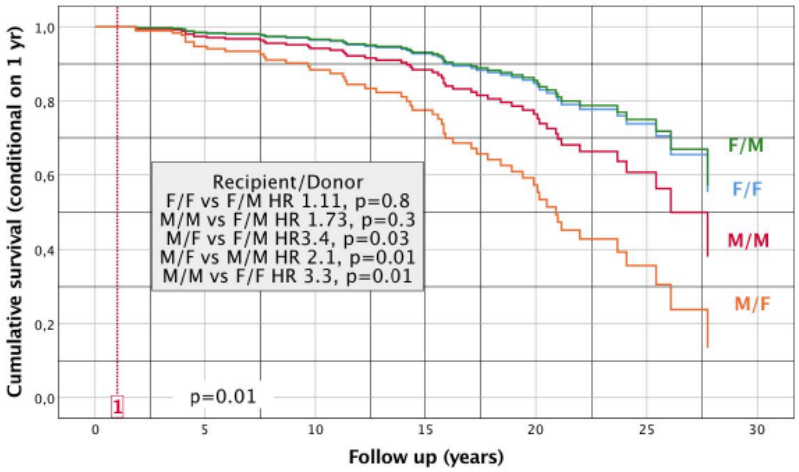

Figure 28: Cumulative survival (conditional on 1 year), in a 30-year follow-up, between gender/donor mismatch

Donor's gender seems to not be related with survival (p= 0.3) (Fig. 29) and neither gender mismatch between donor/recipient (Fig. 30) (p= 0.1).

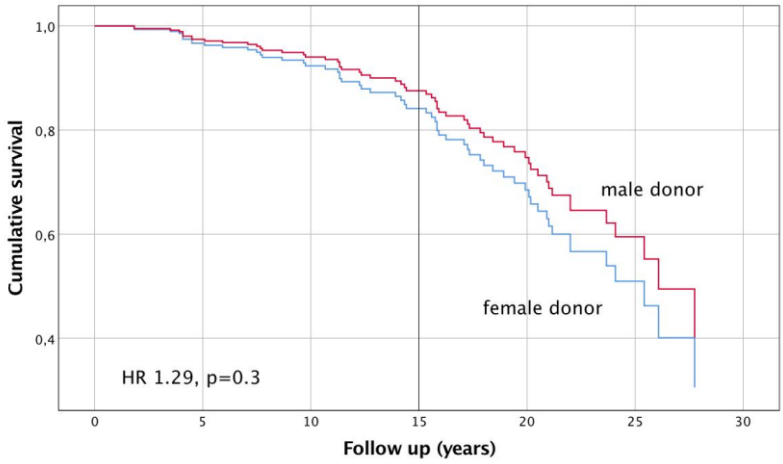

Figure 29: Cumulative survival in 30-follow up according to donors gender

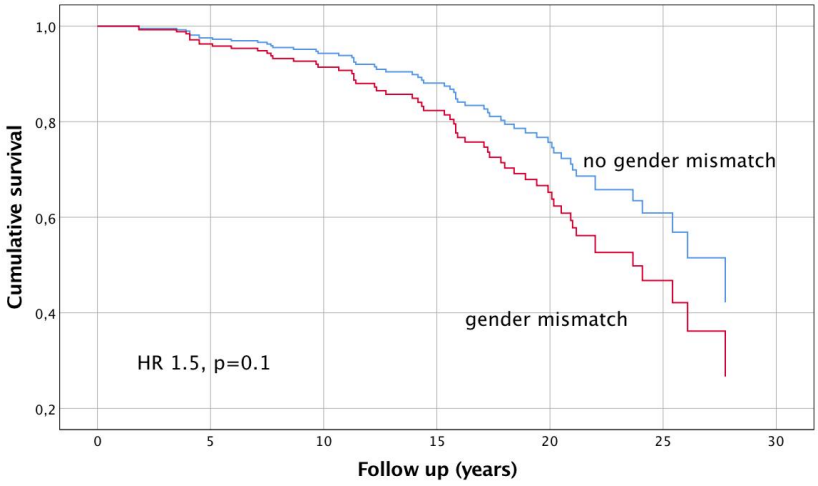

Figure 30: Cumulative survival in 30-follow up according to gender mismatch

***Survival and other factors**:*

The presence of left ventricle assistant device (LVAD) (p <0.0001), ischemic heart disease (IHD) (p <0.001) and pericardial effusion (p <0.0001) showed a statistical relevant relationship to long term survival (Fig. from 31 to 33).

