OPEN

Increase of Cardiac Autoantibodies Against Beta-2-adrenergic Receptor During Acute Cellular Heart Transplant Rejection

Christian Salbach, MD,[1] Philipp Schlegel, MD,[1] Vera Stroikova, MD,[1] Matthias Helmschrott, MD,[1] Anna-Maria Mueller, MD,[1] Christel Weiß, PhD,[2] Evangelos Giannitsis, MD,[1] Norbert Frey, MD,[1] Philip Raake, MD,[3] and Ziya Kaya, MD[1]

Background. Acute cellular rejection (ACR) in heart transplant (HTx) recipients may be accompanied by cardiac cell damage with subsequent exposure to cardiac autoantigens and the production of cardiac autoantibodies (aABs). This study aimed to evaluate a peptide array screening approach for cardiac aABs in HTx recipients during ACR (ACR-HTx). **Methods.** In this retrospective single-center observational study, sera from 37 HTx recipients, as well as age and sex-matched healthy subjects were screened for a total of 130 cardiac aABs of partially overlapping peptide sequences directed against structural proteins using a peptide array approach. **Results.** In ACR-HTx, troponin I (TnI) serum levels were found to be elevated. Here, we could identify aABs against beta-2-adrenergic receptor (β-2AR: EAINCYANETCCDFFTNQAY) to be upregulated in ACR-HTx (intensities: 0.80 versus 1.31, $P = 0.0413$). Likewise, patients positive for β-2AR aABs showed higher TnI serum levels during ACR compared with aAB negative patients (10.0 versus 30.0 ng/L, $P = 0.0375$). Surprisingly, aABs against a sequence of troponin I (TnI: QKIFDLRGKFKRPTLRRV) were found to be downregulated in ACR-HTx (intensities: 3.49 versus 1.13, $P = 0.0025$). A comparison in healthy subjects showed the same TnI sequence to be upregulated in non-ACR-HTx (intensities: 2.19 versus 3.49, $P = 0.0205$), whereas the majority of aABs were suppressed in non-ACR-HTx. **Conclusions.** Our study served as a feasibility analysis for a peptide array screening approach in HTx recipients during ACR and identified 2 different regulated aABs in ACR-HTx. Hence, further multicenter studies are needed to evaluate the prognostic implications of aAB testing and diagnostic or therapeutic consequences.

(*Transplantation* 2024;108: e327–e332).

INTRODUCTION

For a selected group of end-stage heart failure (HF) patients, heart transplantation (HTx) remains the last available treatment option.[1] Here, acute transplant rejection represents a pivotal cause of impaired long-term outcomes. Within the first year after HTx, up to 40% of the patients develop an acute cellular rejection (ACR).[2] Despite the potentially fatal impact of an ACR, there is sparse knowledge about the prevalence and role of cardiac autoantibodies (aABs) in this process. Mechanistically, cardiac damage and cardiomyocyte cell death might cause a presentation of former unknown cellular antigens to the immune system, stimulating the production of cardiac aABs. In other circumstances, such as dilated cardiomyopathy (DCM) or ischemic cardiomyopathy (ICM), cardiac aABs against beta-1-adrenergic receptor (β-1AR) or troponin I (TnI) were shown to be associated with cardiac dysfunction and progressive HF.[3,4] In animal models,

Received 22 December 2023. Revision received 6 March 2024.

Accepted 20 March 2024.

[1] Department of Internal Medicine III, Cardiology, University of Heidelberg, Heidelberg, Germany.

[2] Department of Clinical Statistics, Biomathematics, Information Processing, University of Heidelberg/Mannheim, Mannheim, Germany.

[3] Department of Internal Medicine I, Cardiology, University of Augsburg, Augsburg, Germany.

C.S. and P.S. contributed equally.

C.S., P.S., and Z.K. participated in the research design, writing of the article, performance of the research, and data analysis. V.S. and A.-M.M. participated in research design and data analysis. M.H., E.G., N.F., and P.R. participated in the research design. C.W. participated in data analysis.

