UMEÅ UNIVERSITY

INSIGHTS INTO CARDIAC FUNCTION BY ECHOCARDIOGRAPHY IN ADVANCED HEART FAILURE AND HEART TRANSPLANTATION

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Umeå 2025

Dissertation for PhD
ISBN: 978-91-8070-626-1 (print)
ISBN: 978-91-8070-627-8 (pdf)
ISSN: 0346-6612
New series number: 2348
Cover design by Giulia Elena Mandoli
Electronic version available at: http://umu.diva-portal.org/
Printed by: Scandinavian Print Group
Umea, Sweden 2025

To Francesca, my everyday light, and my husband Matteo, my rock even during the darkest storm.

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Original papers

This thesis is based on research reported in the papers listed.

I. Mandoli GE, Landra F, Chiantini B, Sciaccaluga C, Pastore MC, Focardi M, Cavigli L, D'Ascenzi F, Bernazzali S, Maccherini M, Valente S, Cameli M, Henein M. Tricuspid Regurgitation Velocity and Mean Pressure Gradient for the Prediction of Pulmonary Hypertension According to the New Hemodynamic Definition. Diagnostics (Basel). 2023;13(16).

II. Mandoli GE, Landra F, Chiantini B, Bonadiman L, Pastore MC, Focardi M, D'Ascenzi F, Lisi M, Diviggiano EE, Martini L, Bernazzali S, Valente S, Maccherini M, Cameli M, Henein MY. Myocardial Work Indices Predict Hospitalization in Patients with Advanced Heart Failure. Diagnostics (Basel). 2024;14(11).

III. Mandoli GE, Landra F, Tanzi L, Martini L, Fusi C, Sciaccaluga C, Diviggiano EE, Barilli M, Pastore MC, Focardi M, Bernazzali S, Maccherini M, Cameli M, Henein MY. Reference values of strain-derived myocardial work indices in heart transplant patients. Eur Heart J Imaging Methods Pract. 2024;2(3):qyae091.

IV. Mandoli GE, Coarelli G, Martini L, Cartocci A, Salvatici C, Renzelli A, Minasi V, Cappelli E, Pastore MC, Focardi M, Zito A, Bernazzali S, Maccherini M, Valente S, Söderberg S, Henein MY, Cameli M. NT-proBNP trajectory short and long-term and predictor after heart transplantation. Submitted.

Abstract

Background:

Heart failure (HF) is defined as a clinical syndrome with typical symptoms and signs determined by congenital or acquired abnormalities of the structure or the function of the heart. Several therapeutic options have improved the quality of life and the outcome of patients with HF in recent years. However, also because an increasing number of individuals survives longer, up to the 10% of HF population reaches the advanced stage of the disease. Advanced HF (AdHF) is characterized by persistent severe symptoms despite optimal HF medical and electrical therapy with very poor functional capacity and episodes of unplanned hospitalizations or visits to reduce congestion or improve cardiac output. AdHF patients periodically undergo invasive right heart catheterization (RHC) to estimate pulmonary pressure and vascular resistance. Heart transplantation (HTX) remains the gold standard treatment for AdHF, allowing patients good mid- and long-term survival rates. Main complications after HTX include rejection, more common within the first year after surgery, and cardiac allograft vasculopathy (CAV) in the long term. Echocardiography is a key first line tool for the routine assessment of cardiac function in AdHF and HTX, to monitor effectiveness of therapies and to stratify prognosis. Second level echocardiography techniques, especially Speckle Tracking-derived Myocardial work, are promising in assessing with more sensibility changes in left ventricular function, especially when associated with biomarkers as natriuretic peptides.

Objectives:

This thesis is focused on the added role of echocardiography in AdHF and HTX patients and the main aims include: to study the reliability of echocardiography in the estimation of pulmonary arterial pressures (PAP) and the diagnosis of pulmonary hypertension when compared to gold standard method (i.e. RHC, paper I); to estimate prognostic value of myocardial work in AdHF (paper II); to determine normal reference value of myocardial work indices in the transplanted heart compared to general healthy population (paper III); to describe the trend of NT-

proBNP (the most used natriuretic peptide in HF) after HTX, assessing its possible predictors among pre- and post-operative echocardiographic and clinical variables (paper IV).

Materials and methods:

We retrospectively screened patients with AdHF in regular follow up at our University Hospital. For paper I, we selected all individuals with available RHC data and echocardiographic images recorded on the same day, excluding those with diseases which could represent biases, as chronic obstructive pulmonary disease, and those with poor acoustic window or undetectable tricuspid regurgitation; all patients underwent accurate echocardiographic analysis for the estimation of pulmonary pressures including peak tricuspid regurgitation velocity (TRV) and mean right ventricular–right atrial (RV–RA) pressure gradient. For paper II we included all patients with good acoustic windows and brachial artery cuff systemic blood pressure measured at the same time as the echocardiographic exam, for the calculation of myocardial work indices, excluding those with more than mild heart valve disease or atrial fibrillation. Applying dedicated software, myocardial work indices, including global constructive work (GCW), global work efficiency (GWE); global work index (GWI) and global wasted work (GWW) were calculated in each patient which was then followed up for the development of major events (all-cause mortality, HTX, left ventricular assist devices implantation – primary endpoint – or acute HF hospitalization – secondary endpoint). A population of HTX patients without history of CAV or rejection were screened for paper III and included if the acoustic window was good and brachial artery cuff systemic blood pressure was measured at the same time as the echocardiographic examination. Patients were excluded also in the presence of donor-specific antibodies or atrial fibrillation, more than mild mitral or aortic regurgitation, or abnormal left ventricular function. Myocardial work indices were calculated and compared to general population with similar age and no comorbidities, derived from the European Association of Cardiovascular Imaging (EACVI) NORRE study. Lastly, for paper IV, a wider population of HTX patients with available long term follow up and pre-surgical information were screened and described in terms of NT-proBNP values at 10 different time points including 1 month, 3 months, 6 months and 1 year after the

HTX. Continuous variables were reported as either mean with standard deviation or median with interquartile range according to normal distribution. Receiver Operating Characteristic (ROC) curves were used to evaluate the ability of echocardiographic parameters to predict outcome (PH for Paper I, adverse events for paper II). Pearson's correlation coefficient was utilized to examine the strength of the association between echocardiographic measures and RHC findings in Paper I or NT-proBNP values in paper IV. Univariate and multivariate Cox proportional hazard regression analyses were applied to assess predictors of outcomes in paper II where Kaplan–Meier analysis estimated event-free survival. Linear regression was applied to test possible association with MW indices and population characteristics in paper III.

Results:

Paper I: in the 41 patients enrolled, peak TRV was superior in terms of area under the curve by ROC analysis to mean RV–RA gradient in predicting increased mean PAP at RHC, both when using 20 or 25 mmHg as pathological cut off value. In particular, a peak TRV >2.4 m/s had 65% sensitivity and 100% positive predictive value for predicting PH according to the new guidelines' definition.

Paper II: among 138 enrolled individuals, 35 patients developed at least 1 event at follow up. While myocardial work parameters were not associated with primary endpoint occurrence, the hazard ratio for each increase in GWI by 50 mmHg% was 0.90 ($p = 0.025$) and for each increase in GCW by 50 mmHg% was 0.90 ($p = 0.022$) when estimating the risk of acute HF hospitalization. Patients with GWI ≥ 369 mmHg% had a better event-free survival at Kaplan–Meier analysis.

Paper III: 82 HTx patients, 68.3% male with a median age of 53 (46–62) years were included in a median time lapse for HTX of 5 (2–22) months. No significant differences were described in terms of gender in HTX patients. On the contrary, all the myocardial work indices significantly differed from those reported in the EACVI NORRE study (all P-value <0.001), in particular with lower GWI, GCW, and GWE and higher GWW values in the HTX population.

Paper IV: in a population of 71 HTX patients, major reduction of NT-proBNP was described at month 3 after surgery, with further reduction at 6 months and 1 year after which it tended to remain stable. Among predictors of NT-proBNP values, at regression analysis, 1-year NT-proBNP values was related to RHC measured pulmonary wedge pressure and ischemic etiology but also to post-HTX kidney function and tricuspid regurgitation severity; long term NT-proBNP values were instead predicted by positive Human Leucocyte Antigen (HLA) antibodies, age at HTX and mitral and tricuspid regurgitation severity.

Conclusions:

Standard and advanced echocardiography is confirmed to be an essential and non-invasive tool to describe pathological conditions in AdHF, to determine the best follow up timing to avoid major events or HF hospitalizations but also to early diagnose modification of physiological deformation in case of CAV of rejection or to predict an oincrease of NT-proBNP.

Abbreviations

ACR = acute cellular rejection

AHA = American Heart Association

AMR = antibody mediated rejection

AdHF = advanced heart failure

BBT = bridge to transplantation

BMI = body mass index

BTC = bridge to candidacy

CAV = cardiac allograft vasculopathy

DT = E wave deceleration time

EACVI = European Association CardioVascular Imaging

EF = ejection fraction

EMB = endomyocardial biopsy

ESC = European Society of Cardiology

GLS = global Longitudinal Strain

GCW = global constructive work

GWE = global work efficiency

GWI = global Work Index

GWW = global wasted work

HF = heart failure

HFmrEF = heart failure mid-range ejection fracton

HFpEF = heart failure with preserved ejection fraction

HFrEF = heart failure with reduced ejection fraction

HLA = human leukocyte antigen

HTX = heart transplantation

INTERMACS = Interagency Registry for Mechanically Assisted Circulatory Support

ISHLT = International Society for Heart and Lung Transplantation

IVC = inferior vena cava

LV = left ventricular

LV EF = left ventricular ejection fraction

LVAD = left ventricular assist device

MCS = mechanical circulatory support

mPAP = mean pulmonary arterial pressure

MW = myocardial work

NORRE = Normal Reference Ranges for Echocardiography

NPs = natriuretic peptides

NYHA = New York Heart Association

NT-proBNP = N-terminal pro-peptide brain natriuretic peptide

PAP = pulmonary arterial pressure

PAWP = pulmonary artery wedge pressure

PH = pulmonary hypertension

RA = right atrium

RAAS = renin angiotensin aldosterone system

RHC = right heart catheterization

ROC = Receiver Operating Characteristics

RV = right ventricular

SNS = sympathetic nervous system

STE = Speckle Tracking Echocardiography

TRV = tricuspid regurgitation velocity

VTI = velocity time integral

Enkel sammanfattning på svenska

Bakgrund

Hjärtsvikt (HF) är ett kliniskt syndrom som karakteriseras av hjärtats oförmåga att upprätthålla tillräcklig hjärtminutvolym. Tillståndet innefattar flertalet bakomliggande orsaker så som kardiomyopati, kranskärlssjukdom, klaffel och andra strukturella hjärtavvikelser. Vanligen uppstår ökade pulmonella tryck vid HF sekundärt till progredierande kammardysfunktion. På senare år har framsteg inom farmakologisk behandling förbättrat prognosen för patienter med HF, men upp till 10 % av HF-patienter utvecklar avancerad hjärtsvikt (AdHF), vilket kännetecknas av svåra symtom trots optimal behandling, nedsatt funktionell kapacitet och återkommande sjukhusvistelser. Vid utredning av patienter med AdHF är högersidig hjärtkateterisering en viktig undersökning för att bedöma förekomst av förhöjda pulmonella tryck. Hjärttransplantation (HTX) är den i dagsläget enda botande behandlingen vid AdHF vilket förbättrar överlevnaden både på kort och lång sikt, även om komplikationer såsom avstötning och utveckling av koronar allograft vaskulopati (CAV) kan uppstå. I den kliniska uppföljningen utgör ekokardiografi ett förstahandsval då metoden möjliggör noggrann monitorering av hjärtfunktion hos AdHF- och HTX-patienter, bland annat genom noninvasiv skattning av pulmonella tryck. På senare tid har mer avancerade ekokardiografiska tekniker tagits fram, som Speckle Tracking-baserad Myocardial Work vilket kan ge en förbättrad känslighet. Dessa metoders betydelse vid uppföljning hos patienter med AdHF och HTX är dock sparsamt studerade.

Syfte

Huvudsyftet med denna avhandling var att undersöka ekokardiografins roll hos patienter med AdHF eller genomgången HTX, med specifikt fokus avseende: att utreda tillförlitlighet av ekokardiografi jämfört med RHC vid skattning av lungartärtryck (PAP) och diagnos av pulmonell hypertension (studie I); att studera prognostiskt värde av myocardial work vid AdHF (studie II); att fastställa referensvärden för myocardial work-index i en HTX-population jämfört med friska individer (studie

III); att studera trender och prediktorer för NT-proBNP efter HTX (studie IV).

Material och Metoder

Vi analyserade retrospektivt en population av AdHF- och HTX-patienter vid vårt sjukhus (Azienda Ospedaliera Universitaria Senese, Siena, Italy). Studie I inkluderade patienter med genomgången RHC och ekokardiografisk undersökning utförd under samma dag. I studie II analyserades ekokardiografiska undersökningar med adekvat bildkvalitet hos AdHF patienter med tillgängliga blodtrycksdata, varvid beräkning av myocardial work-index utfördes. Patienter följdes över tid avseende utveckling av händelser såsom HTX och mortalitet, implantation av mekanisk hjärtpump eller akuta sjukhusinläggning. I studie III beräknades myocardial work-index hos HTX-patienter utan komplicerande CAV eller avstötning, och data jämfördes med en åldersmatchad frisk population. I studie IV undersöktes NT-proBNP hos HTX-patienter med tillgänglig långtidsuppföljning, och relationen mellan NT-proBNP och ekokardiografiska och kliniska variabler studerades. Statistiska metoder inkluderade Receiver Operating Characteristics (ROC-analys), Pearsons korrelation, Cox-regression, Kaplan-Meier överlevnadsanalys och linjär regression.

Resultat

Studie I: Bland 41 AdHF-patienter visade systolisk maxhastighet över trikuspidalisklaffen (peak TRV) >2,4 m/s, 65 % sensitivitet och 100 % positivt prediktivt värde för att förutsäga pulmonell hypertension vid RHC.

Studie II: Av 138 inkluderade AdHF-patienter utvecklade 35 patienter minst en händelse vid uppföljning. Högre global work index (GWI) och global constructive work (GCW) var associerade med lägre risk för akut HF-inläggning, där GWI ≥ 369 mmHg% var relaterat till förbättrad överlevnad.

Studie III: 82 HTX-patienter inkluderades och uppvisade signifikant skillnad i myocardial work-index jämfört med kontrollpopulationen, med lägre GWI, GCW, global work efficiency (GWE) och högre global wasted work (GWW).

