ESC HEART FAILURE
ESC Heart Failure (2023)
Published online in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.14442

ORIGINAL ARTICLE

Heart transplantation and anti-HLA antibodY: myocardial dysfunction and prognosis - HeartLAy study

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Abstract

Aims The presence of anti-human leucocyte antigen (HLA) antibodies has been implicated in a higher incidence of complications as well as mortality rate in heart transplantation. The aim of the study was to identify through non-invasive parameters early signs of myocardial dysfunction in the presence of anti-HLA antibodies but without evidence of antibody-mediated rejection (AMR) and its possible prognostic impact.

Methods and results A total of 113 heart-transplanted patients without acute cellular rejection (ACR) and AMR or cardiac allograft vasculopathy (CAV) were prospectively enrolled and divided into two groups ['HLA+' (50 patients) and 'HLA−' (63 patients)], based on the presence of anti-HLA antibodies. Each patient was followed for 2 years after the enrolment, recording episodes of AMR, ACR, CAV, and mortality. Clinical characteristics were similar between the two groups. Among laboratory data, N-terminal pro-B-type natriuretic peptide and high-sensitivity cardiac troponin values were significantly higher in the presence of anti-HLA antibodies ($P < 0.001$ and $P = 0.003$, respectively). The echocardiographic parameters that showed a statistically significant difference between the two groups were deceleration time of E wave (DecT E, $P < 0.001$), left ventricular global longitudinal strain ($P < 0.001$), tricuspid annular plane systolic excursion ($P = 0.011$), tricuspid S' wave ($P = 0.002$), and free wall right ventricular longitudinal strain (fwRVLS, $P = 0.027$), whereas left atrial strain did not differ significantly ($P = 0.408$). Univariate analysis showed that anti-HLA antibodies were associated with the development of CAV at both 1 and 2 year follow-up [odds ratio (OR) 11.90, 95% confidence interval (CI) 1.43–90.79, $P = 0.022$ and OR 3.37, 95% CI 1.78–9.67, $P = 0.024$, respectively]. Bivariate analysis demonstrated that both fwRVLS and DecT E were predictors of CAV development independently from HLA status.

Conclusions The presence of circulating anti-HLA antibodies is correlated with a mild cardiac dysfunction, even in the absence of AMR, and CAV development. Interestingly, reduced values of DecT E and fwRVLS were predictors of future development of CAV, independently from anti-HLA antibody.

Keywords Heart transplantation; Anti-HLA antibody; Antibody-mediated rejection; Prognosis

Received: 29 April 2023; Accepted: 8 June 2023
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Introduction

The immunocompatibility between the donor and recipient in heart transplantation (HTx) is essential to promote a good quality of life as well as a higher long-term survival. The development of anti-human leucocyte antigen (HLA) antibody after transplant, defined as *de novo* antibodies, has been associated with allograft injury. In fact, the presence of these circulating antibodies leads to the activation of the recipient immune response against the graft, principally through the

activation of the complement cascade. This immune-mediated process involves the allograft endothelium, inducing an acute injury, which could finally lead to myocardial injury and graft dysfunction.[1,2] Several studies attested the prognostic role of anti-HLA antibody, in particular donor-specific antibody (DSA),[3,4] whose development is correlated to both antibody-mediated rejection (AMR) and cardiac allograft vasculopathy (CAV).[5,6] Despite their key role in inducing acute endothelial injury, the cornerstone lesion of AMR, their presence is not necessarily required to diagnose AMR, which is based on the histological evidence of acute capillary injury or immunopathological evidence for antibody-mediated injury, in accordance to the International Society for Heart and Lung Transplantation (ISHLT).[7] The development of anti-HLA antibody might be a red flag for an insufficient immunosuppressive regimen and for subclinical rejections,[8] because they are the expression of the activation of the recipient's immune system against the donor graft. In this context, echocardiography, which represents the cornerstone imaging technique for HTx patients' follow-up, has proven to be useful in detecting myocardial alterations induced by acute rejections, in particular acute cellular rejection (ACR) and more recently also AMR, as well as CAV.[9,10] In particular, diastolic function and myocardial strain have emerged as strong prognostic factors in HTx, especially regarding allograft rejection.[11]

Based on these premises, the aim of our study was to identify through non-invasive parameters, such as clinical, laboratory, and echocardiographic ones, early signs of myocardial dysfunction in HTx patients in the presence of anti-HLA antibodies but without AMR assessed through endomyocardial biopsy. Furthermore, the secondary objectives of our study were firstly to assess the prognostic role of anti-HLA antibody and secondly to identify other potential prognostic parameters in HTx patients, in particular if any echocardiographic parameter could be associated with a worse outcome during follow-up.