This work was supported by the German Research Foundation (DFG KA 1797/4-1 and DFG KA 1797/9-1) and the Else-Fresenius Stiftung (2016_T03).

The authors declare no funding or conflicts of interest.

Supplemental visual abstract; http://links.lww.com/TP/D64.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

Correspondence: Ziya Kaya, MD, Department of Internal Medicine III, Cardiology, University of Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany. (zyia.kaya@med.uni-heidelberg.de).

ISSN: 0041-1337/20/10810-e327

DOI: 10.1097/TP.0000000000005062

experimental immunization using peptide sequences of cardiac autoantigens is known to cause experimental autoimmune myocarditis (EAM) accompanied by HF and DCM phenotype within susceptible mouse strains.[5-8] Recent studies could identify certain immunogenic peptide sequences in common autoantigens such as TnI or cardiac myosin (CM), causing autoimmunity in animal models after immunization or exposition to the immune system.[8-11] Additionally, current evidence suggests a worse prognosis in HTx recipients with the presence of cardiac aAB.[12] However, accounting for the immunosuppression for prevention of fatal rejection, HTx patients represent a unique study population for detection of cardiac aABs. Current literature describes both, aAB directed against cardiac autoantigens of the vascular endothelium such as endothelin-1 type A receptor or vimentin, as well as aABs against structural cardiac proteins such as CM or lamin A/C.[12-14] Albeit, the knowledge about cardiac aAB in HTx recipients during ACR directed against structural proteins remains scarce and their potential impact is not yet elucidated.[12-17] Thus, this study aimed (1) to serve as a feasibility analysis for a screening of cardiac aABs directed against structural proteins in HTx patients using a peptide array approach and (2) to serve as hypothesis-generating approach for evidence of differently regulated cardiac aABs during ACR in HTx patients (ACR-HTx) compared with non-ACR-HTx.

MATERIALS AND METHODS

Study Population and Definitions

In this retrospective, single-center study 37 HTx recipients who presented with ACR between April 2007 and February 2016 in the University Hospital of Heidelberg were included. Serum samples for peptide array analysis were collected during endomyocardial biopsy (EMB). Demographic and clinical data from HTx recipients were obtained retrospectively using electronic medical records. All data were checked by 2 physicians not involved in patient care and a third researcher. Healthy age and sex-matched subjects screened for cardiac abnormalities by cardiac MRI were used as control. ACR stage was assessed in EMB using the International Society for Heart and Lung Transplantation (ISHLT) standardized grading system. Here, 0R and 1R were summarized as non-ACR-HTx, and an ISHLT grading >1 R was defined as ACR-HTx. EMB staining ensured the absence of aAB-mediated rejection.[18] ACR-HTx and non-ACR-HTx patients are the same individuals and are used as their own control group. All subjects gave their informed consent for inclusion before they participated in the study. The study was performed in conformation to the principles of the Declaration of Helsinki of the World Medical Association and approved by the local ethics committee of the University of Heidelberg (S-390/2011, 198/1996).

Peptide Array Technique

For detection of aABs, sera from healthy subjects and HTx patients were screened using PEPperPRINT microarray for IgG aABs as described.[19] Serum samples from HTx patients, collected during EMB were frozen at −80 °C. For conduction of the peptide array analysis, serum samples were thawed and centrifuged at 2.500*g* for 10 min at 4 °C before further processing. Fluorescence intensities of selected IgG aABs directed against predefined peptides were measured. For screening of existing aABs, the peptide array was assembled with corresponding autoantigens. Because most of the screened autoantigens were relatively large and as single antigens exceeding the capacities of a peptide array, antigens were divided into partially overlapping peptide sequences. Thus, a total of 130 partially overlapping peptide sequences from a total of 20 antigens were screened for cardiac aABs. Peptides and proteins were selected based on comprehensive literature research for immunogenic peptide sequences. A table of screened sequences is shown in **Table S1** (**SDC,** http://links.lww.com/TP/D63). Peptide array analysis using PepSlideAnalyzer was performed by PEPperPRINT GmbH Heidelberg. Variance-stabilized intensities of peptide array responses were used for statistical analysis.[20]