Studie IV: Bland 71 inkluderade HTX-patienter minskade NT-proBNP signifikant tre månader efter HTX och stabiliserades efter ett år. Prediktorer inkluderade lungkapillärt inkilningstryck, ischemisk etiologi, njurfunktion och klaffläckage. NT-proBNP-värden vid långtidsuppföljning predikterades av positiva HLA-antikroppar, ålder vid HTX samt av mitral- och trikuspidalklaffläckage.

Slutsatser

Transplanterade hjärtan har en komplicerad patofysiologi där analys och monitorering NT-proBNP ej är lika tillförlitligt som vid HF. Ekokardiografi förblir därför avgörande vid hantering av AdHF och HTX, genom att bidra till sjukdomskarakterisering, möjlighet att förutsäga allvarliga händelser och optimera uppföljning. Vidare är det användbart för att identifiera optimal tidpunkt för HTX och identifiera tidiga funktionella förändringar vid CAV eller avstötning.

Introduction

Heart failure: definition and epidemiology

Heart failure (HF) is a complex clinical syndrome characterized by variable symptoms and signs of congestion, resulting from the heart's inability to pump blood adequately to meet the body's demands or only at the cost of elevated filling pressures. HF can be considered the potential end stage of all cardiac diseases. Thanks to advancements in treatments and improved survival rates—especially in ischemic cardiomyopathy, the most common cause of cardiovascular disease—HF has become a true global pandemic, affecting an estimated 15 million adults in Europe and 65 million people worldwide [1,2]. Over recent years, significant progress has been made in managing HF patients, including the introduction of novel disease-modifying and life-prolonging pharmacological therapies. Notable examples include sodium-glucose cotransporter-2 inhibitors and sacubitril-valsartan, both of which have demonstrated the ability to reduce cardiovascular mortality [3,4]. Despite these advancements, 10%–15% of HF patients on optimal medical therapy progresses into advanced heart failure (AdHF). AdHF is a clinical state defined by intolerance to neurohormonal disease-modifying therapies, coupled with the development of cardio-renal dysfunction, a high symptom burden, diminished exercise capacity, and frequent hospitalizations requiring intensive care [5].

Physiopathology

HF represents the end stage of numerous cardiac diseases which can prevalently affect diastolic or systo-diastolic function and left or right heart. Different mechanisms, including cardiomyocytes hypertrophy, neurohumoral activation, vasoconstriction, increased oxidative stress, and inflammation eventually lead to fibrosis and loss of myocardial proper relaxation and contraction [6]. To counterbalance the increased filling pressures and the reduced stroke volume, the body activates compensatory mechanisms, mainly neurohumoral activation. Neurohormonal systems, including the Sympathetic Nervous System (SNS), the Renin-Angiotensin-Aldosterone System (RAAS), and the Natriuretic Peptides (NPs) system increase the myocardial contractility,

the ventricular filling, and the peripheral vasoconstriction with the final aim of maintaining the perfusion of vital organs. However, in the long term the activation of these systems is also responsible for HF progression and unfavorable prognosis. The release of catecholamines and SNS activation induce peripheral vasoconstriction and, via cardiac β-receptors activation, chronotropic and inotropic effect to improve stroke volume [7]. Renin converts angiotensinogen, a plasma protein synthesized in the liver, into angiotensin I, a peptide with mild vasoconstrictive properties. The angiotensin-converting enzyme, a nonspecific enzyme primarily located in the endothelial cells of lung vessels, then converts angiotensin I into angiotensin II. As a potent vasoconstrictor, angiotensin II plays a key role in maintaining circulatory homeostasis but is rapidly degraded by angiotensinases. It interacts with two receptors, angiotensin type 1 (AT1) and angiotensin type 2 (AT2), which exert opposing effects. In cardiac muscle, the AT2-to-AT1 ratio is typically 2:1; however, in HF, AT1 receptor expression increases, leading to enhanced vasoconstriction, aldosterone secretion, cell proliferation, and catecholamine release [8]. NPs are a group of hormones produced by the heart's ventricles in response to changes in blood flow and pressure. Specifically, atrial natriuretic peptide (ANP) is secreted by the atria, while brain natriuretic peptide (BNP) is primarily produced by the ventricles, particularly when the left ventricle is stretched and dilated. In a healthy state, BNP is also produced by the atria. NPs, especially BNP, play a well-established role in HF, offering both diagnostic and prognostic insights. The primary receptor for both ANP and BNP is the natriuretic peptide receptor-A (NPR-A). Activation of this receptor leads to several beneficial effects, including reduced arterial blood pressure through natriuresis and diuresis, vasodilation, and improved endothelial permeability [9]. These actions counterbalance the RAAS, helping to prevent LV hypertrophy and remodeling.

The 4 pillars of HF treatment are currently based on antagonization of sympathetic nervous system and renin-angiotensin-aldosterone system and enhancement of effects of the natriuretic peptides [2].

Heart failure classifications

HF is commonly classified based on left ventricular ejection fraction (LVEF) into three main categories: *reduced ejection fraction* (HFrEF, EF <40%), *preserved ejection fraction* (HFpEF, EF >50%) and *mid-range ejection fraction* (HFmrEF, EF 40–49%) [2]. Patients with HFpEF tend to be older, more commonly women, and often have a history of obesity, hypertension, and/or atrial fibrillation. Patients with HFrEF are more frequently associated with coronary artery disease, such as myocardial infarction, as well as valve disorders like aortic stenosis and mitral regurgitation, or poorly controlled hypertension. Another way for classifying HF is according to clinical presentation. In fact, HF can manifest suddenly (*de novo* HF), often triggered by events like acute myocardial infarction or a hypertensive crisis, but it can also occur in patients with previously stable HF who experience acute decompensation. Many are the possible etiologies of decompensated HF, including excessive fluid intake, anti-inflammatory drug abuse, rapid ventricular response in atrial fibrillation etc. Acute decompensation of chronic HF is the most common presentation of acute HF [2].

According to American Heart Association (AHA) [10], HF can also be described in terms of progression, with 4 different stages (**Figure 1**): *stage A* (at risk for heart failure), including individuals at risk for HF but without symptoms or any structural or functional heart abnormalities. Key risk factors include arterial hypertension, coronary artery disease, diabetes, obesity, genetic predisposition to cardiomyopathy or family history of cardiomyopathy. *Stage B* (pre-heart failure), individuals who have no past or present symptoms of HF but exhibit structural heart abnormalities, elevated cardiac filling pressures, or other contributing risk factors. *Stage C* (symptomatic heart failure), patients with current or previous signs and symptoms of HF. *Stage D* (advanced heart failure), which is described below.

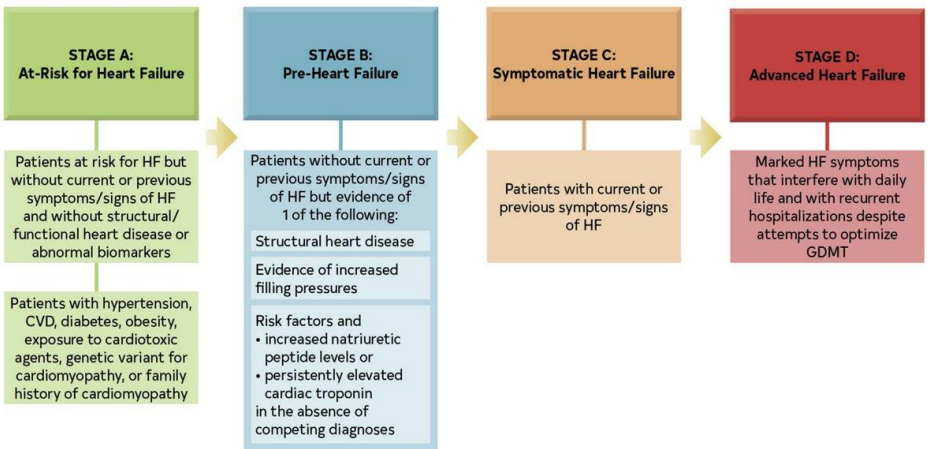


Figure 1. American Heart Association stages of heart failure [11].

Advanced heart failure

Despite improved overall survival in HF patients, a portion of the chronic HF population experiences persistent symptoms despite optimized medical and interventional therapies, ultimately progressing to the end stage of the disease. Patients with AdHF endure severe, persistent symptoms and a poor quality of life due to exercise intolerance [2]. Many also face frequent hospitalizations caused by acute decompensation or low cardiac output. The prognosis for AdHF remains poor. In eligible patients, cardiac replacement therapies, such as durable left-ventricular assist devices (LVADs) or heart transplantation (HTX), are often the only viable options. For those who are not candidates for advanced therapies, the focus should shift to best supportive care to enhance comfort and quality of life.

Definition

Several definitions have been used to define AdHF. The most widely applied derives from the 2018 Position Statement of the Heart Failure Association [12], subsequently adopted by the European Society of Cardiology (ESC) and the AHA in the most recent guidelines for the management of HF patients. In order to diagnose AdHF, all these criteria must be present despite optimal guideline-directed treatment:

1. Severe and persistent symptoms of HF (New York Heart Association – NYHA - class III [advanced] or IV)

2. Severe cardiac dysfunction defined by ≥1 of these:
   - *Left Ventricular Ejection Fraction (LVEF) ≤ 30%*
   - *Isolated right ventricular (RV) failure*
   - *Non-operable severe valve abnormalities*
   - *Non-operable severe congenital heart disease*
   - *LVEF ≥40%, elevated natriuretic peptide levels, and evidence of significant diastolic dysfunction*

3. Hospitalizations or unplanned visits in the past 12 months for episodes of:
   - *Congestion requiring high-dose intravenous diuretics or diuretic combinations*
   - *Low output requiring inotropes or vasoactive medications*
   - *Malignant arrhythmias*

4. Severe impairment of the exercise capacity with inability to exercise or low 6-minute walk test distance (<300 m) or peak VO2 (<12–14 mL/kg/min) estimated to be of cardiac origin.

This definition highlights the multifaceted nature of AdHF. Firstly, it encompasses not only severe left ventricular (LV) systolic dysfunction but also isolated right ventricular (RV) dysfunction and HFpEF. Secondly, it includes various manifestations of advanced stages, going beyond recurrent hospitalizations to account for the loss of hemodynamic stability, characterized by worsening congestion and/or hypoperfusion. Rather than relying on a single parameter, a combination of these clinical features indicates that a patient has become refractory to conventional therapies. The Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) profiles were developed to classify patients who may benefit from durable mechanical circulatory support (MCS) devices. These profiles outline clinical parameters and characteristics that indicate a need for advanced therapies (**Table 1**).

Table 1. INTERMACS profiles [13]

Profile	Description	Details	Time for intervention
1	Critical cardiogenic shock	Life-threatening hypotension despite rapidly escalating inotropic support, critical organ hypoperfusion, often confirmed by worsening acidosis and/or lactate levels. “Crash and burn”	Within hours
2	Progressive decline on inotropic support	Declining function despite intravenous inotropic support, may be manifest by worsening renal function, nutritional depletion, inability to restore volume balance “Sliding on inotropes.” Also describes declining status in patients unable to tolerate inotropic therapy	Within few days
3	Stable but inotrope dependent	Stable but inotrope dependent	Weeks to few months
4	Resting symptoms at home on oral therapy	Patient can be stabilized close to normal volume status but experiences daily symptoms of congestion at rest or during ADL. Doses of diuretics generally fluctuate at very high levels. More intensive management and surveillance strategies should be considered, which may in some cases reveal poor compliance that would compromise outcomes with any therapy. Some patients may alternate between 4 and 5.	Weeks to few months
5	Exertion intolerant	Comfortable at rest and with ADL but unable to engage in any other activity, living predominantly within the house. Patients are comfortable at rest without congestive symptoms, but may	Variable urgency, depends upon

Profile	Description	Details	Time for intervention
		have underlying refractory elevated volume status, often with renal dysfunction. If underlying nutritional status and organ function are marginal, patient may be more at risk than INTERMACS 4 and require definitive intervention.	maintenance of nutrition, organ function, and activity
6	Exertion limited	Without evidence of fluid overload, is comfortable at rest, and with activities of daily living and minor activities outside the home but fatigues after the first few minutes of any meaningful activity. Attribution to cardiac limitation requires careful measurement of peak oxygen consumption, in some cases with hemodynamic monitoring to confirm severity of cardiac impairment. “Walking wounded”	Variable, depends upon maintenance of nutrition, organ function, and activity level.
7	Advanced NYHA Class III symptoms	A placeholder for more precise specification in future, this level includes patients who are without current or recent episodes of unstable fluid balance, living comfortably with meaningful activity limited to mild physical exertion.	Transplantation or circulatory support may not currently be indicated.

They have also proved to be useful in prognosticating outcomes for patients undergoing HTX or LVAD implantation, whether in urgent or ambulatory settings [15,16].

Epidemiology

The age-adjusted incidence of HF in developed countries has declined over recent decades, thanks to improvements in the management of cardiovascular diseases [17]. However, the overall incidence of HF continues to rise due to an aging population [2]. Establishing the true prevalence of AdHF remains challenging, largely due to the lack of a standardized definition and the limited number of epidemiological studies specifically focused on AdHF. Nevertheless, it is estimated that AdHF affects 1–10% of the overall HF population, with prevalence increasing year by year [17]. As mentioned earlier, the prognosis for patients with AdHF remains poor. Despite advances in current therapies, the estimated 1-year mortality rate for these patients ranges from 25% to 75% [18]. In a large population-based study, AdHF patients experienced an average hospitalization rate of 2.91 per person-year (95% CI: 2.78–3.06) during the first year after diagnosis. Notably, there are no significant differences in all-cause mortality or hospitalization rates across different LVEF classes [17]. The clinical course of AdHF is characterized by frequent hospitalizations or unplanned medical visits, often requiring adjustments to diuretic regimens or cycles of inotropic therapy. Identifying patients at higher risk for these adverse events remains a significant challenge, highlighting the need for further studies to address this critical issue.

Long-term treatment of Advanced Heart Failure

Long-term therapies for AdHF should be considered for patients in whom guideline-directed medical and device therapies have failed to sufficiently improve hemodynamics, alleviate symptoms, or preserve end-organ function. HTX remains the gold standard for enhancing both long-term survival and quality of life. However, recent advancements in long-term MCS have brought survival rates closer to those achieved with HTX.

Early referrals of AdHF patients are critical to prevent physical debilitation and end-organ dysfunction typical of prolonged AdHF, as

these complications can disqualify patients from advanced therapies. For those ineligible for durable treatments, long-term inotropic support can be used to alleviate symptoms and improve quality of life. In such cases, palliative and supportive care should also be prioritized to ensure comfort during end-of-life stages.

Long-term mechanical circulatory support devices

LVADs are electromechanical pumps designed to support the failing left heart by maintaining distal perfusion through continuous blood flow from the left ventricle to the ascending aorta. An LVAD system consists of an internal pump, an external controller unit with batteries, and a driveline—a percutaneous cable that connects the internal and external components.

