SEMMELWEIS EGYETEM

DOKTORI ISKOLA

Ph.D. értekezések

3510.

TESZÁK TÍMEA

Szív- és érrendszeri betegségek élettana és klinikuma
című program

Programvezető: Dr. Merkely Béla, egyetemi tanár

Témavezető: Dr. Sax Balázs, egyetemi adjunktus

Modern diagnostics and treatment of heart transplant rejection

PhD thesis

Tímea Teszák, MD

Semmelweis University Doctoral School

Cardiovascular Medicine and Research Division

Supervisor: Balázs Sax, MD, PhD

Official reviewers: Anikó Bohács, MD, PhD
Attila Borbély, MD, PhD

Head of the Complex Examination Committee: Ferenc Horkay, MD, DSc

Members of the Complex Examination Committee:
Andrea Nagy Horváthné, MD, PhD
Balázs Muk, MD, PhD

Budapest
2026

Table of Contents

List of Abbreviations

8-MOP	8-methoxypsoralen
ACES-EMB	A Comparative Effectiveness Study in Heart Transplant Patients of Rejection Surveillance With Cell-free DNA Versus Endomyocardial Biopsy
ACR	acute cellular rejection
AMR	antibody-mediated rejection
APC	antigen-presenting cell
ASFA	American Society for Apheresis
ASTS	American Society of Transplant Surgeons
BNP	B-type natriuretic peptide
Breg	regulatory B cell
CAV	cardiac allograft vasculopathy
cfDNA	cell-free DNA
CMP	cardiomyopathy
dd-cfDNA	donor-derived cell-free DNA
DNA	deoxyribonucleic acid
D-OAR	Utility of Donor-Derived Cell-Free DNA in Association With Gene Expression Profiling
DSA	donor-specific antibody
EACVI	European Association of Cardiovascular Imaging
ECP	extracorporeal photopheresis

EMB	endomyocardial biopsy
ESOT	European Society for Organ Transplantation
GCW	global constructive work
GEP	gene-expression profiling
GRAfT	Genomic Research Alliance for Transplantation
GWE	global work efficiency
GWI	global work index
GWW	global wasted work
HF	heart failure
HLA	human leukocyte antigen
HTx	heart transplantation
IL	interleukin
INFγ	interferon gamma
INTERHEART	Diagnostic and Therapeutic Applications of Microarrays in Heart Transplantation, a Multicenter Study
ISHLT	International Society for Heart and Lung Transplantation
iT35	induced regulatory T cell
LV	left ventricular
LVEF	left ventricular ejection fraction
LVGLS	left ventricular global longitudinal strain

LVOT	left ventricular outflow tract
MDSC	myeloid-derived suppressor cell
MFI	mean fluorescent intensity
MMDx	Molecular Microscope Diagnostic System
MMF	mycophenolate mofetil
NGS	next-generation sequencing
NK cell	natural killer cell
NKreg cell	natural killer regulatory cell
NORRE	Normal Reference Ranges for Echocardiography
NS	not significant
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
OAR	Outcomes AlloMap Registry
pAMR	Pathologic antibody-mediated rejection
PCR	polymerase chain reaction
R	revised
SD	standard deviation
SHORE	Surveillance HeartCare Outcomes Registry
Teff	effector T cell
TGF-β	transforming growth factor beta
Th	helper T cell

TNF	tumour necrosis factor
Treg	regulatory T cell
TRS	total rejection score
UVA	Ultraviolet A

1 Introduction

Heart failure (HF) is a clinical syndrome defined by hallmark symptoms such as shortness of breath, ankle swelling, and fatigue, that may be accompanied by physical signs including elevated jugular venous pressure, pulmonary rales, and peripheral oedema. It results from structural and/or functional cardiac abnormalities that lead to increased intracardiac pressures and/or reduced cardiac output. In Europe, the incidence of HF is approximately 5 cases per 1000 person-years among adults. The estimated prevalence of HF in the adult population ranges between 1% and 2% (1). Although patients with chronic HF have improved outcomes with guideline-directed therapies, an estimated 1-10% of the overall HF population consists of patients with advanced HF. Furthermore, the prevalence of advanced HF is increasing, driven by the growing number of individuals with HF and the improved effectiveness of treatments that enhance survival (2). Advanced HF is characterised by persistent symptoms despite maximal therapy. Its prognosis remains unfavourable, with a 1-year mortality ranging from 25% to 75%. Heart transplantation (HTx) remains the gold standard therapy for patients with advanced HF in the absence of contraindications, significantly improving quality of life and survival (1, 3).

Despite significant improvements in pre- and post-transplant management, the main challenges after HTx are related to the effectiveness and side effects of immunosuppressive therapy, including graft rejection, infections, cardiac allograft vasculopathy (CAV), late graft dysfunction, malignancy, renal impairment, hypertension, and diabetes mellitus (1). Early detection and treatment of acute rejection are cornerstones of post-transplant management to preserve allograft function, improve long-term patient survival, and prevent the development of CAV, a principal contributor to late graft failure and mortality (4).

Endomyocardial biopsy (EMB), pioneered by Phillip Caves and Margaret Billingham at Stanford in the early 1970s, remains a cornerstone in the diagnostic armamentarium for allograft rejection; however, it has notable limitations (5, 6). Evidence indicates that surveillance biopsies performed in asymptomatic patients treated with calcineurin-inhibitor- and mycophenolate-based immunosuppressive therapy have a low diagnostic yield for identifying moderate or severe acute cellular rejection (ACR; 1%-2%), while the incidence of EMB-related complications exceeds the frequency of detected acute

rejection episodes (7). Moreover, there is significant discordance among pathologists in the interpretation of acute rejection (8, 9). EMBs are associated with procedural complications, sampling errors, and patient discomfort, and they require hospital admission (Table 1) (10-12). Furthermore, an inconclusive or misinterpreted EMB may result in repeated procedures, overtreatment, and exposure to unnecessary risks of infection or malignancy (13, 14). Evidence indicates that routine, protocol-driven EMB has a low diagnostic yield as a screening tool for acute allograft rejection beyond six months following HTx (13).

Table 1. Potential complications of endomyocardial biopsy (10-12).

Type of complication	**Manifestation**
Mechanical	Cardiac perforation, hemopericardium, cardiac tamponade
	Tricuspid valve injury
	Papillary muscle or chordae tendineae damage
	Right ventricular free wall rupture
Electrical	Ventricular ectopy/ventricular tachycardia
	Atrial arrhythmias
	Atrioventricular block
	Right bundle branch block
Vascular and access-related	Hematoma or bleeding
	Pseudoaneurysm, arteriovenous fistula
	Venous thrombosis or embolism
	Pneumothorax/hemothorax
	Air embolism
Infectious	Local infection, endocarditis
Procedural	Embolisation of tissue fragment

Thus, many efforts have been made to identify noninvasive procedures and biomarkers to reduce or eliminate the need for surveillance EMBs. Given the low incidence of asymptomatic rejection, a sensitive surveillance strategy with a high negative predictive value may minimise the number of EMBs. Modern surveillance approaches should also enable earlier detection of rejection, rather than relying on histopathological changes that typically indicate more advanced immune activation. Additionally, blood-based tests eliminate interobserver grading inconsistencies, which can affect the accuracy of EMB results. This advancement in clinical practice improves patient satisfaction and safety and enables earlier detection of graft injury (5). The use of B-type natriuretic peptides (BNPs), high-sensitivity troponin, donor-specific antibodies (DSAs), gene-

expression profiling (GEP), and donor-derived cell-free DNA (dd-cfDNA) has been recommended by international guidelines and consensus documents for monitoring HTx rejection (Table 2) (15-17). BNPs and high-sensitivity troponins offer limited sensitivity and specificity (18). Noninvasive peripheral GEP (AlloMap; CareDx, Inc., Brisbane, CA, USA) has emerged as the most promising alternative to EMB. It was designed to distinguish immune quiescence from ACR as early as 55 days after HTx and has demonstrated non-inferiority to EMB in randomised studies evaluating rejection surveillance (19, 20). With a negative predictive value of 99% for ACR, GEP is highly effective at excluding ACR; however, its positive predictive value remains limited, and it was not designed to detect antibody-mediated rejection (AMR) (6). While GEP is not available for clinical use in Europe, increasing evidence supports the use of dd-cfDNA (4, 21-25).

Table 2. Recommendations for biomarker testing (15-17).

Biomarker	**Society**	**Recommendation**	**Level of Evidence**
B-type natriuretic peptide, high-sensitivity troponins	ISHLT ESOT ESOT	Class IIb Weak neutral (Troponin) Weak against (BNP)	C Very low (Troponin) Very low (BNP)
Donor-specific antibody	ISHLT	Class IIa	C
Gene-expression profiling	ISHLT ASTS ESOT	Class I Class I Strong in favour	B Moderate
Donor-derived cell-free DNA	ISHLT ASTS ESOT	Class I Class IIb Weak in favour	B Low

Abbreviations: ASTS, American Society of Transplant Surgeons; BNP, B-type natriuretic peptide; DNA, deoxyribonucleic acid; ESOT, European Society for Organ Transplantation; ISHLT, International Society for Heart and Lung Transplantation.

Although evidence supporting dd-cfDNA testing continues to grow, a prospective randomised trial directly comparing post-HTx outcomes using traditional EMB versus dd-cfDNA monitoring has not yet been published. The ACES-EMB trial (A Comparative Effectiveness Study in Heart Transplant Patients of Rejection Surveillance With Cell-free DNA Versus Endomyocardial Biopsy), a prospective randomised study comparing conventional EMB-based surveillance with noninvasive approaches using the Prospera

dd-cfDNA testing platform (Natera, Inc., Austin, TX, USA), has completed enrolment (18). However, a key consideration in assessing dd-cfDNA test performance is that EMB is a suboptimal gold standard for diagnosing acute rejection (4, 22). Importantly, EMB yields negative results in more than 80% of cases with clinically relevant elevations in dd-cfDNA. EMB may fail to detect pathology despite substantial allograft dysfunction, emphasising its diagnostic limitations (4).

Immunosuppressive therapy leaves patients vulnerable to significant side effects: it lacks specificity, and its use increases the risk of infectious diseases, neurotoxicity, bone marrow depression, renal failure, malignancies, and the development of hypertension, diabetes mellitus and hyperlipidemia, all of which continue to have a profound negative impact on patient and graft survival (26). Therefore, immunosuppressive regimens with fewer toxic side effects are sorely needed for the treatment of HTx recipients.

1.1 Cardiac allograft rejection

Acute rejection remains a significant complication after HTx, with 12.6% of patients transplanted between 2010 and 2016 requiring treatment for rejection between hospital discharge and one year post-transplant (27). For HTx recipients presenting with signs or symptoms of graft dysfunction, the standard of care is to perform an EMB with histopathological evaluation of the cardiac allograft to assess for ACR, AMR, or mixed rejection (16). Precise differentiation between AMR and ACR is essential, as their initial treatment strategies differ significantly and their long-term outcomes also vary. AMR is associated with more pronounced graft dysfunction, an increased likelihood of recurrent rejection, and a higher risk of CAV and mortality (4).

For the assessment of HTx rejection, it is recommended to obtain a minimum of three, and preferably four (or more), adequately evaluable myocardial tissue samples. An evaluable myocardial sample should contain at least 50% myocardium, excluding areas composed of prior biopsy sites, scar tissue, adipose tissue, or blood clots that may constitute the remaining part of the specimen (28).

1.1.1 Acute cellular rejection

ACR is defined by an inflammatory infiltrate primarily composed of lymphocytes, accompanied by macrophages and occasional eosinophils. In 2004, the International Society for Heart and Lung Transplantation (ISHLT) led a multidisciplinary review of the cardiac biopsy grading system to address existing challenges and inconsistencies in

its application (Table 3). The revised (R) categories of cellular rejection are defined as follows: grade 0R indicates no rejection (unchanged from the 1990 classification), grade 1R represents mild rejection (corresponding to 1990 grades 1A, 1B, and 2), grade 2R denotes moderate rejection (equivalent to 1990 grade 3A), and grade 3R represents severe rejection (corresponding to 1990 grades 3B and 4) (28).

Table 3. The 2004 International Society for Heart and Lung Transplantation standardised cardiac biopsy grading scale for acute cellular rejection (28).

Grade	Characteristics
0R No rejection	No evidence of rejection.
1R Mild	Interstitial and/or perivascular infiltrate with up to one focus of myocyte damage.
2R Moderate	Two or more foci of infiltrate with associated myocyte damage.
3R Severe	Diffuse infiltrate with multifocal myocyte damage ± oedema ± haemorrhage ± vasculitis.

Abbreviation: R, revised.

High-dose intravenous corticosteroids should be the first-line therapy for symptomatic ACR, regardless of the EMB grade (1R, 2R, or 3R). High-dose intravenous corticosteroids are recommended for the management of asymptomatic severe ACR (ISHLT grade 3R). Asymptomatic moderate ACR (ISHLT grade 2R) may be treated with either intravenous or oral corticosteroids. Maintenance immunosuppressive therapy should be appropriately adjusted to decrease the risk of recurrent rejection (16).

1.1.2 Antibody-mediated rejection

AMR occurs when recipient antibodies bind to donor human leukocyte antigens (HLAs) expressed on the allograft endothelium. These antibodies trigger complement fixation and activation, leading to tissue injury. Complement activation plays a central role in the pathogenesis of AMR by initiating both innate and adaptive immune responses. The deposition of complement components and immunoglobulins within the allograft microvasculature triggers an inflammatory cascade characterised by endothelial cell activation, cytokine upregulation, macrophage infiltration, increased vascular permeability, and microvascular thrombosis, leading to allograft dysfunction. The characteristic histopathologic findings of AMR on hematoxylin and eosin-stained sections include interstitial capillary injury, characterised by endothelial cell swelling and

intravascular macrophages. The primary immunohistochemistry panel for paraffin-embedded tissue included C4d, with a strong recommendation to incorporate the anti-macrophage marker CD68 (29).

Following the adoption of the 2013 Working Formulation, histologic AMR diagnoses have surpassed those of ACR in frequency, a trend attributable in part to increased recognition of AMR (Table 4) (30).

Table 4. The 2013 ISHLT working formulation for pathologic diagnosis of cardiac antibody-mediated rejection (29, 31).

Grade	Characteristics
pAMR 0 Negative for pathologic AMR	Histologic and immunopathologic studies are both negative.
pAMR 1 (H^+) Histopathologic AMR alone	Histologic findings are present, and immunopathologic findings are negative.
pAMR 1 (I^+) Immunopathologic AMR alone	Histologic findings are negative, and immunopathologic findings are positive ($CD68^+$ and/or $C4d^+$).
pAMR 2 Pathologic AMR	Both histologic and immunopathologic findings are present.
pAMR 3 Severe pathologic AMR	Interstitial haemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis and/or karyorrhexis, and marked oedema with immunopathologic findings are present. It may be associated with severe hemodynamic compromise and poor clinical outcomes.

Abbreviations: AMR, antibody-mediated rejection; ISHLT, International Society for Heart and Lung Transplantation; pAMR, pathologic antibody-mediated rejection.

AMR can manifest as hyperacute rejection within the first 7 days post-transplant in recipients who are sensitised to donor HLAs. Early AMR may also develop within the first month following HTx, due to preexisting or de novo DSAs. Late AMR develops months to years after HTx (31).

The fundamental principles in the management of AMR include the elimination of circulating alloantibodies (plasmapheresis), inhibition of further alloantibodies (intravenous immunoglobulins), suppression or depletion of plasma cells (bortezomib, carfilzomib), inhibition of complement (eculizumab, intravenous gamma globulin), and suppression of both T cell (corticosteroids, mycophenolate mofetil [MMF], anti-lymphocyte antibodies, extracorporeal photopheresis [ECP]) and B cell (corticosteroids,

rituximab) immune responses (31). Daratumumab, tocilizumab, belatacept, and carfilzomib should be considered for patients with recurrent or refractory AMR (32). Most of the therapies listed above are typically administered in combination, either concurrently or sequentially, depending on the patient's clinical response. In addition to cytotoxic or antibody-targeted therapies for managing AMR, optimisation of the baseline immunosuppressive regimen should also be undertaken (31).

1.1.3 Mixed rejection

Mixed rejection consists of concomitant ACR and AMR. It is defined by the presence of cellular infiltrates characteristic of ACR, along with histopathologic and/or immunopathologic features of AMR within the same EMB specimen (29).

Mixed rejection is associated with substantial cardiovascular mortality, incrementally with the severity. In the presence of hemodynamic compromise, aggressive treatment with high-dose intravenous corticosteroids and cytolytic therapy is warranted. Additional interventions targeting AMR should also be considered. For mild forms of mixed rejection without significant clinical symptoms, management should generally follow the protocol for cellular rejection, with the option of adding intravenous immunoglobulin (16).

1.2 Donor-derived cell-free DNA

The presence of cell-free DNA (cfDNA) was first identified in 1948, when it was discovered in the serum of patients with cancer. They are short extracellular DNA fragments released continuously into the circulation by both donor graft and recipient cells during normal cell turnover (16). In healthy individuals, most circulating cfDNA originates from hematopoietic cells, with less than 1% originating from the heart (4). Genetic differences between the donor and recipient enable the quantification of cfDNA derived from the cardiac allograft (21). In the reference population of stable HTx recipients without evidence of rejection, dd-cfDNA is detected at very low levels (22).

During episodes of cellular rejection or AMR, injury to the graft – characterised by myocyte necrosis and apoptosis – results in increased release of dd-cfDNA into the circulation (16). dd-cfDNA serves as an early, highly sensitive marker of graft injury with a high negative predictive value for both ACR and AMR (4, 21, 22). Circulating dd-cfDNA has a very short half-life, ranging from 30 minutes to several hours, allowing for repeat testing within days in the appropriate clinical context for rejection monitoring. The

dd-cfDNA result reflects either the proportion of donor-derived cfDNA relative to the total amount of cfDNA (%) or the absolute quantity of the donor-derived cfDNA (copies/mL, or ng/mL) (Figure 1) (5).