Statistical and Data Analyses

Peptide array data were processed with the Statistical Utility for Microarray and Omics data software, developed at the German Cancer Research Center in Heidelberg Germany. For comparison of aAB intensities, the Wilcoxon rank-sum test was used. Continuous variables were expressed either as mean and SD or as median and interquartile range (IQR). For the comparison of continuous parametric variables Student *t* test was used and for nonparametric data, the Mann-Whitney *U* test was used. For analysis of paired samples, paired samples *t* test for normal distributed data or Wilcoxon rank-sum test for non-normal distributed data were used, respectively. Categorical variables were compared using the χ^2 test or Fishers exact test. A 2-sided *P* value of <0.05 was considered as significant. Statistical analyses of the patient data were performed using MedCalc 20.105 (MedCalc Software bvba, Ostend, Belgium). For generation of heat maps from the peptide array intensities GraphPad Prism 7 (GraphPad Software, La Jolla, CA) was used. The data that support the findings of this study are available from the corresponding author upon reasonable request. The corresponding authors and first authors had full access to all data in the study and took responsibility for its integrity and data analysis.

RESULTS

In this retrospective, single-center, study 37 HTx recipients who developed an ACR were included. Sera of these patients were obtained during ACR as well as non-ACR and screened for aABs against structural proteins described in the literature to be associated with autoimmunity and cardiac dysfunction. Mean interval between sample collections was 9 mo (IQR 1–23). The mean age of patients at the time of HTx was 48.9 y (SD 12), and 28 (75.7%) patients were male. The most frequent underlying diseases for HTx were DCM in 21 (56.6%), amyloidosis in 6 (16.2%), and ICM in 6 (16.2%) patients. Comorbidities of HTx patients included arterial hypertension in 43%, dyslipidemia in 46%, and diabetes mellitus in 24%. A full list of baseline characteristics for HTx patients is shown in Table 1.

A comparison of clinical parameters in HTx patients classified by ACR status (non-ACR-HTx versus ACR-HTx) is shown in Table 2. Except for TnI serum levels, which were significantly higher in ACR-HTx, there were no significant differences between ACR-HTx and non-ACR-HTx patients.

TABLE 1.

Baseline characteristics for HTx patients

Variable	s
Age, mean (SD), y	48.9 (12)
Sex, male, n (%$_{all}$)	28 (76)
BMI, mean (SD) kg/m²	26.1 (4)
Interval from transplant to ACR (mo), median (IQR)	16 (9-40)
End-stage disease	
DCM, n (%$_{all}$)	21 (57)
ICM, n (%$_{all}$)	6 (16)
Amyloidosis, n (%$_{all}$)	6 (16)
Sarcoidosis, n (%$_{all}$)	1 (3)
Valvular heart disease, n (%$_{all}$)	2 (5)
D-TGA, n (%$_{all}$)	1 (3)
Comorbidities	
Arterial hypertension, n (%$_{all}$)	16 (43)
Diabetes mellitus, n (%$_{all}$)	9 (24)
Dyslipidemia, n (%$_{all}$)	17 (46)
COPD, n (%$_{all}$)	2 (5)
Malignant neoplasm, n (%$_{all}$)	4 (11)

ACR, acute cellular rejection; BMI, body mass index; COPD, chronic obstructive pulmonary disease; DCM, dilated cardiomyopathy; D-TGA, transposition of the great vessels; HTx, heart transplantation; ICM, ischemic cardiomyopathy.