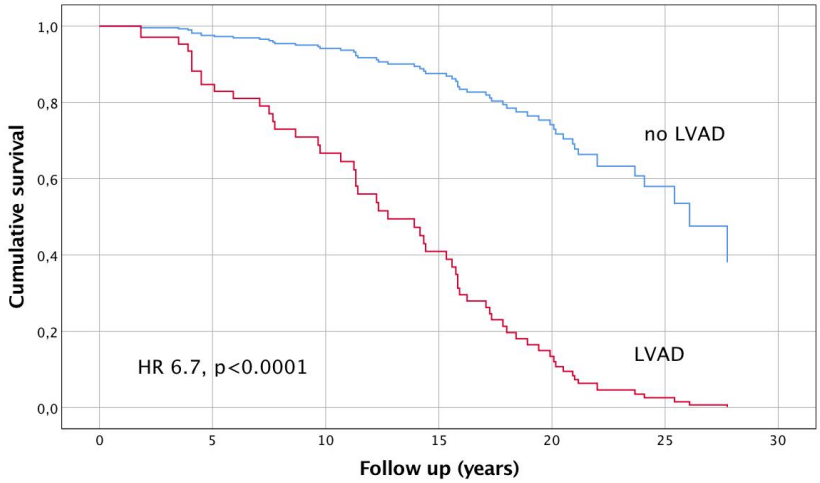

Figure 31: Survival throughout a 30-year follow-up according to patients with LVAD

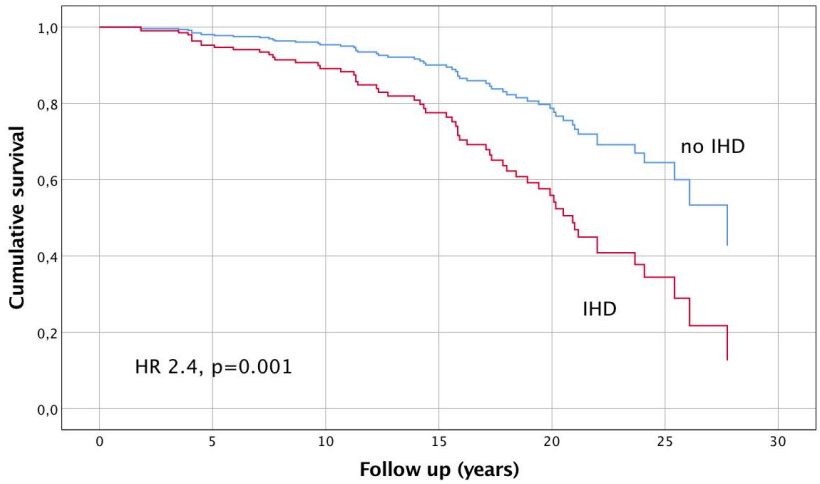

Figure 32: Survival throughout a 30-year follow-up according to patients with ischemic heart disease (IHD)

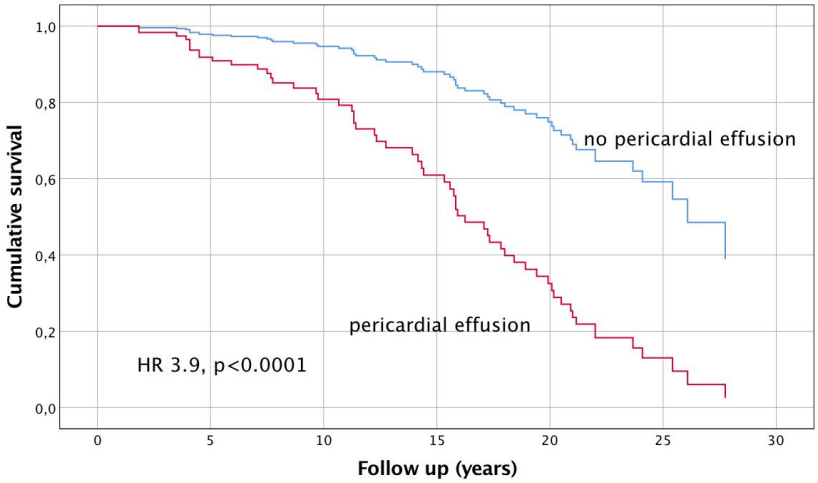

Figure 33: Survival throughout a 30-year follow-up according to patients with pericardial effusion

***Analysis of VAC's determinants**:*

Table X resumes the studied parameters to determine a univariate relationship with VAC as a continuous variable. From this table we evince that high Ea (p=0.026), low Ees (p <0.001), older age at transplant (p= 0.022), rejection score 1 year after transplantation (p <0.001)), severe rejection score at 1 year after transplantation (<0.001), gender recipient (p= 0.025), gender donor (p= 0.046), treatment with everolimus (p=0.013) and arterial hypertension (p= 0.013) are all factors that increase VAC.