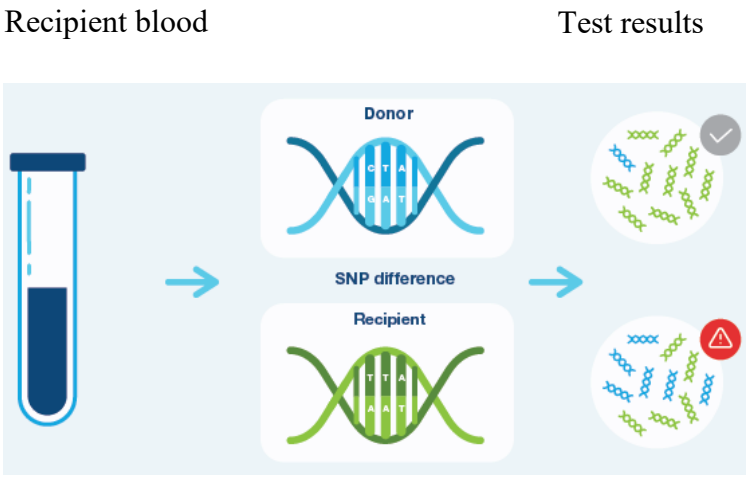

Figure 1. When donor heart injury occurs, myocytes release cell-free DNA into the recipient's plasma, resulting in elevated donor-derived cell-free DNA levels (33). Abbreviations: cfDNA, cell-free DNA; dd-cfDNA, donor-derived cell-free DNA; DNA, DNA, deoxyribonucleic acid; HTx, heart transplantation.

Following the development of the AlloSure dd-cfDNA assay (CareDx, Inc., Brisbane, CA, USA), the D-OAR study (Utility of Donor-Derived Cell-Free DNA in Association With Gene Expression Profiling), which included 740 participants across 26 HTx centres, demonstrated that elevated dd-cfDNA levels correlate with rejection detected by EMB. The D-OAR study established dd-cfDNA as a specific indicator of graft injury, exhibiting a negative predictive value of 97% for both ACR and AMR (5, 22). The assay's high negative predictive value could help minimise the need for EMBs in patients with low clinical suspicion of acute rejection. Conversely, an elevated dd-cfDNA level may increase the likelihood of a positive biopsy result, thereby supporting the initiation of

clinical treatment for acute rejection (22). Previous studies have demonstrated that elevated levels of dd-cfDNA precede the diagnosis of acute rejection, as detected by EMB, by several months (4, 23). These elevated levels indicate early graft injury that precedes detectable myocyte damage on histologic examination. Monitoring with dd-cfDNA may therefore enable early detection of rejection, allowing timely intensification of immunosuppressive therapy to prevent progression to more severe rejection and irreversible graft injury (22). dd-cfDNA represents a paradigm shift in understanding allograft rejection, moving from reliance on histologic assessment of inflammatory infiltrates and myocyte injury to a direct, quantitative evaluation of donor-derived DNA in the recipient's plasma (4). Therefore, the high sensitivity of dd-cfDNA in the early detection of rejection has called into question the designation of EMB as the gold standard diagnostic method (5).

Although dd-cfDNA levels increase in both ACR and AMR in proportion to the severity of rejection, distinct dd-cfDNA patterns exist between ACR and AMR (4, 21, 22, 34). A prospective cohort study by the Genomic Research Alliance for Transplantation (GRAfT) across five transplant centres in the United States found that AMR is associated with longer lead times, a higher area under the receiver operating characteristic curve, and different fragment types and lengths compared with ACR. The guanosine-cytosine base content is higher in AMR than in ACR and non-rejection control samples. In AMR, cfDNA fragments are shorter than in ACR and non-rejection control samples. Median dd-cfDNA levels are higher in patients with AMR than in those with ACR, suggesting that the distinct pathogenic mechanisms underlying these rejection subtypes may account for the observed quantitative differences in dd-cfDNA levels. AMR, characterised by the presence of DSA, leads to widespread macrovascular and microvascular injury that can progress to pancarditis and extensive cellular DNA release. In contrast, ACR typically involves localised lymphocytic infiltration and injury confined to interstitial and perivascular tissues. A dd-cfDNA threshold of 0.25% demonstrated a 99% negative predictive value for acute rejection (4). The dd-cfDNA level decreases following resolution of both ACR and AMR episodes (34). Prior studies have suggested that from post-HTx day 28 onwards, recipients can be monitored for allograft rejection using the dd-cfDNA assay (4, 5, 21). dd-cfDNA assays are designed to serve as screening tools for stable HTx recipients. Patients exhibiting clinical signs or symptoms suggestive

of rejection or evidence of graft dysfunction should undergo a for-cause EMB, irrespective of their inclusion in a noninvasive screening protocol (5).

In the United States, centralised dd-cfDNA testing services are broadly utilised, either as stand-alone dd-cfDNA assays or as a combination of GEP and dd-cfDNA. Patient specimens are shipped to the manufacturer's accredited laboratory for dd-cfDNA analysis, and the resulting data are subsequently reported to the clinicians. Until recently, dd-cfDNA testing was available only through centralised testing services.

Decentralised dd-cfDNA testing kits provide the convenience of on-site laboratory testing, thereby enabling broader access to and adoption of the testing method. A study conducted among kidney transplant recipients at three European centres evaluated a decentralised, CE-IVD-marked dd-cfDNA testing kit against a widely utilised centralised dd-cfDNA testing service in the United States. It proved a strong correlation with the well-characterised, centralised dd-cfDNA assay. Both tests showed a significant association with allograft rejection and similarly good discrimination in predicting rejection (35). Another study aimed to verify the analytical performance of a decentralised, CE-IVD-marked dd-cfDNA assay across three European centres and compare it with manufacturer validation. The overall observed versus expected dd-cfDNA results demonstrated good concordance and a strong positive correlation, as well as high repeatability and reproducibility within and between sites (36).

dd-cfDNA serves as a sensitive marker of immune activation and cellular injury; however, its levels may be influenced by conditions other than acute rejection, such as myocardial ischemia, myocardial trauma, or other graft injury. Interpretation of dd-cfDNA requires caution in cases of sepsis, systemic inflammation, cytomegalovirus infection, HIV, trauma, or cancer, as it may yield a falsely reduced dd-cfDNA percentage due to increased cfDNA release from extracardiac sites or tumour cells. Current evidence on dd-cfDNA in CAV suggests potential utility for detecting preclinical vasculopathy; however, these associations have been inconsistent to date (37). Therefore, interpretation of the dd-cfDNA results should always be made within the clinical context to assess the need for a confirmatory EMB (5, 22, 38).

Elevated dd-cfDNA levels without EMB-proven rejection challenge the management of HTx recipients. Patients who demonstrate concurrent positivity for GEP and dd-cfDNA exhibit an elevated risk of subsequent graft dysfunction and cardiovascular death,

even in the absence of histologic evidence of rejection. These findings indicate that circulating multimodal molecular signatures provide prognostic information that is not captured by conventional histologic assessment (39). Patients with elevated dd-cfDNA have an increased mortality risk compared to patients with negative GEP and dd-cfDNA, regardless of biopsy results. Elevated dd-cfDNA is associated with a higher risk of graft dysfunction and presence of DSA compared to patients with negative GEP and dd-cfDNA (25). Evidence also shows that asymptomatic patients with very high dd-cfDNA levels (>3.0%) are at increased risk of developing subsequent 1-year AMR (40). Patients with persistent elevation of GEP and dd-cfDNA, independent of EMB findings, have an increased risk of developing rejection, graft dysfunction, or cardiovascular death (41). A marked increase in dd-cfDNA may warrant more intensive molecular surveillance and therapeutic intervention, as it can reflect graft injury beyond what is evident from EMB findings alone (34). Addressing subclinical graft injury (i.e., molecular rejection) is the cutting edge of transplant medicine. Ultimately, high levels of dd-cfDNA in the absence of EMB-proven rejection warrant closer surveillance and should prompt careful assessment of the immunosuppressive regimen, evaluation of allograft function, DSAs, and myocardial molecular profiling (Molecular Microscope Diagnostic System [MMDx]) (5, 42). MMDx was initially developed to overcome the limitations of conventional histopathologic criteria by enabling molecular phenotyping of EMB specimens. The platform has since been refined to include molecular injury archetype classifications, as illustrated in the INTERHEART study (Diagnostic and Therapeutic Applications of Microarrays in Heart Transplantation, a Multicenter Study). MMDx quantifies gene expression in biopsy samples. It applies ensembles of machine-learning algorithms to analyse these data, enabling comparison of each new biopsy with a comprehensive reference database of prior specimens (30, 43). MMDx recommends including at least two myocardial tissue samples. The myocardial tissue obtained for MMDx analysis is immediately preserved in RNAlater to inactivate ribonucleases that would otherwise rapidly degrade messenger ribonucleic acid. MMDx samples are shipped to and analysed in central laboratories in North America. Biopsy-derived ribonucleic acid is extracted, labelled, and hybridised to the PrimeView microarray, enabling genome-wide GEP. The platform quantifies expression across approximately 49500 probe sets representing 19462 genes, generating a “CEL file” that provides a robust, though simplified, representation

of transcriptomic activity. This file is subsequently analysed using MMDx software to produce an automated report summarising the key molecular scores for each biopsy. MMDx operates as an iterative discovery-application-discovery framework, in which EMBs from ongoing clinical studies are continuously incorporated to refine the reference dataset and rederive machine-learning models (43). MMDx diagnostic categories are classified into four groups: no rejection, ACR, AMR, and injury. Beyond its role in diagnosing rejection, MMDx enables assessment of parenchymal injury. Although myocardial injury and rejection are closely correlated in cardiac allografts, molecular injury remains a critical component in revealing graft dysfunction and predicting long-term graft failure (44).

The prospective Trifecta-Heart study compared local standard-of-care histologic diagnoses with a triad of centrally performed assays: MMDx, dd-cfDNA, and DSA testing. AMR identified by MMDx correlated more strongly with dd-cfDNA and DSAs than histologic AMR. In cases of MMDx-histology discordance, EMBs showing no histologic rejection but classified as AMR by MMDx were associated with elevated DSA and dd-cfDNA levels. In contrast, biopsies categorised as no rejection by MMDx but interpreted as AMR by histology demonstrated low DSA and dd-cfDNA levels. Accumulating evidence indicates that existing histopathologic criteria are no longer adequate for identifying clinically meaningful AMR. Histologic AMR in isolation was unable to discriminate between high- and low-risk HTx recipients. MMDx enabled reclassification of a subset of histologically positive AMR cases into a “no rejection” category, further supported by correspondingly low levels of dd-cfDNA and DSA. The findings of this study indicate that a combination of diagnostic tests is necessary to support clinical decision-making in these critical contexts, particularly given the high cost and potential adverse effects of emerging therapies for AMR. These inter-test relationships will be valuable for informing AMR management, including monitoring treatment response and detecting relapse, particularly when discrepancies arise between test results. Accordingly, the proposed post-AMR monitoring adopts a multimodal strategy – utilising dd-cfDNA (with or without EMB), echocardiography, DSA assessment, and/or intragraft GEP – conducted at standardised intervals during the first year after completion of therapy (32). However, none of the above diagnostic tests is without limitations, and all four may benefit from continued parallel development and

refinement (30, 44). The study demonstrated that, in addition to cardiac transplant rejection, dd-cfDNA levels were elevated in the MMDx severe injury state. These elevations were particularly pronounced in the late atrophy-fibrosis state, which corresponds to the clinical CAV phenotype. Most cases exhibiting molecular injury with elevated dd-cfDNA also demonstrated concurrent molecular rejection, predominantly AMR, especially in the late atrophy-fibrosis state (44).

1.3 Echocardiographic assessment of the cardiac allograft function

Echocardiography is a primary imaging modality for evaluating patients following HTx, providing accurate assessment of graft anatomy and function and serving as an integral component of routine serial evaluations throughout post-transplant follow-up (16). However, a principal technical challenge is that echocardiographic parameters show greater variability in HTx patients than in the general population. This variability complicates the determination of the normal transplanted heart morphology and function, as well as the determination of appropriate cut-off values for echocardiographic parameters to detect allograft dysfunction. Accordingly, in this unique patient population, the availability of a comprehensive individual baseline echocardiographic study for comparison during serial follow-up assessments is more valuable than reliance on the absolute value of individual measurements. A baseline evaluation should be conducted at least 6 months after HTx. In the early post-transplant period, factors such as adaptation of the donor heart to the thoracic cavity, altered positioning within the chest compared to the native heart, and the presence of confounding factors (e.g., early allograft dysfunction, pericardial effusion, sepsis, and multiorgan dysfunction) may influence echocardiographic findings and reduce the sensitivity of echocardiography for detecting acute graft rejection during follow-up (45).

Left ventricular ejection fraction (LVEF) is a commonly used echocardiographic parameter for assessing cardiac function. Its simplicity and strong correlation with clinical outcomes have established it as a convenient tool in routine clinical practice (46). However, its limitations, including low sensitivity and dependence on loading conditions, reduce its reliability and utility as a comprehensive measure of cardiac function (47). Left ventricular (LV) systolic function is generally preserved following HTx. Although LVEF remains the strongest predictor of outcome in HTx recipients, it is neither an early marker of graft dysfunction nor a sensitive indicator of acute graft rejection and typically does

not correlate with the severity of EMB-proven rejection. A late decline in LVEF is frequently associated with progression of CAV and is linked to an unfavourable prognosis. To address specific clinical questions and enhance sensitivity in detecting morphofunctional changes in transplanted hearts, laboratories may utilise advanced echocardiographic techniques (45).

Two-dimensional speckle-tracking echocardiography is an emerging imaging technique that quantifies LV deformation by measuring myocardial strain across the three planes of chamber motion: longitudinal, circumferential, and radial. Left ventricular global longitudinal strain (LVGLS) provides superior diagnostic and prognostic utility across a broad range of cardiovascular disorders compared with LVEF, demonstrates high reproducibility, and can identify subclinical dysfunction before a decline in ejection fraction. LVGLS is defined as the percentage change in myocardial longitudinal length from end-diastole to end-systole during the cardiac cycle. A large patient-level meta-analysis has suggested that an LVGLS value greater than -16% represents an absolute threshold indicative of myocardial dysfunction, irrespective of vendor-specific differences or clinical covariates, and should prompt clinicians to undertake careful evaluation for underlying cardiac pathology. However, interpretation of LVGLS measurements within the broader clinical context remains essential, particularly for values in the “grey zone” of -16% to -18%, which are generally regarded as borderline or low-normal (48).

LVGLS allows assessment of myocardial deformation and identification of early, subclinical cardiac allograft dysfunction (49, 50). While LVEF is generally preserved until severe damage is established, LVGLS is more significantly associated with myocardial injury in the case of ACR (51, 52). It has also been shown that repeated episodes of ACR are associated with reduced longitudinal systolic function, as assessed by LV longitudinal strain. Data show that global and regional LV longitudinal strain values are lower after HTx than in the control population (53, 54). It remains unclear whether the reduced LV longitudinal strain values reflect the “normal” values of the transplanted heart or whether they are signs of subtle alterations in the contractile function of the allograft (54). The correlation between dd-cfDNA and LV longitudinal strain-derived parameters remains unknown.

Although LVGLS demonstrates less load dependency than LVEF, it remains susceptible to variations in loading conditions (55). Compared with LVGLS and LVEF, myocardial work accounts for loading conditions during myocardial deformation. By integrating LV loading conditions, pressure-strain loop analysis provides a more advanced method for evaluating myocardial performance. It enables quantification of both global and regional myocardial contractile function while offering insight into myocardial energetics and oxygen consumption. This comprehensive assessment supports the identification of subclinical myocardial dysfunction. Myocardial work is defined by four distinct components that contribute to the comprehensive understanding of LV mechanics:

1. global work index (GWI): total work carried out by the left ventricle during systole,
2. global constructive work (GCW): LV work that contributes to LV ejection and quantifies the energy consumed by the myocardium, which effectively contributes to the cardiac output (shortening during systole and lengthening during isovolumetric relaxation),
3. global wasted work (GWW): LV work that does not result in LV ejection and measures the energy wasted due to lengthening during systole and shortening during isovolumetric relaxation,
4. global work efficiency (GWE): reflects the percentage of myocardial work that is converted into cardiac output (i.e., the ratio of constructive and wasted work) (56, 57).

Noninvasive myocardial work represents a promising emerging modality for assessing LV systolic function, with the potential to distinguish impaired myocardial performance resulting from increased afterload from that caused by reduced myocardial contractility. Enhanced detection of subclinical cardiac dysfunction may serve as a valuable surrogate marker of disease, providing mechanistic insight into cardiovascular pathologies and enabling earlier diagnosis (57). Myocardial work has been evaluated in several clinical scenarios to assess its added value relative to LVEF and LVGLS (56-58). However, the significance of myocardial work analysis in assessing HTx injury remains largely unknown.