Identification of Differentially Regulated aABs During ACR

To characterize differently regulated aABs during ACR, sera of non-ACR-HTx and ACR-HTx were screened for IgG aABs. Differentially regulated aABs are shown in Table 3. A heat map of screened IgG aABs is shown in **Figure S1 (SDC,** http://links.lww.com/TP/D63). Our peptide array analysis revealed 2 differences in intensities for IgG aABs in ACR-HTx compared with non-ACR-HTx. A total of 12 (32%) of HTx patients showed upregulated aABs against the peptide sequence of EAINCYANETCCDFFTNQAY corresponding to beta-2-adrenergic receptor (β-2AR). TnI serum levels were elevated compared in ACR-HTx compared with non-ACR-HTx. In patients positive for β-2AR (peptide sequence: EAINCYANETCCDFFTNQAY), TnI serum levels at baseline were similar with a median TnI serum level of 6.45 ng/L (IQR 2.9–25.0) versus 10.0 (IQR 8.2–22.5), P = 0.3293. However, comparing TnI serum levels during ACR revealed higher TnI serum levels in EAINCYANETCCDFFTNQAY aAB positive patients compared with aAB negative patients with a median of 10.0 ng/L (IQR 6.5–35.0) versus 30.0 ng/L (IQR 17.5–100.0), P = 0.0375. Surprisingly, IgG aAB intensities against the peptide sequence QKIFDLRGKFKRPTLRRV corresponding to TnI were significantly downregulated during ACR in 22 (59.5%) patients. Most of the patients with downregulated for TnI aABs were former DCM patients (45.5%), whereas 18.2% had a former ICM or cardiac amyloidosis, respectively. Comparison of other clinical parameters between aAB positive versus aAB negative patients revealed no differences for TnI aAB positive patients.

TABLE 2.

Clinical parameters of HTx patients classified by non-ACR-HTx vs ACR-HTx

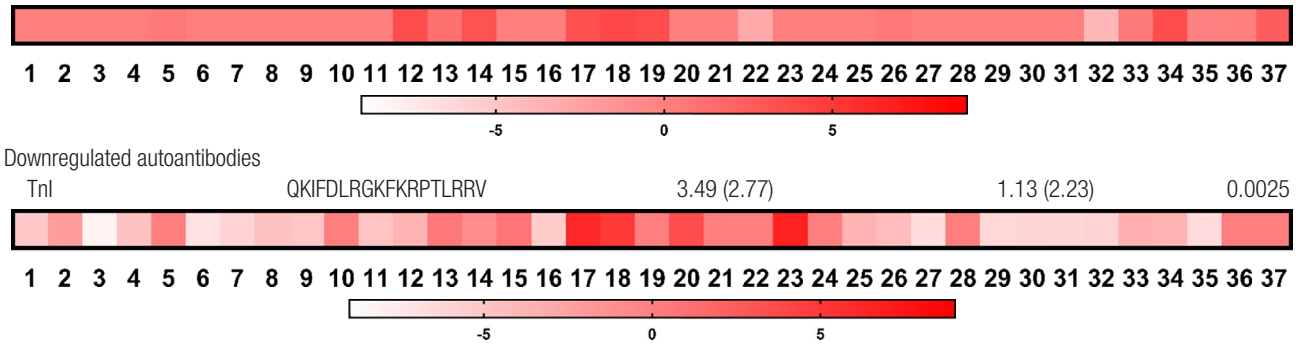
Variable	Non-ACR-HTx, n = 37	ACR-HTx, n = 37	*P*
LVEF			
Preserved, n (%$_{all}$)	29 (78)	33 (89)	0.1357
Moderately impaired, n (%$_{all}$)	5 (14)	2 (5)	0.2354
Severely impaired, n (%$_{all}$)	1 (3)	0 (0)	0.3173
NYHA class			
NYHA I, n (%$_{all}$)	23 (62)	22 (60)	0.3173
NYHA II, n (%$_{all}$)	12 (32)	12 (32)	1.00
NYHA III, n (%$_{all}$)	2 (6)	3 (8)	0.6547
NYHA IV, n (%$_{all}$)	0 (0)	0 (0)	n/a
Immunosuppression			
CSA/Eve, n (%$_{all}$)	4 (11)	3 (8)	1.00
CSA/MMF, n (%$_{all}$)	8 (22)	9 (24)	1.00
MMF/Eve, n (%$_{all}$)	4 (11)	11 (30)	0.1213
Tac/Eve, n (%$_{all}$)	5 (14)	5 (14)	0.7518
Tac/MMF, n (%$_{all}$)	16 (43)	9 (24)	0.2310
Laboratory parameters			
NTproBNP, ng/L, median (IQR)	768 (296–1571), n = 36	1024 (476–1784), n = 36	0.7729
TnI, ng/L, median (IQR)	10.0 (2.9–22.5), n = 37	30.0 (10.0–90.0), n = 36	0.0072
Leucocytes, /nL, median (IQR)	6.1 (4.5–7.4), n = 35	6.0 (4.9–8.1), n = 36	0.1260
CRP, mg/L, median (IQR)	3.9 (2–12), n = 37	2.8 (2–14), n = 37	0.9075
Hb, g/dL median (IQR)	11.3 (9–13), n = 35	11.5 (10–13), n = 36	0.1889
Creatinine, mg/dL, median (IQR)	1.52 (1.1–1.6), n = 37	1.29 (1.0–1.7), n = 37	0.2363