LVADs are utilized in AdHF for various purposes:

1. **Bridge to Transplantation (BTT):** to sustain the patient until HTX.

2. **Bridge to Candidacy (BTC):** to address reversible contraindications to HTX.

3. **Destination Therapy (DT):** for patients with irreversible contraindications to HTX.

According to international guidelines, long-term MCS should be considered for patients with INTERMACS profiles 2 to 4. It may also be appropriate for patients with INTERMACS profiles 5–6 who exhibit high-risk characteristics, such as frequent hospitalizations, worsening end-organ dysfunction, refractory congestion, or inability to perform cardiopulmonary exercise tests with adequate oxygen consumption. Additionally, patients recovering from INTERMACS profile 1 while on short-term MCS, provided they have no irreversible non-cardiac organ failure, may also qualify for long-term MCS [2].

A clinical consensus statement has been recently published by the European Association Cardiovascular Imaging (EACVI) for the multimodality assessment of patients before and after LVAD implantation [19]. A thorough evaluation of RV function is essential for determining LVAD eligibility and is a critical predictor of post-

implantation survival and outcomes (**Figure 2**). This assessment should be multiparametric, incorporating clinical observations, laboratory tests, echocardiographic findings, and invasive hemodynamic measurements, including right heart catheterization (RHC) [20]

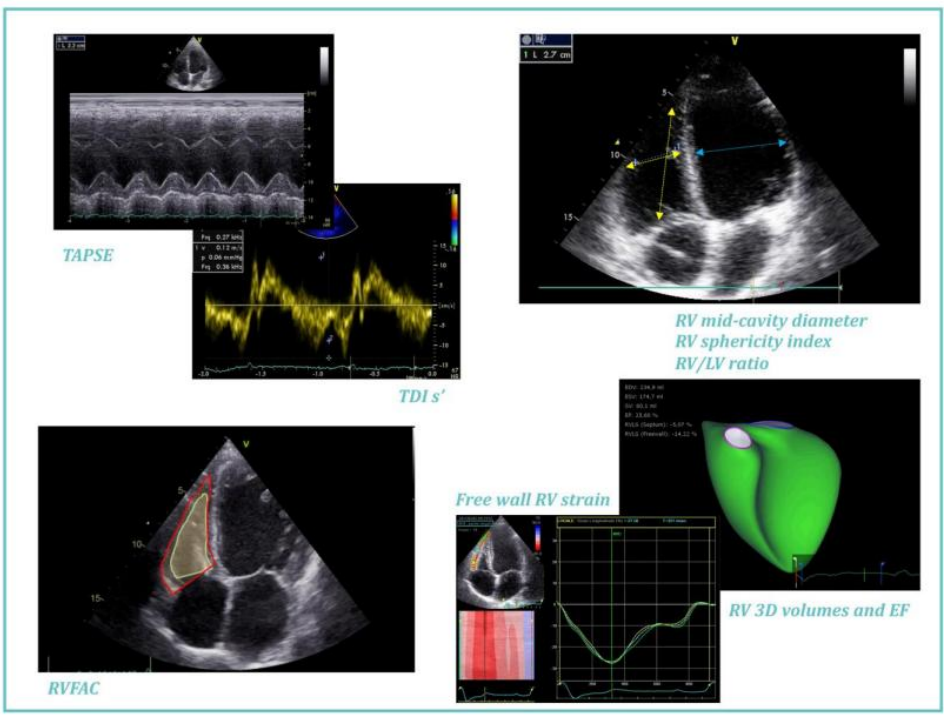


Figure 2. Multiparametric assessment of the right ventricle before LVAD implantation (from [19]).

The HeartMate III (Abbott, Chicago, Illinois, **Figure 3**), a fully magnetically levitated centrifugal pump, is the latest LVAD model approved across various countries. Compared to its predecessor, the axial-flow HeartMate II, the HeartMate III has shown a significantly lower incidence of hemocompatibility-related complications, including non-surgical bleeding, thromboembolic events, pump thrombosis, and neurological events [21]. Additionally, a 5-year follow-up analysis confirmed that centrifugal-flow pumps outperformed axial-flow pumps in overall survival to transplantation, recovery, or complication-free support [22].

Figure 3. HeartMate III, approved LVAD for AdHF patients (from Abbott webpage).

Patients with continuous-flow LVADs have reported survival rates of approximately 80% at 1 year and 70% at 2 years. In a recent study comparing 3-year survival between HeartMate 3 recipients and HTX patients aged 18–49 years, survival was similar (96.9% vs. 95.9%, p = 1.00). However, among patients aged ≥50 years, LVAD survival was slightly lower (75.0% vs. 83.9%, p = 0.60) [23]. The ELEVATE registry, which includes real-world data on HeartMate 3 LVADs, reported 2-year survival of 74.5%, with rates of gastrointestinal bleeding (9.7%), stroke (10.2%), and pump thrombosis (1.5%) [24].

Despite advancements, LVAD-related infections remain a significant concern, with driveline exit site infections being the most common complication, affecting 10–52% of recipients [19]. To address this issue, future developments are focusing on fully implantable devices, such as

the Leviticus FiVAD (Leviticus-Cardio, Israel), which features wireless internal battery recharging systems.

Total artificial heart

The Total Artificial Heart is a MCS device designed to replace both ventricles, serving as an alternative to HTX for patients with severe biventricular failure. To date, the Total Artificial Heart has been successfully implanted in over 1,700 patients as a temporary, life-saving technology used exclusively as a BTT [19]. Despite more than six decades of research, a Total Artificial Heart suitable for DT remains unavailable. Several challenges, including high complication rates, large device size, limited durability, and reduced quality of life for patients, continue to restrict the broader use of Total Artificial Heart.

A recent innovation showing promise is the CARMAT Total Artificial Heart. This device incorporates features that simulate more physiologically accurate cardiac function:

- Blood compatibility: It uses bovine pericardium to reduce clotting and improve biocompatibility.

- Pulsatile flow: Hydraulic pumps mimic natural systole and diastole, providing pulsatile blood flow.

- Self-regulation: The device adjusts output based on the patient's physiological needs, enhancing responsiveness.

While these advancements bring hope for the future of Total Artificial Heart technology, further development is needed to make these devices viable for long-term use.

Orthotopic heart transplantation

HTX is the gold standard treatment for AdHF, offering significant improvements in both quality of life and functional status [2]. The history of HTX began with the groundbreaking work of Dr. Norman Shumway and Dr. Richard Lower, and Dr. Christiaan Bernard, which performed the first successful HTX in Cape Town in South Africa in 1967. Since then, surgical techniques have undergone substantial refinement, resulting in better outcomes and increased survival rates.

A major milestone in the field was the introduction of cyclosporine, which revolutionized post-transplant care and marked the beginning of the modern era of heart transplantation. Today, HTX provides the best survival benefits for patients with AdHF, with the International Society for Heart and Lung Transplantation registry reporting a median survival of over 12 years [25].

The bicaval technique (**Figure 4**) is currently the most widely used surgical approach, offering greater efficiency and lower risk compared to standard methods [26]. Following transplantation, over 90% of patients achieve NYHA functional class I or II status within 1 to 3 years, with a marked improvement in symptoms and overall quality of life [27].

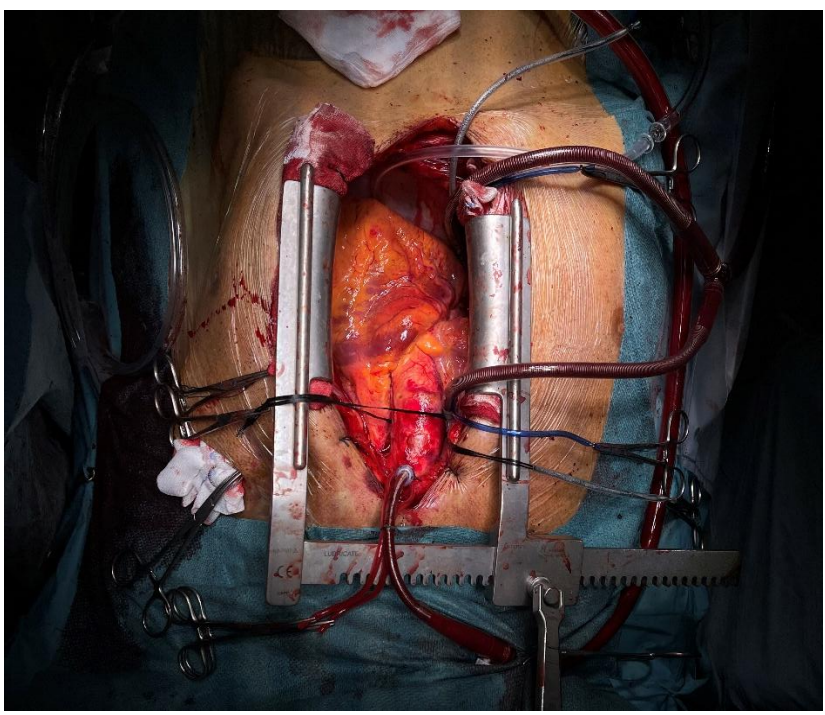


Figure 4. Heart transplantation by bicaval technique.

Outcomes following HTX are influenced by a variety of recipient's and donor's factors. For recipient-related factors, the need for end-organ support, such as extracorporeal membrane oxygenation (ECMO) or

mechanical ventilation, before HTX significantly increases the risk of 1-year mortality [28]. In a study from the Spanish National Heart Transplant Registry, patients listed for urgent HTX in INTERMACS categories 1 and 2 were found to have the highest risk of complications, including primary graft failure, need for renal replacement therapy, and in-hospital mortality [15].

Several recipient characteristics are also linked to increased 1-year mortality after HTX. These include reduced kidney function (as indicated by low estimated glomerular filtration rates), elevated total bilirubin levels, increased pulmonary vascular resistance, and a high level of reactive antibodies at the time of transplantation [28].

On the donor side, factors like age, LV dysfunction, and sex mismatch can also impact outcomes. According to International Society for Heart and Lung Transplantation (ISHLT), donor age and ischemic time are the most significant factors affecting HTX outcomes. Clinical experience shows that ischemic times longer than 4 hours have the most noticeable impact on outcomes, and donor hearts with an ischemic time expected to exceed 6 hours should generally be avoided [29].

Matching the donor and recipient appropriately is crucial for a successful transplant. This includes ensuring ABO blood compatibility, immunocompatibility, human leukocyte antigen (HLA) matching (especially in cases of polysensitization), and size matching.

The decision between HTX and LVAD therapy is complex and depends on the individual patient. Factors such as clinical condition, body size (which can change over time), and other factors unrelated to the patient—like time on the transplant waiting list, the experience of the transplant center, and regional demographic characteristics (like blood group distribution and HLA) can all influence the choice of therapy.

The main indications and contraindications for HTX are summarized in **Table 2**.

Table 2. Indications and contraindications for HTX [2]

Indications
Advanced heart failure
No other therapeutic option, except for LVAD as BTT
Contraindications
Active infection (relative)
Severe peripheral arterial or cerebrovascular disease
Pharmacologic irreversible pulmonary hypertension (LVAD should be considered to reverse elevated pulmonary vascular resistance with subsequent re-evaluation to establish candidacy)
Malignancy with poor prognosis (a collaboration with oncology specialists should occur to stratify each patient as regards their risk of tumor progression or recurrence which increases with the use of immunosuppression)
Irreversible liver dysfunction (cirrhosis) or irreversible renal dysfunction (e.g. creatinine clearance <30 mL/min/1.73 m^2). *Combined heart-liver or heart-kidney transplant may be considered*
Systemic disease with multiorgan involvement
Other serious comorbidities with poor prognosis
Pre-transplant BMI >35 kg/m^2 (weight loss is recommended to achieve a BMI <35 kg/m^2)
Current alcohol or drug abuse
Psychological instability that jeopardizes proper follow-up and intensive therapeutic regime after heart transplantation
Insufficient social supports to achieve compliant care in the outpatient setting

BMI = body mass index; BTT = bridge to transplantation; LVAD = left ventricular assist device.

Pulmonary hypertension: definition, diagnosis and role in advanced heart failure

Pulmonary hypertension (PH) is characterized by an increase in the mean pulmonary artery pressure (PAP) at rest. The threshold for diagnosing PH has recently been lowered from 25 mmHg to 20 mmHg, according to the 2022 ESC guidelines [30]. This decision was made by the ESC Task Force to improve the sensitivity of PH detection, allowing for earlier intervention and more timely initiation of specific therapies, as even mildly elevated PAP has significant prognostic implications. This change was supported by studies examining the upper limit of normal PAP in healthy individuals [31-33] and by research on the prognostic relevance of increased PAP [34-36]. Accurately diagnosing PH is especially important in the context of AdHF, as PH can affect eligibility for HTX. Unreversible PH in AdHF patients significantly raises the risk of graft failure, making early identification crucial for successful outcomes [30].

Table 3 outlines the clinical classification of PH.

Table 3. Clinical classification of PH (from [30])

GROUP 1 Pulmonary arterial hypertension (PAH)	
Idiopathic	Non-responders at vasoreactivity testing
	Acute responders at vasoreactivity testing
Heritable	
Associated with drugs and toxins	
Associated with	Connective tissue disease
	HIV infection
	Portal hypertension
	Congenital heart disease
	Schistosomiasis
PAH with features of venous/capillary (PVOD/PCH) involvement	
Persistent PH of the newborn	
GROUP 2 PH associated with left heart disease	
Heart failure	with preserved ejection fraction
	with reduced or mildly reduced EF
Valvular heart disease	
Congenital/acquired cardiovascular conditions leading to post-capillary PH	
GROUP 3 PH associated with lung diseases and/or hypoxia	
Obstructive lung disease or emphysema	
Lung disease with mixed restrictive/obstructive pattern	
Hypoventilation syndromes	
Hypoxia without lung disease (e.g. high altitude)	
Developmental lung disorders	
GROUP 4 PH associated with pulmonary artery obstructions	
Chronic thrombo-embolic PH	
Other pulmonary artery obstructions	
GROUP 5 PH with unclear and/or multifactorial mechanisms	
Hematological disorders	

Systemic disorders
Metabolic disorders
Chronic renal failure with or without hemodialysis
Pulmonary tumor thrombotic microangiopathy
Fibrosing mediastinitis

In patients with AdHF, PH is typically classified as Group 2, which includes those with left heart disease [37]. RHC remains the gold standard for assessing pulmonary circulation pressures and resistances [38]. While RHC is invaluable for making an accurate diagnosis and identifying specific PH phenotypes, it is an invasive procedure that carries potential complications, including issues with vascular access, tricuspid valve injury, right ventricular perforation, infective endocarditis, and arrhythmias [39].