1.4 Extracorporeal photopheresis

ECP is a type of apheresis technique. The leukocyte-targeted cell therapy involves withdrawing the patient's peripheral blood, isolating the leukocyte-rich plasma, treating

it with a photosensitising agent (8-methoxypsoralen; 8-MOP), and then irradiating it with ultraviolet A (UVA) light. UVA-mediated activation of 8-MOP induces DNA, deoxyribonucleic acid (DNA) cross-link formation, leading to programmed cell death via apoptosis, which is recognised as the primary direct mechanism underlying ECP. Following reinfusion, the buffy coat – composed of leukocytes and platelets – contains a mixture of viable cells, apoptotic and necrotic cells, cellular debris, and soluble factors. UVA-treated leukocytes are phagocytosed by activated monocytes, which then differentiate into dendritic cells. The uptake of the leukocytes initiates a cascade of indirect immunomodulatory effects (31, 59, 60). ECP reduces the production of effector T cells, promotes the expansion of regulatory T cells, which suppress the immune system in an antigen-specific manner, and induces plasmacytoid (tolerogenic) dendritic cells (61). Evidence from multiple studies indicates that reinfused leukocytes treated with ECP undergo apoptosis over a period of one to two weeks, stimulating an autologous suppressor response – partly mediated by T cells – that acts against nonirradiated T cells of similar clonal origin. Furthermore, these leukocytes promote the tolerogenic activity of quiescent dendritic cells (and likely macrophages) with which they interact (31). ECP exerts a sustained immunomodulatory effect, appearing to beneficially influence both humoral and cellular immune responses directed against the allograft. Although the precise mechanism by which ECP prevents or treats HTx rejection remains incompletely understood, the prevailing hypothesis attributes its effect primarily to the immunosuppression induced by irradiated helper T cells (Figure 2). Unlike immunosuppressive drugs, ECP does not induce generalised immunosuppression (31, 59, 60, 62).

ECP is used on a chronic basis, with each treatment cycle consisting of two sessions on consecutive days. Therapeutic regimens vary widely, with series performed weekly or every 2-8 weeks over several months. Treatments are typically performed until improvement and/or stabilisation of both allograft function and symptoms occur (61, 63). There is no consensus on the optimal treatment protocol for ECP, including initiation, session number and frequency, tapering, and discontinuation strategies. Nonetheless, multiple studies have highlighted its favourable safety profile, reporting a very low incidence of adverse effects. The most frequently observed adverse effect is photosensitivity, which is generally well controlled with appropriate sun protection

measures. The primary challenge associated with ECP is achieving reliable vascular access. Although most patients can be treated via peripheral veins, some require insertion of a central venous catheter, a procedure associated with a low but recognised risk of infection, bleeding, and thrombosis (59). The literature reports anaemia, thrombocytopenia, hypotension, fatigue, and ECP-related fever (64, 65).

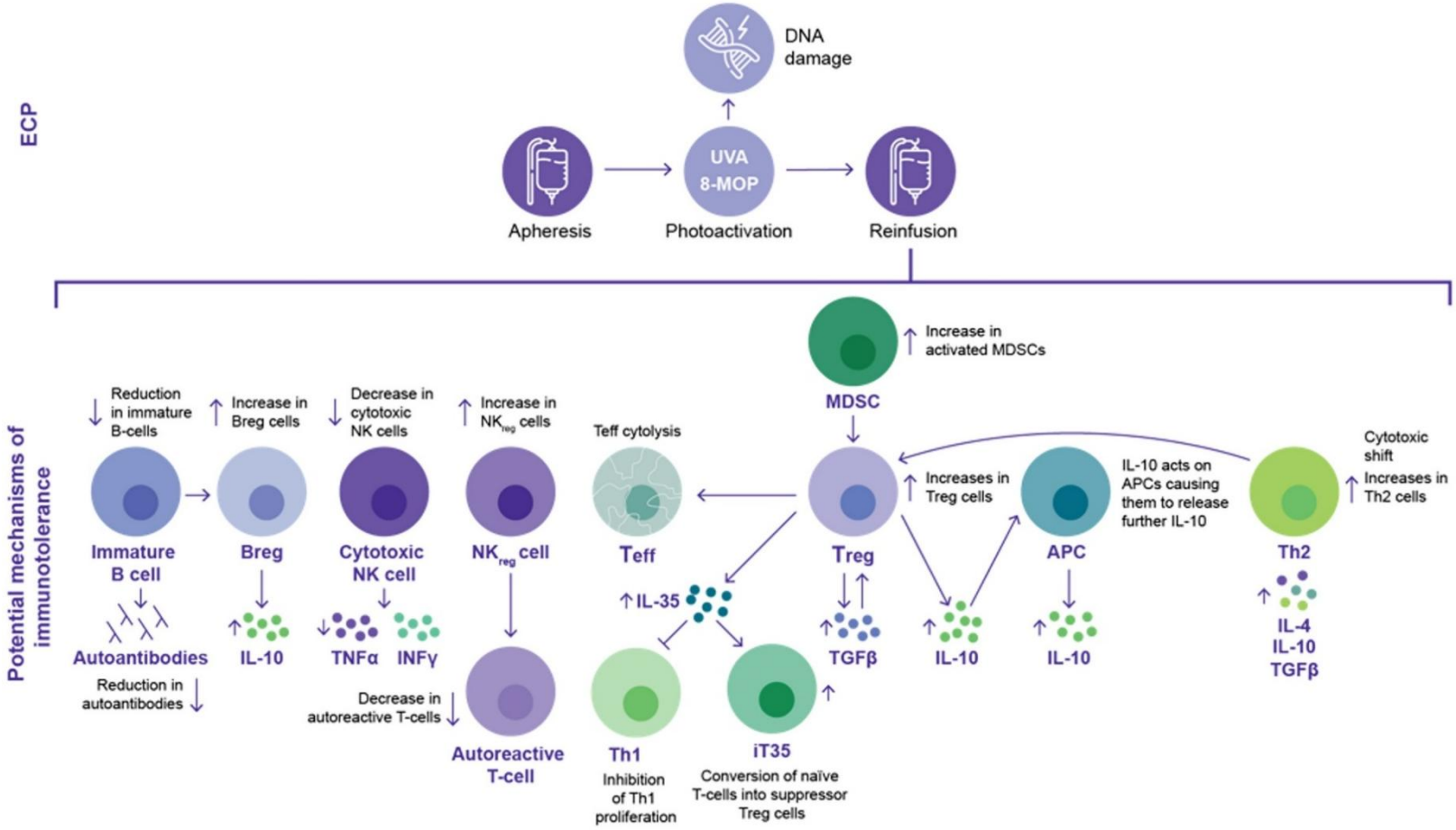
ECP
DNA damage
UVA 8-MOP
Apheresis
Photoactivation
Reinfusion
Potential mechanisms of immunotolerance
Reduction in immature B-cells
Increase in Breg cells
Decrease in cytotoxic NK cells
Increase in NKreg cells
Immature B cell
Breg
Cytotoxic NK cell
NKreg cell
Autoantibodies
IL-10
TNFα
INFγ
Reduction in autoantibodies
Decrease in autoreactive T-cells
Autoreactive T-cell
Teff cytolysis
Teff
↑IL-35
Th1
Inhibition of Th1 proliferation
iT35
Conversion of naïve T-cells into suppressor Treg cells
MDSC
Increase in activated MDSCs
Treg
Increases in Treg cells
TGFβ
IL-10
APC
IL-10
IL-10 acts on APCs causing them to release further IL-10
Th2
Cytotoxic shift
Increases in Th2 cells
IL-4
IL-10
TGFβ

Figure 2. Proposed mechanisms of action of extracorporeal photopheresis (62).
Abbreviations: APC, antigen-presenting cell; Breg, regulatory B cell; DNA, deoxyribonucleic acid; ECP, extracorporeal photopheresis; IL, interleukin; INFγ, interferon gamma; iT35, induced regulatory T cell; MDSC, myeloid-derived suppressor cell; NK cell, natural killer cell; NKreg cell, natural killer regulatory cell; Teff, effector T cell; TGF-β, transforming growth factor beta; Th, helper T cell; Treg, regulatory T cell; TNF, tumour necrosis factor.

The limited evidence suggests that ECP is more commonly used in cardiothoracic solid-organ transplantation than in abdominal transplantation. However, across all organ types, the available evidence is limited by the scarcity of randomised controlled trials (66). The indication for ECP has expanded to include cardiac allograft rejection; although most previous studies used a triple-drug maintenance immunosuppressive regimen comprising cyclosporine, azathioprine, and corticosteroids, combined with ECP (Table 5) (59, 60, 63-65, 67-80). The scientific societies' recommendations for ECP therapy include cellular rejection, recurrent rejection, and rejection prophylaxis (Table 6) (16, 61). Based on current data, ECP may be indicated as prophylaxis for selected patients at high risk of rejection due to previous failed transplants, prior pregnancies, a high number of blood transfusions, high panel reactive antibody levels, and poor HLA matching. ECP can also be considered as a therapeutic option for allograft rejection in patients who require a reduction in conventional immunosuppression due to complications such as severe infections, renal impairment, or an increased risk of cancer recurrence (60, 72).

Table 5. The review of the literature on extracorporeal photopheresis in heart transplantation.

Study (year)	Study design	Study population, ECP regimen	Key outcomes
Barten et al. (2023) (64)	Multicentre retrospective study	105 HTx recipients treated with ECP at 7 European centres for ACR, AMR, or mixed rejection; mean follow-up 22.5 months	Significant histologic improvement in ACR, AMR, and mixed rejection groups, with many patients demonstrating partial or complete resolution of rejection on follow-up EMBs
Gökler et al. (2022) (72)	Prospective study	28 high-risk HTx recipients received 6 months of ECP combined with reduced immunosuppression; intensive early schedule followed by tapering sessions (24 treatments)	Low incidence of ACR within the first year; no rebound rejection following ECP cessation; acceptable infection rates despite high baseline risk; no cancer recurrence during long-term follow-up
Kirklin et al. (2006) (80)	Retrospective study	36 HTx patients with rejection with hemodynamic compromise, recurrent rejection, or DSAs, treated with ECP for a minimum of 3 months	Decreased rejection risk after ECP; significantly reduced risk for subsequent rejection with hemodynamic compromise or death
Barr et al. (2000) (70)	Prospective randomised study	23 HTx recipients randomised to standard immunosuppression alone or immunosuppression plus ECP	ECP is associated with a significant reduction in calculated panel reactive antibodies and reduced coronary artery intimal

Study (year)	Study design	Study population, ECP regimen	Key outcomes
			thickness at 1- and 2-year follow-up, suggesting a protective effect against CAV
Dall'Amico et al. (2000) (60)	Retrospective study	11 HTx patients with recurrent rejection underwent ECP for 6 months	The fraction of EMBs with a grade 0/1A rejection increased, while EMBs with a 3A/3B rejection decreased; ECP allowed a reduction in immunosuppressive therapy
Barr et al. (1998) (67)	Multicentre randomised controlled trial	60 HTx recipients randomised to standard immunosuppression with or without adjunctive ECP during the first 6 months post-transplant (24 procedures per patient)	Comparable survival between groups; significantly fewer acute rejection episodes and lower cytomegalovirus detection in the ECP group; no increase in infection rates
Meiser et al. (1994) (75)	Prospective study	15 HTx recipients received standard immunosuppression alone or combined with low-dose or high-dose ECP	Patients treated with ECP experienced significantly fewer acute rejection episodes and infections compared with controls; no ECP-related adverse effects

Abbreviations: ACR, acute cellular rejection; AMR, antibody-mediated rejection; CAV, cardiac allograft vasculopathy; DSA, donor-specific antibody; ECP, extracorporeal photopheresis; EMB, endomyocardial biopsy; HTx, heart transplantation.

Table 6. Recommendations for extracorporeal photopheresis in heart transplantation (16, 61).

Society	Indication	Recommendation
ASFA	Cellular rejection	Category II, Grade 1B
	Recurrent rejection	Category II, Grade 1B
	Rejection prophylaxis	Category II, Grade 2A
ISHLT	Recurrent or resistant ACR	Class IIA, Level of evidence: B

Abbreviations: ACR, acute cellular rejection; ASFA, American Society for Apheresis; ISHLT, International Society for Heart and Lung Transplantation.

Although promising results have been reported for ECP therapy in treating cardiac allograft rejection, data on its use in HTx recipients are still needed. Studies on ECP in conjunction with a tacrolimus-based immunosuppressive protocol, with either MMF or mycophenolic acid, and methylprednisolone, as well as data on its use in patients with persistent ISHLT grade 1R cellular rejection, remain scarce (71).

2 Objectives

Although the frequency of treated rejection has decreased, allograft rejection remains a major determinant of post-transplant morbidity and mortality (27). EMB has historically been regarded as the gold standard for rejection surveillance; however, its limitations restrict the timely and accurate detection and management of rejection. In contrast, dd-cfDNA has emerged as a sensitive, reliable, and reproducible biomarker that may identify allograft injury months before histopathological changes become evident on EMB (4, 23, 42). Advances in immunosuppressive therapy have substantially improved survival and quality of life among HTx recipients. However, the current standard-of-care immunosuppressive regimen is associated with significant adverse effects, including opportunistic infections, secondary malignancies, metabolic complications, and end-organ damage. In this context, ECP represents an immunomodulatory treatment option with a favourable safety profile and no increased risk of infection (61, 66).

Against this background, we sought to address key aspects of contemporary HTx rejection surveillance and treatment. First, we described the initial experience with the clinical implementation of a local laboratory-run, commercially available, CE-IVD-marked dd-cfDNA assay-based monitoring programme for routine HTx rejection surveillance. Second, we examined whether a reduction in LV longitudinal strain parameters could result from early HTx injury, as assessed by dd-cfDNA (Rejection surveillance). Third, we investigated the efficacy of ECP in combination with a tacrolimus-based maintenance immunosuppressive therapy for treating cardiac allograft rejection in various clinical scenarios (Rejection treatment).

3 Methods

3.1 Recruitment of patients

3.1.1 Rejection surveillance

Since October 2022, consecutive HTx recipients who have not experienced graft rejection requiring intensification of immunosuppressive therapy have been transitioned from our EMB-based surveillance protocol (approximately 12-15 EMBs performed during the first post-transplant year) to a monthly dd-cfDNA-guided rejection monitoring approach. Exclusion criteria included multi-organ transplant recipients, a blood transfusion containing white blood cells within the past 30 days, pregnancy, and an EMB performed within the previous 24 hours. We also identified a cohort of consecutive HTx recipients who underwent treatment for AMR (n=9). Informed consent was obtained from each patient, and the study was conducted in accordance with the principles of the Declaration of Helsinki.

3.1.2 Rejection treatment

We conducted a retrospective, single-centre study on adult HTx recipients who underwent ECP between September 2013 and October 2020. Informed consent was obtained from all patients before the initiation of the ECP course.

3.2 Collection of data

We retrospectively collected patients' clinical data from institutional electronic records and stored them in a separate database.

3.3 Immunosuppression

Induction therapy with antithymocyte globulin (1.5 mg/kg) was initiated on the first post-transplant day and maintained for up to 3 days, except when an active infection was present at the time of HTx. All patients received a calcineurin-inhibitor-based maintenance immunosuppressive regimen. Tacrolimus was initiated on the fifth postoperative day and administered twice daily. Target trough levels were maintained at 10-15 ng/mL during the first 1-3 months post-transplant or after a moderate or severe rejection episode, 8-11 ng/mL between months 3-6, 7-10 ng/mL from months 6-12, and 5-7 ng/mL beyond 12 months. In the patient cohort monitored with dd-cfDNA, one recipient was switched to cyclosporine due to side effects. All HTx recipients received mycophenolate as an antiproliferative agent. The target dose of MMF was 3000 mg/day until the tacrolimus target trough level was achieved; thereafter, the MMF

dose was adjusted to 2000 mg/day. Methylprednisolone was tapered from the perioperative induction doses to 8 mg/day for the first 6 months; thereafter, gradual tapering was initiated, and it was weaned off at the discretion of the clinicians, either by the first post-transplant year or 1 year after the allograft rejection episode. Both ISHLT grade 2R rejections and mixed rejection episodes accompanied by graft dysfunction were treated with intravenous pulse methylprednisolone therapy (500 mg or 1000 mg o.d.) for three days. Patients with AMR and cardiac allograft dysfunction additionally received three to five therapeutic plasma exchanges in combination with intravenous immunoglobulin therapy (1 g/kg) and completed with rituximab (375 mg/m^2) if needed. In case of a rejection episode, the maintenance immunosuppressive regimen was increased to the early post-transplant level.

3.4 Laboratory analysis

Serum N-terminal prohormone of brain natriuretic peptide (NT-proBNP) measurements were performed during routine follow-up visits and as clinically indicated.

Donor-specific anti-HLA class I and II antibodies were assessed at one, three, six, and twelve months post-transplant, then annually and as clinically indicated. DSA surveillance was intensified during episodes of allograft rejection. Detection and quantification were carried out using solid-phase microbead-based assays. A DSA was considered positive when the mean fluorescent intensity (MFI) exceeded 3000, while a high-titre DSA was defined by an MFI greater than 10000.

3.5 Donor-derived cell-free DNA analysis

Before introducing dd-cfDNA surveillance into clinical practice, we conducted a cross-sectional, retrospective pilot study to assess the feasibility of a local laboratory-run, targeted, next-generation sequencing-based, commercially available, CE-IVD-marked dd-cfDNA assay (AlloSeq, based on the well-characterised AlloSure central laboratory assay, CareDx, Inc., Brisbane, CA, USA). The study included 27 samples from 23 patients, with and without rejection, collected at various times after HTx. The pilot analysis demonstrated the assay's reliability and reproducibility, with internal control samples consistently returning the predefined values (data not shown) (81).

We performed the first dd-cfDNA measurement before simultaneous surveillance EMB in 24 cases (34%). For dd-cfDNA analysis, 8 mL of peripheral venous blood was collected into Streck cfDNA blood collection tubes (Streck, La Vista, NE, USA). The dd-

cfDNA analysis was performed at months 2, 3, 4, 5, 6, 7, 8, 10, and 12 following HTx, alongside clinical evaluation, electrocardiography, echocardiography, and routine laboratory testing. In the patient cohort treated for AMR, dd-cfDNA was collected either before surveillance EMB or during routine follow-up visits. The dd-cfDNA analysis was performed according to the manufacturer's instructions for use (AlloSeq cfDNA Instructions for Use IFU090, Version Number: 9.0, CareDx, Inc., Brisbane, CA):

1. preservation of plasma,
2. quantification of the input DNA,
3. cfDNA extraction,
4. amplification of cfDNA,
5. sequencing of the polymerase chain reaction (PCR) products,
6. quantification of dd-cfDNA values and quality control (35, 82).