Table X: Univariate analysis of VAC determinants using VAC as a continuous variable

Determinant	B	Confidence interval	p value
Ea	0.048	0.006 – 0.091	**0.026**
Ees	-0.092	0.106 – -0.077	**<0.001**

Time_TX_VAC	0.001	-0.003 – 0.005	0.566
BMI	-0.002	-0.009 – 0.004	0.472
CKD	0.010	-0.071 – 0.090	0.810
Age_TX	-0.002	-0.003 – 0.000	**0.022**
RS 1year	0.085	0.042 – 0.129	**<0.001**
TRS	0.051	-0.022 – 0.0125	0.167
Sev RS 1year	0.114	0.060 – 0.168	**<0.001**
Sev TRS	0.094	-0.003 – 0.191	0.058
Gender_recepient	0.069	0.009 – 0.130	**0.025**
Gender_donator	0.055	0.001 – 0.108	**0.046**
Sex mismatch	-0.024	-0.079 – 0.032	0.400
Ischemic time	0.000	0.000 – 0.001	0.083
VAD	0.028	-0.085 – 0.141	0.625
Heart and renal transplant	0.047	-0.128 – 0.222	0.599
IHD	-0.004	-0.060 – 0.52	0.892
Donor age	-0.001	-0.003 – 0.001	0.310
Donor BMI	-0.005	-0.012 – 0.002	0.191
Recipient_BMI	-0.002	-0.009 – 0.004	0.472
CAV	0.022	-0.047 – 0.090	0.531
Pericardial effusion	0.072	-0.008 – 0.152	0.078
Everolimus	0.082	0.017 – 0.146	**0.013**
Cyclosporin	-0.011	-0.161 – 0.138	0.883
Tacrolimus	0.009	-0.147 – 0.166	0.908
Azathioprine	-0.041	-0.100 – 0.019	0.183
Prednisone	0.027	-0.027 – 0.080	0.323
Methylprednisolone	0.003	-0.343 – 0.349	0.986
Mycophenolate mofetil	0.008	-0.047 – 0.063	0.770
ACEI_SART	0.019	-0.043 – 0.080	0.548
CCB	-0.015	-0.093 – 0.064	0.710

Aldosterone antagonist	-0.032	-0.117 – 0.053	0.458
Diuretic	-0.006	-0.071 – 0.060	0.859
Statin	-0.029	-0.088 – 0.29	0.325
Beta blocker	0.042	-0.060 – 0.143	0.423
Diabetes	0.003	-0.066 – 0.071	0.933
Hypertension	-0.069	-0.123 – -0.15	**0.013**
Hypercholesterolemia	-0.003	-0.060 – 0.054	0.913
Obesity	-0.032	-0.116 – 0.052	0.453
0=FF, 1=MM, 2=FM, 3=MF	0.002	-0.024 – 0.028	0.873
FMvs.MF	0.016	-0.055 – 0.088	0.651

Ea: Effective arterial elastance. Ees: End-systolic LV elastance. Time_TX_VAC: Time from HT to VAC measurement. BMI: Body mass index. CKD: Chronic kidney disease. Age_TX: Age of HT. RS 1year: Rejection score at 1 year after HT. TRS: Total rejection score. Sev RS 1year: Severe rejection score at 1 year after HT. VAD: Ventricular assistant device. IHD: Ischemic heart disease. CAV: Cardiac allograft vasculopathy. ACEI_SART: Angiotensin-converting-enzyme inhibitors_Sartans. CCB: Calcium channel blockers. FMvs.MF: Female male vs. male female.

We proceeded to do a multivariate analysis (Table XI) using the significant data obtained in the previous univariate analysis. From this research it emerged that high Ea ($p < 0.001$), low Ees ($p < 0.001$) and severe rejection score 1 year after transplantation ($p < 0.001$) were independent determinants of VAC, while all the other variables were excluded.

