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Article

Recipient Pericardial Apolipoprotein Levels Might Be an Indicator of Worse Outcomes after Orthotopic Heart Transplantation

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Abstract: End-stage heart failure (ESHF) leads to hypoperfusion and edema formation in the whole body and is accompanied by neurohormonal and immunological alterations. Orthotopic heart transplantation (HTX) has been used as a beneficial option for ESHF. Due to the shortage of donor hearts, the ideal matching and timing of donors and recipients has become more important. Our aim was to explore the relationship between the clinical outcome of HTX and the cytokine and apolipoprotein profiles of the recipient pericardial fluid obtained at transplantation after opening the pericardial sac. The clinical data and the interleukin, adipokine and lipoprotein levels in the pericardial fluid of twenty recipients were investigated. Outcome variables included primer graft dysfunction (PGD), need for posttransplantation mechanical cardiac support (MCS), International Society for Heart and Lung Transplantation grade ≥ 2R rejection and mortality. Recipient risk scores were also investigated. Leptin levels were significantly lower in patients with PGD than in those without PGD (median: 6.36 [IQR: 5.55-6.62]; versus 7.54 [IQR=6.71-10.44]; p = 0.029). Higher ApoCII levels (median: 14.91 [IQR: 11.55-21.30] versus 10.31 [IQR=10.02-13.07]; p = 0.042) and ApoCIII levels (median: 60.32 [IQR: 43.00-81.66] versus 22.84 [IQR=15.84-33.39]; p=0.005) were found in patients (n=5) who died in the first 5 years after HTX. In patients who had rejection (n=4) in the first month after transplantation, the levels of adiponectin (median: 74.48 [IQR: 35.51-131.70] versus 29.96 [IQR: 19.86-42.28]; p=0.039), ApoCII (median: 20.11 [IQR: 13.06-23.54] versus 10.32 [IQR: 10.02-12.84]; p=0.007) and ApoCIII (median: 70.97 [IQR: 34.72-82.22] versus 26.33 [IQR: 17.18-40.17; p=0.029) were higher than those in the nonrejection group. Moreover, the pericardial thyroxine (T4) levels (median: 3.96 [IQR: 3.49-4.46] versus 4.69 [IQR: 4.23-5.77]; p=0.022) were lower in patients with rejection than in patients who did not develop rejection. Our results indicate that apolipoproteins can facilitate the monitoring of rejection and could be a useful tool in the forecast of early and late complications.

Keywords: organ recipient; heart transplantation; primary graft dysfunction; interleukin; apolipoprotein

1. Introduction

Orthotopic heart transplantation (HTX) has been used as a beneficial option for end-stage heart failure (ESHF).[1-3] Improvement of the selection criteria, a more accurate determination of urgency, and optimization of preoperative conditions has helped to decrease adverse outcomes. [4] Nevertheless, HTX still has a considerable complication rate, including primer graft dysfunction (PGD), early graft loss, rejection and mortality. A recent comparison of available risk scores raised concern about their discriminative ability for one-year mortality. [5]

In contrast, several biomarkers have been used in risk estimation. These diagnostic tests are part of the routine pretransplantation evaluation of factors such as natriuretic peptides, the glomerular filtration rate, bilirubin levels, etc. [4] Interleukins (IL) and apolipoproteins (Apo) have also been advocated to play important roles in the development and progression of ESHF. [6,7] These new biomarkers seem to have promising prognostic roles in the development of diabetes and atherosclerosis. Only sparse data exist regarding the application of these new markers in transplantation medicine. [8] Based on the known donor shortage, optimization of patient selection, use of aged donors in borderline indications, and adding these new biomarkers to recipient selection might help to improve the risk prediction of the available risk scores.

We hypothesized that the extent of immunological injury can be quantified and that we can obtain a closer picture of heart function if aliquots are sampled from pericardial fluid. The aim of this study was to compare the levels of different interleukins and apoproteins in the pericardial fluid with the possible adverse outcomes of recipients after orthotopic heart transplantation. The primary outcome was primary graft dysfunction. Mortality and rejection were also investigated. Additionally, the inotropic score (IS), vasoactive-inotropic score (VIS), United Network for Organ Sharing (UNOS) recipient risk score and The Index for Mortality Prediction After Cardiac Transplantation (IMPACT) score were also correlated with pericardial hormone levels.

2. Results

2.1. Recipient characteristics

The median age of the recipients was 58.5 years, and all recipients were male (n=20). There was gender mismatch in 20% (n=4) of the transplantations, and the medians of the recipients' and donors' body mass index (BMI) values were 25.6 and 25.05 kg/m2, respectively. The etiology of heart failure was ischemic in 75% (n=15) and idiopathic in 25% (n=5) of the transplants. Further descriptive characteristics of the recipients are summarized in Table 1.