The AlloSeq cfDNA assay utilises differences in 202 single-nucleotide polymorphism loci, which vary between donor and recipient, to determine the proportion of donor-derived cfDNA relative to the total cfDNA from a plasma sample. It does not require a prior donor and recipient genotyping (83). After sequencing is complete, the data is analysed using the AlloSeq cfDNA software, which reports the percentage of the donor-derived cfDNA (Table 7). To ensure the accuracy and reliability of reported dd-cfDNA results, several quality-control metrics are established, each with a defined cutoff (82). Results that fell below or exceeded these thresholds were neither reported nor used in clinical decision-making. For each measurement, at least one control sample was analysed alongside the clinical samples to ensure data consistency across independent assessments. The turnaround time for results was three working days. A dd-cfDNA level of <0.25% was considered quiescence, whereas ≥0.35% indicated severe injury (22). The 0.25-0.35% dd-cfDNA range was regarded as a grey zone (4).

Table 7. Workflow and equipment for the local laboratory-run donor-derived cell-free DNA analysis.

Workflow	Equipment
Preservation of plasma	Cell-free DNA blood collection tube (Streck) Centrifuge
Quantification of the input DNA	Qubit fluorometer (Thermo Fisher Scientific)
Cell-free DNA extraction	QIAamp Circulating Nucleic Acid Kit (QIAGEN) QIAvac vacuum system (QIAGEN)
Amplification of cell-free DNA	AlloSeq cfDNA kit (CareDx) Thermal Cycler (Veriti, Thermo Fisher Scientific) DynaMag-2 Magnet (Thermo Fisher Scientific)
Sequencing of the PCR products	Next-generation sequencing instrument (MiSeq, Illumina) MiSeq Reagent Kit (Illumina)
Quantification of dd-cfDNA values and quality control	AlloSeq cell-free DNA software (CareDx)

Abbreviations: cfDNA, cell-free DNA; dd-cfDNA, donor-derived cell-free DNA; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction.

3.6 Transthoracic echocardiography

Experienced operators performed transthoracic echocardiography using Philips Epiq Elite or Epiq CVx (Philips, Andover, MA, USA) (n = 151) or Vivid E95 (GE HealthCare, Horten, Norway) (n = 43) machines, with an electrocardiogram continuously recorded (84). All echocardiographic images were stored in DICOM format. Echocardiographic studies were performed on the same day, after blood was drawn for dd-cfDNA analysis. For echocardiographic assessments, patient selection was limited to individuals who had undergone HTx at least 6 months before inclusion (45).

LVEF was calculated using the biplane method of disc summation. Allograft dysfunction was defined as an LVEF <54% in women and <52% in men (84).

For strain analysis, each study used the 18-segment LV segmentation model. LVGLS and myocardial work indices were calculated offline after automated tracking of myocardial deformation (TomTec Imaging Systems, Munich, Germany). Echocardiographic examinations were excluded when tracking quality was inadequate in more than one segment. Regional longitudinal strain parameters, including LV basal, mid, and apical strain as well as LV lateral and septal strain, were evaluated. The apical sparing ratio was calculated by dividing the apical LV longitudinal strain by the sum of the

average basal and average mid LV longitudinal strain values. An apical sparing ratio of 1 or greater was considered indicative of apical sparing (85). Myocardial work was evaluated noninvasively using pressure-strain loop analysis, from which GWI, GCW, GWW, and GWE were derived.

LV longitudinal strain and myocardial work parameters were compared with results from the Normal Reference Ranges for Echocardiography (NORRE) study, conducted across 22 laboratories accredited by the European Association of Cardiovascular Imaging (EACVI) and one laboratory in the United States. The first large-scale, prospective, multicentre European study established contemporary normal reference values for all two-dimensional echocardiographic measurements of cardiac chambers, including two-dimensional LV strain. The final NORRE study population comprised 734 healthy individuals. For LV strain analysis, the final study population consisted of 549 normal subjects, while for myocardial work analysis, it comprised 226 healthy subjects (86, 87).

3.7 Endomyocardial biopsy

Surveillance for HTx rejection was conducted through serial transvenous EMBs during the first year post-transplant, throughout episodes of rejection, and as clinically indicated. For each biopsy procedure, three to six myocardial tissue samples were collected from the right ventricular septum.

We performed a for-cause EMB whenever dd-cfDNA results exceeded 0.35%. In the absence of clinical suspicion of rejection, we continued regular dd-cfDNA testing in patients with dd-cfDNA levels of 0.25%-0.35% (Table 8).

Table 8. Interpretation of donor-derived cell-free DNA values in our routine clinical practice.

Donor-derived cell-free DNA range	Interpretation
≤0.25%	Quiescence
0.25-0.35%	Grey zone
≥0.35%	Injury

Abbreviation: DNA, deoxyribonucleic acid.

The ISHLT grading scale was used to evaluate EMB specimens (16, 28, 29). Acute moderate cellular rejection corresponds to ISHLT grade 2R, whereas acute severe cellular rejection denotes ISHLT grade 3R. Persistent mild cellular rejection is defined as four or more consecutive episodes of ISHLT grade 1R rejection. Mixed rejection refers to a combination of cellular rejection and AMR, or cellular rejection in the presence of high-titre donor-specific anti-HLA antibodies. To compare cellular rejection status in the cardiac allograft, the total rejection score (TRS) was calculated for each patient in both the pre- and post-ECP therapy periods. TRS was calculated for each patient according to the ISHLT criteria (0R=0, 1R=1, 2R=2, 3R=3) and normalised by dividing by the total number of biopsies performed during the specified period (88).

3.8 Extracorporeal photopheresis

ECP was initiated following the episode of cardiac allograft rejection and administered alongside pharmacological anti-rejection therapy. The procedure utilised a closed-system ECP device (UVAR-XTS in three patients and CELLEX in the remaining patients, both by Therakos Ltd., Staines-Upon-Thames, UK). 8-MOP was administered extracorporeally. ECP treatments were performed in cycles over two consecutive days. The therapy was more intensive in the period immediately following allograft rejection, with weekly or biweekly sessions. It was tapered to a monthly schedule based on allograft function and rejection severity. All observed adverse events were documented throughout the treatment.

3.9 Statistical analysis

Data were expressed as count (percentage), mean±standard deviation (SD), or median (min-max), as appropriate. The dataset's distribution was assessed using the D'Agostino-Pearson and Shapiro-Wilk tests. Between-group differences were evaluated using the paired t-test, the Mann-Whitney test, or the Wilcoxon matched-pairs signed-rank test, depending on the distribution of the dataset. Groups were compared using either the one-way ANOVA or the Kruskal-Wallis test. For correlation analysis, Pearson's or Spearman's correlation coefficient was used, as appropriate. The Kaplan-Meier method was used to analyse survival. We considered $p < 0.05$ to be statistically significant. GraphPad Prism 10.4.0 software (GraphPad Software, Inc., La Jolla, CA, USA) was used for the statistical analyses.

4 Results

4.1 Rejection surveillance

4.1.1 Donor-derived cell-free DNA analysis

A total of 71 patients were transitioned from our EMB-based rejection surveillance protocol to the dd-cfDNA-guided monitoring approach. Between October 2022 and November 2024, 449 dd-cfDNA samples were analysed across 26 assay runs (Table 9).

Table 9. Patient characteristics (89).

Variable	Population
Number of patients	71
Number of samples	449
Pre-transplant diagnosis	
Non-ischemic cardiomyopathy	39 (55%)
Ischemic cardiomyopathy	24 (34%)
Hypertrophic cardiomyopathy	5 (7%)
Arrhythmogenic right ventricular dysplasia	1 (1%)
Valvular heart disease	1 (1%)
Restrictive cardiomyopathy	1 (1%)
Female sex (%)	18 (25%)
Mechanical support at the time of heart transplantation	
Durable circulatory support (%)	4 (6%)
Temporary circulatory support (%)	3 (4%)
Age at time of heart transplantation [median (min-max); years]	54 (18-67)
Days post-transplant at enrolment [median (min-max); days]	62 (17-367)
Enrolment without simultaneous endomyocardial biopsy (%)	47 (66%)
Immunosuppression	
Tacrolimus/mycophenolate/methylprednisolone (%)	68 (96%)
Cyclosporine/mycophenolate/methylprednisolone (%)	1 (1%)
Tacrolimus/mycophenolate (%)	2 (3%)
Tacrolimus trough level at enrolment [median (min-max); (ng/mL)]	13.5 (3.0-23.7)
Patients presenting with donor-specific antibody at enrolment (%)	14 (20%)
Maximum MFI [median (min-max)]	1492 (737-15771)
NT-proBNP level at enrolment [median (min-max); (pg/mL)]	1271 (193-35000)
Creatinine level at enrolment [median (min-max); umol/L]	106 (57-709)

Abbreviations: MFI, mean fluorescent intensity; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

In clinically stable patients, the dd-cfDNA fraction remained low (0.13±0.06%) (Figure 3).

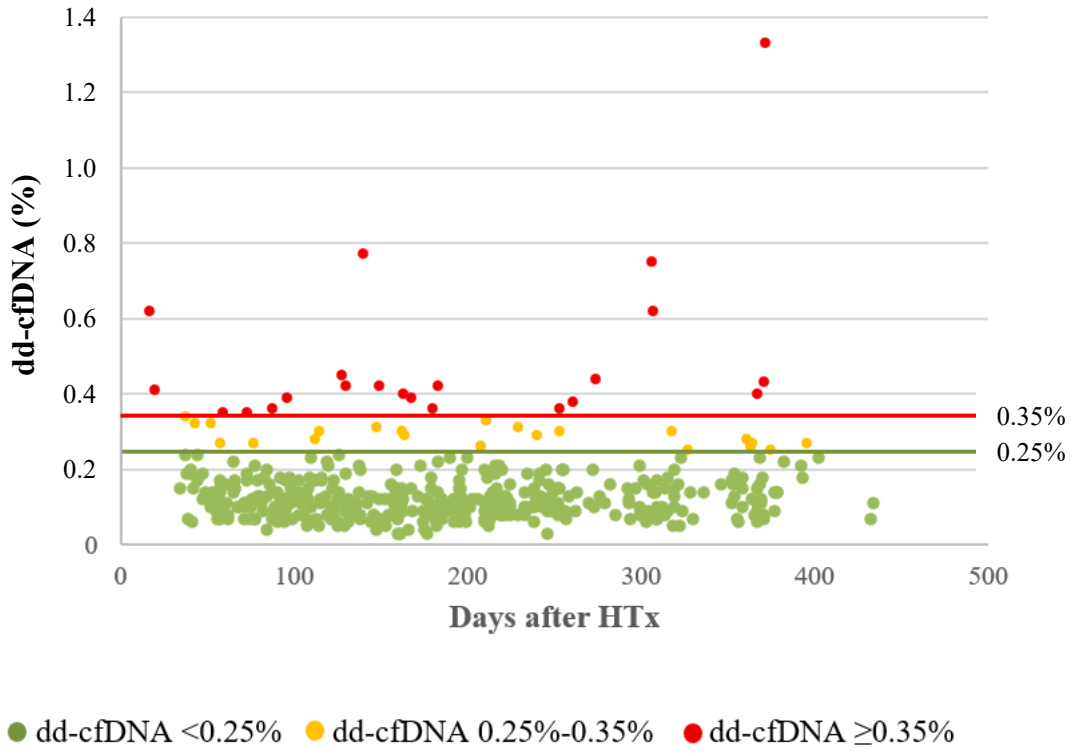

Figure 3. Distribution of donor-derived cell-free DNA data points in the function of time after heart transplantation.

Abbreviations: dd-cfDNA, donor-derived cell-free DNA; DNA, deoxyribonucleic acid; HTx, heart transplantation.

Nine dd-cfDNA measurements failed to meet quality control criteria. In one case, we performed an EMB because the patient was in the early post-transplant period (<4 months after HTx). For the remaining eight cases, dd-cfDNA testing was repeated the following month because substantial HTx rejection could be excluded based on other routine clinical parameters. Elevated dd-cfDNA levels, suggestive of myocardial injury, prompted 26 for-cause EMBs. Of these, one EMB identified moderate cellular rejection requiring intravenous steroid therapy. Six EMBs showed mild cellular rejection, and one EMB confirmed mild AMR. Eighteen EMBs showed no rejection: two dd-cfDNA results corresponded to perioperative injury (testing was performed early, <1 month post-transplant), while one result was presumably caused by pre-analytical error. Furthermore, two for-cause EMBs were performed before dd-cfDNA results became available, owing to suspicion of rejection based on other clinical findings (NT-proBNP level, tacrolimus trough level, echocardiography). These confirmed moderate cellular rejection episodes

that required intravenous corticosteroid therapy. Patients with EMB-proven rejection were transitioned back to regular EMB testing. Three hundred and ninety-six (88%) routine surveillance EMBs that would otherwise have been performed were avoided.

4.1.2 Echocardiographic analysis

For the echocardiographic analysis, 194 echocardiograms from 51 patients were included. On average, 4±1 echocardiographic studies were analysed per patient. The median donor age was 45 years (range, 24-62 years), and 24% of the donors were female. The median recipient age at the time of imaging was 55 years (range, 18-67 years), and 20% of the recipients were female. Echocardiographic assessments were performed at a median of 249 days after HTx (range, 180-434 days). The mean LVEF remained within the normal range at 57.2±7.0%. In contrast, the mean LVGLS and the longitudinal strain values obtained from the apical 4-chamber, 2-chamber, and 3-chamber views were reduced compared to reference values reported in the literature (Table 10) (86).

Table 10. Two-dimensional echocardiographic parameters of left ventricular longitudinal strain (n=194 echocardiograms of 51 patients) compared to the results of the EACVI NORRE study (n=549, one echocardiogram per patient) (89).

	Study examinations mean±SD	**EACVI NORRE mean±SD**
longitudinal strain, %		
apical 4-chamber	-16.0±3.3	-22.6±3.0
apical 2-chamber	-14.8±4.2	-23.2±3.3
apical 3-chamber	-15.4±3.5	-21.6±3.2
LVGLS	-15.6±2.8	-22.5±2.7

Abbreviations: EACVI, European Association of Cardiovascular Imaging; LVGLS, left ventricular global longitudinal strain; NORRE, Normal Reference Ranges for Echocardiography; SD, standard deviation.

Regarding regional strain values, we found a significant difference between lateral and septal longitudinal strains (-17.3±3.6% vs. -12.5±4.1%; $p<0.0001$). Furthermore, longitudinal strain differed significantly across the basal, mid, and apical segments (-16.2±4.8% vs. -12.7±3.5% vs. -14.9±6.0%; $p<0.0001$). We found no correlation between LVGLS and dd-cfDNA values (n=156; $p=0.762$). Similarly, no significant association was identified between regional longitudinal strain parameters and dd-cfDNA levels.

There was no significant difference in LVEF, LVGLS, or apical sparing ratio at 6 or 12 months after HTx. The regional longitudinal strain values were comparable between the two time points, except for lateral longitudinal strain, which showed a significant difference between indices measured at 6 and 12 months post-HTx (-16.6±2.9% vs. -18.6±3.4%; p=0.031) (Table 11).

Table 11. Left ventricular longitudinal strain values and left ventricular ejection fraction were measured 6 and 12 months after heart transplantation (89).

	6 months after HTx mean±SD n=20	**12 months after HTx mean±SD n=20**	**p**
longitudinal strain, %			
apical 4-chamber	-16.0±3.1	-17.6±3.7	0.155
apical 2-chamber	-14.7±3.6	-15.7±3.6	0.389
apical 3-chamber	-14.8±3.4	-16.1±3.4	0.188
LVGLS	-15.2±2.4	-16.5±2.9	0.176
lateral strain	-16.6±2.9	-18.6±3.4	0.031
septal strain	-11.7±3.0	-11.9±7.1	0.465
basal strain	-15.4±4.4	-16.0±3.9	0.823
mid strain	-12.4±2.9	-13.9±3.4	0.436
apical strain	-14.3±5.6	-16.8±4.8	0.083
apical sparing ratio	0.53±0.21	0.58±0.21	0.223
LVEF, %	56.9±7.1	58.7±8.3	0.596

Abbreviations: HTx, heart transplantation; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; NS, not significant; SD, standard deviation.

Six recipients had an apical sparing ratio ≥1. There was no significant difference in dd-cfDNA levels between patients with apical sparing and those without an apical sparing ratio of 1 or greater.

To evaluate myocardial work, 59 echocardiograms from 22 HTx recipients were examined. The median time from HTx was 293 (183-402) days. Compared with healthy subjects from the EACVI NORRE study, transplanted hearts exhibited reduced GWI, GCW, and GWE values, and increased GWW values (Table 12) (87). No significant associations were observed between myocardial work parameters and dd-cfDNA levels.

Table 12. Strain-derived myocardial work parameters (n=59 echocardiograms of 22 patients) compared to the results of the EACVI NORRE study (n=226, one echocardiogram per patient) (89).

	Study examinations mean±SD or median (min-max)	**EACVI NORRE mean±SD or median (min-max)**
GWI (mmHg%)	1420.3±361.8	1896±308
GCW (mmHg%)	1790.5±425.6	2232±331
GWW (mmHg%)	142 (16-954)	78.5 (53-122.2)
GWE (%)	93 (70-99)	96 (94-97)

Abbreviations: EACVI, European Association of Cardiovascular Imaging; GWI, global work index; GCW, global constructive work; GWW, global wasted work; GWE, global work efficiency; NORRE, Normal Reference Ranges for Echocardiography; SD, standard deviation.

We created three subgroups based on dd-cfDNA values: dd-cfDNA <0.25% (n=171), 0.25%-0.35% (n=11), and ≥0.35% (n=7). No significant differences were observed between the groups in terms of LVEF or LV longitudinal strain indices. However, NT-proBNP values differed significantly between the groups (Table 13). When analysing the predefined dd-cfDNA categories (<0.25%, 0.25%-0.35%, and ≥0.35%), no correlations were identified between dd-cfDNA levels and LVEF, strain-derived echocardiographic parameters, or serum NT-proBNP levels.