Methods

Population and study design

The HeartLAy study (Heart transplantation and anti-HLA antibodY: myocardial dysfunction and prognosis) is a monocentric prospective observational study conducted at the Heart Transplantation Center of University of Siena. A total of 138 heart-transplanted patients requiring a routine endomyocardial biopsy, according to our centre's follow-up protocol, were screened between 1 March 2017 and 1 October 2020. Patients were excluded in the presence of recent HTx (<3 months), baseline left ventricular (LV) ejection fraction (LVEF) ≤50%, wall motion abnormality, inadequate acoustic window, evidence of histological AMR or ACR, CAV, atrial fibrillation at the time of the exam, severe heart valve disease, severe renal disease with an estimated glomerular filtration rate <30 mL/min according to Cockcroft–Gault equation, and anti-HLA antibodies before HTx. All the subjects underwent a transthoracic echocardiographic examination the same day of the endomyocardial biopsy. Based upon the presence or absence of circulating anti-HLA antibodies, the study population was divided into two groups: 'HLA+' and 'HLA−', respectively. Each patient was prospectively followed for 2 years from the time of the enrolment in the study, so the study ended on 1 October 2022. During follow-up, each event defined as ACR or AMR episodes, diagnosis of CAV, or cardiovascular death was recorded at both 1 and 2 years. Finally, major adverse cardiovascular events (MACEs) were defined as ACR and AMR episodes, CAV development, and mortality during the 2 year follow-up. The diagnosis of CAV after the enrolment was diagnosed at routine angiography performed as follow-up. The presence of coronary atherosclerosis was excluded before HTx or at the 1 year follow-up angiography. The patients in which CAV or donor atherosclerosis was still unknown were excluded. The research design complied with the Declaration of Helsinki, and the study was approved by our local ethics committee. All subjects gave a written informed consent for participation in the study.

Echocardiography

The echocardiographic examination was performed by an expert cardiologist, using a high-quality echocardiograph (Vivid E9, GE, Milwaukee, WI, USA) equipped with a 3 MHz transducer. The echocardiographic exam was performed on the same day of the endomyocardial biopsy and new determination of anti-HLA antibodies status. The echocardiographic exam included the assessment of diastolic function through the measurement of peak early (E) and late (A) diastolic transmitral flow velocity and E/A ratio, deceleration time (DecT) of E wave, pulsed-wave tissue Doppler systolic (S') and early diastolic (E') velocities at both septal and lateral mitral annulus, and E/e' ratio as an estimate of LV filling pressure. Right ventricular (RV), LV, and left atrial (LA) dimensional parameters were evaluated according to the American Society of Echocardiography (ASE) recommendations.[12] Furthermore, parameters of RV function such as RV fractional area change (RVFAC), tricuspid annular plane systolic excursion (TAPSE), and tricuspid pulsed-wave tissue Doppler systolic (S') velocity were assessed as well as LVEF, which was measured using the biplane method (modified Simpson's rule) in apical four- and two-chamber views.[12]

A semi-automated two-dimensional strain software (EchoPAC, GE, Milwaukee, WI, USA) was used for speckle tracking echocardiography analysis, which the same cardiolo-

gist with high experience performed. The frame rate was set between 60 and 80 frames per second, and the images were acquired from the apical four-, three-, and two-chamber images using conventional two-dimensional greyscale echocardiography during brief breath-hold and with a stable electrocardiogram (ECG) recording. The software automatically traced the endocardial border, and it was manually corrected if necessary. Regarding LV strain analysis, LV global longitudinal strain (LV-GLS) was calculated averaging the value of longitudinal strain measured in apical four-, two-, and three-chamber views. RV strain analysis assessed RV longitudinal strain of free wall in an RV-focused apical four-chamber view. Both peak atrial longitudinal strain (PALS) and peak atrial contraction strain (PACS), obtained by averaging the value of LA strain measured in apical four- and two-chamber views, were assessed. The segments with insufficient tissue tracking were first readjusted manually, and if this was ineffective, they were excluded from further analysis. All echocardiographic parameters were averaged over three consecutive cardiac cycles.