Table 1. Descriptive characteristics of the recipient and heart transplantations.

Factor	n median	% (IQR 25-75)
UNOS recipient score	1.00	(1.00-2.25)
0	3	15.00
1	11	55.00
2	1	5.00
3	3	15.00
4	1	5.00
5	1	5.00
IMPACT score	5.00	(2.00-9.00)
Demographic parameters		
Age	58.5	(53.00-60.00)
Weight	80	(75.00-84.50)
Height	178	(173.00-182.00)
BMI	25.6	(24.70-26.60)
Sex:		
male	20	100.00
female	0	0.00

sex mismatch	4	20.00
NYHA		
3	6	30.00
4	14	70.00
Diagnosis		
Idiopathic Heart Disease	5	25.00
Ischemic Heart Disease	15	75.00
Bridge to transplant	0	0.00
Amiodarone	3	15.00
Echocardiography parameters		
LVLDD	71.00	(61.75-78.25)
LVLSD	60.00	(54.75-67.50)
Posterior Wall Diastolic Diameter	40.00	(37.00-44.00)
Ascending Aorta Diameter	34.00	(31.25-35.75)
Septum Diastolic Diameter	9.00	(7.00-9.75)
Left Atrial Longitudinal Diameter	47.00	(42.50-54.50)
Left Atrial Horizontal Diameter	60.00	(51.50-63.50)
Right Atrial Longitudinal Diameter	44.00	(41.00-49.00)
Right Atrial Horizontal Diameter	54.00	(43.00-63.00)
Aortic Root Systolic Diameter	22.00	(19.50-25.00)
AoVmax	0.95	(0.70-1.10)
Preoperative laboratory values		
Sodium (mmol/L)	138.00	(134.25-139.00)
Potassium (mmol/L)	4.20	(4.10-4.60)
Creatinine (μmol/L)	102.00	(96.25-151.00)
INR	1.79	(1.26-2.21)
AST (UI/L)	27.00	(21.50-31.25)
ALT (UI/L)	26.50	(16.50-33.00)
GGT (UI/L)	74.00	(47.25-159.25)
LDH (UI/L)	349.00	(291.00-382.00)
ALP (UI/L)	92.50	(68.75-107.25)
Total Bilirubin (μmol/L)	11.45	(6.97-20.95)
Total Protein (g/L)	72.10	(68.32-75.65)
Albumin (g/L)	45.55	(42.55-47.25)
Cholesterol (mmol/L)	4.10	(3.65-5.40)
Triglycerides (mmol/L)	1.08	(1.00-1.68)
Thyroid function		
Hypothyroidism	0	0.00
Hyperthyroidism	2	10.00
Endocrine management		
L-thyroxine	1	5.30

ALP: alkaline phosphatase; ALT: alanine transaminase; AST: aspartate aminotransferase; BMI: body mass index; GGT: gamma-glutamyl transpeptidase; IMPACT: Index for Mortality Prediction After Cardiac Transplantation; INR: international normalized ratio; IQR: interquartile range; LDH: lactate dehydrogenase; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter NYHA: New York Heart Association; UNOS: United Network for Organ Sharing.

2.2. Recipient risk scores and laboratory parameters

The median scores of the patients were 1.00 (IQR: 1.00-2.25) and 5.00 (IQR: 2.00-9.00) for the UNOS recipient score and IMPACT score, respectively. The UNOS-R score showed correlation with the adipsin (p=0.020) and adiponectin (p=0.008) levels. IMPACT scores were not related to the measured panels. ApoAII levels were higher in patients who had bilirubin levels above 1 mg/dL compared to those who had values < 1 mg/dL (p= 0.035). ApoAI showed a correlation with preoperative creatinine, aspartate aminotransferase (AST) and alanine transaminase (ALT) levels (p=0.040; p=0.004; p=0.005, respectively). Preoperative AST levels were also correlated with ApoCII

(p=0.040) and ApoCIII (p=0.040) levels. Postoperative bilirubin levels were correlated with IL-21 (p=0.002), IL-6 (p=0.001), IL-9 (p<0.001) and adiponectin (p=0.001) levels. Further correlations of laboratory parameters are summarized in Table S7-S8.

2.3. Complications and adverse reactions

After transplantation, five patients needed postoperative MCS, and four patients had PGD. Four patients had ISHLT Grade 2R rejection, and vasoplegia was diagnosed in four cases. The detailed postoperative complications and laboratory parameters are shown in Table 2.

Table 2. Descriptive characteristics of complications and adverse reactions.