Table XI: Multivariate analysis of VAC determinants using VAC as a continuous variable

Determinant	B	Confidence interval	p value
Ea	0.276	0.242- 0.310	**<0.001**
Ees	-0.159	-0.173 – -0.145	**<0.001**
Sev RS 1year	0.068	0.034 – 0.102	**<0.001**

Ea: Effective arterial elastance. Ees: End-systolic LV elastance. Sev RS 1year: Severe rejection score at 1 year after HT

We then studied VAC, using a logistic regression, no longer as a continuous variable but as a dichotomic value with the cut-off set to the median VAC value of 0.59. Considering VAC over 0.59 as uncoupled and under 0.59 as coupled. As seen in Table XII, low Ees (p <0.001), time from HT to VAC measurement (p= 0.027), gender recipient (p= 0.054) and gender donor (p= 0.014) were determinant factors for a high VAC and therefor an uncoupled system.

Table XII: Univariate analysis of VAC determinants according to VACs median value (0.59)

	Odds ratio (OR)	Confidence interval	p value
Ea	1.375	0.969 – 1.951	0.075
Ees	0.325	0.249 – 0.425	**<0.001**
Time_TX_VAC	0.963	0.932 – 0.996	**0.027**
BMI	1.004	0.953 – 1.057	0.886
CKD	1.219	0.637 – 2.330	0.550
Age_TX	0.996	0.983 – 1.008	0.496
RS 1year	0.859	0.604 – 1.222	0.399
TRS	0.611	0.337 – 1.109	0.105

Sev RS 1year	0.855	0.554 – 1.319	0.479
Sev TRS	0.448	0.198 – 1.012	**0.054**
Gender_recepient	1.867	1.133 – 3.078	**0.014**
Gender_donor	0.782	0.508 – 1.203	0.263
Sex mismatch	0.880	0.564 – 1.373	0.572
Ishcemic time	0.999	0.996 – 1.003	0.739
VAD	0.663	0.264 – 1.664	0.381
Heart and renal transplant	3.000	0.066 – 1.665	0.180
IHD	0.901	0.576 – 1.410	0.648
Donor age	0.997	0.983 – 1.011	0.642
Donor BMI	1.059	0.982 – 1.142	0.135
CAV	1.642	0.969 – 2.783	0.065
Pericardial effusion	0.465	0.236 – 0.918	**0.027**
Everolimus	0.570	0.335 – 0.968	**0.038**
Cyclosporina	1.207	0.361 – 4.034	0.760
Tacrolimus	1.000	0.284 – 3.519	1.000
Azathioprine	0.785	0.484 – 1.273	0.326
Prednisone	1.264	0.825 – 1.939	0.282
Methylprednisolone	1.000	0.062 – 16.119	1.000
Mycophenolate mofetil	1.016	0.655 – 1.577	0.943
ACEI_SART	0.904	0.560 – 1.458	0.678
CCB	0.839	0.457 – 1.540	0.570
Aldosterone antagonist	0.875	0.453 – 1.691	0.691
Diuretic	1.025	0.617 – 1.703	0.924
Beta blocker	1.187	0.536 – 2.631	0.672
Statin	0.666	0.422 – 1.051	0.081
Diabetes	1.277	0.737 – 2.213	0.383
Hypertension	0.743	0.479 – 1.153	0.185
Hypercholesterolemia	1.249	0.794 – 1.966	0.337
Obesity	0.865	0.441 – 1.700	0.675

TSI	0.996	0.990 – 1.002	0.156
0=FF, 1=MM, 2=FM, 3=MF	1.179	0.958 – 1.450	0.120
MISMATCH=1 NO MISMATCH=0	1.137	0.728 – 1.774	0.572
MM=0, MF=1	1.026	0.611 – 1.723	0.924
FMvsMF	1.366	0.605 – 3.082	0.453

Ea: Effective arterial elastance. Ees: End-systolic LV elastance. Time_TX_VAC: Time from HT to VAC measurement. BMI: Body mass index. CKD: Chronic kidney disease. Age_TX: Age of HT. RS 1year: Rejection score at 1 year after HT. TRS: Total rejection score. Sev RS 1year: Severe rejection score at 1 year after HT. VAD: Ventricular assistant device. IHD: Ischemic heart disease. CAV: Cardiac allograft vasculopathy. ACEI_SART: Angiotensin-converting-enzyme inhibitors_Sartans. CCB: Calcium channel blockers. FMvs.MF: Female male vs. male female

The multivariate analysis of these data showed that only low Ees ($p < 0.001$) and time from HT to VAC measurement ($p = 0.043$) resulted independent factors for an uncoupled heart with VAC above 0.59.