Due to these risks, RHC is reserved for specific clinical scenarios, such as assessing adult congenital heart disease, cardiac evaluation before HTX or LVAD implantation, and quantifying intracardiac shunts. To assess PH risk without these complications, current guidelines recommend using echocardiographic estimated peak tricuspid regurgitation velocity (TRV) as a non-invasive alternative, with a threshold of >2.8 m/s to estimate the likelihood of PH [30,40]. However, the accuracy of echocardiographic estimates of PAP has been debated in recent years, and conflicting evidence has emerged regarding its reliability [41-44]. Despite recent revisions to the hemodynamic definition of PH, the threshold for peak TRV remains unchanged at 2.8 m/s, though further validation from literature is still needed [30,43,44-47].

Complications and mortality after HTX

The causes of death following HTX vary depending on the time since surgery.

Primary graft failure is the leading cause of mortality within the first 30 days after HTX. This is a complex clinical syndrome with multiple potential causes, including pre-existing myocardial dysfunction in the donor, catecholamine-induced injury to the graft, surgical complications such as air embolism, cardioplegia, or delayed reperfusion injury, and recipient-related factors like unrecognized PH.

Later complications are typically related to immunosuppressive therapy, which must follow a delicate balance between preventing graft rejection and minimizing the risk of infections and cancer. Infections, both common and opportunistic, are the leading cause of death between six months and one year after HTX. These include typical bacterial and viral infections as well as opportunistic infections common in immuno-compromised patients.

Cellular (acute cellular rejection, ACR) and antibody-mediated rejection (AMR) are significant causes of mortality within the first 2-3 years after HTX, contributing to about 10% of deaths during this period [48]. Acute cellular rejection, or T-cell mediated rejection, occurs when the recipient's T-cells attack the graft, leading to direct myocardial injury [49]. On the other hand, AMR happens when the recipient's antibodies target the donor's HLA antigens, activating the complement system and causing microcirculation stress, which gradually leads to parenchymal atrophy and fibrosis [50].

Currently, endomyocardial biopsy (EMB) is the gold standard for detecting cardiac rejection, but it is an invasive procedure with significant risks, such as wall rupture and pericardial tamponade. Additionally, interpreting EMB results can be challenging and somewhat subjective.

Beyond the first year after HTX, cardiac allograft vasculopathy (CAV) and malignancies become more common. CAV is particularly the leading cause of death after 1–3 years post-transplant. The immunosuppressive drugs used to prevent graft rejection, as well as cytomegalovirus infections, play a major role in the development and progression of CAV [51].

Similar to rejection, early diagnosis of CAV is typically done via invasive methods like coronary angiography, which carries risks. The most recent guidelines recommend annual coronary angiography for the first five years after transplantation, followed by every two years thereafter. However, coronary angiography has significant limitations, especially for younger patients due to radiation exposure and for those with chronic kidney disease due to the risks of iodine contrast agents, which are commonly used in transplant recipients [52].

Given these challenges, the ability to monitor cardiac function using non-invasive strategies, such as advanced echocardiographic techniques,

would be highly beneficial to reduce the risks associated with EMB and coronary angiography, and deserve dedicated research.

Natriuretic peptides in heart transplantation

In HTX patients, BNP and its precursor, N-terminal pro-peptide (NT-proBNP), are often elevated, particularly within the first two months after transplant. Some studies have shown that higher BNP levels are associated with ACR and AMR. Additionally, RV dysfunction and tricuspid regurgitation have been identified as predictors of elevated BNP levels in connection to CAV [53].

Elevated NT-proBNP levels in HTX patients are also linked to renal failure and liver dysfunction [54]. However, the specific patterns of NT-proBNP changes after HTX, particularly in relation to comorbidities, and how they can guide treatment or prognosticate have not been fully explored in large patient populations.

Advanced echocardiography: Speckle Tracking

Global longitudinal strain in HF and HTX

Global longitudinal strain (GLS) measured by Speckle Tracking Echocardiography (STE) provides a precise, angle-independent, and highly reproducible assessment of LV myocardial deformation during the cardiac cycle [55]. It offers valuable insights into the function of the endocardial fibers (**Figure 5**). A decrease in GLS indicates subclinical myocardial dysfunction, often preceding overt systolic dysfunction, making it an extremely sensitive and early marker of cardiac changes.

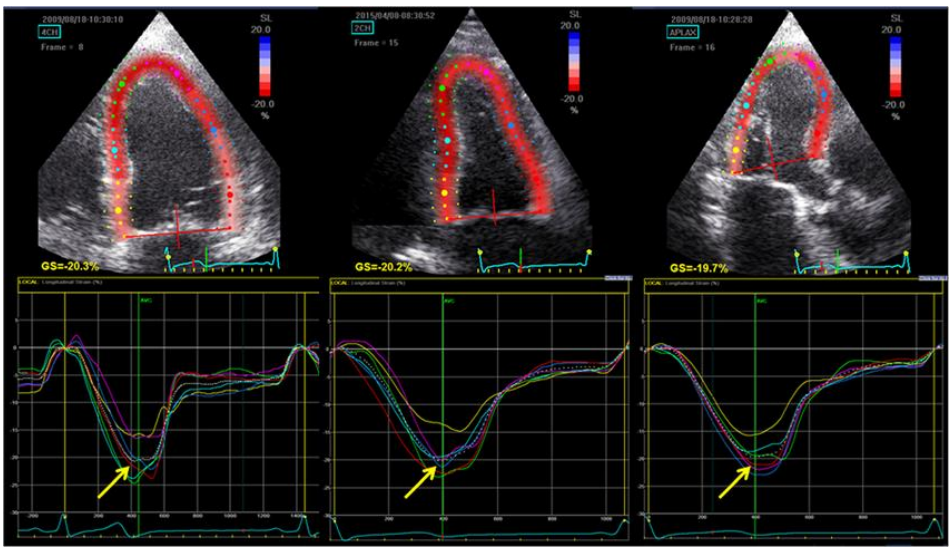


Figure 5. Global longitudinal strain is calculated as the average value of 4-, 2- and 3-chamber views peak longitudinal strain at end systole.

GLS has been shown to strongly correlate with outcomes such as cardiac and all-cause mortality in HF patients, regardless of clinical profile, medications, NYHA class, or overall cardiac function [56]. This makes it a powerful tool for risk stratification.

In pediatric patients, GLS plays a significant role as well. It has been demonstrated to be a sensitive and specific marker for detecting rejection after HTX in children [57]. Similarly, GLS has been studied in the adult HTX population [58], where it is useful for the early detection of developing AMR, potentially improving patient management [59]. In HTX patients with anti-HLA antibodies, GLS is significantly reduced [60]. Additionally, layer-specific GLS and the endocardial-epicardial strain gradient are abnormal in HTX patients with CAV [61].

Despite its numerous advantages, GLS has an important limitation: it is primarily afterload-dependent. This means its accuracy can be influenced by changes in systemic vascular resistance.

Normal GLS values in the general population range from -15.9% to -22.1%, with a mean of -19.7% (95% CI, -20.4% to -18.9%). Normal ranges for GLS can also vary based on common clinical factors such as age, weight (β –0.03, $p < 0.01$), and systolic blood pressure (β –0.02, $p < 0.01$) [62].

Myocardial work

Myocardial work (MW) is a promising non-invasive echocardiographic tool that combines LV afterload with GLS techniques, based on the concept of pressure-volume loops. MW has been studied in various clinical contexts, showing its ability to provide additional value in assessing cardiac function compared to traditional measures like LVEF and GLS [63].

While GLS offers a more sensitive quantification of LV function, both GLS and LVEF are influenced by afterload [64]. MW, on the other hand, normalizes LV function for afterload – measured through arterial blood pressure – providing an afterload-independent measure. By incorporating afterload into the calculation, MW yields a value expressed in mmHg%, representing the work of each cardiac segment.

MW assessment is performed using a vendor-specific algorithm (General Electric, GE). The process starts by acquiring three apical transthoracic views for GLS analysis. The software automatically processes any valve events, although they can also be manually adjusted. Once GLS is obtained, a bull's-eye plot is generated, displaying GLS values. Blood pressure, measured non-invasively with a sphygmomanometer, is recorded during image acquisition. The software incorporates the systolic and diastolic blood pressure values (in mmHg), and a bull's-eye plot is then created to show MW values [64,65] (**Figure 6**).

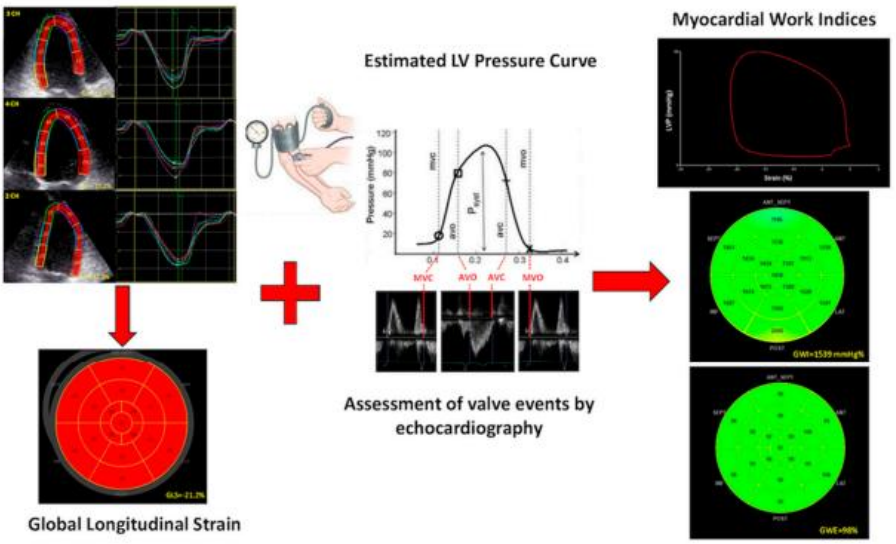


Figure 6. Steps for the calculation of LV myocardial work, incorporating blood pressure values into GLS algorithm [65].

MW offers a more accurate and load-independent assessment of myocardial function, enhancing our ability to evaluate cardiac performance and detect early signs of dysfunction.

Four values of MW are calculated:

1. **Global constructive work (GCW):** Work performed by the ventricle during systole that the contributes to LV function. Positive work (longitudinal shortening) during systole, negative work during isovolumetric relaxation (lengthening).

2. **Global work index (GWI):** total work done by the ventricle during systole, including isovolumetric relaxation.

3. **Global wasted work (GWW):** Work performed by the LV which does not contribute to LV function. Negative work during systole, positive during isovolumetric relaxation.

4. **Global work efficiency (GWE):** obtained as GCW/(GCW+GWW).

Figure 7 lists the normal range values of MW indices in a population of 226 healthy subjects according to gender and age ranges, as obtained in the EACVI Normal Reference Ranges for Echocardiography (NORRE) study [66].

The usefulness of MW in AdHF prognostication still needs to be proven. Equally, in the HTX population, normal reference values or dedicated studied with MW are missing.

Table 3 2DE parameters of myocardial work and blood pressure values according to gender and age

	Age 20–40 years ($n = 95$)		Age 40–60 years ($n = 97$)		Age ≥60 years ($n = 34$)		*P*-value		Male		Female	
	Male, mean ± SD or median (IQR)	Female, mean ± SD or median (IQR)	Male, mean ± SD or median (IQR)	Female, mean ± SD or median (IQR)	Male, mean ± SD or median (IQR)	Female, mean ± SD or median (IQR)	Male	Female	*R*	*P*-value	*R*	*P*-value
SBP (mmHg)	120 ± 10	108 ± 10*	124 ± 8	115 ± 13*	121 ± 7	122 ± 12	0.1	<0.001	0.12	0.3	0.4	<0.001
DBP (mmHg)	73 ± 9	69 ± 8*	76 ± 6	74 ± 9	74 ± 8	76 ± 8	0.1	0.002	0.12	0.2	0.3	0.001
GWI (mmHg%)	1758 ± 270	1800 ± 251	1900 ± 317	2027 ± 341	1866 ± 286	2002 ± 270	0.2	<0.001	0.16	0.1	0.25	0.002
GCW (mmHg%)	2186 ± 240	2109 ± 289	2267 ± 327	2329 ± 365	2226 ± 328	2338 ± 386	0.5	0.001	0.09	0.3	0.22	0.007
GWW (mmHg%)	99 (68–144.5)	90 (48–145)*	89 (58–122.5)	76 (51–118)	85 (49–129)	90 (48–145)	0.5	0.6	-0.13	0.2	0.06	0.4
GWE (mmHg%)	95 (93–97)	95 (94–97)*	96 (95–97)	96 (95–97)	96 (94–97)	95 (94–97)	0.6	0.8	0.12	0.2	-0.03	0.7

CI, confidence interval; DBP, diastolic blood pressure; GCW, global constructive work; GWE, global work efficiency; GWI, global work index; GWW, global work waste; IQR, interquartile range; SBP, systolic blood pressure; SD, standard deviation.
**P*-value < 0.05 vs. male.

Figure 7. MW indices in general population [66].

Aims

The main purpose of the thesis is to enlighten the key role of echocardiography in a selected group of patients with HF and advanced stage of the disease and patients after HTX, to improve diagnostic and prognostic insights required to fill in the current gaps and improve management.

In particular, detailed purposes were as follows:

- **Paper I**: in patients with AdHF undergoing echocardiography and RHC in the work-up for HTX or LVAD implantation, to assess the ability of peak TRV to predict PH, using both the new cutoff value for mean PAP of >20 mmHg and the old cutoff of >25 mmHg. In addition to compare the results with the mean right ventricular–right atrial (RV–RA) gradient, which is considered to correlate more closely with mean PAP.

- **Paper II**: in patients with AdHF, to evaluate the prognostic value of LV MW indices by testing their mid-term risk stratification ability, including all-cause mortality, LVAD implantation, HTX and acute HF hospitalization.

- **Paper III**: to establish reference ranges for 2D echocardiographic MW indices in healthy adult (without surgery-related complications, i.e. CAV and/or rejection) HTX patients and compare them with data from the EACVI NORRE healthy population.

- **Paper IV**: to track changes in NT-pro BNP levels over time in HTX patients and to identify potential predictors of short- and long-term variations in NPs levels to understand their utility in management of HTX patients.

Materials and Methods

Study populations

Advanced heart failure cohort

For paper I and II, we retrospectively screened patients with AdHF followed up at Siena University Hospital (Azienda Ospedaliera Universitaria Senese) for end-stage HF therapies suitability.

In particular, for paper I, we considered all patients who underwent a RHC between 2016 and 2021 with available echocardiographic images, excluding those with chronic obstructive pulmonary disease, any septal defects, poor acoustic window, or undetectable tricuspid regurgitation and absent informed consent.

