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

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1 Introduction

Despite significant improvements in pre- and post-transplant management, the main challenges after heart transplantation (HTx) are related to the effectiveness and side effects of immunosuppressive therapy.

Endomyocardial biopsy (EMB) remains a cornerstone in the diagnostic armamentarium for allograft rejection; however, it has notable limitations. Thus, many efforts have focused on identifying noninvasive biomarkers to reduce or eliminate the need for surveillance EMBs. International guidelines and consensus documents recommend using donor-derived cell-free DNA (dd-cfDNA) to monitor HTx rejection, alongside other biomarkers such as donor-specific antibodies or gene expression profiling. In stable HTx recipients without evidence of rejection, dd-cfDNA is detected at very low levels. During episodes of cellular rejection or antibody-mediated rejection (AMR), graft injury leads to increased release of dd-cfDNA into the circulation. Elevated dd-cfDNA levels correlate with rejection detected by EMB. dd-cfDNA has a negative predictive value of 97-99% for both acute cellular rejection (ACR) and AMR, which

could help minimise the need for EMBs in patients with low clinical suspicion of acute rejection.

Echocardiography is the primary imaging modality for evaluating cardiac allograft function. Data show that global and regional left ventricular (LV) longitudinal strain values are lower after HTx than in the control population. It remains unclear whether they reflect the "normal" values of the transplanted heart or represent subtle alterations in the allograft's contractile function. The correlation between dd-cfDNA and LV longitudinal strain-derived parameters is unknown. The significance of myocardial work analysis in assessing HTx injury remains largely unclear.

Immunosuppressive therapy leaves patients vulnerable to significant side effects; therefore, immunosuppressive regimens with fewer toxic side effects are sorely needed. Extracorporeal photopheresis (ECP) exerts a sustained immunomodulatory effect, and it does not induce generalised immunosuppression.

2 Objectives

We sought to address key aspects of contemporary HTx rejection surveillance and treatment.

2.1 Rejection surveillance

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.

2.2 Rejection treatment

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.

3 Methods

3.1 Recruitment of patients

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 during the first post-transplant year) to a monthly dd-cfDNA-guided rejection monitoring approach. We also identified a cohort of consecutive HTx recipients who underwent treatment for AMR (n=9) (Rejection surveillance).

We conducted a retrospective, single-centre study on adult HTx recipients who underwent ECP between September 2013 and October 2020 (Rejection treatment).

3.2 Donor-derived cell-free DNA analysis

dd-cfDNA analysis was performed at months 2, 3, 4, 5, 6, 7, 8, 10, and 12 following HTx. A dd-cfDNA level of <0.25% was considered quiescence, whereas ≥0.35% indicated severe injury. The 0.25-0.35% dd-cfDNA range was regarded as a grey zone.

3.3 Transthoracic echocardiography

For echocardiographic assessments, patient selection was limited to individuals who had undergone HTx at least 6 months before inclusion. Left ventricular global longitudinal strain (LVGLS) and myocardial work indices were calculated offline after automated tracking of

myocardial deformation. Myocardial work was evaluated noninvasively using pressure-strain loop analysis, from which global work index (GWI), global constructive work (GCW), global wasted work (GWW), and global work efficiency (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.

3.4 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. 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 1).

Table 1. 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 International Society for Heart and Lung Transplantation (ISHLT) grading scale was used to evaluate EMB specimens. Acute moderate cellular rejection corresponds to ISHLT grade 2R, whereas 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 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 EMBs performed during the specified period.

3.5 Extracorporeal photopheresis

ECP was initiated following the episode of cardiac allograft rejection and administered alongside pharmacological anti-rejection therapy. 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.

4 Results

4.1 Rejection surveillance

Between October 2022 and November 2024, 449 dd-cfDNA samples from 71 patients were analysed across 26 runs. In clinically stable patients, the dd-cfDNA fraction remained low (0.13±0.06%) (Figure 1).

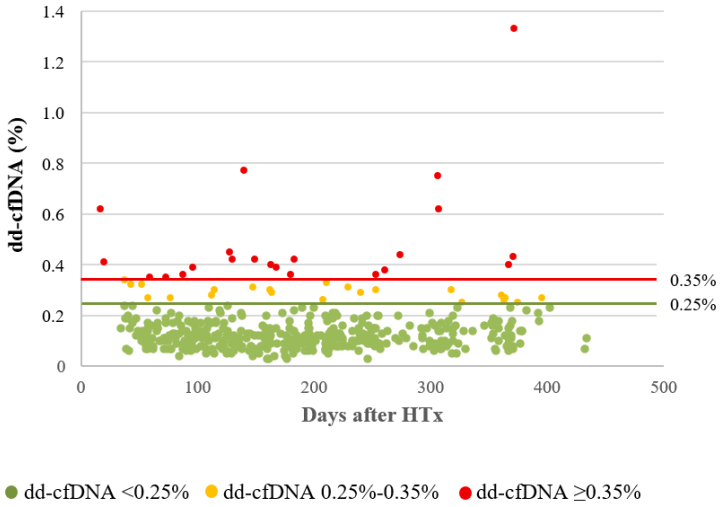

Figure 1. Distribution of donor-derived cell-free DNA data points in the function of time after heart transplantation.