ACR-HTx, acute cellular rejection in heart transplantation recipients; CRP, C-reactive protein; CSA, ciclosporin A; Eve, everolimus; Hb, hemoglobin; IQR, interquartile range; LVEF, left ventricular ejection fraction; MMF, mycophenolate mofetil; NTproBNP, n-terminal brain natriuretic peptide; NYHA, New York Heart Association; Tac, tacrolimus; TnI, troponin I.

TABLE 3.

Differential IgG aABs in patients with ACR-HTx and non-ACR-HTx

Protein	Peptide sequences	Non-ACR-HTx, mean (SD)	ACR-HTx, mean (SD)	*P*
Upregulated autoantibodies				
β-2AR	EAINCYANETCCDFFTNQAY	0.80 (1.65)	1.31 (1.91)	0.0413
Downregulated autoantibodies				
TnI	QKIFDLRGKFKRPTLRRV	3.49 (2.77)	1.13 (2.23)	0.0025



P value was calculated using the Wilcoxon rank-sum test.
aABs, autoantibodies; ACR, acute cellular rejection; ACR-HTx, heart transplantation recipients with ACR; β-2AR, beta-2-adrenergic receptor; TnI, troponin I.

Downregulation of aABs in Healthy Subjects Compared With Non-ACR HTx

Because HTx recipients represent a group of immunosuppressed patients, we sought to compare the prevalence of cardiac aABs in non-ACR-HTx recipients compared with healthy nonimmunosuppressed subjects. A table of significantly different peptide array intensities is shown in **Table S2 (SDC,** http://links.lww.com/TP/D63). A heat map for the comparison of healthy subjects and non-ACR-HTx is shown in **Figure S2 (SDC,** http://links.lww.com/TP/D63). A total of 32 (24.4%) of the screened aABs were downregulated in HTx recipients compared with healthy subjects. However, aABs against TnI peptide sequence (QKIFDLRGKFKRPTLRRV) were significantly upregulated in HTx patients compared with healthy subjects ($P = 0.0205$).

DISCUSSION

Despite significant improvements in recent years, acute rejection and subsequent graft loss of the HTx still remain a life-threatening complication for HTx recipients.[21] Thus, identifying novel factors affecting graft function remains of utmost importance. In recent years, the characterization of the humoral immune response during HTx rejection attained more attention. Peptide arrays offer the possibility of screening for multiple antibodies using a small amount of blood sample.[17] However, screening approaches for cardiac aABs in HTx patients are seldom implemented in clinical routine. Thus, this study aimed to evaluate a peptide array analysis for the detection of cardiac aABs in HTx patients during ACR.