***Analysis of pressure-volume derived parameters on Ea and Ees**:*

For the following analysis we divided our population according to the median value of Ea and Ees (2.237mmHg/ml and 6.750mmHg/ml respectivlely). Then we studied how cardiac mechanics varied in conditions above or under these median values.

Hearts with higher arterial elastance (>2.237mmHg/ml), showed a higher Ees and Eed, lower SW, PE and PVA (all values presented a $p < 0.001$). VAC and efficiency remained non-significant. See Table XIII.

Table XIII: Pressure-volume derived parameters according to Ea median

	Ea Median				**p value**
	≤2.237 mmHg/ml		>2.237 mmHg/ml		
	Mean	SD	Mean	SD	
Ea	1.825	±0.256	2.793	±0.470	**<0.001**
Ees	3.322	±1.22	4.751	±1.478	**<0.001**
Eed	0.161	±0.360	0.234	±0.059	**<0.001**
VAC	0.610	±0.272	0.634	±0.221	0.373
SW	7827.170	±1868.521	5810.095	±1449.412	**<0.001**
PE	2375.826	±1084.845	1788.279	±546.641	**<0.001**
PVA	10202.997	±2576.124	7598.374	±1791.158	**<0.001**
Efficiency	77.203	±5.956	76.354	±5.314	0.163

Ea: Effective arterial elastance. Ees: End-systolic elastance. VAC: Ventricular arterial coupling. SW: Stroke work. PE: Potential energy. PVA: Pressure-volume area. Eed: End-diastolic elastance.

Hearts with lower Ees (≤6.750mmHg/ml) presented lower Ea, Eed and efficiency and higher VAC, SW, PE and PVA, all with a p value <0.001. See Table XIV

Table XIV: Pressure-volume derived parameters according to Ees median

	Ees Median				**p value**
	≤6.750 mmHg/ml		>6.750 mmHg/ml		
	Mean	SD	Mean	SD	
Ea	2.047	±0.498	2.589	±0.605	**<0.001**
Ees	3.020	±0.739	5.129	±1.316	**<0.001**
Eed	0.174	±0.058	0.224	±0.050	**<0.001**
VAC	0.717	±0.295	0.519	±0.118	**<0.001**
SW	7210.873	±2115.713	6401.750	±1665.166	**<0.001**
PE	2507.309	±1014.945	1625.262	±446.733	**<0.001**
PVA	9718.182	±2796.414	8027.013	±1964.713	**<0.001**
Efficiency	74.204	±5.907	79.557	±3.745	**<0.001**

Ea: Effective arterial elastance. Ees: End-systolic elastance. VAC: Ventricular arterial coupling. SW: Stroke work. PE: Potential energy. PVA: Pressure-volume area. Eed: End-diastolic elastance.

DISCUSSION

It is reasonable to think that adaptation of a new heart to a previously existent arterial system might not be perfect because the new heart must adapt to the recipient's afterload, considering that the heart has been exposed to particular conditions of pressure and volume during the time spent in the donor's body. Ventricular-arterial coupling allows to somehow give a further explanation to this organ crosstalk.

In our investigation, transplanted heart patients after a year from surgery, seemed to have a normal ventricular-arterial interaction since no significant statistical difference was found in VAC values when compared to healthy control group. When evaluating VACs components separately we saw that both, arterial afterload (Ea) and LV contractility (Ees), were increased. This translates in a heart that contracts vigorously against a higher arterial resistance which allows the graft to work appropriately at first, but it is hardly sustainable in the long term, and with time, uncoupling will undercome. This is precisely why, even though VAC is an important determinant of global cardiovascular performance, we must not ignore the fact that it is a ratio and therefor if both components change in the same direction VAC remains in normal range. For this reason, we advise to also study Ea and Ees separately.

On the other hand, when studying cardiac energetics, we observed that efficiency remained unvaried in transplanted patients compared to controls. This phenomenon is explained by the fact that, the heart, in order to preserve its energetic efficiency, enhanced its ventricular contractility. This reinforces the concept that the VA coupling present in heart transplanted patients is an unfavorable coupling.

When we divided these transplanted patients in two groups, according to the median VAC value (0.59), we noticed that those above this cutoff had higher VAC at expenses of a lower Ees, while Ea had no influence. This means that the uncoupled hearts presented mainly a contractility deficit.