Anti-human leucocyte antigen antibody testing

HLA antibodies and their specificity were determined using Luminex technology (Luminex Corporation). Sensitized patients were tested for both Class I (HLA-A, HLA-B, and HLA-C) and Class II (HLA-DR, HLA-DQ, and HLA-DP) anti-HLA antibodies using Luminex single-antigen beads technique (One Lambda). The blood sample to assess the presence of circulating anti-HLA antibodies was drawn on the same day of the echocardiographic exam. Only patients with *de novo* anti-HLA antibodies were enrolled, whereas sensitized patients prior to HTx were excluded. Anti-HLA antibodies were considered positive if the mean fluorescence intensity (MFI) was 1000 or more. Patients with anti-HLA antibodies were treated in accordance to our protocol in the presence of very high MFI values (e.g. above 10 000) or in the presence of MFI associated with signs of heart failure or decline in LVEF.

Endomyocardial biopsy

At our centre, endomyocardial biopsies are performed every week during the first month after HTx, every 2 weeks until the sixth month, and then every year after HTx. All biopsies were performed with right heart catheterization from standard percutaneous femoral or internal jugular venous approaches using a 5.5 F bioptome positioned along the RV septum. From each patient, a total of four endomyocardial tissue samples were obtained and immediately placed in formalin. Three of the four samples were then fixed in paraffin and were subjected to haematoxylin–eosin staining. The fourth tissue sample was used for immunopathological study, in particular, to test the presence of C4d and CD68+ macrophages through the immunoperoxidase technique. An anatomopathologist expert in the field proceeds then to the interpretation and classification of both ACR and AMR according to the latest guidelines.[7,13]

Outcomes

ACR was defined in the presence of grade ACR $\geq$ 1R, according to the latest ISHLT classification.[13] The diagnosis of AMR was made in the presence of histopathological and/or immunopathological criteria and was considered in the presence of pathologic AMR (pAMR) $\geq$1.[7] Finally, CAV was diagnosed by coronary angiography and defined in the presence of CAV $\geq$ 1, in accordance to the latest ISHLT classification.[14] Finally, cardiovascular mortality was considered. The occurrence of each event was reported for the first year and the second year of follow-up.

Statistical analysis

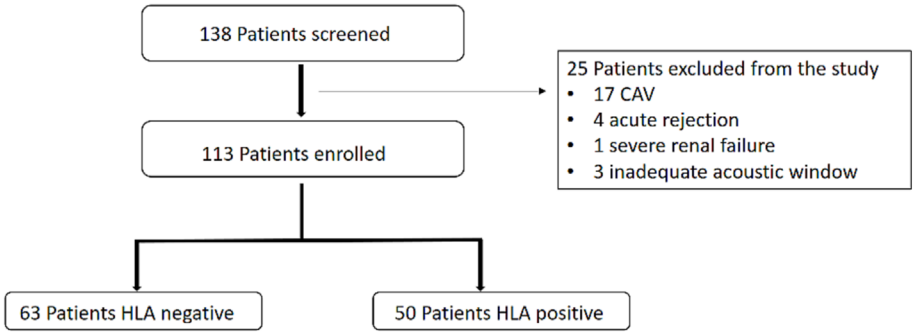
Continuous data are presented as mean and standard deviation or as median and inter-quartile range (IQR), as appropriate. Kolmogorov–Smirnov test was used to verify normal distribution of variables. Categorical data are summarized as absolute and relative frequencies. Continuous variables were compared using the unpaired *t*-test for normally distributed variables and the nonparametric Mann–Whitney *U* test for non-normally distributed variables. The χ^2 test was used for categorical variables. Univariate and bivariate regression analysis was performed to calculate the odds ratio (OR) for each outcome for the independent variables of interest. A *P* value $<$0.05 was considered statistically significant. Analysis was performed using SPSS, Version 26 (SPSS, Chicago, IL, USA).