We screened the sera of 37 HTx recipients during ACR using a peptide array technique for a predefined subset of cardiac aABs against proteins or peptides that were described to be associated with autoimmunity in animal models or humans. Mechanistically, cardiac cell damage during ACR reveals formerly unknown autoantigens to the immune system, stimulating the production of cardiac aABs. Due to underlying immunosuppression, HTx recipients represent a unique patient population when focusing on the measurement of immune responses. In our study, we were able to detect an upregulation of aABs corresponding to a peptide sequence of β2-AR, which was significantly upregulated in ACR-HTx. To the best of our knowledge, there is no evidence for cardiac aABs against β2-AR in HTx recipients yet. Recently, aABs against β2-AR were described to be prevalent in patients with acute ST-elevation myocardial infarction with proximal left anterior descending lesions.[22] Although the authors studied a partially overlapping epitope of β2-AR corresponding to amino acids 172 – 192, they hereby discuss that β2-AR could be abnormally expressed in unphysiological conditions such as hypoxia in acute myocardial infarction, causing the formation of cardiac aABs against β2-AR. This mechanism might be transferred to ACR-HTx and could also in part explain our results of an upregulation of β2-AR during ACR. Additionally, for cardiac aABs against β1-AR, there is strong evidence in HF patients; however, results of β1-AR could not be transferred to aABs against β2-AR.[23] During ACR-HTx we also observed elevated TnI serum levels in β2-AR positive, compared with β2-AR-negative patients, which supports the hypothesis that a myocardial injury triggered aAB formation. Cardiac damage during ACR is described to be associated with elevated troponin levels.[24] However, a systematic review on troponin elevation during ACR concluded a poor diagnostic accuracy for stand-alone troponin elevation in ACR-HTx. Thus, additional biomarkers such as cardiac aABs against β2-AR might help identify patients developing an ACR. Additionally, the amount of calcineurin inhibitor (CNI)-free immunosuppression in ACR-HTx was higher compared with non-ACR-HTx. This is in accordance with previous data from the MANDELA trial, where CNI-free immunosuppression protocols were found to be associated with more frequent ACRs.[25] Surprisingly, we identified aABs targeting the sequence of TnI QKIFDLRGKFKRPTLRRV to be downregulated in ACR-HTx and found aABs against the same peptide sequence to be upregulated in non-ACR-HTx compared with healthy individuals. The

TnI peptide sequence of QKIFDLRGKFKRPTLRRV, corresponding to amino acids 130–147 of the TnI protein was already described to induce EAM in susceptible mice strains and corresponds to the upstream of helix H2 of whole TnI protein.[8,26] Using an ELISA, Doesch et al[3] previously investigated TnI aABs in DCM and ICM patients and described an unsuspected ambivalent role of TnI aABs in their study since the presence of TnI aABs in 23% of DCM patients was associated with increased survival. The authors assumed the protective effect of TnI aABs to be mediated by a more robust immune system or a higher capacity of reverse remodeling in DCM patients. In our cohort, however, 45.5% of TnI aAB positive ACR-HTx patients had a former DCM before HTx. Thus, an underlying disease-specific effect in our study remains unlikely. The role and mechanism of action of the observed upregulation of TnI aABs in DCM patients in the study from Doesch et al[3] and the downregulation in our peptide array screening during ACR-HTx remains controversial. Animal data suggest contrary effects since in mice, immunization using the TnI protein or TnI peptide sequences triggers the development of TnI aABs which induce HF with a DCM phenotype.[6,8,27] However, one of the reasons for the detected downregulation of TnI aABs during ACR could be an interference of cardiac TnI aABs and released cardiac TnI during ACR. As already described by Eriksson et al,[28] TnI antibodies are able to bind serum TnI and cause a delay and confounding in TnI measurement in patients with acute myocardial infarction. Because the peptide array technique also relies on ELISA, one could assume an interference. Here, TnI aABs could cause binding of the released TnI protein, and a subsequent false negative peptide array result for this TnI aAB sequence. The fact that the observed effect of TnI sequence corresponds to the upstream of TnI helix H2, which is a noncryptic part of the TnI protein, where aABs could potentially bind supports this hypothesis.[26] However, the observed result of a downregulated subproportion of TnI aABs during ACR highlights the importance of further research on cardiac TnI aABs in HTx patients.