Factor	n median	% (IQR 25-75)
Mortality		
30 days	0	0.00
1 year	2	10.00
2 years	3	15.00
5 years	5	25.00
Postoperative MCS	5	25.00
Perioperative Complications		
Vasoplegia	4	20.
Primary Graft Dysfunction	4	20
Rejection	4	20
Reoperation	5	25
Retransplantation	1	5
Transfusion		
RBC (units)	6.50	(3.75-19.75)
Platelet Count (units)	3.50	(3.00-12.75)

IQR: interquartile range; MCS: mechanical circulatory support; RBC: red blood cell.

In the pericardial fluid of the recipients, leptin levels were significantly lower in patients with PGD than in those without it (p= 0.029). Leptin levels were also significantly lower in the MCS group (p = 0.042). The detailed comparisons are shown in Table 3, Figure 1, and Tables S1 and S2.

Table 3. Relationship between recipients' pericardial leptin levels and postoperative complications.

	No PGF		PGF		No MCS		MCS	
	median	IQR (25-75)	median	IQR (25-75)	median	IQR (25-75)	median	IQR (25-75)
leptin (ng/mL)	7.54	(6.71-10.44)	6.36	(5.55-6.62)	7.61	(6.70-10.50)	6.53	(5.77-6.81)
p value		0.029				0.042		

IQR: interquartile range; MCS: mechanical circulatory support; PGD: primary graft dysfunction.

Figure 1. (**a**) Relationship between recipients' pericardial leptin levels and primary graft dysfunction after heart transplant. (**b**) Relationship between recipients' pericardial leptin levels and postoperative mechanical circulatory support after heart transplant.

In the recipient pericardial fluids, ApoD levels were significantly higher in patients who had postoperative vasoplegia than in those who had no vasoplegia (median: 28.69 [15.85–37.66] vs. 12.34 [8.92–20.34] μg/mL, p=0,022 in the vasoplegic and nonvasoplegic patients, respectively). (Table S3, Figure2)
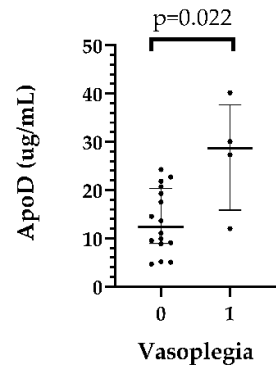

Figure 2. Relationship between recipients' pericardial ApoD levels and vasoplegia after heart transplant.

Higher ApoCII (p= 0.042) and ApoCIII (p=0.005) levels were detected in patients (n=5) who died in the first 5 years after HTX (n=5). (Table 4 and S4, Figure 3)

Table 4. Relationship between recipients' pericardial ApoCII and ApoCIII levels and 5 years mortality.

	Not died within 5 years		Died within 5 years		
	median	IQR (25-75)	median	IQR (25-75)	p value
ApoCII (ug/mL)	10.31	(10.02-13.07)	14.91	(11.55-21.30)	0.042
ApoCIII(ug/mL)	22.84	(15.84-33.39)	60.32	(43.00-81.66)	0.005

Apo: apolipoprotein, IQR: interquartile range.

(a)   (b)
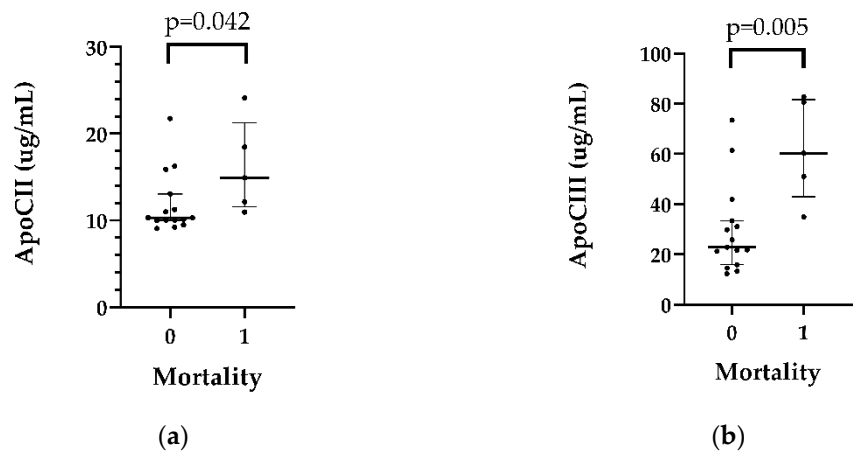
Figure 3. Relationship between recipients' pericardial ApoCII (**a**) and ApoCIII (b) levels and 5 years mortality.

In patients who had rejection (n=4) in the first month after transplantation, adiponectin (p=0.039), ApoCII (p=0.007) and ApoCIII (p=0.029) levels were higher than those in the nonrejection group, while pericardial T4 levels were lower in patients with rejection than in those who did not develop rejection. The data are shown in Table 5, Figure 4 and Table S5.