Results

Study population

Among the initial 138 screened HTx patients, a total of 25 patients were excluded due to inadequate acoustic window (*n* = 3), evidence of AMR or ACR (*n* = 4), CAV (*n* = 17), and severe renal disease (*n* = 1). Thus, 113 patients were finally enrolled in the study. Fifty of 113 patients were included in the HLA+ group, whereas 63 of 113 patients were included in the HLA− group, based upon the presence or absence of circulating anti-HLA antibodies, respectively. *Figure 1* shows the design study.

Figure 1 Design study. CAV, cardiac allograft vasculopathy; HLA, human leucocyte antigen.



Demographic characteristics

Table 1 summarizes the main clinical and laboratory features of the study population also stratified for the HLA status. The majority of the patients were male (77%), and the median age was 62 years (IQR 54–69 years) and did not differ significantly between HLA− and HLA+ patients (P = 0.952). Both the age of the graft and the distance from HTx did not show any difference between the two groups (P = 0.540 and P = 0.913, respectively). Among the whole study population, only one patient had a successful pregnancy and two patients were bridged with an LV assist device (LVAD) before HTx, and each of these patients belonged to HLA+ group. Around 23% of the total population had cytomegalovirus (CMV) donor/recipient mismatch. No difference in CMV donor/recipient mismatch was found between the two groups (P = 0.300). Regarding the laboratory parameters, both N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin I showed significantly higher values in the HLA+ group ($P < 0.001$ and P = 0.003, respectively). Among HLA+ patients, Class I anti-HLA antibodies were present in 26 of 50 patients whereas Class II anti-HLA antibodies were found in 38 of 50 patients. A total of 23 out of 50 patients had DSA. The mean MFI for Class I and II anti-HLA antibodies was 1391 and 4662, respectively. Finally, the median time of anti-HLA antibody development after HTx was 3 years (IQR 1–8 years). During 2 year follow-up, in HLA− group, five patients developed anti-HLA antibodies during follow-up, and two of them showed DSAs, whereas six patients showed only the transitory presence of anti-HLA antibodies in single determination.

Echocardiographic findings

Table 1 reports the echocardiographic parameters of the study population. Among the two groups, either biventricular dimensions or atrial dimensions did not show any differences. Regarding diastolic indexes, deceleration time of E wave (DecT E) was the parameter that showed a statistically significant difference between the two groups [188 ms (IQR 180–209 ms) in HLA− vs. 163 ms (IQR 140–180 ms) in HLA +, $P < 0.001$]. Differences in lateral and septal E′ and S′ tissue Doppler imaging (TDI)-derived velocities reached statistical significance between the two groups (lateral S′ P = 0.040, lateral E′ P = 0.035, septal S′ P = 0.004, and septal E′ P = 0.002). RV systolic indexes, such as TAPSE and tricuspid S′ velocity, showed significantly lower values in HLA+ group (P = 0.011 and P = 0.002, respectively). Regarding the analysis of longitudinal strain, a significant difference between the two groups was registered for both LV-GLS [−19.6% (IQR −20.5 to −18.7) vs. −15.8% (IQR −18.5 to −14.3), $P < 0.001$] and free wall RV longitudinal strain (fwRVLS) (−19.6 ± 4.0 vs. −17.8 ± 3.5, P = 0.027), with lower absolute values in patients with anti-HLA antibodies. LA strain analysis did not show any differences between the two groups (PALS P = 0.408 and PACS P = 0.702).

Outcomes

During the 2 year follow-up, 65 events occurred in the total population, of which 49 occurred in HLA+ group and 16 occurred in HLA− group, as summarized in *Table 2*. All AMR episodes, of which nine cases occurred at 1 year and six cases within 2 years, occurred in HLA+ group ($P < 0.001$ and P = 0.004, respectively). No cardiovascular death was registered at 1 year, whereas two patients with anti-HLA antibodies died at 2 year follow-up. The incidence of CAV was higher in the HLA+ group compared with HLA− group, with 8 cases vs. 1 case at 1 year and 11 cases vs. 8 cases at 2 year follow-up (P = 0.004 and P = 0.015, respectively). In the HLA− group, all CAV cases were graded as CAV 1, whereas in the HLA+ group, seven cases were graded as CAV 1, three cases as CAV 2, and only one case of severe CAV defined as CAV 3.