Limitations

This retrospective single-center study serves as feasibility analysis of a peptide array screening approach for cardiac aABs in HTx patients developing an ACR. This study is neither designed nor powered to validate peptide arrays for screening tools in HTx patients.

The current analysis could not provide confirmatory data to prove the peptide array reactivity with true specificity for the described results. Owing to the pivotal descriptive, hypothesis-generating design, a larger multicenter approach is needed, which should also include confirmatory data for the peptide array specificity. The study was neither designed nor powered to access a pathophysiologic relevance and provide adequate powered longitudinal data. Thus, no pathophysiologic relevance of our results could be concluded. Because of the retrospective design of the study, clinical data were accessed from electronic patient records with the respective limitations in data quality and control. Therefore, a systematic underestimation of the results cannot be fully excluded.

Strengths

Because HTx patients represent a small but special patient population, this study was able to characterize differentially regulated cardiac aABs in post-HTx patients in the presence and absence of ACR. Herein, we could show the feasibility of a screening approach for cardiac aABs in HTx patients, enabling further research for autoimmune mechanisms within this field.

CONCLUSIONS

Our study was able to collect evidence for differentially regulated cardiac aABs against structural proteins in HTx patients during ACR. We designed this study as feasibility analysis for a peptide array screening approach in HTx recipients and were able to identify 2 differentially regulated aABs against β2-AR and TnI in ACR-HTx patients. These data pave the ground for further studies assessing aABs in this patient population. High throughput screening might help to better understand the clinical significance of aABs against structural cardiac proteins potentially identifying patients at risk.

ACKNOWLEDGMENTS

The authors acknowledge Renate Oettl for technical assistance.

REFERENCES

1. Kim IC, Youn JC, Kobashigawa JA. The past, present and future of heart transplantation. *Korean Circ J*. 2018;48:565–590.
2. Costanzo MR, Dipchand A, Starling R, et al; International Society of Heart and Lung Transplantation Guidelines. The International Society of Heart and Lung Transplantation guidelines for the care of heart transplant recipients. *J heart lung transplant*. 2010;29:914–956.
3. Doesch AO, Mueller S, Nelles M, et al. Impact of troponin I-autoantibodies in chronic dilated and ischemic cardiomyopathy. *Basic Res Cardiol*. 2011;106:25–35.
4. Dungen HD, Dordevic A, Felix SB, et al. beta1-Adrenoreceptor autoantibodies in heart failure: physiology and therapeutic implications. *Circ Heart Fail*. 2020;13:e006155.
5. Neu N, Rose NR, Beisel KW, et al. Cardiac myosin induces myocarditis in genetically predisposed mice. *J Immunol*. 1987;139:3630–3636.
6. Kaya Z, Katus HA, Rose NR. Cardiac troponins and autoimmunity: their role in the pathogenesis of myocarditis and of heart failure. *Clin Immunol*. 2010;134:80–88.
7. Kaya Z, Leib C, Katus HA. Autoantibodies in heart failure and cardiac dysfunction. *Circ Res*. 2012;110:145–158.
8. Kaya Z, Goser S, Buss SJ, et al. Identification of cardiac troponin I sequence motifs leading to heart failure by induction of myocardial inflammation and fibrosis. *Circulation*. 2008;118:2063–2072.
9. Pummerer CL, Luze K, Grassl G, et al. Identification of cardiac myosin peptides capable of inducing autoimmune myocarditis in BALB/c mice. *J Clin Invest*. 1996;97:2057–2062.
10. Okazaki T, Tanaka Y, Nishio R, et al. Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. *Nat Med*. 2003;9:1477–1483.
11. Muller AM, Bockstahler M, Hristov G, et al. Identification of novel antigens contributing to autoimmunity in cardiovascular diseases. *Clin Immunol*. 2016;173:64–75.
12. See SB, Mantell BS, Clerkin KJ, et al. Profiling non-HLA antibody responses in antibody-mediated rejection following heart transplantation. *Am J Transplant*. 2020;20:2571–2580.
13. Nunoda S. Impact of pretransplant antibodies on outcomes after heart transplantation. *Curr Opin Organ Transplant*. 2019;24:220–226.
14. Nath DS, Ilias Basha H, Tiriveedhi V, et al. Characterization of immune responses to cardiac self-antigens myosin and vimentin in human cardiac allograft recipients with antibody-mediated rejection and cardiac allograft vasculopathy. *J Heart Lung Transplant*. 2010;29:1277–1285.