Table 5. Relationship between recipients' pericardial Adiponectin, ApoCII, ApoCIII and T4 levels and rejection.

	No rejection		Rejection		
	median	IQR (25-75)	median	IQR (25-75)	p value
Adiponectin (ng/mL)	29.96	(19.86-42.28)	74.48	(35.51-131.70)	0.039
ApoCII (ug/mL)	10.32	(10.02-12.84)	20.11	(13.06-23.54)	0.007
ApoCIII (ug/mL)	26.33	(17.18-40.17)	70.97	(34.72-82.22)	0.029
T4 (ug/mL)	4.69	(4.23-5.77)	3.96	(3.50-4.46)	0.022

Apo: apolipoprotein; IQR: interquartile range; T4: thyroxine.

(**a**)

(**b**)

(**c**)

(**d**)
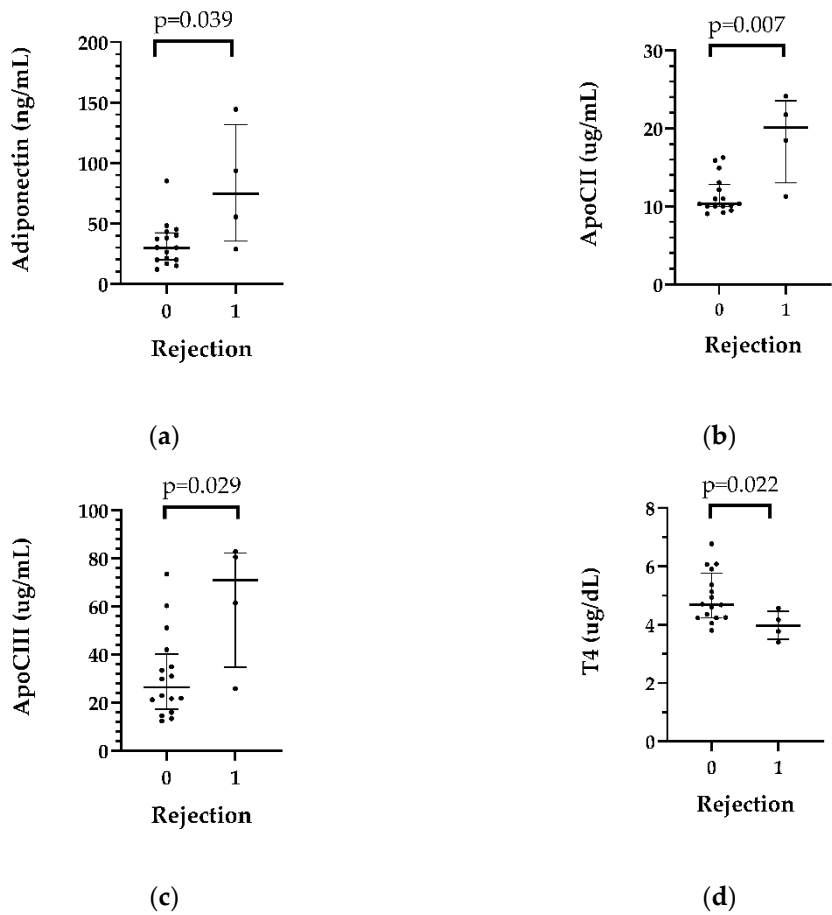
Figure 4. Relationship between recipients' pericardial Adiponectin (**a**), ApoCII (**b**), ApoCIII (**c**) and T4 (**d**) levels and rejection after heart transplant.

2.4. Vasoactive-inotropic score and inotropic score

Significant correlations were found between maximum vasoactive-inotropic score, inotropic score and several immunological parameters. The detailed results are shown in Table S9.

2.5. K-means clustering based on the molecular composition of the pericardial fluid

K-means clustering (Figure 5) has identified 3 subgroups of immunological parameters (cluster A: Adipsin, Leptin, T4, IL-21, IL-6, IL-9 and oxLDL; cluster B: T3, ApoD, ApoAII, ApoCIII, ApoCII, ApoB100, ApoAI, ApoM and Adiponectin; cluster C: IL-4, IL-17A, IL-2, IL-13, IL-17F, IFN, TNF, IL-5, IL-10 and IL-22) and of patients (clusters 1-3). Patients of cluster 1 have shown overall elevated levels of the interleukin rich cluster C, while cluster 3 was characterized by higher degrees of pericardial apolipoprotein content (cluster B). Furthermore, patient cluster 1 and cluster 3 have