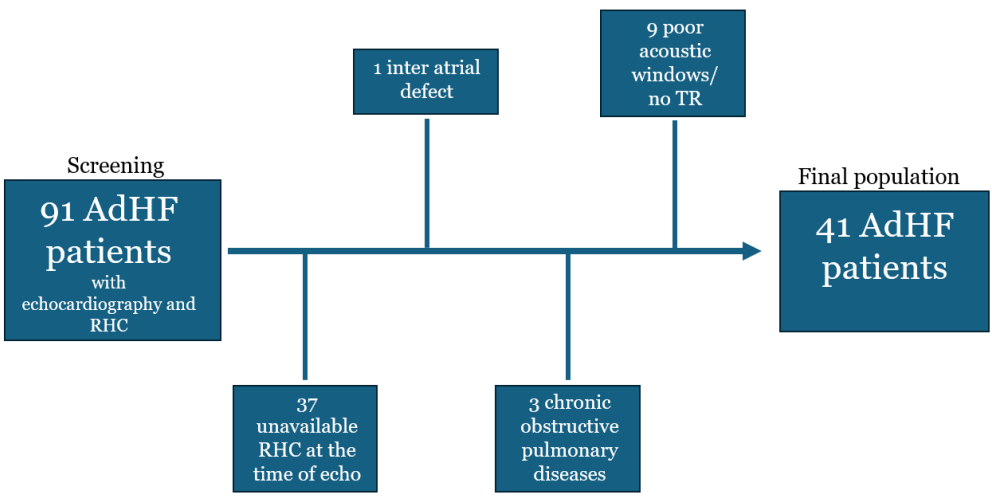


Figure 8. Paper I study population. AdHF = advanced heart failure; RHC = right heart catheterization; TR = tricuspid regurgitation.

For paper II, the screened population was bigger, including all AdHF patients referred to Siena clinic between January 2018 and December 2022, excluding only those with poor echocardiographic acoustic window, inadequate echocardiographic exams, unavailable brachial artery cuff pressure measured at the same time as the echocardiographic exam; atrial fibrillation; relevant heart valve diseases (i.e. more than mild mitral or aortic regurgitation); and absent informed consent.

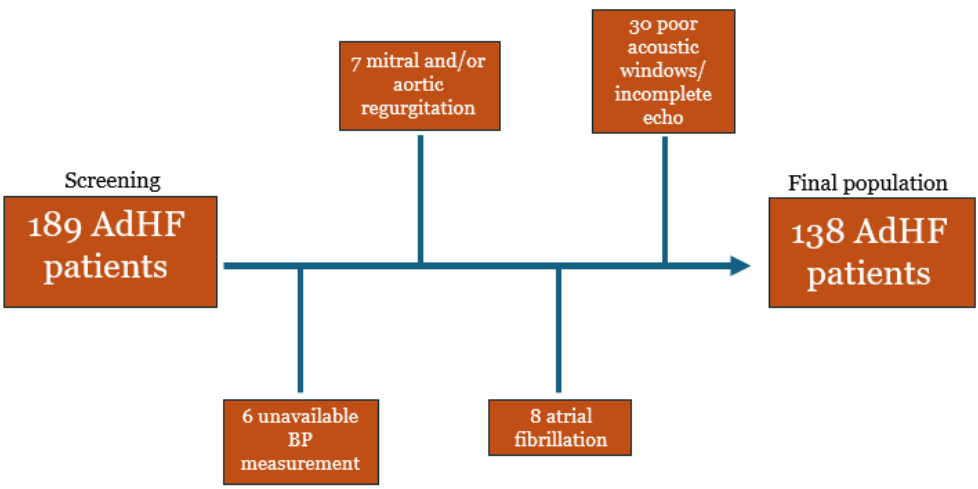


Figure 9. Paper II study population. AdHF = advanced heart failure; BP = blood pressure.

Heart transplanted patients' cohort

For paper III and 4 we selected patients regularly followed up after HTX at Siena University Hospital, in the protocol of screening for rejection and/or CAV.

In particular, for paper III we reviewed all consecutive HTX patients from September 2019 to May 2022 who underwent EMB. Patients were excluded if they lacked a complete echocardiographic evaluation, had insufficient image quality for STE LV analysis, or if brachial artery cuff pressure measurements were unavailable. Additional exclusions included patients with a known history of rejection, CAV, ACR, antibody-AMR at EMB, or the presence of donor-specific antibodies. Furthermore, patients with atrial fibrillation, more than mild mitral or aortic

regurgitation, or reduced/mildly reduced LVEF <50% were also excluded. For patients who underwent multiple EMBs during the study period, only the first biopsy was included in the analysis.

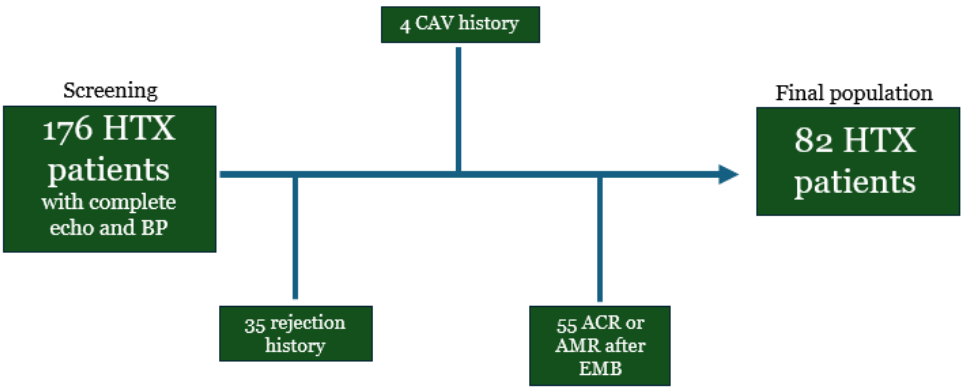


Figure 10. Paper III study population. ACR = acute cellular rejection; AMR = antibodies mediated rejection; EMB = endomyocardial biopsy; HTX = heart transplantation.

For paper IV, we reviewed all consecutive HTX with at least 10 evaluations available from one month after surgery, including anamnestic data, laboratory parameters, and standard echocardiographic assessment. We excluded patients with unavailable pre-HTX data (RHC, etiology of HF), unavailable NT-proBNP values at each time point, inadequate echocardiographic acoustic window, absent informed consent.

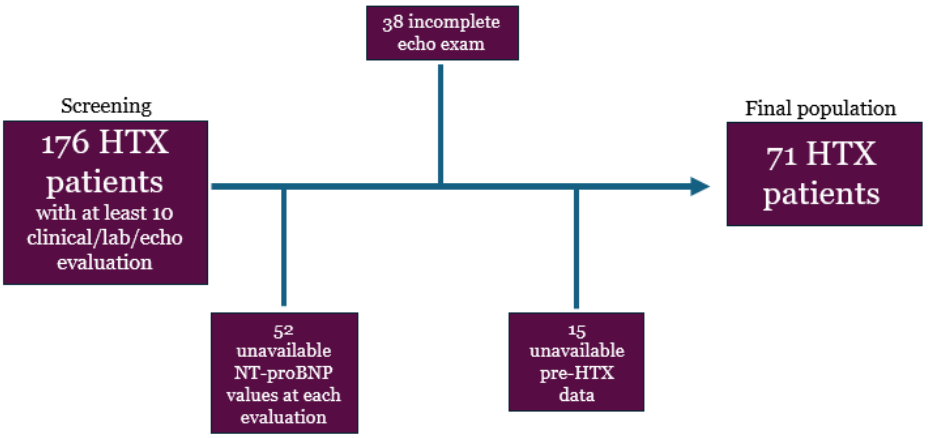


Figure 11. Paper IV study population. BNP = brain natriuretic peptide; HTX = heart transplantation.

Data collection and echocardiography

For each patient included in the thesis, clinical, demographic and laboratory data obtained from the institution's electronic records were reviewed and included in dedicated databases.

All echocardiographic assessments were conducted by skilled operators using a GE Vivid E80/E95 system, equipped with a 1.5–4.3 MHz phased-array transducer. Continuous ECG monitoring was performed throughout the examination, following the guidelines established by the American Society of Echocardiography and the EACVI.

Pulmonary pressure estimation

Comprehensive echocardiographic evaluations were conducted on the same day, immediately preceding RHC. TR was assessed using color Doppler imaging in the apical four-chamber view. A well-defined continuous-wave Doppler TR signal was recorded to precisely measure peak TRV and velocity–time integral (VTI). Cine imaging of the inferior vena cava (IVC) during a full respiratory cycle was also performed to estimate right atrial (RA) pressure based on its diameter and collapsibility. The mean pressure gradient between the right ventricle and right atrium (RV–RA) was retrospectively calculated using the TR VTI. Mean pulmonary artery pressure (mPAP) was determined by

adding the mean RV–RA gradient to the estimated mean RA pressure [68]. Additionally, the maximum RV–RA gradient was derived from the peak TRV using the modified Bernoulli equation ($4V^2$). Systolic PAP was calculated by adding the maximum RV–RA gradient to the estimated RA pressure, while diastolic PAP was obtained using the formula: diastolic PAP = 1.5 × [mean PAP – (systolic PAP/3)] [69].

Myocardial work analysis

The STE-LV strain analysis was performed semi-automatically using dedicated software across three apical views, with manual adjustments by the operator to optimize the region of interest width and positioning for accurate endomyocardial tracking. For MW assessment, markers indicating the opening and closure of the aortic and mitral valves were placed to define the start and end of each main phase of the cardiac cycle, based on the apical long-axis view [65].

Brachial blood pressure measurements were recorded during the echocardiographic exam and used to calibrate the reference curve for LV pressure estimation in terms of both timing and amplitude. The software (EchoPAC v204, GE Medical, Milwaukee, WI, USA) generated the key parameters described in the introduction:

- **Global Work Index (GWI):** The total work performed by the heart from mitral valve closure to mitral valve opening.
- **Global Constructive Work (GCW):** The work performed during systolic shortening combined with the work during lengthening in isovolumetric relaxation.
- **Global Wasted Work (GWW):** The work performed during systolic lengthening combined with the work during shortening in isovolumetric relaxation.
- **Global Work Efficiency (GWE):** The ratio of constructive work to the sum of GCW and GWW.

Right heart catheterization

In paper I, RHC was conducted immediately following the echocardiographic examination on the same day. A Swan–Ganz pulmonary artery catheter was utilized to measure mean RA pressure, as well as systolic, diastolic, and mean PAP, along with pulmonary arterial

wedge pressure (PAWP), following standard international procedures [70]. Pressure waveforms were recorded and analyzed by an experienced operator, who averaged the values over ten cardiac cycles, regardless of respiratory phase.

The cardiac index was calculated by adjusting the cardiac output, obtained using the indirect Fick method, for body surface area. Pulmonary vascular resistances was then estimated indirectly using pressure and flow measurements.

Endomyocardial biopsies

Patients enrolled in paper III and IV underwent EMB on a regular basis, standardized by international guidelines [71]. EMB were conducted on a day-hospital basis, as usual planned in our hospital. Vascular access was obtained using ultrasound guidance via the right internal jugular vein under local anesthesia, employing the Seldinger technique. The bioptome's correct positioning at the mid-septum level was confirmed using fluoroscopy before collecting the biopsy samples. Typically, four or more tissue samples were taken per patient. Once the procedure was completed, the biopsy specimens were immediately sent to the pathology department for analysis. Routine staining with hematoxylin and eosin was performed, with additional trichrome staining used in specific cases.

To exclude AMR, immunofluorescent or immunoperoxidase staining was employed, supplemented by solid-phase or cell-based assays to detect donor-specific antibodies. The International Society for Heart and Lung Transplantation definitions and diagnostic criteria were applied to identify both ACR and AMR.

Statistical analysis

Continuous variables were reported as either mean with standard deviation or median with interquartile range, depending on their distribution. The Kolmogorov–Smirnov test was applied to assess the normality of data distribution. Categorical variables were expressed as absolute numbers and percentages. Receiver Operating Characteristic (ROC) curves were used to evaluate the ability of echocardiographic parameters to predict outcomes (PH for Paper I, adverse events for paper II), with the Youden index employed to identify optimal cutoff values. Pearson's correlation coefficient was utilized to examine the strength of the association between echocardiographic measures and RHC findings in Paper I or NT-proBNP values in paper IV. Univariate and multivariate Cox proportional hazard regression analyses were

applied to assess predictors of outcomes in paper II where Kaplan–Meier analysis estimated event-free survival. Linear regression was applied to test possible association with MW indices and population characteristics in paper III. Regressions were estimated by first considering only pre-HTX parameters and then parameters immediately after HTX, to predict the nt-proBNP value at 1 year and at the last follow-up in paper IV. Stepwise procedures based on AIC criteria were performed.

A p-value of less than 0.05 was considered indicative of statistical significance. All analyses were performed using SPSS software, version 26 (SPSS Inc., Chicago, IL, USA).

Ethical considerations

The studies underlying this thesis were conducted in compliance with the Declaration of Helsinki. All patients signed an informed consent. All data were collected retrospectively so patients did not undergo any test or procedure differently from the planned routine work-up and follow-up.

Results

Paper I

The study population was made of 41 patients with AdHF. The median age of the study population was 58 years (interquartile range 52–62), with males comprising 82.9% (34 individuals). The average body surface area (BSA) was 1.97 ± 0.21 m^2. Among the participants, 41.4% (17 individuals) were smokers, 65.9% (27 patients) had dyslipidemia, and 24.4% (10 patients) were diagnosed with diabetes. Hypertension was observed in 34.1% (14 patients), while obesity (BMI > 30 kg/m^2) was present in 26.8% (11 patients). Chronic kidney disease (CKD), defined as an eGFR < 60 mL/min/1.73 m^2, was identified in 19.5% (8 patients).

Atrial fibrillation affected 39.0% (16 individuals), and 85.4% (35 individuals) had an implanted cardioverter-defibrillator (ICD). Regarding the etiology of HF, the majority, 61.0%, was nonischemic.

Prevalently, the population presented with a NYHA class II (61.0%) or III (24.4%). NT-proBNP concentrations had a median value of 1522 pg/mL (interquartile range 649–2550).

From an echocardiographic point of view, the whole population had a severely reduced LV EF (28 [23–35] %) with dilated left atrium (126 [98–146] ml) and increased LV filling pressures (E/e' 13 [10–17]). In total, 26.8% of the patients had a severe mitral regurgitation and a mild (51.3%) to moderate (29.2%) TR.