15. Zhang Q, Cecka JM, Gjertson DW, et al. HLA and MICA: targets of antibody-mediated rejection in heart transplantation. *Transplantation*. 2011;91:1153–1158.
16. Grafft CA, Cornell LD, Gloor JM, et al. Antibody-mediated rejection following transplantation from an HLA-identical sibling. *Nephrol Dial Transplant*. 2010;25:307–310.
17. Chruscinski A, Huang FY, Nguyen A, et al. Generation of antigen microarrays to screen for autoantibodies in heart failure and heart transplantation. *PLoS One*. 2016;11:e0151224.
18. Stewart S, Winters GL, Fishbein MC, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J heart lung Transplant*. 2005;24:1710–1720.
19. Maguy A, Tardif JC, Busseuil D, et al. Autoantibody signature in cardiac arrest. *Circulation*. 2020;141:1764–1774.
20. Huber W, von Heydebreck A, Sultmann H, et al. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. *Bioinformatics*. 2002;18(suppl_1) :S96–104.
21. Tonsho M, Michel S, Ahmed Z, et al. Heart transplantation: challenges facing the field. *Cold Spring Harb Perspect Med*. 2014;4:a015636.
22. Dang W, Cao N, Zhang Y, et al. Association among beta2-adrenergic receptor autoantibodies and proximal left anterior descending artery lesions in patients with initial ST-segment elevation myocardial infarction. *Clin Cardiol*. 2023;46:1371–1379.
23. Düngen HD, Dordevic A, Felix SB, et al. β(1)-Adrenoreceptor autoantibodies in heart failure: physiology and therapeutic implications. *Circ Heart Fail*. 2020;13:e006155.
24. Liu Z, Perry LA, Penny-Dimri JC, et al. Elevated cardiac troponin to detect acute cellular rejection after cardiac transplantation: a systematic review and meta-analysis. *Transpl Int*. 2022;35:10362.
25. Barten MJ, Hirt SW, Garbade J, et al. Comparing everolimus-based immunosuppression with reduction or withdrawal of calcineurin inhibitor reduction from six months after heart transplantation: the randomized MANDELA study. *Am J Transplant*. 2019;19:3006–3017.
26. Marston S, Zamora JE. Troponin structure and function: a view of recent progress. *J Muscle Res Cell Motil*. 2020;41:71–89.
27. Goser S, Andrassy M, Buss SJ, et al. Cardiac troponin I but not cardiac troponin T induces severe autoimmune inflammation in the myocardium. *Circulation*. 2006;114:1693–1702.
28. Eriksson S, Hellman J, Pettersson K. Autoantibodies against cardiac troponins. *N Engl J Med*. 2005;352:98–100.