demonstrated numerically greater incidences of MCS, PGD and mortality (2/3, 2/3, 1/3 and 3/6, 2/6, 4/6 respectively) compared to patients of cluster 2 (0/11, 0/11, 0/11).
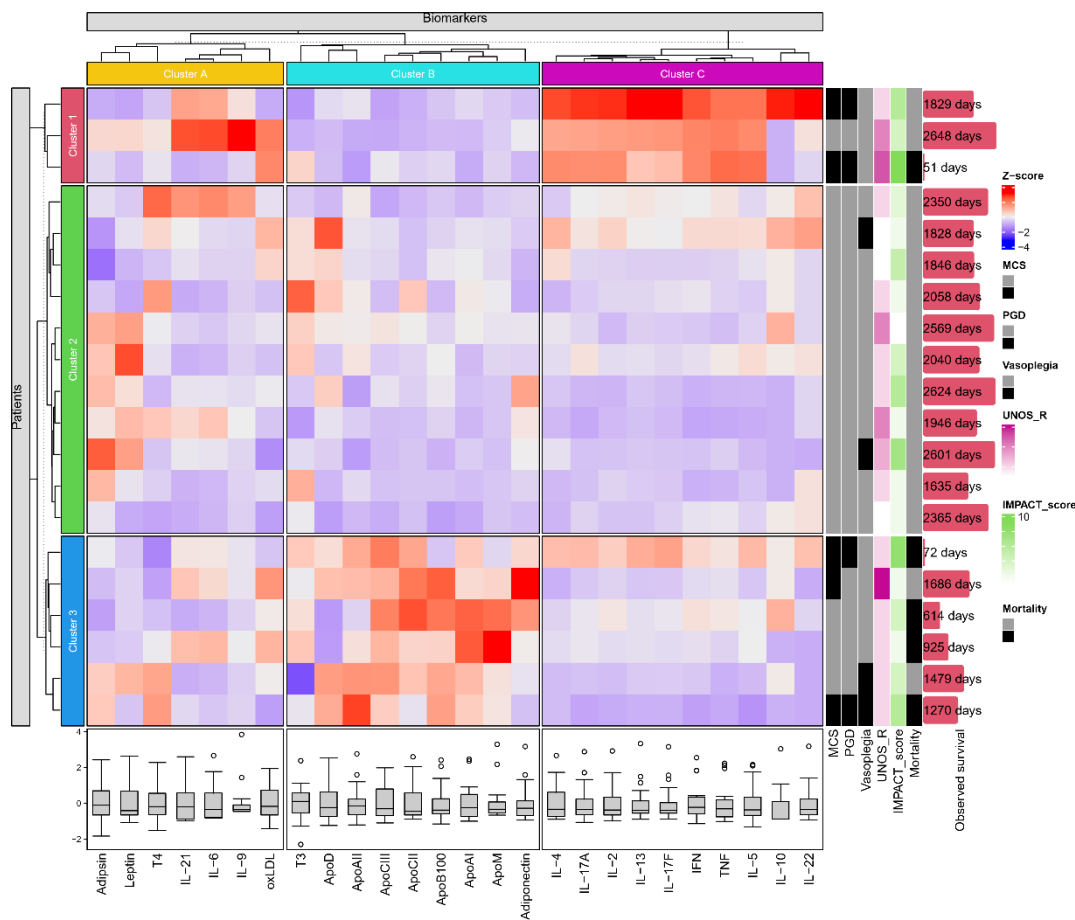

Figure 5. Heatmap with K-means clustering based on immunological parameters of the pericardial fluid of HTX recipients.

3. Discussion

We found that recipients who subsequently had PGD or required ventricular assist device support had lower pericardial leptin levels than recipients who did not. Higher pericardial ApoD levels were associated with vasoplegia, and higher adiponectin, ApoB100, ApoCII, and ApoCIII levels and lower T4 levels were associated with rejection. Furthermore, higher pericardial ApoCII and ApoCIII levels showed a significant correlation with mortality.

The pathophysiology of ESHF is complex, characterized by volume overload, different extents of end organ dysfunction and neurohormonal changes and inflammation. [9] Several risk scores have been developed to determine the urgency of heart transplantation or for usage of MCS before transplantation to bridge the unstable state or severe hypoperfusion to candidacy. [10,11,12] Systemic inflammation and cytokine release can further worsen hemodynamic instability and thus can serve as a diagnostic tool in risk estimation. Our results also indicate the strong relationship between the UNOS score and markers of kidney and liver dysfunction.