Table 13. Two-dimensional echocardiographic parameters of left ventricular longitudinal strain, left ventricular ejection fraction, and serum N-terminal prohormone of brain natriuretic peptide levels in the function of prespecified ranges of donor-derived cell-free DNA (n=189 echocardiograms of 51 patients) (89).

	dd-cfDNA <0.25% mean±SD	**dd-cfDNA 0.25%-0.35% mean±SD**	**dd-cfDNA ≥0.35% mean±SD**	**p**
longitudinal strain, %				
apical 4-chamber	-16.0±3.2	-16.0±3.4	-17.6±4.8	0.690
apical 2-chamber	-14.8±4.3	-14.5±4.2	-14.8±3.6	0.750
apical 3-chamber	-15.4±3.5	-15.4±4.3	-17.3±3.2	0.271
LVGLS	-15.6±2.7	-15.3±3.5	-16.6±3.7	0.823
lateral strain	-17.2±3.5	-16.6±3.5	-19.4±4.0	0.314
septal strain	-12.6±3.5	-12.9±3.7	-15.0±4.2	0.172
basal strain	-16.2±4.8	-16.3±5.3	-16.3±4.6	0.924
mid strain	-12.6±3.5	-13.1±4.1	-13.3±3.6	0.548
apical strain	-14.7±5.9	-14.1±5.7	-19.4±8.1	0.265
LVEF, %	57.1±7.0	56.1±5.2	63.2±6.2	0.420
NT-proBNP, pg/mL	1451±4212	952±971	1855±1667	0.044

Abbreviations: dd-cfDNA, donor-derived cell-free DNA; DNA, deoxyribonucleic acid; LVEF, left ventricular ejection fraction; LVGLS, left ventricular global longitudinal strain; NS, not significant; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; SD, standard deviation.

In patients with HTx rejection on for-cause EMB (n=10 rejection episodes of 9 patients), the mean dd-cfDNA level rose to 0.77±0.79%. The mean LVEF was preserved (60.8±8.5%). The mean LVGLS was -15.6±5.3%. LVGLS was not pathognomonic in EMB-proven rejection cases either.

The predefined patient cohort treated for AMR consisted of 9 HTx recipients (4 females; mean age at the time of measurement was 52±10 years). None of the patients exhibited hemodynamic instability. We analysed 26 dd-cfDNA samples from this patient cohort 40±23 months after HTx. The median dd-cfDNA level was 2.76% (0.68%-10.01%). The mean LVEF remained preserved at 58.8±9.8%, while the mean LVGLS was -17.4±3.3%. We found no correlation between dd-cfDNA values and either LVEF or LV longitudinal strain parameters.

4.2 Rejection treatment

4.2.1 Patient and ECP characteristics

A total of 22 HTx recipients received a median of 11 ECP cycles (22 treatments). All patients completed at least one course of ECP therapy. Two individuals were classified as non-compliant after declining further treatment following the second and tenth sessions, respectively. No ECP-related adverse effects were observed (Table 14).

Table 14. Characteristics of the heart transplant recipients and extracorporeal photopheresis therapy (90).

Variable	HTx patients (n=22)
Female (%)	7 (32%)
Indication of HTx (%)	
Dilated CMP	14 (64%)
Idiopathic	11 (50%)
Myocarditis-related CMP	1 (5%)
Toxic CMP	2 (9%)
Ischemic CMP	4 (18%)
Hypertrophic CMP	1 (4.5%)
Restrictive CMP	1 (4.5%)
Non-compaction CMP	1 (4.5%)
LVOT thrombus formation following valvular surgery	1 (4.5%)
Age at HTx (mean±SD; years)	46±10
Age at initial ECP (mean±SD; years)	47±10
Time from HTx to initial ECP [median (min-max); days]	179 (15-3523)
Initiation of ECP within the first year following HTx (%)	15 (68%)
Initiation of ECP in the second post-transplant year, (%)	2 (9%)
Initiation of ECP after the second post-HTx year, (%)	5 (23%)
Duration of ECP [median (min-max); days]	172.5 (1-465)
ECP treatments [median (min-max)]	22 (2-44)
Venous access (%)	
Peripheral	21 (95.5%)
Central	1 (4.5%)

Abbreviations: CMP, cardiomyopathy; ECP, extracorporeal photopheresis; HTx, heart transplantation; LVOT, left ventricular outflow tract; SD, standard deviation.

Since 2013, the use of ECP has gradually increased in our HTx population (Figure 4). Indications for ECP included acute moderate cellular rejection (9 patients), mixed rejection (8 patients), and persistent mild cellular rejection (5 patients).

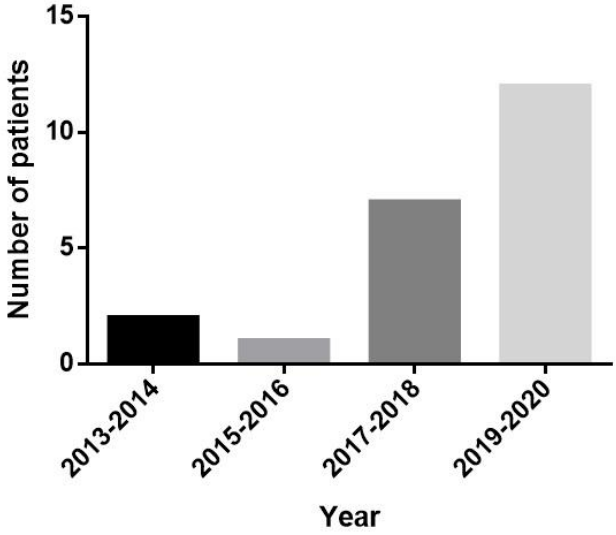

Figure 4. The use of extracorporeal photopheresis in our heart transplant recipients is shown by year (90).

4.2.2 Immunosuppression

Induction therapy was administered in twenty-one patients (95.5%). Immunosuppressive treatment was intensified at the time of allograft rejection before the initiation of ECP. Following the diagnosis of rejection, 15 patients received additional immunosuppressive therapy (i.e., intravenous methylprednisolone, rituximab, therapeutic plasma exchange, and intravenous immunoglobulin), with 5 receiving more than one agent.

At the time allograft rejection was diagnosed, tacrolimus trough levels were within the therapeutic range in nineteen recipients (86%). A statistically significant difference in mean tacrolimus trough levels was observed across the four time points evaluated. The mean tacrolimus trough level at the final follow-up visit was significantly lower than at both the initial and final ECP time points. A statistically significant difference in the mean daily mycophenolate doses was observed across the four time points. At the time of allograft rejection, eighteen patients (82%) were receiving methylprednisolone. The number of patients maintained on corticosteroids decreased over time, and by the final follow-up visit, methylprednisolone had been discontinued in fourteen patients. From the initiation of ECP, a reduction in the daily methylprednisolone dose was achieved by the end of the ECP course in 79% of patients and in all recipients by the last follow-up. The

mean daily methylprednisolone dose at the final follow-up was significantly lower compared to all three preceding time points (Table 15).

Table 15. Change in maintenance immunosuppressive therapy throughout extracorporeal photopheresis treatment (90).

Immunosuppressive therapy	Rejection	Start of ECP	End of ECP	Last visit	p
Tacrolimus trough level (ng/mL)	11.3±4.1	13.4±2.2	12.8±4.0	7.9±2.5[#]	<0.0001
Mycophenolate dose (mg/day)	1900±447	2300±571	2025±112	1800±441	0.0013
Methylprednisolone dose (mg/day)	6.6±3.4	10.8±3.1	6.1±2.2[§]	1.2±1.7[$]	<0.0001

Abbreviation: ECP, extracorporeal photopheresis.

Data are presented as mean±standard deviation.

[#]On post hoc analysis, the mean daily dose of tacrolimus was significantly lower by the last follow-up visit compared to both initial and last ECP treatments.

[§]On post hoc analysis, the mean daily dose of methylprednisolone was significantly lower by the last ECP treatment compared to the initial ECP treatment.

[$]On post hoc analysis, the mean daily dose of methylprednisolone was significantly lower at the last follow-up visit compared to all three preceding specific time points.

4.2.3 Cardiac allograft rejection

Throughout the study period, no episodes of ISHLT grade 3R cellular rejection were noted. The combination of ECP with additional immunosuppressive agents successfully reversed all ISHLT grade 2R cellular rejection episodes. Following completion of ECP therapy, no allograft rejections exceeding ISHLT grade 1R were observed, and no further rejection episodes requiring additional immunosuppressive treatment were detected. The proportion of biopsies negative for cellular rejection significantly increased following the ECP treatment course (0.33±0.27 vs. 0.63±0.40; p=0.031). TRS was significantly lower in the period following the ECP course than in the preceding period (0.86±0.49 vs. 0.37±0.40; p=0.007) (Figure 5).

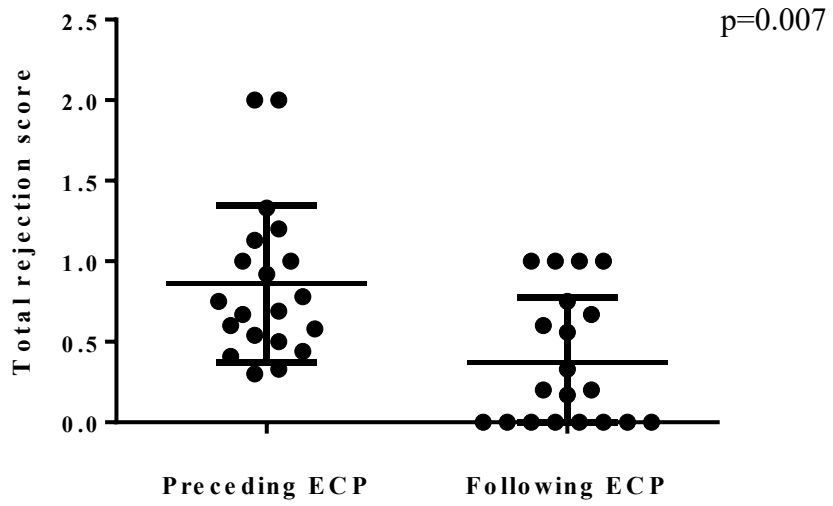

Figure 5. Total rejection score at initial and last extracorporeal photopheresis (90). Abbreviation: ECP, extracorporeal photopheresis.

4.2.4 Echocardiography

Six patients experienced cardiac allograft dysfunction due to a rejection episode before starting ECP therapy. Among those who completed the full ECP treatment course, allograft function normalised by the end of therapy. No patients developed hemodynamic instability as a result of the rejection episode. Median LVEF remained stable throughout the ECP treatment period [62% (43%-76%) vs. 60% (52%-70%); p=0.948].

4.2.5 Serum NT-proBNP level

A statistically significant difference in median NT-proBNP levels was observed across the four time points evaluated. By the final follow-up visit, median NT-proBNP levels were significantly lower compared to those recorded at the initiation of ECP therapy (Figure 6).

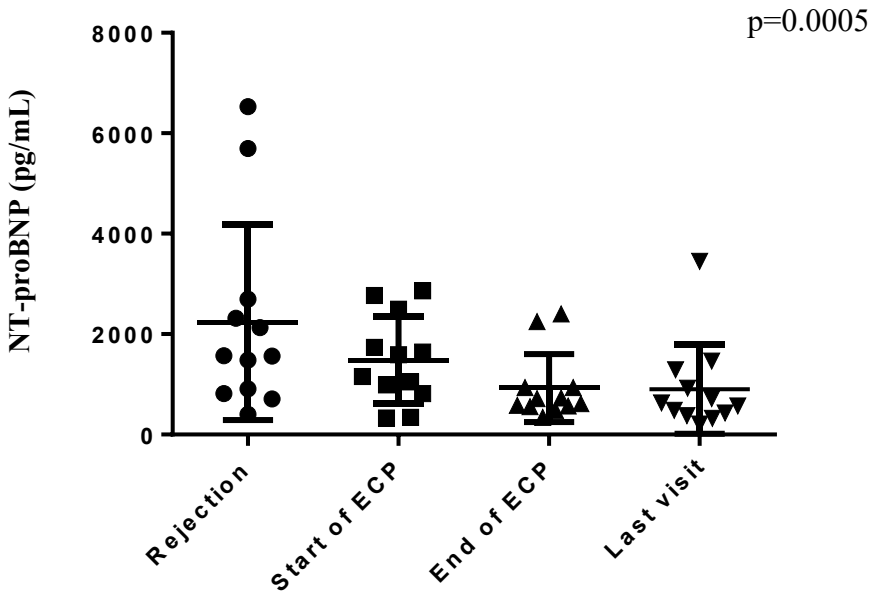

Figure 6. Median NT-proBNP levels during the study period (90).
Abbreviations: ECP, extracorporeal photopheresis; NT-proBNP, N-terminal prohormone of brain natriuretic peptide.

4.2.6 Donor-specific antibodies

At the start of ECP therapy, six recipients (27%) had positive DSAs, including one patient with both HLA class I and class II DSAs. High-titre DSAs were observed in four recipients. By the final follow-up, ECP therapy was associated with a non-significant decrease in DSA levels among patients who completed the entire treatment course (mean MFI: 9096±4743 vs. 6834±4665; p=0.302) (Figure 7).

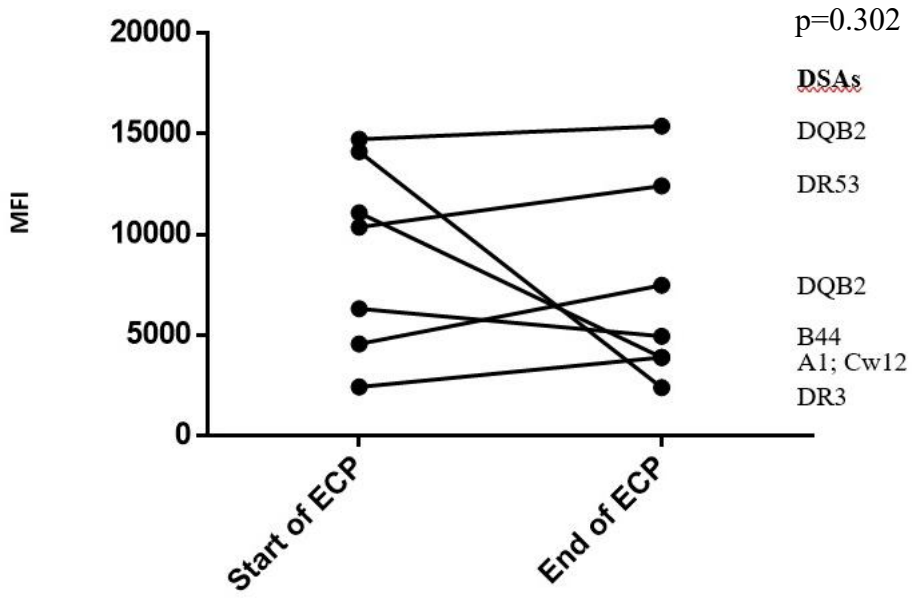

Figure 7. Donor-specific antibody levels in patients who completed the extracorporeal photopheresis therapy (90).

Abbreviations: DSA, donor-specific antibody; ECP, extracorporeal photopheresis; MFI, mean fluorescent intensity.

4.2.7 Survival

The median follow-up time after completion of ECP therapy was 20 months (range, 8-87 months). All patients who received ECP for cellular rejection were alive at follow-up, demonstrating optimal functional status. Among those treated for mixed rejection, 6 of 8 patients were alive with optimal graft function. Two patients died a median of 40.5 days after initiating ECP due to progression of AMR; one of these patients was classified as non-compliant, having discontinued therapy after the second session. Both short- and long-term survival following ECP were favourable and aligned with ISHLT registry survival data for HTx recipients (91% at both 1 and 5 years) (27).

5 Discussion

Heart transplantation has been challenged for over five decades by allograft rejection, which significantly impairs allograft survival. EMB has traditionally served as the cornerstone of rejection diagnosis; however, its diagnostic performance is limited by low sensitivity and substantial interobserver variability. Furthermore, EMB is associated with a risk of potentially serious procedural complications (8, 42). Conversely, dd-cfDNA provides a sensitive, reliable, and reproducible method for detecting allograft injury in both EMB-positive and EMB-negative contexts (42).

Although advances in immunosuppressive therapy have substantially improved survival after HTx, the contemporary standard-of-care immunosuppressive regimen is associated with significant side effects, including opportunistic infection, malignancy, renal failure, and neurotoxicity. Furthermore, controlling the immune mechanisms driving persistent or recurrent rejection may be insufficient, thereby contributing to long-term graft failure. In contrast, clinical studies have demonstrated that ECP is an effective therapeutic option for cardiac allograft rejection, with a favourable safety profile. Its utility has been documented in the management of both ACR and AMR (65).

To address these limitations, we first proposed using a local laboratory-run dd-cfDNA assay-based HTx rejection surveillance programme and integrated it into our routine clinical practice. Second, we facilitated the implementation of ECP for the management of HTx rejection across different clinical scenarios.

5.1 Novel biomarker in cardiac transplant rejection surveillance

We have reported the results of the first clinical experience with a standardised, analytically validated, local laboratory-run dd-cfDNA assay-based rejection surveillance programme in HTx recipients. The theory of the AlloSeq dd-cfDNA assay is the same as that of the widely used AlloSure test, having a strong concordance between test results (35). Comparable to previous studies on centralised testing, median dd-cfDNA levels were high early after HTx, corresponding to perioperative injury, then decreased to low levels and remained stable in patients with no overt, clinically significant allograft rejection (Table 16) (4, 21, 22, 91).

Table 16. Donor-derived cell-free DNA levels in patients with no heart transplant rejection (89).