Table 1 Baseline clinical, laboratory, and echocardiographic characteristics of general population

Clinical characteristics	All (n = 113)	HLA− (n = 63)	HLA+ (n = 50)	P value
Age (years)	62 (54–69)	62 (54–67)	62 (51–69)	0.952
Male sex	87 (77)	50 (79)	37 (74)	0.501
BSA (m^2)	1.94 ± 0.19	1.97 (1.82–2.08)	1.94 (1.77–2.03)	0.091
Heart rate (b.p.m.)	79 ± 8.7	78 ± 8.5	80 ± 8.9	0.689
SBP (mmHg)	131 ± 11.8	129 ± 10.2	132 ± 13.3	0.320
DBP (mmHg)	78 (72–84)	77 (70–83)	78 (72–86)	0.489
Time from HTx (years)	7 (3–12)	7 (3–12)	7 (2–12)	0.913
Graft age (years)	48 (37–58)	52 (37–61)	46 (39–56)	0.540
CMV D+/R− mismatch	26 (23)	13 (21)	13 (26)	0.300
Diabetes (%)	31 (27)	13 (20)	18 (36)	0.059
Arterial hypertension (%)	87 (77)	50 (79)	37 (74)	0.514
Dyslipidaemia (%)	66 (58)	32 (51)	34 (68)	0.047
Smoke (%)	4 (3)	1 (1.5)	3 (6)	0.199
Corticosteroids	71 (63)	35 (55)	36 (72)	0.051
CNI	105 (93)	57 (90)	48 (96)	0.105
mTORi	21 (18)	10 (16)	11 (22)	0.376
MMF	71 (63)	39 (62)	32 (64)	0.711
Antiplatelet therapy	91 (80)	50 (80)	41 (82)	0.438
Statin	71 (63)	38 (63)	33 (66)	0.422
eGFR[a] (mL/min)	72.0 ± 29.9	73.0 (50.0–93.0)	61 (49–89)	0.257
Altered hepatic function[a]	1 (0.9)	0 (0)	1 (2)	0.367
NT-proBNP (pg/mL)	501 (291–1185)	357 (252–689)	769 (395–1651)	<0.001
Hs troponin I (ng/L)	17 (11–28)	13.0 (9.7–22.7)	21 (14–37)	0.003
IVS (mm)	1.2 (1.0–1.2)	1.1 (1.0–1.2)	1.2 (1.1–1.3)	0.024
PW (mm)	1.1 (1.0–1.2)	1.0 (1.0–1.2)	1.1 (1.0–1.2)	0.111
LV-EDD (mm)	45 (42–49)	44 (42–49)	45 (41–47)	0.640
LV-ESD (mm)	25 (23–29)	24 (23–29)	27 (22–29)	0.430
LV-EDV (mL)	78 (68–94)	79.5 (70.0–97.2)	72.0 (63.0–85.5)	0.089
LV-ESV (mL)	30 (25–36)	34.0 (26.5–40.0)	26.0 (25.0–34.0)	0.019
LV-EDVi (mL/mq)	42.1 ± 11.9	42.7 ± 14.3	41.3 ± 8.8	0.590
LV-ESVi (mL/mq)	15.3 (12.5–19.5)	15.8 (12.7–19.5)	15.0 (12.2–18.5)	0.359
SV (mL)	46 (41–60)	48 (41–60)	43 (39–52)	0.087
LVEF (%)	60 (58–60)	60 (57–60)	60 (58–61)	0.211
RV-EDDm (mm)	31.(28.0–34.0)	32.0 (29.5–34.0)	31.0 (28.0–34.0)	0.542
TAPSE (mm)	15 (13–17)	15 (14–18)	14 (12–16)	0.011
Tricuspid S′ wave	0.10 (0.08–0.12)	0.10 (0.09–0.12)	0.09 (0.07–0.11)	0.002
RVFAC (mm)	45 (40–45)	45 (40–45)	44 (44–49)	0.690
sPAP (mmHg)	26 (25–30)	25 (25–30)	27 (25–31)	0.408
LA area (cm^2)	26 (22–30)	26 (23–30)	25 (22–29)	0.489
LA volume (mL)	84 (67–100)	87 (70–100)	81 (64–99)	0.328
LAVI (mL/mq)	42.3 (35.7–52.7)	43.5 (35.9–51.3)	41.9 (34.8–55.8)	0.984
RA area (cm^2)	18.1 (14.2–21.0)	17 (14–21)	18.5 (15.2–21.3)	0.261
E wave	0.82 ± 0.20	0.79 ± 0.21	0.86 ± 0.19	0.936
A wave	0.40 (0.34–0.47)	0.39 (0.33–0.45)	0.42 (0.36–0.51)	0.062
E/A ratio	1.9 (1.7–2.5)	1.9 (1.6–2.4)	1.9 (1.6–2.4)	0.806
DecT E (ms)	180 (162–198)	188 (180–209)	163 (140–180)	<0.001
Lateral S′ wave	0.09 (0.08–0.12)	0.10 (0.09–0.12)	0.09 (0.07–0.11)	0.040
Lateral E′ wave	0.15 (0.12–0.16)	0.15 (0.14–0.16)	0.13 (0.10–0.16)	0.035
Septal S′ wave	0.08 (0.07–0.09)	0.10 (0.09–0.12)	0.07 (0.06–0.08)	0.004
Septal E′ wave	0.08 (0.07–0.10)	0.08 (0.07–0.11)	0.07 (0.08–0.09)	0.002
E/e′ ratio	8 (6–10)	7 (6–8)	8 (6–10)	0.110
PALS (%)	12.6 ± 4.4	13.0 ± 3.7	12.2 ± 5.1	0.408
PACS (%)	4.7 (3.0–6.5)	4.4 (3.2–6.3)	4.8 (2.9–6.5)	0.702
LV-GLS (%)	−18.9 (−19.9 to −16.4)	−19.6 (−20.5 to −18.7)	−15.8 (−18.5 to −14.3)	<0.001
fwRVLS (%)	−18.9 ± 3.9	−19.6 ± 4.0	−17.8 ± 3.5	0.027