The current challenges of HTX are the increased age of donors and recipients, the large number of patients who have MCS or are operated on in high urgency status, and increased allosensitization. [13,14] The occurrence of early and late complications has also increased. Therefore, the use of biomarkers to detect the ideal matching seems rational. Leptin is a nonglycosylated protein produced mainly by adipose tissue that plays an important role in normal cardiovascular function. [15] In young, healthy men, there is an inverse correlation between the thickness of the carotid wall and the circulating leptin concentration, which indicates the vascular protective effect of leptin. [16]   In

patients with nonischemic cardiomyopathy, lower leptin levels were associated with an increased likelihood of HTX. [17] ApoD has recently been the subject of research on neurodegenerative diseases and cardiovascular function. Circulating apoD levels have been observed to be increased in heart failure patients, so this apoprotein can even be used as a biomarker. Apolipoprotein D is associated with both developing and mature blood vessels and plays a role in inflammation. [18, 19] Pericardial recipient levels were found to differ significantly from the normal level in early complications such as PGD, need for MCS and vasoplegia, preceding and forecasting these hemodynamic problems.

Rejection has become more important in the current era; as older age, previous MCS, previous pregnancies, and transfusion frequently lead to allosensitization. ApoCIII is a small 8.8 kDa apolipoprotein produced in the liver and found on the surface of very low density lipoprotein, low density lipoprotein (LDL) and high-density lipoprotein (HDL). ApoCIII has both indirect atherogenic effects (causes hypertriglyceridemia) and direct atherogenic effects (stimulates monocyte adhesion to endothelial cells, promotes the production of inflammatory mediators in these cells and increases LDL retention in the arterial wall). [20, 21] ApoCII is also mainly produced in the liver and interacts with systemic inflammation. [22] One piece of Apo B-100 produced by hepatocytes is found on the surface of every atherogenic particle. [23, 24] The circulating level is an effective measure of cardiovascular risk and coronary artery disease.[24, 25] A postmortem study revealed that the pericardial fluid of people with severe atherosclerosis contained higher amounts of ApoB than those without atherosclerosis.[26] Apolipoproteins can be better predictive markers of cardiovascular events than serum lipids used in everyday practice; however, conventional immunoassays used to determine apolipoprotein levels can carry serious limiting factors (for example, interfering antibodies and cross-reactivity). Apolipoproteins have important roles in the maintenance of endothelial function, blood pressure and hemostasis, and adipokines contribute to the regulation of appetite and energy turnover by regulating the secretion of insulin-sensitive tissues and pancreatic β-cells. Our results also indicate that the amount of apolipoproteins is associated with the development of rejection and mortality. On the other hand, monitoring a panel of valid biomarkers may provide patient stratification and better immunosuppression treatment selection. After transplantation, the therapy should be adjusted based on the prediction of rejection episodes (maintained alloreactivity), prognosis of allograft damage progression, and personal drug response. [27]

Adiponectin is mainly produced by adipose tissue, and low levels of adiponectin are associated with ischemic heart diseases and peripheral arterial diseases. However, a high adiponectin level does not always mean a better outcome because severe cardiovascular patients with liver or kidney disease often have elevated levels due to insufficient clearance as a secondary consequence of cardiovascular disease.[15] Patients with nonischemic cardiomyopathy plus higher adiponectin levels are more likely to receive a transplant than those with low adiponectin levels.[17]

3.1. Limitation of the study

The main limitation of this study is that the pericardial fluid samples of the recipients were not available. There are no data about the serum levels. Moreover, the small population size and the heterogeneity of the recipients resulted in lower statistical power.

4. Materials and Methods

4.1. Study design, setting and participants

Well-characterized pseudonymized human pericardial fluid and cell-depleted pericardial fluid samples of 20 randomly chosen recipients from the period between February 2013 and December 2017 were obtained from the Transplantation Biobank of the Heart and Vascular Center at Semmelweis University, Budapest, Hungary. Between January 2013 and April 2017, 206 orthotopic transplantations were performed, and 118 donor pericardial fluid specimens were available (57.3% of the transplantations). Pericardial fluid samples contaminated with blood were an exclusion criterion in the study. The procedure of sample procurement was reviewed and approved by the institutional and national ethics committee (ethical permission numbers: ETT TUKEB 7891/2012/EKU

[119/PI/12.] and ETT TUKEB IV/10161-1/2020). Clinical patient data were obtained from the database of the Transplantation Biobank. Our study was conducted in accordance with the Eurotransplant standards for organ sharing and with the Hungarian National Blood Transfusion Service. The last check on the follow-up data was made on January 22th, 2023. [28]