Study	Assay	Median dd-cfDNA (%)
D-OAR (22)	AlloSure (targeted NGS)	0.07
GRAfT (4)	Shotgun sequencing	0.03
DEDUCE (21)	Prospera (targeted NGS)	0.04
FreeDNA-CAR (91)	Allonext (targeted NGS)	0.095
Heart and Vascular Centre, Semmelweis University (89)	AlloSeq (targeted NGS)	0.11

Abbreviations: dd-cfDNA, donor-derived cell-free DNA; DNA, deoxyribonucleic acid; NGS, next-generation sequencing.

A single-centre retrospective study evaluated adult HTx recipients by comparing a rejection-surveillance cohort utilising GEP with biopsy (transplanted between January 2015 and December 2017) with a cohort monitored using combined GEP and dd-cfDNA testing (transplanted between July 2018 and December 2020). Implementation of a surveillance strategy incorporating paired GEP and dd-cfDNA testing was associated with a significant reduction in the number of EMBs performed. No significant differences were observed between cohorts in three-year survival, AMR-free survival, graft function, or the incidence of DSA development (92). These results align with the favourable patient outcomes reported in the multicentre Surveillance HeartCare Outcomes Registry (SHORE), which demonstrated a 2-year survival exceeding 90% and fewer biopsies than conventional rejection surveillance strategies (24). Similarly, we demonstrated that the dd-cfDNA assay can effectively rule out clinically significant acute rejection and reduce the need for routine invasive EMBs. In our clinical practice, 88% of routine surveillance EMBs that would otherwise have been performed were avoided. While EMBs continue to provide valuable diagnostic information, this strategy would reserve their use for high-risk scenarios, such as abnormal noninvasive test results, clinical symptoms, or evidence of graft dysfunction (92). Current EMB surveillance protocols may warrant re-evaluation in the modern era (7, 18).

In our patient cohort treated for AMR, the median dd-cfDNA level was elevated at 2.76%. Similarly, data from the SHORE Registry showed that dd-cfDNA levels are significantly higher in patients with biopsy-proven AMR than in those without AMR (0.57% vs. 0.06%; P <0.001) (34). While histologic and immunohistochemical analysis of EMB remains the established standard for identifying AMR, the diagnostic

determination remains characterised by substantial uncertainty. To improve diagnostic precision, EMB findings are increasingly integrated with molecular diagnostic modalities, including intragraft GEP and dd-cfDNA. This growing reliance on noninvasive biomarkers and molecular testing signals a significant shift toward a less invasive, more sensitive approach to early diagnosis of AMR (32). Findings from the SHORE Registry demonstrate that a dd-cfDNA threshold of 0.50% yields higher specificity for AMR (92.8%) compared with a lower threshold of 0.20% (84.1%). Applying a higher dd-cfDNA threshold of ≥0.50% may be appropriate when the pretest probability of biopsy-confirmed AMR is low, such as in recipients with preserved graft function and no detectable DSAs. Conversely, a lower dd-cfDNA threshold of 0.20% may be more suitable in contexts where the pretest probability of biopsy-confirmed AMR is higher and a positive EMB result is more likely to prompt therapeutic intervention. Specifically, EMBs performed solely in response to dd-cfDNA levels between 0.20% and 0.49%, in the absence of DSAs or graft dysfunction, are highly unlikely to demonstrate AMR. In the absence of clinical or molecular evidence of ACR, these biopsies can largely be avoided (34). These findings support a nuanced Bayesian approach to interpreting molecular testing, suggesting that a single dd-cfDNA threshold for triggering EMB may not be universally appropriate for all HTx recipients. This work represents a meaningful step forward in integrating dd-cfDNA into clinical care, supporting a paradigm shift away from invasive biopsy-based monitoring toward precise, dynamic molecular surveillance (42).

We demonstrated that dd-cfDNA can be reliably analysed at the local level and successfully implemented as the default noninvasive rejection surveillance strategy in clinical practice. Implementing a dd-cfDNA-based rejection surveillance programme requires careful consideration of the centre's specific patient population and available resources. As dd-cfDNA assays are primarily designed as screening tools for stable transplant recipients, programmes may initially apply noninvasive surveillance to lower-risk patients, with subsequent extension to higher-risk cohorts as institutional experience increases. Thresholds defining abnormal dd-cfDNA levels continue to evolve and vary across centres, depending on patient risk of rejection, clinician expertise, and whether the focus is on optimising negative or positive predictive values. Accordingly, centres should

routinely evaluate and refine their protocols in response to centre-specific outcomes, emerging evidence, and society recommendations (5).

5.2 Evaluation of left ventricular longitudinal strain-derived parameters in assessment of cardiac transplant injury

LVEF generally remains preserved until severe injury has evolved in the transplanted heart (53, 54). Accordingly, in our cohort, the mean LVEF was maintained, and changes in LVEF did not suggest graft injury, as indicated by dd-cfDNA. However, no episodes of hemodynamically significant rejection occurred among the patients in this study. Similarly, in the OAR (Outcomes AlloMap Registry) study, LVEF did not provide additional diagnostic value in evaluating ACR (93). Allograft dysfunction resulting from rejection often represents a late-stage manifestation.

Compared with LVEF, LVGLS is more strongly associated with myocardial injury in ACR (51, 52). After HTx, ACR is associated with local oedema, an inflammatory infiltrate, myocyte damage, and the development of myocardial fibrosis, which can affect subendocardial myocardial fibres and reduce longitudinal myocardial function, as assessed by strain analysis (50). In HTx recipients with ACR, LVGLS is a significant predictor of mortality (50, 94). One study demonstrated that GEP correlated with LV longitudinal strain parameters, independent of HTx rejection, CAV, or other clinical variables (53). However, other studies found that LV longitudinal strain remained reduced regardless of biopsy-confirmed episodes of cellular rejection and that both global and regional LV longitudinal strain values were lower in HTx recipients than in control populations (53, 54, 95). We found that the mean LVGLS and the apical 4-chamber, 2-chamber, and 3-chamber longitudinal strain values were lower than reference values reported in healthy individuals and were comparable to those in previous studies of HTx recipients (53, 54, 86). We observed that the LVGLS values remained consistent and did not change between months 6 and 12 post-HTx (as measured in a small cohort of patients), underscoring the stability of these indices over time. In a previous study, LVGLS values were reported to be very similar from year 1 through a median of 10.6 years post-transplant (54).

Although apical sparing has been evaluated in an increasing number of clinical scenarios, its utility in assessing the cardiac allograft remains unclear (96). In one study, regional longitudinal strain analysis revealed a gradient of longitudinal strain values,

increasing from the basal to the apical segments. Mean basal and mid segments showed abnormal longitudinal strain parameters, while apical segments were normal (94). Conversely, another study found that LV apical longitudinal strain remained persistently attenuated at one year post-transplant (53). When assessing regional LV longitudinal strain parameters, we observed consistently reduced mean basal, mid, and apical strain values in our HTx patient cohort. However, we found no correlation between the apical sparing ratio and myocardial injury assessed by dd-cfDNA. A statistically significant difference was noted between lateral and septal longitudinal strain values. This difference is likely attributable to conduction abnormalities causing dyssynchrony, a well-documented phenomenon in HTx recipients (97).

No association was observed between any LV longitudinal strain parameters and myocardial injury measured by dd-cfDNA. Since dd-cfDNA directly assesses graft injury and its elevation may precede EMB-confirmed rejection, the lack of correlation with LV longitudinal strain indices may indicate that these strain-derived parameters are less sensitive for detecting early transplant injury (23). Additionally, multiple factors can contribute to reduced LV systolic function after HTx. The decline in LV longitudinal strain parameters may be attributed to factors such as surgical procedure, pericardiotomy, allograft ischemic time, and both donor and recipient characteristics (50, 97). Donor-related factors include advanced age, LV hypertrophy, and gender mismatch, while recipient-related factors involve alloimmune responses, rejection episodes, and CAV (53). During the early post-transplant period, factors such as graft adaptation and altered positioning, early graft dysfunction, pericardial effusion, sepsis, and multi-organ failure can influence these echocardiographic parameters (45). Non-immune contributors include sympathetic denervation following surgery and conduction disturbances, both of which can result in mechanical dyssynchrony. Additional contributing factors include hyperlipidemia, hypertension, diabetes mellitus, hyperhomocysteinemia, advanced age, and infections. Myocardial fibrosis has been associated with restrictive cardiac physiology and advanced CAV. Furthermore, neurohormonal activation during rejection episodes can alter loading conditions, thereby affecting LVGLS measurements (50, 53, 95, 97). Nonetheless, since LVGLS has been demonstrated to remain stable from month six post-transplant onwards, its use as a complementary, noninvasive monitoring tool is emphasised. Establishing a baseline LVGLS measurement provides a valuable reference

point for identifying future post-transplant complications. The likelihood of HTx rejection increases when multiple parameters are abnormal during recipient evaluation (45).

The use of LVGLS is limited by its load-dependency, which can be influenced by factors such as systemic hypertension and neurohormonal activation. In contrast, myocardial work analysis incorporates LV afterload, thus reducing its sensitivity to load variations and potentially offering additional insights into the evaluation of the HTx population (95). Myocardial work has been evaluated across various clinical scenarios to determine its added diagnostic value compared to LVEF and LVGLS (56-58). Emerging evidence suggests its potential utility in detecting CAV, acute rejection, and right ventricular dysfunction (95, 98, 99). A study investigated the association between echocardiography-derived myocardial work parameters and EMB-proven ACR in adult HTx recipients within their first post-transplant year. The results showed that GWE values were elevated in patients with ISHLT grade ≥2R cellular rejection (95). Another study evaluated right ventricular functional adaptation following HTx and derived the right ventricular myocardial work index. Pulmonary artery pressure was measured invasively via right heart catheterisation. Episodes of HTx rejection were associated with a significant reduction in myocardial work index, which was an independent predictor of EMB-proven rejection-related myocardial damage (99). A study assessed the correlation between LV myocardial work indices and the presence of CAV in pediatric HTx recipients. These patients underwent coronary angiography within three months of their echocardiographic assessment. Echocardiograms were analysed using speckle-tracking echocardiography to derive myocardial work indices. The study found that reduced GWE values were associated with pediatric CAV (98).

It has also been demonstrated that myocardial work parameters in HTx recipients differ significantly from those reported in the EACVI NORRE study of healthy volunteers (97). Consistent with prior research, our findings showed that transplanted hearts exhibited lower GWI, GCW, and GWE values and higher GWW values than healthy volunteers in the EACVI NORRE study (95, 97). This finding may be attributed to the lower LVGLS values observed in the transplanted hearts (45, 50, 53, 97). Conduction disturbances may lead to mechanical dyssynchrony, which may account for the reduced GWE values and the elevated GWW value (97). No association was observed between

myocardial work parameters and dd-cfDNA levels in the small patient cohort. Badano et al. highlighted that the risk of allograft rejection increases when multiple parameters are abnormal during the evaluation of HTx recipients. When an abnormality is identified, a comprehensive review of both current and baseline imaging studies is recommended (45). Intraindividual variations in myocardial work parameters may indicate changes in graft integrity and warrant further diagnostic evaluations to identify the underlying cause.

Additional research is needed to generate long-term outcome data, clarify the clinical value and diagnostic role of strain-derived myocardial work indices for detecting HTx injury, and evaluate their utility for monitoring cardiovascular response to therapy.

5.3 Extracorporeal photopheresis for cardiac transplant rejection

We demonstrated that ECP, whether combined with standard tacrolimus-based maintenance immunosuppression or administered following additional immunosuppressive therapies, was effective in reducing the severity of subsequent allograft rejection episodes. After completing ECP therapy, HTx recipients included in this single-centre retrospective study did not experience any rejection episodes exceeding mild cellular rejection during follow-up. As in previous studies, following the ECP course, a greater proportion of patients had either no rejection or ISHLT grade 1R cellular rejection in <50% of EMBs, compared with the pre-ECP period (67, 77).

Previous prospective studies found that prophylactic ECP, when used alongside maintenance immunosuppressive therapy, reduced the incidence of acute rejection (67, 70, 75, 100). In a retrospective analysis, Kirklin et al. reported that the use of ECP as secondary prophylaxis in patients with rejection and hemodynamic compromise reduced the incidence of rejection to levels comparable to those observed in the control HTx group (80). Previous research demonstrated that ECP administration facilitated an improved management of both recurrent and refractory or persistent cardiac allograft rejection (60, 68, 69, 76, 78, 79, 101-103). Evidence suggests that recurrent ISHLT grade 1R cellular rejection is associated with adverse long-term outcomes, including increased risk of subsequent major rejection episodes, higher rates of ISHLT grade 2R rejection, and reduced rejection-free survival among this subgroup of HTx recipients (104). In our study, patients with persistent ISHLT grade 1R cellular rejection were treated with ECP. After completion of ECP therapy, we observed a trend toward a higher proportion of

EMBs that were negative for cellular rejection, and there were no episodes of rejection requiring additional or intensified immunosuppressive treatment.

We achieved a significant reduction in the daily doses of both methylprednisolone and tacrolimus by the end of the ECP treatment period without an associated increase in rejection episodes. Corticosteroid tapering is substantial due to its wide range of potential adverse effects, thereby reducing long-term morbidity and improving overall survival in HTx recipients. Previous studies showed that immunosuppressive therapy could be safely reduced in patients receiving ECP (60, 69, 76-79, 102). Evidence also exists supporting the use of prophylactic ECP as a strategy to avoid induction therapy in HTx patients (72).

Following the ECP treatment course, HTx recipients maintained optimal functional status and graft function. Among patients who developed cardiac allograft dysfunction secondary to rejection before initiating ECP, allograft function normalised by the end of therapy. In an observational study, Kobashigawa et al. reported echocardiographic improvement in cardiac function among patients with chronic LV dysfunction (103).

We verified the safety of ECP therapy in managing cardiac allograft rejection. ECP demonstrated an excellent safety profile, with minimal adverse effects and good tolerability (26). In our study, the survival of HTx recipients who received ECP for allograft rejection was comparable to the median post-transplant survival and aligned with ISHLT registry data on overall survival among HTx recipients (one-year survival rates exceeding 85% for transplants performed between 2009 and 2015) (27). This favourable survival outcome may be attributed to reduced risk and severity of subsequent allograft rejection episodes, as well as the safe tapering of immunosuppressive therapy following ECP treatment.

5.4 Limitations

Interpretation of LV longitudinal strain-derived echocardiographic parameters is limited by our study's single-centre design, the relatively small number of eligible echocardiographic examinations – many of which were excluded according to recommendations to perform baseline assessments six months after HTx – and the small sample sizes in subgroup analyses, including those evaluating myocardial work indices. These factors limit more extensive analyses and reduce the generalisability of the findings. The primary limitations of our study on ECP, as in most other investigations, include a small sample size, the absence of a control group, limited access to ECP

(currently available only for outpatients), and variability in treatment protocols. Additionally, differences in immunosuppressive regimens administered alongside ECP among patients may confound the outcomes.

5.5 Future perspectives

5.5.1 Rejection surveillance

We confirmed the feasibility of conducting dd-cfDNA analysis at the local level and its successful implementation as the default noninvasive surveillance strategy in clinical practice, resulting in a substantial reduction in the need for invasive surveillance EMBs. Our findings support the expectation that a noninvasive rejection surveillance strategy using local laboratory-run dd-cfDNA testing will be more widely implemented in routine clinical practice in the future.

In the current era of highly precise genomic assays, clinical researchers should re-evaluate the validity of EMB as the gold standard for diagnosing rejection and consider alternative strategies to assess the effectiveness of novel biomarkers, such as dd-cfDNA, for detecting the earliest stages of allograft rejection (4). The use of dd-cfDNA, alongside MMDx and/or complementary biomarkers, may ultimately supplant EMB as the reference standard for evaluating allograft injury (4, 42).

Discrepancies between MMDx and histologic AMR may warrant a renewed reconsideration of AMR definitions in the era of molecular diagnostics, particularly as MMDx and dd-cfDNA are increasingly adopted in HTx centres. With the expanding adoption of these approaches in HTx surveillance and the strong concordance observed between their results, they are likely to contribute meaningfully to clinical decision-making regarding AMR (44). Improved understanding of the relationships among AMR-related diagnostic test results may facilitate the development of standardised treatment strategies for AMR, particularly in the context of emerging therapeutic options. Given the reliability of dd-cfDNA as a marker of allograft injury, it is anticipated that dd-cfDNA will serve as an endpoint in future studies evaluating treatments for AMR in cardiac transplant recipients (34).

Elevated dd-cfDNA levels in the absence of EMB-confirmed rejection represent a significant challenge in the clinical management of cardiac transplant recipients. These levels may reflect early graft injury and have prognostic value (25, 34, 39-41). Future

studies may clarify whether intensified immunosuppressive therapy benefits this patient group. In such cases, the immunomodulatory ECP may have a role.

As an early, highly sensitive marker of graft injury, dd-cfDNA may enable targeted preventive and personalised anti-rejection therapy. Immunosuppressive agents, including corticosteroids and calcineurin inhibitors, could be tapered more rapidly in patients without evidence of graft injury and intensified in those with elevated dd-cfDNA levels. Consequently, immunosuppression could be optimised for recipients at increased risk of acute rejection, while minimising the adverse effects and toxicities of these medications in stable patients (22). Ultimately, dd-cfDNA may provide a more accurate representation of allograft injury, enabling clinicians to interpret EMB findings more precisely and individualise treatment decisions (34). It is anticipated that dd-cfDNA testing will facilitate a shift in care for HTx recipients from a reactive to a preventive approach (105).

Key areas for future research include differentiating between ACR and AMR, evaluating absolute versus relative quantification of dd-cfDNA, assessing the test's utility in multi-organ transplant recipients, and exploring applications beyond rejection surveillance (5). Further testing of dd-cfDNA is required to evaluate its diagnostic performance for CAV (37).

The cost-effectiveness of dd-cfDNA testing in comparison with conventional EMB-based surveillance strategies has not yet been evaluated. Although a reduction in healthcare costs was modelled for the AlloMap test, assessment is likely to be complicated by heterogeneity among healthcare systems, insurance models, and reimbursement policies across different countries (5, 106).