The median estimated RA pressure was 5 mmHg, with an interquartile range of 5–10 mmHg. The median TRV was 2.5 m/s (range 2.2–3.0 m/s). Systolic pulmonary arterial pressure (sPAP) had a median value of 34 mmHg (interquartile range 25–45 mmHg), while an acceleration time (AcT) averaged 101 ± 27 milliseconds. The mean RV–RA gradient was 18 mmHg (range 10–24 mmHg). Diastolic pulmonary arterial pressure (dPAP) had a median value of 18 mmHg (interquartile range 10–29 mmHg), and mPAP was 23 mmHg (range 15–35 mmHg). **Table 4** lists the results of RHC in the study population.

Table 4. Right heart catheterization indices in study population (from [72])

Parameter	
Mean RA pressure (mmHg)	7.5 ± 4.1
Systolic PA pressure (mmHg)	39 ± 12
Diastolic PA pressure (mmHg)	19 [12–24]
Mean PA pressure (mmHg)	27 ± 9
Pulmonary arterial wedge pressure (mmHg)	17 [10–22]
Cardiac index (L/min/mq)	2.07 ± 0.38
Pulmonary vascular resistance (WU)	2.51 [1.95–2.98]

Data are expressed as mean±SD or median [IQR].

Testing the performance of echocardiographic measurements for the prediction of pulmonary hypertension according to the new (**Figure 8**) and the old (**Figure 9**) guidelines definitions, we found that in the first scenario, peak TRV had the best AUC while in the second case estimated systolic PAP had a slightly superior specificity and sensitivity.

The cutoff value of 2.4 m/s of peak TRV had 65% sensitivity and 100% positive predictive value while 2.8 m/s a 44% sensitivity and 100% positive predictive for new predicted PH.

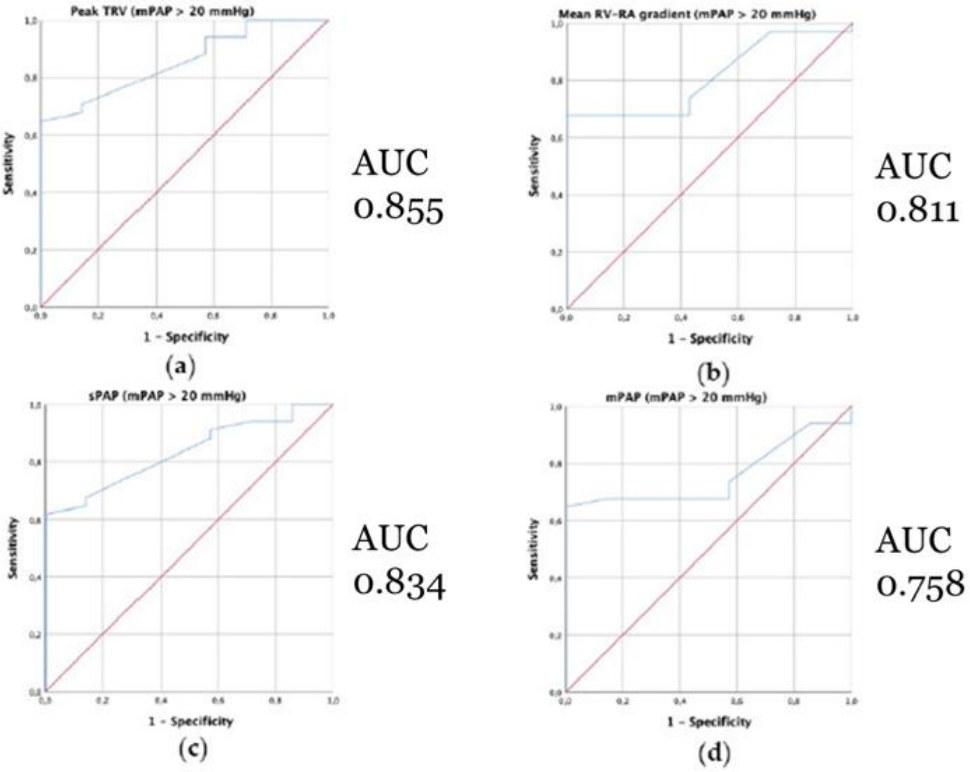


Figure 8. Comparison of Area Under the Curve (AUC) of TRV, mean RV-RA gradient, sPAP and mPAP for the prediction of PH according to the new definition [72].

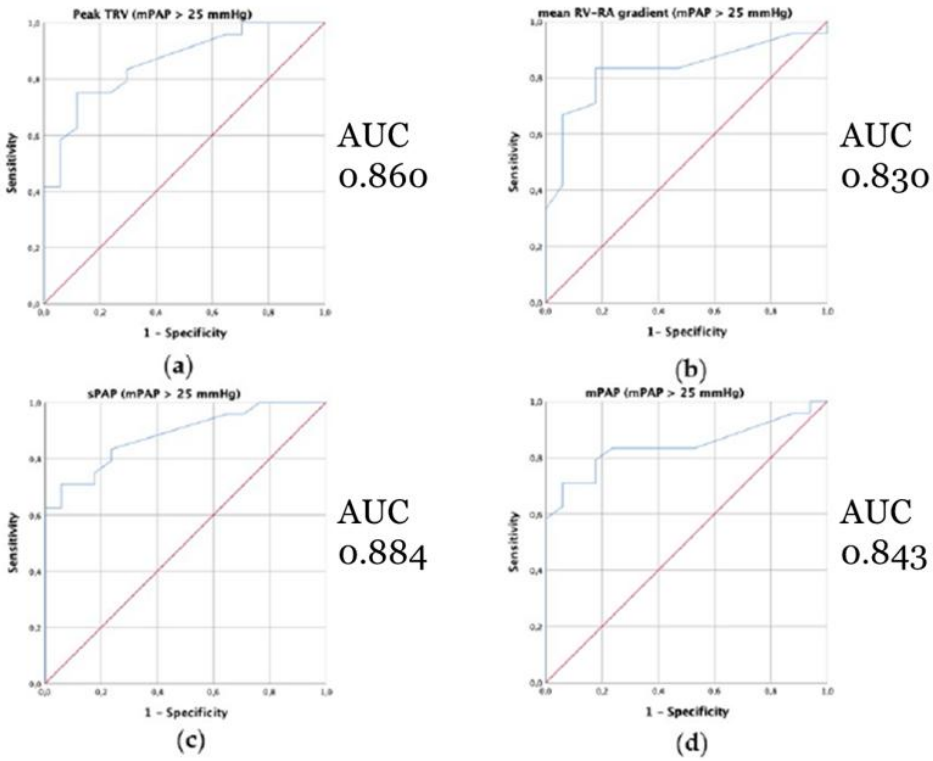


Figure 9. Comparison of Area Under the Curve (AUC) of TRV, mean RV-RA gradient, sPAP and mPAP for the prediction of PH according to the old definition [72].

Paper II

Among the screened AdHF patients of our center, we included 138 patients, with a median age of 58 [50-62] years, mostly males (80%) with nonischemic cardiomyopathies (66%) and a NYHA class at the time of the echocardiographic evaluation of II (60%) and III (27%) prevalently. 88% of individuals (122) had ICD at baseline. The study population was in optimal medical therapy, with a variable combination of ACE inhibitors/sartans/angiotensin receptors neprilysin inhibitors, B-blockers, mineralocorticoids receptors antagonists and sodium-glucose cotransporter 2 inhibitors, according to personal tolerance. In particular, sodium-glucose cotransporter 2 inhibitors use had a low prevalence among patients because they were not considered as a recommended first line therapy for HF at the time of enrollment.

The population was divided according to the occurrence of composite endpoint. Median LV EF was 30% [range: 23–35%], 23% [range: 20–28%] in the group with events vs 30% [range: 25–37%] with no events, showing a highly significant difference ($p < 0.001$). LA volume was also bigger in patients who met the endpoint (121 mL [range: 94–138 mL] vs 93 mL [range: 72–116 mL], $p = 0.001$) with poorer chamber function (LA strain 8.8% [range: 6.7–13.1%] vs 15.0% [range: 9.7–21.5%], respectively, $p = 0.004$). E/e' ratio as an estimate of LV filling pressures, was instead similar in the two groups 15 [range: 9–22] vs 11 [range: 7–14], $p = 0.064$. **Table 5** compares the values of STE LV parameters, including MW in the two groups.

Table 5. GLS and myocardial work parameters in study population according to primary events occurrence (from [73])

Parameter	***Composite end-point***	***No composite end-point***	***p-value***
LV GLS (%)	−5 [−8−−3]	−8 [−11−−5]	<0.001
LV GWE (%)	74 ± 11	77 ± 11	0.202
LV GWI (mmHg%)	346 [239–612]	660 [424–908]	<0.001
LV GCW (mmHg%)	573 [433–803]	939 [653–1184]	<0.001
LV GWW (mmHg%)	168 [97–229]	209 [142–287]	0.016

Data are expressed as mean±SD or median [IQR].

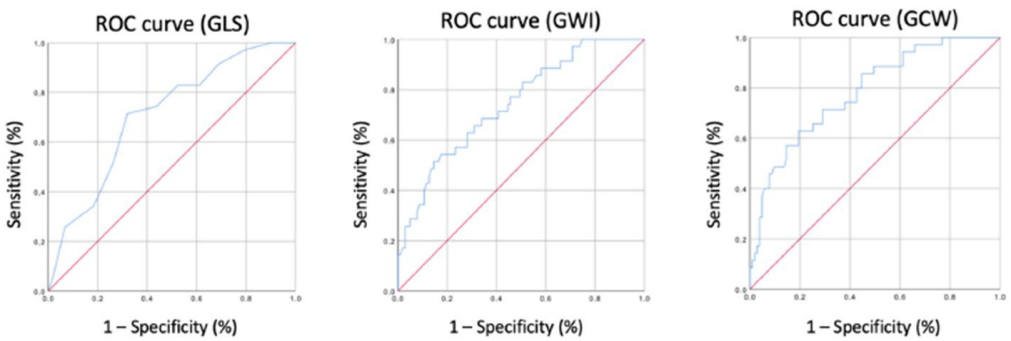
The population was also compared according to secondary endpoint occurrence. The LVEF was again lower in patients with events 25 [20–30]% vs 30 [23–35]%, p = 0.039, while LA size and function was similar between the two groups (volume 126 [90–156] ml vs 95 [75–121], p = 0.084; LA strain 7.2 [5.5–17.0] % vs 13.4 [9.0–19.6] %, p =0.050). **Table 6** lists the GLS and MW indices in comparison.

Table 6. GLS and myocardial work parameters in study population according to secondary event occurrence (form [73])

Parameter	***Composite secondary end-point***	***No composite secondary end-point***	***p-value***
LV GLS (%)	−6 [−8−−4]	−8 [−11−−5]	0.033
LV GWE (%)	76 ± 9	76 ± 11	0.849
LV GWI (mmHg%)	481 [287–651]	609 [362–880]	0.065
LV GCW (mmHg%)	614 [573–970]	814 [592–1182]	0.050
LV GWW (mmHg%)	167 [89–293]	198 [139–280]	0.575

Data are expressed as mean±SD or median [IQR].

At univariate analysis, some variables resulted associated with primary end-point, including kidney function, bilirubin, NYHA class, NT-proBNP but none of them remained associated with outcome at multivariate analysis. ROC analysis showed good predictive value of LV GLS, GWI and GCW for the composite endpoint (see **Figure 10**).



AUC 0.709 AUC 0.745 AUC 0.783

Figure 10. AUC comparison between GLS, GWI and GCW for the prediction of outcome [73].

GWE did not show good performance in stratifying the risk of primary events at Kaplan Meier analysis, while GWI, with a cut-off value of ≥613%mmHg according to the results of ROC curves, was able to prognosticate (**Figure 11**).

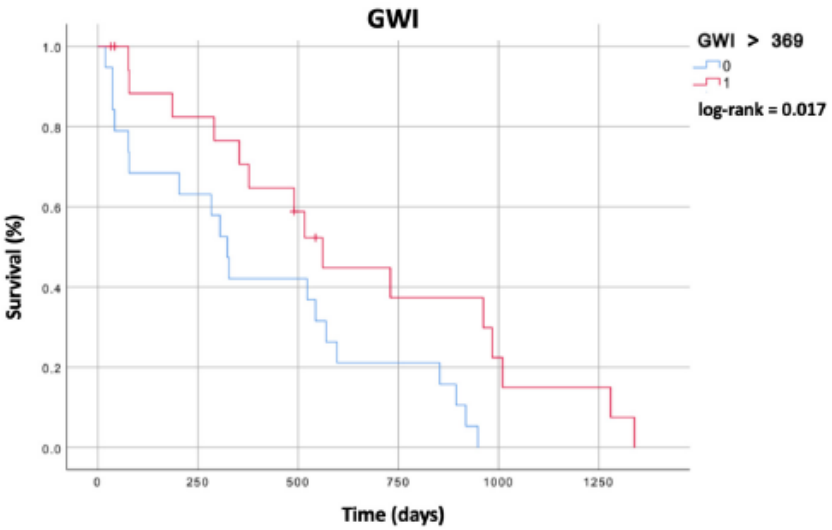


Figure 11. Kaplan Meier curves for event-free survival according to GWI value.

Paper III

The 83 patients enrolled after HTX had a mean age of 53 years [range: 46-62], prevalently men (56, 68.3%). No differences emerged in women vs men in terms of systolic and diastolic pressure, months from surgery, hemoglobin, immunosuppression regimens, LV EF and GLS, tricuspid annular plane systolic excursion (TAPSE) and sPAP. Also, MW parameters were similar for the two sexes (**Table 7**).

Table 7. Myocardial work parameters in study population according to sex (from [74])

Parameter	Women	Men	p-value
GWI (mmHg%)	1538 ± 415	1407 ± 403	0.189
GCW (mmHg%)	2143 ± 281	2034 ± 470	0.293
GWW (mmHg%)	296 (209–501)	315 (222–426)	0.894
GWE (%)	84 ± 9	84 ± 8	0.897

Data are expressed as mean±SD or median [IQR].

Data were compared to the known reference values of MW in general healthy population from the EACVI NORRE study. We found that patients after HTX, even with normal LV EF and no complications in term of rejection or CAV, had lower values of GWI, GCW and GWE and higher GWW (except in women which had similar GCW). **Table 8** details differences.

Table 8. Comparison between myocardial work indices in HTX and general population from EACVI NORRE (from [74])

Parameter	HTX patients	EACVI NORRE	p-value
GWI (mmHg%)	1447 ± 409	1896 ± 308	<0.001
GCW (mmHg%)	2067 ± 423	2232 ± 331	0.002
GWW (mmHg%)	310 (217–499)	79 (53–122)	<0.001
GWE (%)	84 ± 8	96 (94–97)	<0.001

Data are expressed as mean±SD or median [IQR].