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

Elevated dd-cfDNA levels, suggestive of myocardial injury, led to 26 for-cause EMBs. Of these, one EMB identified moderate ACR 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 <1 month post-transplant), while one result was presumably caused by pre-analytical error. Patients with EMB-proven rejection were transitioned back to routine EMB testing. Three hundred and ninety-six (88%) routine surveillance EMBs that would otherwise have been performed were avoided.

The mean LVGLS and longitudinal strain values obtained from the apical 4-chamber, 2-chamber, and 3-chamber views were lower than reference values reported in the literature. 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.

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 groups in LV longitudinal strain indices. However, NT-proBNP values differed significantly between the groups (Table 2).

Table 2. 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).

	dd-cfDNA <0.25%	**dd-cfDNA 0.25-0.35%**	**dd-cfDNA ≥0.35%**	**p**
longitudinal strain, %				
apical 4-chamber	-16.0±3.2	-16.0±3.4	-17.6±4.8	NS
apical 2-chamber	-14.8±4.3	-14.5±4.2	-14.8±3.6	NS
apical 3-chamber	-15.4±3.5	-15.4±4.3	-17.3±3.2	NS
LVGLS	-15.6±2.7	-15.3±3.5	-16.6±3.7	NS
lateral strain	-17.2±3.5	-16.6±3.5	-19.4±4.0	NS
septal strain	-12.6±3.5	-12.9±3.7	-15.0±4.2	NS
basal strain	-16.2±4.8	-16.3±5.3	-16.3±4.6	NS
mid strain	-12.6±3.5	-13.1±4.1	-13.3±3.6	NS
apical strain	-14.7±5.9	-14.1±5.7	-19.4±8.1	NS
LVEF, %	57.1±7.0	56.1±5.2	63.2±6.2	NS
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.
Data are presented as mean±standard deviation.

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.

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 LVGLS was -15.6±5.3%. LVGLS was not pathognomonic in EMB-proven rejection cases either.

In the patient cohort treated for AMR, the median dd-cfDNA level was 2.76% (0.68%-10.01%). The mean LVGLS was -17.4±3.3%. We found no correlation between dd-cfDNA values and LV longitudinal strain parameters.

Compared with healthy subjects from the EACVI NORRE study, transplanted hearts exhibited reduced GWI, GCW, and GWE values, and increased GWW

values (Table 3). No significant associations were observed between myocardial work parameters and dd-cfDNA levels.

Table 3. 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).

	Study examinations	**EACVI NORRE**
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.

Data are presented as either mean±standard deviation or median (min-max).

4.2 Rejection treatment

A total of 22 HTx recipients received a median of 11 ECP cycles (22 treatments). No ECP-related adverse effects were observed. Indications for ECP included acute moderate cellular rejection (9 patients), mixed rejection (8 patients), and persistent mild cellular rejection (5 patients).

The mean tacrolimus trough level at the final follow-up visit was significantly lower than at both the initial and final ECP time points. 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 dose of methylprednisolone decreased significantly from the initial to the last ECP treatment and was significantly lower at the final follow-up than at all three preceding time points.

The combination of ECP with additional immunosuppressive agents successfully reversed all ISHLT grade 2R cellular rejection episodes. Following completion of ECP, neither allograft rejections exceeding ISHLT grade 1R, nor rejection episodes requiring additional immunosuppressive treatment were detected.

The proportion of biopsies negative for cellular rejection significantly increased following the ECP (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 2).

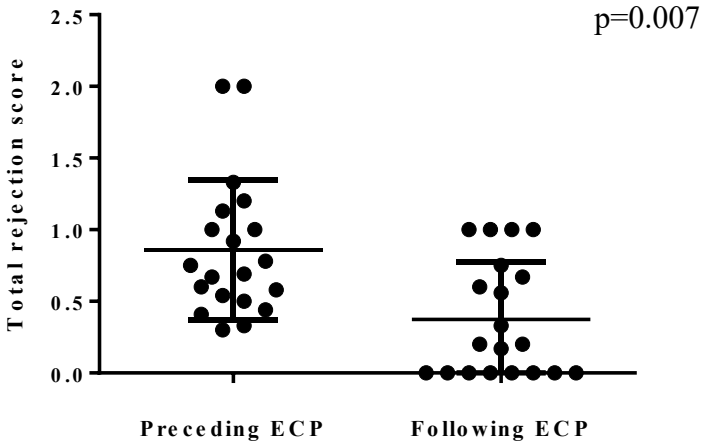

Figure 2. Total rejection score at initial and last extracorporeal photopheresis.

Abbreviation: ECP, extracorporeal photopheresis.

5 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.

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 range of clinical contexts. Methylprednisolone dose reduction was 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 high-titre donor-specific antibodies. Overall survival among HTx recipients treated with ECP was consistent with data from the ISHLT registry.

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.