5.5.2 Rejection treatment

Identifying optimal responders, determining the most appropriate timing for initiating ECP, and establishing the ideal treatment frequency, tapering schedule, and therapy duration will require broader clinical experience. It remains unclear whether patients would derive additional long-term benefits from continuing ECP after clinical stabilisation, or whether synergistic effects exist with other treatment modalities. At present, there is no standardised monitoring strategy for ECP, and the precise immunological mechanisms underlying its effects remain incompletely understood. A deeper understanding of ECP-induced immune modulation in HTx recipients could support more targeted patient selection and individualised treatment approaches,

ultimately improving clinical outcomes. We speculate that, in the future, dd-cfDNA may serve as a biomarker for monitoring the efficacy of ECP therapy and for optimising treatment indication, frequency and duration. There remains a clear need for further evidence generated from large-scale randomised controlled trials.

6 Conclusions

For the first time, we presented the clinical application of a standardised, analytically validated, local laboratory-run dd-cfDNA assay for routine HTx rejection monitoring. This approach reliably excludes clinically significant acute rejection and reduces the need for routine invasive EMB procedures. Our results set the stage for further clinical utility studies of the local laboratory-run dd-cfDNA assay in the management of solid-organ transplant recipients.

LVGLS appears to be less sensitive than the dd-cfDNA assay for detecting early myocardial injury in HTx recipients at low risk of rejection. Further studies are necessary to clarify the clinical role of strain-derived myocardial work indices in identifying graft injury.

We concluded that HTx recipients receiving ECP alongside standard immunosuppressive therapy experienced successful reversal of allograft rejection, fewer subsequent rejection episodes, and restoration of allograft function. We demonstrated that ECP could be implemented effectively and safely across a broad range of clinical contexts. Methylprednisolone dose reduction was shown to be safe throughout the ECP treatment course. In our clinical practice, indications for ECP include acute moderate and persistent mild cellular rejection, AMR, mixed rejection, and the presence of high-titre DSAs.

7 Summary

Heart transplantation has significantly improved the quality of life and survival of patients with advanced HF. Significant advances have been made in the diagnosis and treatment of HTx rejection. dd-cfDNA serves as a biomarker of graft injury, rising during episodes of acute rejection and demonstrating excellent negative predictive value. LVGLS and strain-derived myocardial work indices are emerging echocardiographic parameters with expanding clinical utility. ECP, which does not cause generalised immunosuppression, has been proposed as a therapy for HTx rejection.

First, we described the first clinical implementation of a local laboratory-run, commercially available dd-cfDNA assay-based molecular monitoring programme for routine HTx rejection surveillance. Second, we investigated whether reductions in LV longitudinal strain parameters could reflect early HTx injury, as assessed by dd-cfDNA (Rejection surveillance). Third, we evaluated the efficacy of ECP in combination with a tacrolimus-based maintenance immunosuppressive therapy for treating cardiac allograft rejection across various clinical scenarios (Rejection treatment).

Since October 2022, HTx recipients have been transitioned from our EMB-based rejection surveillance protocol to a monthly dd-cfDNA-guided monitoring strategy. The mean dd-cfDNA fraction remained low (0.13±0.06%). By November 2024, this strategy had eliminated 88% of otherwise indicated surveillance EMBs. dd-cfDNA is effective at excluding clinically significant acute rejection and reduces the need for invasive surveillance EMBs. No correlation was observed between dd-cfDNA and LVGLS. LVGLS appears less sensitive than dd-cfDNA for detecting early myocardial injury in low-risk HTx recipients. ECP was indicated for acute moderate-to-severe cellular rejection, persistent mild cellular rejection, or mixed rejection. When combined with conventional anti-rejection therapy, ECP effectively reversed cardiac allograft rejection, reduced the incidence of subsequent rejection episodes, and restored allograft function in patients who completed the treatment course. Methylprednisolone dose reduction was safely achieved throughout the ECP treatment period. ECP can be safely utilised alongside standard immunosuppressive regimens for both the treatment and prevention of cardiac allograft rejection.

8 References

1. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Bohm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J. 2021;42(36):3599-726.
2. Crespo-Leiro MG, Metra M, Lund LH, Milicic D, Costanzo MR, Filippatos G, et al. Advanced heart failure: a position statement of the Heart Failure Association of the European Society of Cardiology. Eur J Heart Fail. 2018;20(11):1505-35.
3. Heidenreich PA, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, et al. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation. 2022;145(18):e895-e1032.
4. Agbor-Enoh S, Shah P, Tunc I, Hsu S, Russell S, Feller E, et al. Cell-Free DNA to Detect Heart Allograft Acute Rejection. Circulation. 2021;143(12):1184-97.
5. Holzhauser L, DeFilippis EM, Nikolova A, Byku M, Contreras JP, De Marco T, et al. The End of Endomyocardial Biopsy?: A Practical Guide for Noninvasive Heart Transplant Rejection Surveillance. JACC Heart Fail. 2023;11(3):263-76.
6. Kittleson MM, Garg S. Solid Gold, or Liquid Gold?: Towards a New Diagnostic Standard for Heart Transplant Rejection. Circulation. 2021;143(12):1198-201.
7. Cusi V, Vaida F, Wettersten N, Rodgers N, Tada Y, Gerding B, et al. Incidence of Acute Rejection Compared With Endomyocardial Biopsy Complications for Heart Transplant Patients in the Contemporary Era. Transplantation. 2024;108(5):1220-7.
8. Crespo-Leiro MG, Zuckermann A, Bara C, Mohacsi P, Schulz U, Boyle A, et al. Concordance among pathologists in the second Cardiac Allograft Rejection Gene Expression Observational Study (CARGO II). Transplantation. 2012;94(11):1172-7.
9. Mehta A, Goldberg J, Bagchi P, Marboe C, Shah KB, Najjar SS, et al. Pathologist interrater reliability and clinical implications of elevated donor-derived cell-free DNA beyond heart transplant rejection, on behalf of the GRAfT investigators. J Heart Lung Transplant. 2025;44(5):803-12.
10. Bhat G, Burwig S, Walsh R. Morbidity of endomyocardial biopsy in cardiac transplant recipients. Am Heart J. 1993;125(4):1180-1.

11. Baraldi-Junkins C, Levin HR, Kasper EK, Rayburn BK, Herskowitz A, Baughman KL. Complications of endomyocardial biopsy in heart transplant patients. J Heart Lung Transplant. 1993;12(1 Pt 1):63-7.

12. Saraiva F, Matos V, Goncalves L, Antunes M, Providencia LA. Complications of endomyocardial biopsy in heart transplant patients: a retrospective study of 2117 consecutive procedures. Transplant Proc. 2011;43(5):1908-12.

13. Oh KT, Mustehsan MH, Goldstein DJ, Saeed O, Jorde UP, Patel SR. Protocol endomyocardial biopsy beyond 6 months-It is time to move on. Am J Transplant. 2021;21(2):825-9.

14. Marboe CC, Billingham M, Eisen H, Deng MC, Baron H, Mehra M, et al. Nodular endocardial infiltrates (Quilty lesions) cause significant variability in diagnosis of ISHLT Grade 2 and 3A rejection in cardiac allograft recipients. J Heart Lung Transplant. 2005;24(7 Suppl):S219-26.

15. Nikolova A, Agbor-Enoh S, Bos S, Crespo-Leiro M, Ensminger S, Jimenez-Blanco M, et al. European Society for Organ Transplantation (ESOT) Consensus Statement on the Use of Non-invasive Biomarkers for Cardiothoracic Transplant Rejection Surveillance. Transpl Int. 2024;37:12445.

16. Velleca A, Shullo MA, Dhital K, Azeka E, Colvin M, DePasquale E, et al. The International Society for Heart and Lung Transplantation (ISHLT) guidelines for the care of heart transplant recipients. J Heart Lung Transplant. 2023;42(5):e1-e141.

17. ASTS Statement on donor derived cell-free DNA (dd-cfDNA) – Updated October 2024 [Available from: https://www.asts.org/docs/default-source/position-statements/asts-statement-on-donor-derived-cell-free-dna-(dd-cfdna)---updated-oct.-2024.pdf?sfvrsn=19314fd3_3.

18. Isath A, Mehra MR. "Surveillance without scars": The quest for noninvasive detection of cardiac allograft rejection. J Heart Lung Transplant. 2025;44(4):570-2.

19. Pham MX, Teuteberg JJ, Kfoury AG, Starling RC, Deng MC, Cappola TP, et al. Gene-expression profiling for rejection surveillance after cardiac transplantation. N Engl J Med. 2010;362(20):1890-900.

20. Kobashigawa J, Patel J, Azarbal B, Kittleson M, Chang D, Czer L, et al. Randomized pilot trial of gene expression profiling versus heart biopsy in the first year

after heart transplant: early invasive monitoring attenuation through gene expression trial. Circ Heart Fail. 2015;8(3):557-64.

21. Kim PJ, Olymbios M, Siu A, Wever Pinzon O, Adler E, Liang N, et al. A novel donor-derived cell-free DNA assay for the detection of acute rejection in heart transplantation. J Heart Lung Transplant. 2022;41(7):919-27.

22. Khush KK, Patel J, Pinney S, Kao A, Alharethi R, DePasquale E, et al. Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study. Am J Transplant. 2019;19(10):2889-99.

23. De Vlaminck I, Valantine HA, Snyder TM, Strehl C, Cohen G, Luikart H, et al. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. Sci Transl Med. 2014;6(241):241ra77.

24. Khush K, Hall S, Kao A, Raval N, Dhingra R, Shah P, et al. Surveillance with dual noninvasive testing for acute cellular rejection after heart transplantation: Outcomes from the Surveillance HeartCare Outcomes Registry. J Heart Lung Transplant. 2024;43(9):1409-21.

25. Moeller CM, Valledor AF, Oren D, Rahman S, Rahman A, Regan M, et al. Clinical Utility of Combined Donor-Derived Cell-Free DNA and Peripheral Gene-Expression-Profiling in Heart Transplant Recipients. Clin Transplant. 2025;39(10):e70340.

26. Marques MB, Schwartz J. Update on extracorporeal photopheresis in heart and lung transplantation. J Clin Apher. 2011;26(3):146-51.

27. Khush KK, Cherikh WS, Chambers DC, Harhay MO, Hayes D, Jr., Hsich E, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-sixth adult heart transplantation report - 2019; focus theme: Donor and recipient size match. J Heart Lung Transplant. 2019;38(10):1056-66.

28. Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. J Heart Lung Transplant. 2005;24(11):1710-20.

29. Berry GJ, Burke MM, Andersen C, Bruneval P, Fedrigo M, Fishbein MC, et al. The 2013 International Society for Heart and Lung Transplantation Working Formulation

for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation. J Heart Lung Transplant. 2013;32(12):1147-62.

30. The tissue is the issue: Going beyond histopathology in antibody-mediated rejection in heart transplant patients [Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC12747125/.

31. Colvin MM, Cook JL, Chang P, Francis G, Hsu DT, Kiernan MS, et al. Antibody-mediated rejection in cardiac transplantation: emerging knowledge in diagnosis and management: a scientific statement from the American Heart Association. Circulation. 2015;131(18):1608-39.

32. Mehta A, DeFilippis EM, Stehlik J, Jackson AM, Kobashigawa JA, Shah P. Contemporary Antibody-Mediated Rejection in Heart Transplantation: JACC: Heart Failure Position Statement. JACC Heart Fail. 2025;13(10):102614.

33. Natera. 2026 [Available from: https://www.natera.com/organ-health/prospera-heart-transplantation-assessment/.

34. Kim PJ, Alam AH, Teuteberg JJ, Khush KK, Pinney SP, Cheng RK, et al. Donor-Derived Cell-Free DNA in Antibody-Mediated Rejection: An Analysis of the Surveillance HeartCare Outcomes Registry. JACC Heart Fail. 2025:102716.

35. Loupy A, Certain A, Tangprasertchai NS, Racape M, Ursule-Dufait C, Benbadi K, et al. Evaluation of a Decentralized Donor-Derived Cell-Free DNA Assay for Kidney Allograft Rejection Monitoring. Transpl Int. 2024;37:13919.

36. Casas S, Tangprasertchai NS, Oikonomaki K, Mathers S, Sollet ZC, Samara S, et al. Multi-centre analytical performance verification of an IVD assay to quantify donor-derived cell-free DNA in solid organ transplant recipients. HLA. 2024;103(5):e15518.

37. Cardiac allograft vasculopathy in the era of precision medicine: The role of biomarkers and advanced imaging [Available from: https://www.jhltonline.org/article/S1053-2498(25)02477-5/fulltext.

38. Alam AH, Van Zyl J, Shakoor HI, Farsakh D, Abdelrehim AB, Maliakkal N, et al. The impact of active cytomegalovirus infection on donor-derived cell-free DNA testing in heart transplant recipients. Clin Transplant. 2024;38(3):e15287.

39. Multimodal molecular testing provides prognostic value for heart transplant recipients [Available from: https://www.jhltonline.org/article/S1053-2498(25)02400-3/fulltext.

40. Stern L, Kittleson M, Patel J, Kransdorf E, Kanungo A, Deckerman P, et al. High Level Donor Derived Cell-Free DNA in Asymptomatic Patients After Heart Transplant - Does It Matter? American Journal of Transplantation. 2025;25(8):S160.
41. Cheng RK, Vidic A, Alharethi R, Nathan S, Uriel N, Kobulnik J, et al. Impact of Persistently Elevated Simultaneous Gene Expression Profiling and Donor-Derived Cell-Free DNA on Future Clinical Events. American Journal of Transplantation. 2025;25(8):S162.
42. Baranowska J, Agbor-Enoh S. A Biopsy-Free Future: Science Fiction or Science Reality? JACC Heart Fail. 2025:102782.
43. Halloran PF, Madill-Thomsen KS. The Molecular Microscope Diagnostic System: Assessment of Rejection and Injury in Heart Transplant Biopsies. Transplantation. 2023;107(1):27-44.
44. Defining the relationships among four tests for assessing antibody-mediated rejection in heart transplants in a prospective, observational study [Available from: https://www.jhltonline.org/article/S1053-2498(25)02363-0/fulltext.
45. Badano LP, Miglioranza MH, Edvardsen T, Colafranceschi AS, Muraru D, Bacal F, et al. European Association of Cardiovascular Imaging/Cardiovascular Imaging Department of the Brazilian Society of Cardiology recommendations for the use of cardiac imaging to assess and follow patients after heart transplantation. Eur Heart J Cardiovasc Imaging. 2015;16(9):919-48.
46. Marwick TH. Ejection Fraction Pros and Cons: JACC State-of-the-Art Review. J Am Coll Cardiol. 2018;72(19):2360-79.
47. Konstam MA, Abboud FM. Ejection Fraction: Misunderstood and Overrated (Changing the Paradigm in Categorizing Heart Failure). Circulation. 2017;135(8):717-9.
48. Mihos CG, Liu JE, Anderson KM, Pernetz MA, O'Driscoll JM, Aurigemma GP, et al. Speckle-Tracking Strain Echocardiography for the Assessment of Left Ventricular Structure and Function: A Scientific Statement From the American Heart Association. Circulation. 2025;152(10):e96-e109.
49. Cruz C, Hajjar LA, Bacal F, Lofrano-Alves MS, Lima MSM, Abduch MC, et al. Usefulness of speckle tracking echocardiography and biomarkers for detecting acute cellular rejection after heart transplantation. Cardiovasc Ultrasound. 2021;19(1):6.

50. Chamberlain R, Edwards NFA, Doyle SN, Wong YW, Scalia GM, Sabapathy S, et al. Prognostic Value of Left and right ventricular deformation strain analysis on Acute Cellular rejection in Heart Transplant recipients: A 6-year outcome study. Int J Cardiovasc Imaging. 2022;38(11):2271-81.

51. Rodriguez-Diego S, Ruiz-Ortiz M, Delgado-Ortega M, Kim J, Weinsaft JW, Sanchez-Fernandez JJ, et al. The Role of Left Atrial Longitudinal Strain in the Diagnosis of Acute Cellular Rejection in Heart Transplant Recipients. J Clin Med. 2022;11(17).

52. Ciarka A, Cordeiro F, Droogne W, Van Cleemput J, Voigt JU. Speckle-tracking-based global longitudinal and circumferential strain detect early signs of antibody-mediated rejection in heart transplant patients. Eur Heart J Cardiovasc Imaging. 2022;23(11):1520-9.

53. Eleid MF, Caracciolo G, Cho EJ, Scott RL, Steidley DE, Wilansky S, et al. Natural history of left ventricular mechanics in transplanted hearts: relationships with clinical variables and genetic expression profiles of allograft rejection. JACC Cardiovasc Imaging. 2010;3(10):989-1000.

54. Syeda B, Hofer P, Pichler P, Vertesich M, Bergler-Klein J, Roedler S, et al. Two-dimensional speckle-tracking strain echocardiography in long-term heart transplant patients: a study comparing deformation parameters and ejection fraction derived from echocardiography and multislice computed tomography. Eur J Echocardiogr. 2011;12(7):490-6.

55. Russell K, Eriksen M, Aaberge L, Wilhelmsen N, Skulstad H, Remme EW, et al. A novel clinical method for quantification of regional left ventricular pressure-strain loop area: a non-invasive index of myocardial work. Eur Heart J. 2012;33(6):724-33.

56. Moya A, Buytaert D, Penicka M, Bartunek J, Vanderheyden M. State-of-the-Art: Noninvasive Assessment of Left Ventricular Function Through Myocardial Work. J Am Soc Echocardiogr. 2023;36(10):1027-42.

57. Marzlin N, Hays AG, Peters M, Kaminski A, Roemer S, O'Leary P, et al. Myocardial Work in Echocardiography. Circ Cardiovasc Imaging. 2023;16(2):e014419.