Possible predictors of MW values were tested via univariate and multivariate analysis. GWE showed correlations with weight, systolic blood pressure, diastolic blood pressure, and glycate hemoglobin in univariate analysis. However, in multivariate analysis, significant associations were observed only with weight ($\beta = -0.410$, $p = 0.002$) and glycate hemoglobin ($\beta = -0.375$, $p = 0.005$). GWI was linked to weight, systolic blood pressure, and diastolic blood pressure in univariate analysis. Multivariate analysis revealed that only weight ($\beta = -0.205$, $p = 0.031$) and systolic blood pressure ($\beta = 0.529$, $p < 0.001$) remained significantly associated. GCW was associated with both systolic and diastolic blood pressure in univariate analysis, but only systolic blood pressure ($\beta = 0.497$, $p < 0.001$) retained a significant association in multivariate analysis. GWW demonstrated links with weight and glycate hemoglobin in univariate analysis. In multivariate analysis, glycate hemoglobin ($\beta = 0.406$, $p = 0.006$) was the only factor significantly associated.

Paper IV

After screening 176 patients, the study included a total of 71 participants, with 710 complete post-HTX laboratory and echocardiographic assessments. A total of 38 patients were excluded due to incomplete echocardiographic examinations, 52 due to unavailable 10 NT-proBNP results and 15 due to missing pre-HTX data. All patients underwent HTX using the bicaval technique.

Assessments were conducted at 10 different time points: at 1 month (1st NT-proBNP), 3 months (2nd NT-proBNP), 6 months (3rd NT-proBNP), and 1 year (4th NT-proBNP) post-transplant, followed by annual or semiannual evaluations, resulting in 10 total laboratory and clinical assessments.

The majority of patients were male (69.6%), with a mean age at HTX of 53.1±9.8 years. In 28.8% of cases, the transplant was performed as an emergency procedure. **Figure 12** illustrates the trend of NT-proBNP levels during follow-up.

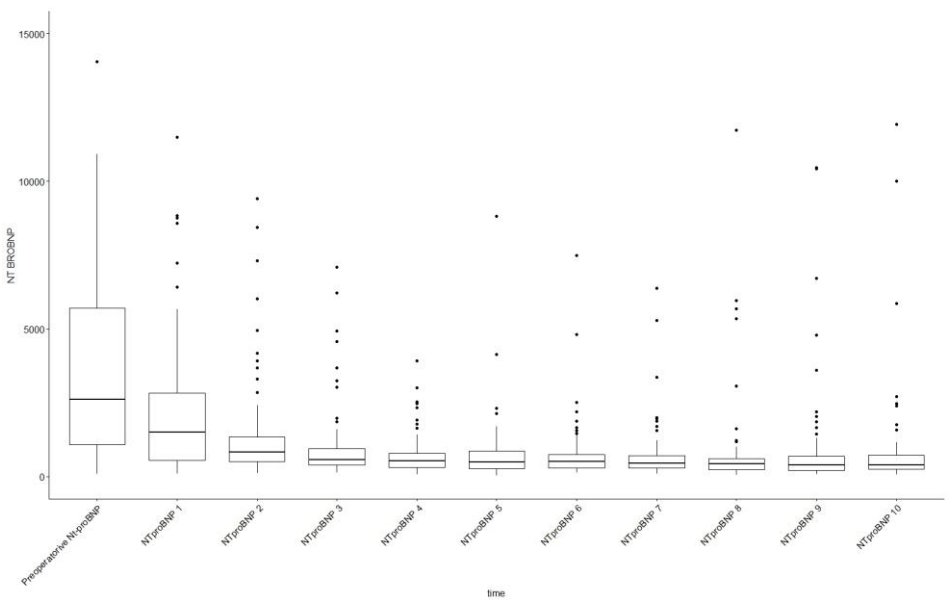


Figure 12. NT-proBNP trajectory after HTX

The 8th NT-proBNP measurement was obtained at a median postoperative time of 1423.0 [904.5; 1979.5] days, while the 9th was taken at a median of 1793.0 [1082.5; 2312.0] days. The longest available

follow-up (10th NT-proBNP) was recorded at a median of 2116.0 [1263.0; 2692.0] days, corresponding to nearly six years post-HTX.

At all outpatient visits, patients remained in NYHA class ≤II. However, NT-proBNP levels were generally higher than the normal reference values used for HF populations. Compared to the first assessment at 1 month, NT-proBNP levels gradually decreased at 3, 6, and 12 months, then remained relatively stable in the long-term follow-up.

Figures 13, 14, and 15 illustrate the differences in NT-proBNP levels based on sec, rejection episodes, and the development of CAV. Over the long-term follow-up, female patients, as well as those who experienced rejection or developed CAV, showed higher NPs levels.

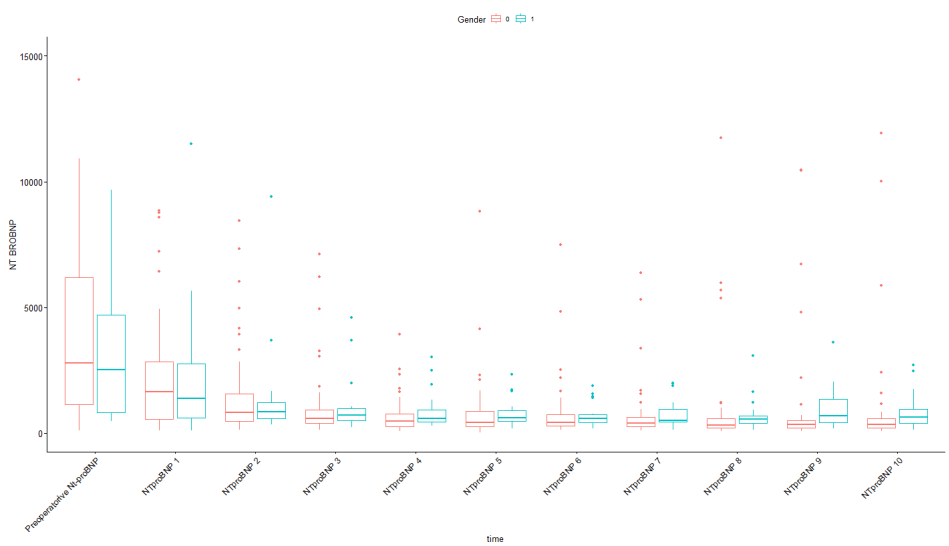


Figure 13. Comparison of NT-proBNP levels according to sex at different time points.

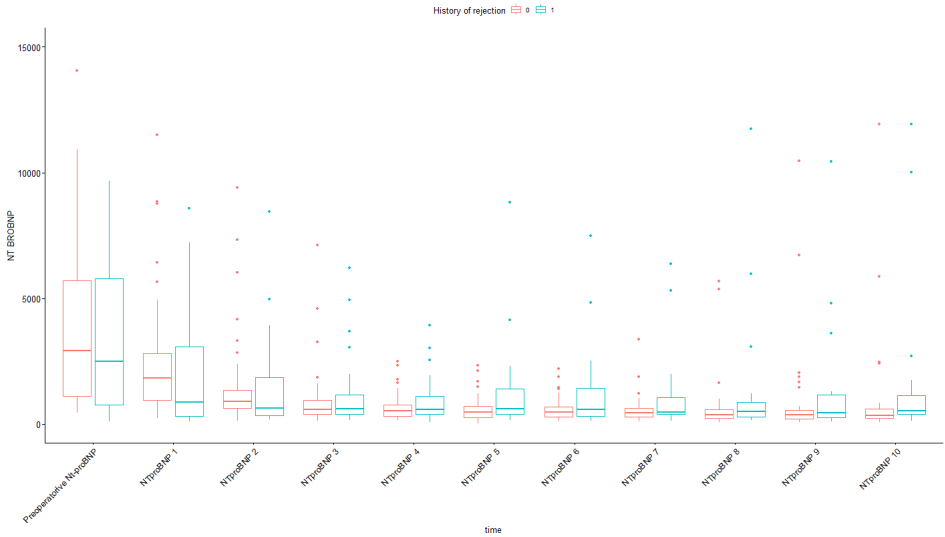


Figure 14. Comparison of NT-proBNP levels according to rejection at different time points.

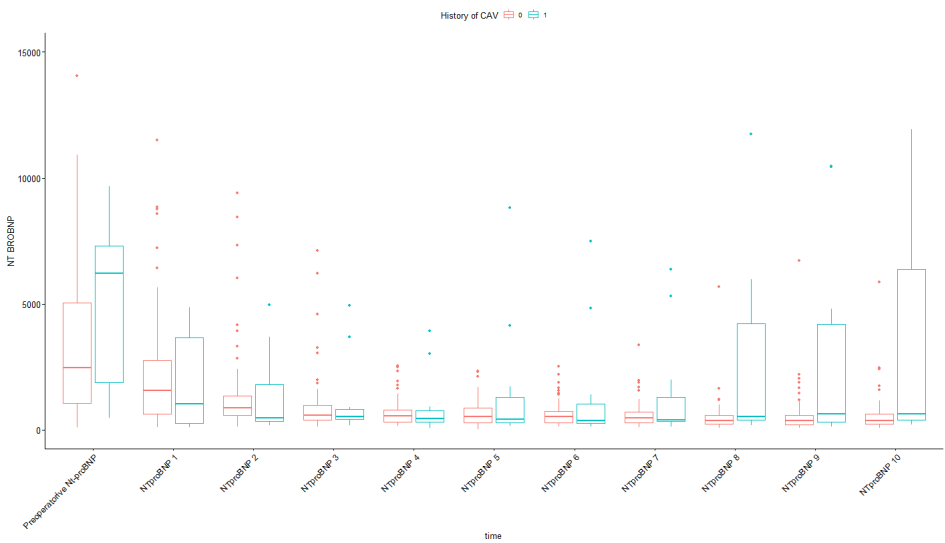


Figure 15. Comparison of NT-proBNP levels according to CAV at different time points.

When analyzing preoperative factors, we identified a statistically significant correlation between NT-proBNP levels during the first-year post-transplant (measured at three different time points) and both mPAP and PCWP. In contrast, patient age at the time of transplant was linked to long-term NT-proBNP levels.

Regarding time-dependent variables, NT-proBNP levels at each time point were inversely correlated with body weight and hemoglobin (Hb), while estimated glomerular filtration rate (eGFR) was associated only in the mid-term (1-year) and long-term follow-up. No significant relationship was found between NT-proBNP levels and bilirubin, cholesterol, or glycated hemoglobin.

Long-term NT-proBNP levels also showed a correlation with LV EF, which remained however preserved in the whole population, and sPAP. Among diastolic function markers, the E/e' ratio consistently showed a correlation. No significant relationship was observed between NT-proBNP and either mitral regurgitation severity or global right ventricular function, as assessed by RV fractional area change.

At multiple linear regression analysis, including a combination of pre- and early post-operative factors, only ischemic etiology, PAWP, eGFR at and TR severity at month 1 were independent predictors of 1-years NT-proBNP values (see **Table 9**). On the contrary, **Table 10** shows independent predictors of long term follow up.

Table 9. Linear regression results for the prediction of 1-year NT-proBNP levels

Variable	coefficient	p-value
Post-ischemic CMD	-0.526334	0.006
WEDGE	0.039676	<0.001
eGFR (ml/min/1.73 m2)	-0.006935	0.044
IT (1-4+/4+)	0.209	0.036

R^2=0.329

Table 10. Linear regression results for the prediction of long term NTproBNP levels

Variable	coefficient	p-value
HLA positivity	0.73143	0.003
Age at HTX	0.02518	0.029
IM (1-4+/4+)	0.32176	0.045
IT (1-4+/4+)	0.25892	0.035

R^2=0.249

Discussion

Methodological considerations

Background considerations

The main idea of the papers included in the present thesis was born from my everyday clinical practice. I have been working at advanced heart failure and heart transplantation clinic in my University Hospital for a few years, including a mixed population of patients with end stage disease listed for heart transplantation or LVAD implantation and patients who already underwent heart transplantation or LVAD implantation. These patients need dedicated and tailored care, also considering the huge amount of hospital visits, non-invasive and invasive tests they must perform every year. Despite all the currently available diagnostic and prognostic tools, we still require more research on these patients to improve management, also considering that part of the physio-pathological pathways we know in standard hearts is not applicable after heart transplantation. Echocardiography is a key technique in heart failure which I perform in every patient every working day, from which I obtain more and more information, based on which we set early diagnosis, adjust therapies, ask for further tests, and identify potential patients who need closer follow up. My willingness to ameliorate my capability of taking care of patients and find possible knowledge gaps in current applications of echocardiography in advanced heart failure and heart transplantation pushed me to collect data and to do research.

Selection of patients

All enrolled patients were selected from the advanced heart failure and heart transplantation clinic of my hospital, focusing on two different groups: the first consisted of patients with AdHF referred to the clinic from peripheral hospital and followed regularly while waiting for HTX or LVAD, which were selected for Paper I and 2; and the second group was composed of patients who had already undergone HTX and received regular clinical and instrumental follow up at the clinic, included in Paper III and 4. For paper I, patients should have had an available echocardiography and RHC performed on the same day, excluding all

individuals with potential biases in terms of RHC or echo results (chronic obstructive pulmonary disease, any septal defects, poor acoustic window or undetectable tricuspid regurgitation). For paper II, we excluded only patients with inadequate acoustic window, unavailable brachial artery cuff pressure for the calculation of myocardial work, atrial fibrillation or relevant heart valve diseases. For paper III, a selected population of HTX patients was included to assess normal values of myocardial work indices in this setting, excluding known CAV or rejection. A group of HTX patients with available long term follow up with echocardiography and NT-proBNP was finally selected for paper IV.

Standard and advanced echocardiography

As mentioned, echocardiography is an ally for the clinician working at advanced heart failure laboratories. Standard echocardiographic measurements are internationally recognized to discriminate between a normal and a pathological condition. However, when definitions change, or novel modalities become available, new data is needed to confirm previous results or improve management. The modification of the definition of pulmonary hypertension is relevant for AdHF patients, since an irreversible pulmonary hypertension represents a contraindication of HTX. Since the definition has changed to improve diagnostic accuracy, paper I was born with the aim to improve echocardiographic accuracy in detecting abnormal pulmonary pressure compared to RHC, which is the gold standard for the diagnosis. For paper II and 3, I wanted to apply a novel echocardiographic Speckle Tracking-derived technique, myocardial work, which has been given good support in other clinical settings, in advanced heart failure and healthy heart transplanted populations, respectively. Lastly, in paper IV, standard echocardiography was used as a predictor of NT-proBNP levels in short and long-term follow up after HTX, since in my everyday clinical practice I have noticed varying correlations between echocardiography parameters and natriuretic peptides in this group of patients.