Fulfilling the objective of our investigation we studied clinical and echocardiographic parameters a year after heart transplantation looking for those factors that could predict mortality. From the clinical aspects, it emerged that 70% of patients that had died were men. When analyzing in depth this phenomenon we saw that primarily those with worse outcomes had received a female heart. This finds an explanation based on immunological and mechanical theories. Men seem to have higher afterload than women, consequently, usually male hearts are more predisposed to systolic dysfunction while women to diastolic dysfunction. So, when a male recipient receives a female heart, the graft may present diastolic impairment that adapts poorly to an elevated Ea. An immunological theory seems less feasible since women receiving male hearts do not have lower survival.

We evaluated mismatch recipient/donator and survival in a 30-year span and effectively, men receiving female hearts had a lower long-term survival compared to men receiving male hearts and women no matter the mismatch.

As to echocardiographic characteristics- one year after transplantation- patients presented hypertrophic hearts with smaller volumes and diastolic dysfunction. This coincides to what is reported in literature, since immunosuppressive drugs and steroids influence the development of arterial hypertension. When studying the echocardiographic factors as mortality predictors we notice that 20% of the patients that died had higher end-diastolic and end-systolic volumes. This means that the heart in final stages loses its pump function and start to develop heart failure.

Using these clinical and echocardiographic data, we wanted to see if there was an association with VAC. In fact, male patients and patients with higher volumes measured by echocardiography showed higher VAC values indicating that patients with these characteristics are more prone to develop VA uncoupling.

Based on the fact that the before mentioned parameters were related to higher VAC we wanted then to analyze, if, in return, VAC itself was useful to predict survival in the long-term. As a matter of fact, VAC over 0.59 predicted mortality in the univariate analysis in a 30-year follow-up, with a 60% increase in mortality compared to those with VAC under this cutoff. Yet, VAC showed no significance in the multivariate analysis and can't be considered an independent mortality predictor.

Nevertheless, Ea and Ees were significant on the univariate and multivariate analysis which means that an Ea >4mmHg/ml or an Ees ≤6.75mmH/ml are independent prognostic risk factors for cardiovascular death in heart transplanted patients. Patients with Ea above this cutoff present twice a risk of mortality (HR= 2.01), while patients with Ees under this value present almost 3 times the risk (HR= 2.72)

To summarize, even though VAC loses its ability to predict death when adjusted to other variables, its components studied separately, over the cutoff previously discussed, do help to predict mortality in this population.

Cardiac energetics, understood as SW, PE, PVA and efficiency showed no influence on survival.

Patients under and above LVEF median (62%) showed no difference in survival in our population and the reason for this is that we included only patients with normal EF in our study.

Other factors related to a worse survival in a 30-year lapse were the presence of LVAD, ischemic heart disease and pericardial effusion.

Our second objective was to study VACs determinants. The determinants that increased VAC, when considered as a continuous variable at the univariate analysis, were high arterial afterload, contractility deficit, severe rejection score 1 year after transplantation, rejection score at 1 year after transplantation, older age, male patients, male donors, having received treatment with everolimus and hypertension. From these variables just high arterial afterload, contractility deficit and severe rejection score at 1 year after transplantation showed to be independent factors of high VAC, which means that the solely presence of these determinants are enough to increase VAC.

When we considered uncoupled patients as those with VAC over the median value of 0.59, we observed again that contractility deficit, male patients and male donors were responsible of this uncoupling, beside a prolonged time from heart surgery to the moment in which VAC was measured. Only a contractility deficit and a prolonged time from surgery to VAC measurement were independent factors of a high VAC.

Contractility deficit was present either when evaluating VAC as a continuous variable than as a dichotomic value, stressing the importance of Ees on VAC.

CONCLUSIONS

Ventricular-arterial coupling predicts long-term outcomes in transplanted heart patients only as a univariate variable, however, Ea and Ees when studied separately proved to be independent prognostic risk factors. These parameters are particularly useful since they can be easily determined with simple calculations and echocardiographic measurements that are routinely evaluated by cardiologists. It can be helpful to apply them in cases where EF is in normal ranges because it constitutes a further tool in assessing prognosis in these types of patients.

The limitation of VAC is that being a ratio it is bound to result normal when both its components change in the same direction, therefore, evaluating arterial afterload (Ea) and ventricular contractility (Ees) separately is advisable.

In our investigation, uncoupled patients had predominantly a contractility impairment while arterial afterload didn’t seem to have any influence on VAC.

Lastly, it is important to highlight that one of the strengths of this investigation is the elevated cohort of transplanted heart patients and the extended time in which they were followed.

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