4.2. Local Protocols

Donor and recipient variables were retrieved from the National and Eurotransplant-based donor data report form and from electronic medical records from our institutional databank. A description of the study is shown in Figure 6.
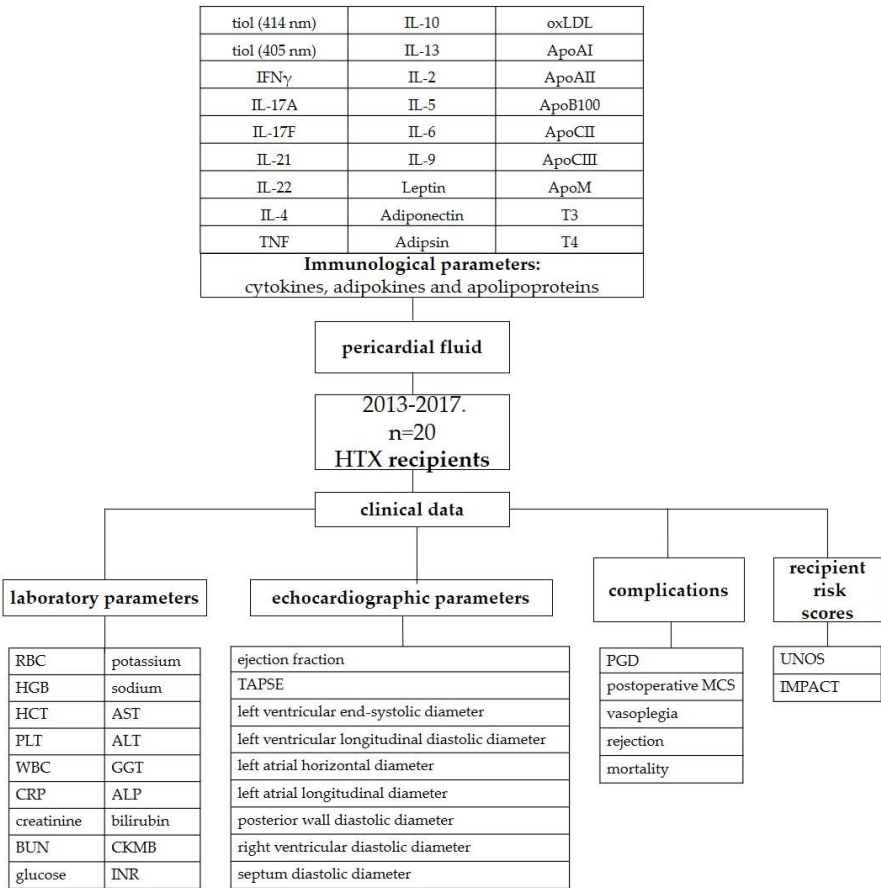
Figure 6. Description of the study (ALP: alkaline phosphatase; ALT: alanine transaminase; Apo: apolipoprotein; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CKMB: creatinine kinase-MB isoform; CRP: C-reactive protein; GGT: gamma-glutamyl transferase; HCT: hematocrit; HGB: hemoglobin; HTX: heart transplantation; IFN γ: interferon-γ; IL: interleukin; IMPACT: Index for Mortality Prediction After Cardiac Transplantation; INR: international normalized ratio; MCS: mechanical circulatory support; oxLDL: oxidized low-density lipoprotein; PGD: primary graft dysfunction; PLT: platelet count; RBC: red blood cell; T3: triiodothyronine; T4: thyroxine; TAPSE: tricuspid annular plane systolic excursion; TNF: tumor necrosis factor; UNOS: United Network for Organ Sharing; WBC: white blood cells).

4.3. Major study parameters

Due to the relatively small sample size of our study, the UNOS score was calculated for donors, recipients and overall individuals. The donor-specific UNOS (UNOS D) score includes donor age (1 point if aged between 50 and 55 years or 2 points if aged above 55 years), total ischemic time (above 4 hours 2 points), sex mismatch (1 point) and donor diabetes mellitus (1 point). According to these criteria, the UNOS-D score was calculated, and three risk groups were formed: low (score: 0),

intermediate (score: 1 or 2) or high (score: ≥3) UNOS-D risk groups. The recipient-specific UNOS score considered the following parameters: age (above 65 years: 1 point), body mass index (30-35 kg/m2: 1 point, >35 2 points), mean pulmonary artery pressure (above 30 mmHg: 1 point), total bilirubin (1.5-1.9 mg/dL: 1 point, >1.9 mg/dL: 2 points), creatinine (1.5-2.0 mg/dL: 1 point; >2 mg/dL: 2 points), previous transplant, previous cancer and pretransplant mechanical ventilation (each 2 points) or mechanical circulatory support (noncontinuous-flow: 2 points).[29] The total score is the sum of donor- and recipient-specific scores, and this score was used in the multivariable logistic regression analyses for adjustment.