General discussion on main findings

Paper I

Exclusion of pulmonary hypertension is part of the diagnostic work up of patients with AdHF screened for HTX, representing an exclusion criterium [2]. AdHF usually determines a type 2 pulmonary hypertension, secondary to chronic left heart disease [27]. In the 2022 ESC Guidelines for the diagnosis and treatment of pulmonary hypertension [30], the diagnostic threshold for pulmonary hypertension was reduced from 25 to 20 mmHg with the aim of improving sensitivity and eventually anticipating specific therapies. The gold standard diagnostic test for pulmonary pressure estimation is RHC, which also allows to study pulmonary vascular resistances and cardiac output and to test possible reversibility. However, RHC requires a dedicated catheterization laboratory and is an invasive procedure with possible complications. Among echocardiographic indices, the ESC Guidelines indicates measurement of TRV as the best indirect estimator of systolic pulmonary pressure even if the cut-off of 2.8 m/s was not modified according to the new definition threshold [30]. In our small group of AdHF patients, we confirmed that TRV performed well in predicting RHC-diagnosed PH both when using 25 or 20 mmHg as a reference value, however with 2.4 m/s as the cut-off presenting with the best sensitivity and positive predicting value. The prediction capability of the mean RV-RA gradient and sPAP estimation was lower and this was probably attributed to the application of formulas and the use of inferior vena cava dimension and collapsibility as central venous pressure estimators. Echocardiography has certainly limitations when estimating pulmonary pressures. First, TRV is obtained by continuous Doppler interrogation of the tricuspid valve so at least a mild regurgitation with good TR envelope is required; on the contrary a severe or more than severe TR, as well as RV dysfunction can lead to an underestimation of TRV. The results of this study helped me to be more precise when describing the different indices of pulmonary pressures in the echocardiographic report and we have started to adopt a lower threshold of 2.4 m/sec in clinical practice to describe abnormal TRV in AdHF patients waiting for HTX. No data is available yet regarding the impact of the different mPAP cut-offs on successful HTX.

Paper II

Second level Speckle Tracking analysis of the left ventricle, for the calculation of GLS, has extensively been studied in the heart failure population both in acute and chronic settings, demonstrating good capability for diagnostic and prognostic purposes [75,76]. Compared to LV EF, GLS is not dependent on preload but remains dependent on afterload. To overcome this limitation, myocardial work analysis was created by integrating LV afterload (derived by systemic arterial pressure) with GLS and it can generate pressure-strain curves similarly to pressure-volume curves [65]. Being a relatively novel tool, MW data in the context of AdHF were lacking. On a selected group of AdHF patients, I tested the prognostic capability of MW indices. Even if severely reduced, the left atrial strain and high pulmonary pressures were best predictors of major endpoints (LVAD implantation, HTX and death), and MW-derived GWI and GCW were useful predictors for acute HF hospitalizations, with a GWI ≥ 369 mmHg% as the best cut-off. Despite optimized therapies, the trajectory of patients with HF is characterized by intermittent exacerbations which become more frequent approaching advanced disease and are challenging to predict. The accurate research of updated and novel tools to guarantee tailored medicine is mandatory for the best care of HF patients. Left atrial function and estimated sPAP were associated with the compositive outcome because of the extension of the disease from the left ventricle to the left atrium and the pulmonary circulation, thus representing patients with more compromised hearts. The number of hospitalizations in AdHF is high and the majority of patients are admitted for congestion or low output states. with significant costs for the health care systems [77]. We have recently started to test our results prospectively at our centra. In addition to clinical signs and known standard echocardiographic predictors, we have noticed that thanks to MW indices, especially GWI, we can increase the dosage of diuretics or arrange periodical intravenous administration of loop diuretics or levosimendan reducing the rate of unplanned HF hospitalizations. I will continue to collect these types of data.

Paper III

The transplanted heart has a very peculiar physiopathology which requires dedicated study and approach in clinical practice. Starting from the surgical technique, which is currently prevalently as “bicaval”

approach, while the right atrium is derived from the donor, the left atrium is cut and sewed between the pulmonary veins and atrial walls [26]. Moreover, between aortic clamping and organ reperfusion in the donor, there is a variable ischemic timeframe which can interfere with the transplant survival. The life of a transplanted patient is characterized by medication of immunosuppressor agents, which are responsible for onset of comorbidities onset like arterial hypertension and chronic kidney disease, and repeated invasive endomyocardial biopsies and coronary angiographies to rule out rejection and CAV [71]. Due to nervous deafferentation, these patients do not suffer from typical angina and instead, usually complain of dyspnea or other angina equivalents. From an echocardiographic point of view, LV EF usually remains preserved except in cases of severe rejection or CAV, while diastolic function is the first to be affected by pathological triggers. Diastolic function is not easily assessed by echo, since the atrial components are lost due to the surgical procedure [78]. GLS and twisting mechanics are reduced in HTX patients, possibly due to incomplete or heterogeneous cardiac reinnervation after surgery, inadequate stimulation of LV myocardial beta-receptors, hypoxia-induced pro-angiogenic signals and ultrastructural remodeling even in healthy grafts. Reference values of echocardiographic indices are mandatory to define what is normal and pathological in this group. There were no reference values for MW parameters in HTX. In a group of HTX patients with no history of rejection or CAV, we found that MW indices are different from the general population with lower GWI, GCW, and GWE and higher GWW values, irrespective of sex. After the publication of paper III, I compared the MW results in HTX patients with three other groups of patients who underwent cardiac surgery (coronary artery bypass, aortic valve replacement and mitral valve repair). As shown in **Table 11**, values of GLS, GCW and GWE are better in HTX patients than in the other groups while GWW were comparable. The main factor determining a worse GWI in HTX is probably the loss of longitudinal deformation of the heart which is partly dependent on mitral annulus and left atrium, altered by the surgical procedure. However, ischemic timeframe, pericardiectomy and comorbidities of the donor can influence, as well as the immunosuppressor regime which increases blood pressure and induce subendocardial damage. From a clinical perspective, the observation of abnormal MW can be helpful in the early diagnosis of rejection or CAV

with the aim of reducing the number of invasive procedures in these patients.

Table 11. Comparison of myocardial work indices between HTX and other cardiac surgery groups

Variable	CABG	AVR	MR surgery	HTX	Overall p
LV EF (%)	55 [50-60]	55 [55-60]	55 [55-59]	60 [57-62]	<0.001
GLS (%)	-13.3±3.9	-13.6±3.9	-13.3±3.7	-16.1±3.5	<0.001
LV GWI (mmHg%)	1291±444	1180±400	1299±472	1447±409	<0.001
LV GCW (mmHg%)	1881±514	1667±516	1902±587	2067±423	<0.001
LV GWW (mmHg%)	308 [218-357]	248 [178-357]	370±158	310 [217-499]	0.316
LW GWE (mmHg%)	83.0 [79.0-88.0]	84.3±5.9	82.3±5.4	83.7±7.9	0.017

Data are expressed as mean±SD or median [IQR].

AVR = aortic valve replacement; CABG = coronary artery bypass grafting; MR = mitral regurgitation.

Paper IV

Following the discussion about peculiar HTX characteristics, the usefulness of natriuretic peptides, and in particular NT-proBNP is different compared to HF patients. From previous publications we know that circulating NT-proBNP levels in HTX population are generally higher than in controls [53], but we do not have recently updated data about long-term trajectories and correlation between NT-proBNP levels and echocardiographic indices in the transplanted heart. We selected HTX patients with ten repeated NT-proBNP measurements and complete echocardiographic examinationsand we could confirm that despite absence of or minimal HF symptoms and a preserved ejection fraction, NT-proBNP levels were higher than expected. NT-proBNP levels were reduced during the first year after HTX, remaining substantially stable at mid and long-term follow up and were poorly related with kidney function especially early after HTX, while being correlated with weight and Hb levels. Multivariate analysis demonstrated that pre-HTX HLA sensibilization, age and severity of MR and TR within the first year after surgery were independent predictors of long-term NTproBNP levels. LV hypertrophy could be one determinant of lack of normalization of NT-proBNP in patients with preserved LV EF and graft complications. LV hypertrophy is determined in the early phases by surgical procedure and ischemia-reperfusion damage while in the long term by arterial hypertension induced by immunosuppressors, immune disorders, possible viral infections or rejection episodes [79,80]. Rejection and CAV diagnosed in our population, although not severe, induced a long-term increase of NT-proBNP levels, which related to LV EF, even if within the normal range. This should be considered when discussing NT-proBNP values in these patients.

Clinical perspectives and future applications

Patients with end stage HF represent a minority of the whole population of patients with HF but they concentrate the highest rate of events, including recurrent hospitalizations, necessity of long-term inotrope support, short and long term circulatory mechanical supports, urgent HTX. Literature papers dedicated to AdHF usually include small study populations with selected individuals and we generally have less data

available. The hub centers working with AdHF patients require a dedicated team with high expertise and the continuous update by research products is improving everyday care in terms of better quantity and quality of life for patients, early complications diagnosis and lower cost for national health systems.

My thesis was born with the aim of supporting clinicians and patients, especially enhancing the central role of non-invasive diagnostic tools as echocardiography. Results on estimation of pulmonary hypertension by echocardiography could help in accurate characterization of patients also when RHC is not available or is successively planned but also in case patients are screened at spoke center before the referral to an hub hospital. Myocardial work seems to be promising, when available, to selected outpatients who require a closer follow up or more aggressive pharmacological therapy in ambulatory setting and, if confirmed on larger numbers, reduce hospitalization rates. With regards of HTX, the incessant research of possible predictors of early damage can be supportive to integrate a non-invasive diagnosis of rejection or CAV to lengthen the distance between endomyocardial biopsies or coronary angiographies, considering the physical stress of the patients and the possible complications, related for example to pericardial effusion or contrast-induced nephropathy. Reference values of MW and expected results of NT-proBNP values according to laboratory and echocardiographic findings could represent an additional support to the definition of physiological graft function or the suspect of complications for which the clinician would require further investigations.

Study limitations

Paper I

This is a single-center study, and the data analysis was done retrospectively. The patient population included in the study was highly selected, which may restrict the generalizability of the findings to other stages of HF. However, since comparisons were made between two measurement methods within the same individuals, this point is somewhat mitigated. Although the participants were AdHF patients being evaluated for HTX, one of the main drawbacks of the study is the relatively small sample size. Because of the retrospective nature of the study, no sample size calculation was conducted. Instead, the analysis was performed using the available data from our institution, which ultimately proved sufficient for the study's objectives.

Paper II

Similarly to Paper I, this study is a retrospective analysis conducted at a single center with a limited sample size. As a result, the findings may not be broadly applicable until they are validated in a larger cohort, ideally across multiple centers. However, to the best of our knowledge, this represented the largest group of patients with AdHF evaluated using MW analysis. I need to clarify that I classified AdHF patients as inclusion criteria if they were referred to my University Hospital for screening for AdHF therapies, i.e. LVAD implantation or HTX, rather than strictly following the ESC Guideline AdHF definition [1,5]. Consequently, many of our patients were in a relatively good functional class, differing from the NYHA III/IV classification typically required for an AdHF diagnosis. In our clinical practice, we assess patients for AdHF therapies at a quite early stage of the disease to allow sufficient time for comprehensive cardiac and non-cardiac evaluation before significant hemodynamic deterioration.

Paper III

First, the study was limited by its single-center retrospective design. However, to minimize potential bias related to operator-dependent calculations, we conducted a reproducibility analysis. Second, the study included a relatively small number of patients, most of whom were Caucasian. Nevertheless, the sample size was comparable to or even larger than previous studies involving HTX recipients. The median time since HTX was relatively short, given that baseline echocardiographic assessments are typically recommended around six months post-surgery. Additionally, data on donor heart function before transplantation was unavailable, limiting further analysis. However, the broad range of post-HTX time points in our cohort could provide valuable insights, particularly for patients in the early post-HTx period when the risk of acute rejection is higher. Lastly, a feasibility analysis was not conducted, as patients with inadequate image quality for speckle-tracking echocardiography were excluded from the population.

Paper IV

Also paper IV was a single center study with retrospective data collection with a relatively small numbers of patients even if the total number of evaluations was high. Most of the patients were male Caucasians. Moreover, only standard echocardiographic parameters were tested, without available GLS or MW indices. Finally, beyond the first year (whose evaluation were standardized in terms of time for the whole study population), the successive follow up timing was variable according to HTX period.

Main conclusions

- Even if echocardiography has only modest agreement when estimating pulmonary artery pressures versus right heart catheterization (gold standard), tricuspid regurgitation velocity is the best predictor of a mean pulmonary arterial pressure > 20 mmHg, the new cut-off for the diagnosis of pulmonary hypertension according to international guidelines.

- A lower threshold of tricuspid regurgitation velocity, 2.4 m/sec, has equal positive predictive value but higher sensitivity compared to previously indicated 2.8 m/sec.

- Left atrial function and estimated pulmonary pressure are stronger predictors of major cardiovascular events in advanced heart failure, as previously demonstrated in literature.

- Myocardial work indices seem to be additional tools in the stratification of hospitalization risk in advance heart failure, helping in a tailored medicine.

- As described for Global Longitudinal Strain, myocardial work parameters in patients after heart transplantation and no history of rejection or cardiac allograft vasculopathy are lower than in general population in both sexes.

- NT-proBNP values after heart transplantation reduce in the first year after surgery but remain higher also at long term follow up even if the patient has preserved left ventricular function and no or poor symptoms.

- Among echocardiographic predictors of long-term NT-proBNP values, early (first year after transplantation) significant mitral or tricuspid regurgitation had higher statistical significance.

Acknowledgement

These PhD years have been particularly noteworthy for my growth as a Researcher, but also tough and strenuous for many concomitant factors, including COVID-19 pandemic which changed for a very long-time clinical activities and required lots of extra-work to support Intensive Care Units. I would love to thank many who supported me on this long journey:

Prof. Micheal Y Henein – my principal supervisor for the majority of PhD program. He has known me for a very long time now and his brilliant mind inspired me for several research ideas. Always ready to give good suggestions and recommendations, I would never have carry on this PhD without him;

Prof. Stefan Söderberg – I had the chance to meet him at my mid-term examination and he supported me in the last phase of PhD as new principal supervisor. In this relatively short period of time, he demonstrated huge clinical and research competence but also kindness and devotion to his students; thank you so much for being there for me;

Prof. Matteo Cameli – my local assistant supervisor, he has pushed me a lot to be the best version of myself in all these years, he taught me to avoid giving up and to always be enthusiastic about works and projects. I learned a lot from him, including the way of being kind and accommodating with patients which makes a physician a special doctor;

Prof. Sandra Arvidsson – my Swedish assistant supervisor, brilliant and always available for giving the perfect solution for each problem;

Gunilla Mårald and Maria Sandström – Coordinators;

Elin Lindahl – Administrator;

All the PhD colleagues and Teachers I had the chance to meet in all these years – from each of you I learned a lot;

All my co-authors for the great support in these years of work together on paper.

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