58. van der Bijl P, Kostyukevich M, El Mahdiui M, Hansen G, Samset E, Ajmone Marsan N, et al. A Roadmap to Assess Myocardial Work: From Theory to Clinical Practice. JACC Cardiovasc Imaging. 2019;12(12):2549-54.

59. Patel J, Klapper E, Shafi H, Kobashigawa JA. Extracorporeal photopheresis in heart transplant rejection. Transfus Apher Sci. 2015;52(2):167-70.
60. Dall'Amico R, Montini G, Murer L, Andreetta B, Zacchello G, Gambino A, et al. Extracorporeal photochemotherapy after cardiac transplantation: a new therapeutic approach to allograft rejection. Int J Artif Organs. 2000;23(1):49-54.
61. Connelly-Smith L, Alquist CR, Aqui NA, Hofmann JC, Klingel R, Onwuemene OA, et al. Guidelines on the Use of Therapeutic Apheresis in Clinical Practice - Evidence-Based Approach from the Writing Committee of the American Society for Apheresis: The Ninth Special Issue. J Clin Apher. 2023;38(2):77-278.
62. Barten MJ, Fisher AJ, Hertig A. The use of extracorporeal photopheresis in solid organ transplantation-current status and future directions. Am J Transplant. 2024;24(10):1731-41.
63. Cho A, Jantschitsch C, Knobler R. Extracorporeal Photopheresis-An Overview. Front Med (Lausanne). 2018;5:236.
64. Barten MJ, Sax B, Schopka S, Amarelli C, Epailly E, Natali B, et al. European multicenter study on the real-world use and clinical impact of extracorporeal photopheresis after heart transplantation. J Heart Lung Transplant. 2023;42(8):1131-9.
65. Rubinstein G, Valledor AF, Moeller CM, Baranowska J, Oren D, Oh DKT, et al. Extracorporeal Photopheresis in the Treatment of Cardiac Allograft Rejection in the Modern Era: A Single-Center Experience. Clin Transplant. 2025;39(8):e70247.
66. Augusto JF, Benden C, Diekmann F, Zuckermann A. The value of extracorporeal photopheresis as an immunosuppression-modifying approach in solid organ transplantation: a potential solution to an unmet medical need. Front Immunol. 2024;15:1371554.
67. Barr ML, Meiser BM, Eisen HJ, Roberts RF, Livi U, Dall'Amico R, et al. Photopheresis for the prevention of rejection in cardiac transplantation. Photopheresis Transplantation Study Group. N Engl J Med. 1998;339(24):1744-51.
68. Lehrer MS, Rook AH, Tomaszewski JE, DeNofrio D. Successful reversal of severe refractory cardiac allograft rejection by photopheresis. J Heart Lung Transplant. 2001;20(11):1233-6.

69. Savignano C, Rinaldi C, Tursi V, Dolfini C, Isola M, Livi U, et al. Extracorporeal photochemotherapy in heart transplant rejection: A single-center experience. Transfus Apher Sci. 2017;56(4):520-4.
70. Barr ML, Baker CJ, Schenkel FA, McLaughlin SN, Stouch BC, Starnes VA, et al. Prophylactic photopheresis and chronic rejection: effects on graft intimal hyperplasia in cardiac transplantation. Clin Transplant. 2000;14(2):162-6.
71. Slomovich S, Bell J, Clerkin KJ, Habal MV, Griffin JM, Raikhelkar JK, et al. Extracorporeal photopheresis and its role in heart transplant rejection: prophylaxis and treatment. Clin Transplant. 2021;35(7):e14333.
72. Gokler J, Aliabadi-Zuckermann A, Zuckermann A, Osorio E, Knobler R, Moayedifar R, et al. Extracorporeal Photopheresis With Low-Dose Immunosuppression in High-Risk Heart Transplant Patients-A Pilot Study. Transpl Int. 2022;35:10320.
73. Rose EA, Barr ML, Xu H, Pepino P, Murphy MP, McGovern MA, et al. Photochemotherapy in human heart transplant recipients at high risk for fatal rejection. J Heart Lung Transplant. 1992;11(4 Pt 1):746-50.
74. Costanzo-Nordin MR, Hubbell EA, O'Sullivan EJ, Johnson MR, Mullen GM, Heroux AL, et al. Photopheresis versus corticosteroids in the therapy of heart transplant rejection. Preliminary clinical report. Circulation. 1992;86(5 Suppl):II242-50.
75. Meiser BM, Kur F, Reichenspurner H, Wagner F, Boos KS, Vielhauer S, et al. Reduction of the incidence of rejection by adjunct immunosuppression with photochemotherapy after heart transplantation. Transplantation. 1994;57(4):563-8.
76. Dall'Amico R, Livi U, Milano A, Montini G, Andreetta B, Murer L, et al. Extracorporeal photochemotherapy as adjuvant treatment of heart transplant recipients with recurrent rejection. Transplantation. 1995;60(1):45-9.
77. Dall'Amico R, Montini G, Murer L, Andreetta B, Tursi V, Feltrin G, et al. Benefits of photopheresis in the treatment of heart transplant patients with multiple/refractory rejection. Transplant Proc. 1997;29(1-2):609-11.
78. Giunti G, Schurfeld K, Maccherini M, Tanganelli P, Rubegni P, Alfani D, et al. Photopheresis for recurrent acute rejection in cardiac transplantation. Transplant Proc. 1999;31(1-2):128-9.

79. Maccherini M, Diciolla F, Laghi Pasini F, Lisi G, Tanganelli P, D'Ascenzo G, et al. Photopheresis immunomodulation after heart transplantation. Transplant Proc. 2001;33(1-2):1591-4.
80. Kirklin JK, Brown RN, Huang ST, Naftel DC, Hubbard SM, Rayburn BK, et al. Rejection with hemodynamic compromise: objective evidence for efficacy of photopheresis. J Heart Lung Transplant. 2006;25(3):283-8.
81. Teszak T, Bodor C, Hegyi L, Levay L, Nagy B, Fintha A, et al. Local laboratory-run donor-derived cell-free DNA assay for rejection surveillance in heart transplantation-first six months of clinical experience. Clin Transplant. 2023;37(9):e15078.
82. Teszak T, Bodor C, Hegyi L, Levay L, Nagy B, Fintha A, et al. [Noninvasive rejection surveillance after solid organ transplantations: analysis of the donor-derived cell-free DNA]. Orv Hetil. 2024;165(33):1275-85.
83. Grskovic M, Hiller DJ, Eubank LA, Sninsky JJ, Christopherson C, Collins JP, et al. Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients. J Mol Diagn. 2016;18(6):890-902.
84. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2015;16(3):233-70.
85. Kyrouac D, Schiffer W, Lennep B, Fergestrom N, Zhang KW, Gorcsan J, 3rd, et al. Echocardiographic and clinical predictors of cardiac amyloidosis: limitations of apical sparing. ESC Heart Fail. 2022;9(1):385-97.
86. Sugimoto T, Dulgheru R, Bernard A, Ilardi F, Contu L, Addetia K, et al. Echocardiographic reference ranges for normal left ventricular 2D strain: results from the EACVI NORRE study. Eur Heart J Cardiovasc Imaging. 2017;18(8):833-40.
87. Manganaro R, Marchetta S, Dulgheru R, Ilardi F, Sugimoto T, Robinet S, et al. Echocardiographic reference ranges for normal non-invasive myocardial work indices: results from the EACVI NORRE study. Eur Heart J Cardiovasc Imaging. 2019;20(5):582-90.
88. Raichlin E, Edwards BS, Kremers WK, Clavell AL, Rodeheffer RJ, Frantz RP, et al. Acute cellular rejection and the subsequent development of allograft vasculopathy after cardiac transplantation. J Heart Lung Transplant. 2009;28(4):320-7.

89. Teszak T, Barcziova T, Bödör C, Hegyi L, Levay L, Nagy B, et al. Donor-Derived Cell-Free DNA Versus Left Ventricular Longitudinal Strain and Strain-Derived Myocardial Work Indices for Identification of Heart Transplant Injury. Biomedicines. 2025;13(4):841.

90. Teszak T, Assabiny A, Kiraly A, Tarjanyi Z, Parazs N, Szakal-Toth Z, et al. Extracorporeal photopheresis in the treatment of cardiac allograft rejection: A single-centre experience. Transpl Immunol. 2023;79:101853.

91. Jimenez-Blanco M, Crespo-Leiro MG, Garcia-Cosio Carmena MD, Gomez Bueno M, Lopez-Vilella R, Ortiz-Bautista C, et al. Donor-derived cell-free DNA as a new biomarker for cardiac allograft rejection: A prospective study (FreeDNA-CAR). J Heart Lung Transplant. 2025;44(4):560-9.

92. Henricksen EJ, Kim JS, Khush KK, Njoroge J, Luikart H, Zhang MB, et al. Three-year outcomes with the use of dual non-invasive rejection surveillance after heart transplantation. JHLT Open. 2026;11:100456.

93. Moayedi Y, Foroutan F, Miller RJH, Fan CS, Posada JGD, Alhussein M, et al. Risk evaluation using gene expression screening to monitor for acute cellular rejection in heart transplant recipients. J Heart Lung Transplant. 2019;38(1):51-8.

94. Barakat AF, Sperry BW, Starling RC, Mentias A, Popovic ZB, Griffin BP, et al. Prognostic Utility of Right Ventricular Free Wall Strain in Low Risk Patients After Orthotopic Heart Transplantation. Am J Cardiol. 2017;119(11):1890-6.

95. Otto MEB, Martins AMA, Campos Dall'Orto AOM, Leite SF, de Queiroz Mauricio Filho MAF, Martins NT, et al. Acute Cellular Rejection in Heart Transplant Patients: Insights of Global Longitudinal Strain, Myocardial Work, and an Exclusive Group of Chagas Disease. Front Cardiovasc Med. 2022;9:841698.

96. Leitman M, Tyomkin V. Apical Sparing in Routine Echocardiography: Occurrence and Clinical Significance. J Cardiovasc Dev Dis. 2024;11(9):262.

97. Mandoli GE, Landra F, Tanzi L, Martini L, Fusi C, Sciaccaluga C, et al. Reference values of strain-derived myocardial work indices in heart transplant patients. Eur Heart J Imaging Methods Pract. 2024;2(3):qyae091.

98. Pradhan S, Mullikin A, Zang H, Ollberding NJ, Stark S, Hill GD, et al. Decreased Global Myocardial Work Efficiency Correlates with Coronary Vasculopathy in Pediatric Heart Transplant Patients. Pediatr Cardiol. 2022;43(3):515-24.

99. Sade LE, Colak A, Duzgun SA, Hazirolan T, Sezgin A, Donal E, et al. Approach to optimal assessment of right ventricular remodelling in heart transplant recipients: insights from myocardial work index, T1 mapping, and endomyocardial biopsy. Eur Heart J Cardiovasc Imaging. 2023;24(3):354-63.
100. Dieterlen MT, Bittner HB, Pierzchalski A, Dhein S, Mohr FW, Barten MJ. Immunological monitoring of extracorporeal photopheresis after heart transplantation. Clin Exp Immunol. 2014;176(1):120-8.
101. Wallner S, Gokler J, Just U, Knobler R, Horner-Golden T, Zuckermann A. Extracorporeal Photopheresis in the Treatment of Complicated Acute Rejection after Heart Transplantation. Transpl Int. 2015;28:356-.
102. Schurfeld K, Giunti G, Maccherini M, Rubegni P, D'Ascenzo G, Diciolla F, et al. Photopheresis after cardiac transplantation induces apoptosis. Transplant Proc. 1999;31(1-2):125-7.
103. Kobashigawa J, Watanabe J, Shafi H, Patel J, Kittleson M, Jordan S, et al. Clinical and Mechanistic Outcomes of Photopheresis after Heart Transplantation. Am J Transplant. 2013;13:453-.
104. Peled Y, Lavee J, Ram E, Kassif Y, Peled A, Freimark D, et al. Recurrent acute cellular rejection graded ISHLT 1R early after heart transplantation negatively affects long-term outcomes: The prognostic significance of 1990 ISHLT grades 1B and 2. Transpl Immunol. 2019;55:101204.
105. Khush KK. Clinical utility of donor-derived cell-free DNA testing in cardiac transplantation. J Heart Lung Transplant. 2021;40(6):397-404.
106. Evans RW, Williams GE, Baron HM, Deng MC, Eisen HJ, Hunt SA, et al. The economic implications of noninvasive molecular testing for cardiac allograft rejection. Am J Transplant. 2005;5(6):1553-8.

9 Bibliography of the candidate's publications

Publications related to the thesis

Teszak T, Barcziova T, Bödör C, Hegyi L, Levay L, Nagy B, Fintha A, Szijarto A, Kovacs A, Merkely B, Sax B. Donor-Derived Cell-Free DNA Versus Left Ventricular Longitudinal Strain and Strain-Derived Myocardial Work Indices for Identification of Heart Transplant Injury. Biomedicines. 2025 Apr 1;13(4):841. doi: 10.3390/biomedicines13040841. IF: 3.9. Quartile: Q1.

Teszak T, Bödör C, Hegyi L, Levay L, Nagy B, Fintha A, Merkely B, Sax B. Local laboratory-run donor-derived cell-free DNA assay for rejection surveillance in heart transplantation-first six months of clinical experience. Clin Transplant. 2023 Sep;37(9):e15078. doi: 10.1111/ctr.15078. Epub 2023 Jul 25. IF: 1.9. Quartile: Q2.

Teszak T, Assabiny A, Kiraly A, Tarjanyi Z, Parazs N, Szakal-Toth Z, Hartyanszky I, Szabolcs Z, Racz K, Reti M, Merkely B, Sax B. Extracorporeal photopheresis in the treatment of cardiac allograft rejection: A single-centre experience. Transpl Immunol. 2023 Aug;79:101853. doi: 10.1016/j.trim.2023.101853. Epub 2023 May 16. IF: 1.6. Quartile: Q2.

Teszák T, Bödör C, Hegyi L, Lévay L, Nagy B, Fintha A, Merkely B, Sax B. Noninvazív rejekció utánkövetése szolid szervek átültetését követően: a donoreredetű sejtmentes DNS vizsgálata [Noninvasive rejection surveillance after solid organ transplantations: analysis of the donor-derived cell-free DNA]. Orv Hetil. 2024 Aug 18;165(33):1275-1285. Hungarian. doi: 10.1556/650.2024.33106. IF: 0.9. Quartile: Q4.

Teszák T, Bödör Cs, Hegyi L, Lévay L, Nagy Á, Fintha A, Pólos M, Hartyánszky I, Merkely B, Sax B. Szívtranszplantációt követő kilökődés noninvazív diagnosztikája: A donoreredetű sejtmentes DNS mérésének lehetősége a gondozás során. CARDIOLOGIA HUNGARICA. 2023; 53 (4). pp. 399-405. IF: 0.

Publications not related to the thesis

Edvi B, Assabiny A, **Teszák T**, Tolvaj M, Fábián A, Hartyánszky I, Pólos M, Lakatos BK, Vágó H, Sax B, Merkely B, Kovács A. Trajectory of Diastolic Function after Heart Transplantation as Assessed by Left Atrial Deformation Analysis. Diagnostics (Basel). 2024 May 29;14(11):1136. doi: 10.3390/diagnostics14111136. IF: 3.3. Quartile: Q2.

Barten MJ, Sax B, Schopka S, Amarelli C, Epailly E, Natali B, **Teszák T**, Gökler J, Borchert K, Theil J, Ingram A, Zuckermann A. European multicenter study on the real-

world use and clinical impact of extracorporeal photopheresis after heart transplantation. J Heart Lung Transplant. 2023 Aug;42(8):1131-1139. doi: 10.1016/j.healun.2023.03.005. Epub 2023 Mar 15. IF: 6.4. Quartile: D1.

Parázs N, Lakatos B, Kovács A, Assabiny A, Király Á, Tarjányi Z, Szakál-Tóth Zs, **Teszák T**, Tokodi M, Ujvári A, Kugler Sz, Szücs N, Merkely B, Sax B. Jobbszívfél-elégtelenség évekkel a szívtranszplantációt követően: Egy ritka etiológiai tényező esete. CARDIOLOGIA HUNGARICA. 2021; 51 (1). pp. 69-72. IF: 0.

10 Acknowledgements

I want to express my deepest gratitude to my PhD supervisor, Balázs Sax, MD, PhD, for his unwavering support, insightful guidance, and constant encouragement throughout this journey. His vast knowledge and wisdom have profoundly influenced my academic development. I am sincerely grateful for the countless ways he has contributed to both my academic and professional growth. It has been a true privilege to learn under his mentorship, and I feel immensely fortunate to have had his guidance during this formative stage of my career.

I wish to express my sincere appreciation to Prof. Béla Merkely for his exceptional support, guidance, and inspiration, which were crucial to the successful completion of my doctoral studies.

I want to extend my sincere appreciation to Prof. Csaba Bödör for the immense work shared in paving the way for the implementation of the local laboratory-run dd-cfDNA assay. I want to thank Luca Lévay, Lajos Hegyi, and Ákos Nagy, PhD, from the Department of Pathology and Experimental Cancer Research, for their meticulous analytical work. I am incredibly grateful to all the nurses, including Csilla Csobánné Oláh and Tünde Cseke, as well as the staff of the Department of Heart Surgery and Transplantation, for their involvement in the care of HTx patients, whose contributions were invaluable.

I sincerely thank Tímea Barcziová, MD, and Attila Kovács, MD, PhD, for their support and technical expertise, which contributed to the successful completion of the echocardiographic study.

I am deeply grateful to the staff involved in ECP treatments at the Department of Apheresis of the National Institute for Haematology and Infectious Diseases, including Marienn Réti, MD, Zita Farkas, MD, Ágnes Király, MD, Andrea Várkonyi, MD, and Julianna Baán.

I am profoundly grateful to my family for their constant support, encouragement, and unwavering faith in my abilities.