4.3.1. The IMPACT score

The IMPACT score included the following 12 recipient characteristics: age (above 60 years: 1 point), serum bilirubin (0-0.99 mg/dL: 0 point, 1-1.99 mg/dL: 1 point, 2-3.99 mg/dL: 3 points, ≥4 mg/dL: 4 points), creatinine clearance (≥50 mL/min: 0 point, 30-49 mL/min: 2 points, <30 mL/min: 5 points), dialysis between listing and transplant (4 points), female sex (3 points), heart failure etiology (idiopathic 0 point, ischemic 2 points, congenital 5 points, other 1 point), recent infection (3 points), intra-aortic balloon pump (3 points), mechanical ventilation pretransplant (5 points), race (Caucasian: 0 points, African American: 3 points, Hispanic: 0 points, other: 0 points), temporary circulatory support (7 points), and ventricular assist device (older-generation pulsatile: 3 points, newer-generation continuous: 5 points, Heartmate II: 0 point).[10]

4.4. Sample collection and preparation

After sternal splitting and opening of the pericardium, ACD (trisodium citrate with citric acid and dextrose) vacutainer tubes (BD Vacutainer® System, BD Biosciences, San Jose, California, USA) were used for the collection of pericardial fluid samples. After pelleting cells (300 g, 10 min, 4 °C), the supernatant was centrifuged at 2000 g for 10 min at 4 °C to remove large particles. The cell-free supernatants were collected, divided into three 500 μL aliquots and stored in liquid nitrogen until use.

4.5. Flow cytometric multiplexed bead-based immunoassays

LEGENDplex™ bead-based immunoassays (BioLegend, San Diego, CA, USA) were used for the quantification of pericardial fluid cytokines, adipokines and apolipoproteins according to the manufacturer's instructions.

4.6. Enzyme-linked immunosorbent assays (ELISA)

Pericardial fluid oxidized low-density lipoprotein (oxLDL), triiodothyronine (T3) and thyroxine (T4) concentrations were determined by ELISA (human OxLDL ELISA® Kit of Cloude Clone; T3 and T4 Human ELISA Kits Abcam, Cambridge, UK) according to the manufacturer's instructions. Oxidative damage of pericardial fluid proteins was assessed by determining the free SH content expressed in cysteine equivalents via the DTNB (5,5'-dithio-bis-(2-nitrobenzoic acid = Ellman reagent) method (Thermo Scientific Pierce Ellman's Reagent).

4.7. Outcomes

Our primary outcome was primary graft dysfunction (PGD) in recipients. PGD was defined according to the consensus criteria of the International Society of Heart and Lung Transplantation (ISHLT).[30] The decision regarding mechanical circulatory support (MCS) implantation was made by a team of experts (including a cardiologist, a cardiac surgeon and a cardiac anesthesiologist), according to international guidelines.[31] Acute rejection was defined as an event that necessitated increased immunosuppression with an ISHLT grade ≥ 2R endomyocardial biopsy result or with non-cellular reactions with hemodynamic compromise. Patients underwent routine surveillance for allograft function via endomyocardial biopsy and echocardiography at 1, 2, 3 and 4 weeks, as well as

at 3, 6, 9 and 12 months after transplantation. Posttransplantation vasoplegia was defined according to the ISHLT criteria.[32] Mortality was also assessed, and survival was checked at April 30, 2023.

The maximum vasoactive-inotropic score (VIS) and the length of their administration were also analyzed. VIS was calculated using vasopressor (norepinephrine, epinephrine, vasopressin) and inotropic (dobutamine, dopamine, levosimendan, milrinone) medication doses. For the calculation of IS, the medication doses of dopamine, dobutamine and epinephrine were used.[33]

4.8. Statistical analysis

Data are expressed as the median and interquartile range (first–third [IQR]). Differences between the groups were assessed by the nonparametric Mann–Whitney U test for continuous variables. The association between outcomes and inflammatory variables was tested via Spearman's correlation. Comparisons between interleukins (IL), apolipoproteins and recipient scores were conducted by performing 1-way analysis of variance on the ranks test. Data were analyzed by using IBM-SPSS 22.0 software (International Business Machines Corporation, Armonk, New York, USA). K-means clustering was performed in R (R Foundation for Statistical Computing, Vienna, Austria) using the ComplexHeatmap package and Z-score normalization [34], utilizing the algorithm of Hartigan and Wong [35] with the number of expected clusters defined as 3. Elements of the resulting clusters were further clustered hierarchically. All of the statistical tests were two-sided, and a p value < 0.05 was considered to indicate statistical significance.

5. Conclusions

As the number of patients on the waiting list exceeds the potential of HTX, new prognostic scores or more sensitive biomarkers are needed to determine the optimal candidates for transplantation. The ideal immunesuppression would be a noninvasive strategy that can reliably discriminate the presence and absence of rejection and overimmunosuppression. [36]

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent was obtained from the patient(s) to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy reasons.

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