BSA, body surface area; CMV, cytomegalovirus; CNI, calcineurin inhibitors (tacrolimus/ciclosporin); DBP, diastolic blood pressure; DecT E, deceleration time of E wave; eGFR, estimated glomerular filtration rate with Cockcroft–Gault equation; fwRVLS, free wall right ventricular longitudinal strain; HLA, human leucocyte antigen; Hs, high-sensitivity; HTx, heart transplantation; IVS, interventricular septum; LA, left atrial; LAVI, left atrial volume index; LV-EDD, left ventricular end-diastolic diameter; LV-EDV, left ventricular end-diastolic volume; LV-EDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LV-ESD, left ventricular end-systolic diameter; LV-ESV, left ventricular end-systolic volume; LV-ESVi, left ventricular end-systolic volume index; LV-GLS, left ventricular global longitudinal strain; MMF, mycophenolate mofetil; mTORi, mammalian target of rapamycin inhibitors (sirolimus/everolimus); NT-proBNP, N-terminal pro-B-type natriuretic peptide; PACS, peak atrial contraction strain; PALS, peak atrial longitudinal strain; PW, posterior wall; RA, right atrial; RV-EDDm, medium right ventricular end-diastolic diameter; RVFAC, right ventricular fractional area change; SBP, systolic blood pressure; sPAP, systolic pulmonary artery pressure; SV, stroke volume; TAPSE, tricuspid annular plane systolic excursion.
[a]Defined in the presence of transaminase levels above the upper limit of normality.

Table 2 Univariate analysis between anti-HLA antibodies and outcomes

Events	All (n = 113)	HLA− (n = 63)	HLA+ (n = 50)	P value	OR (95% CI)	P value
AMR 1 year	9 (8)	0 (0)	9 (18)	<0.001	—	—
ACR 1 year	14 (12)	5 (8)	9 (18)	0.089	2.57 (0.80–8.23)	0.113
CAV 1 year	9 (8)	1 (2)	8 (16)	0.004	11.90 (1.43–90.79)	0.022
Mortality 1 year	0	0	0	—	—	—
AMR 2 years	6 (5)	0 (0)	6 (12)	0.004	—	—
ACR 2 years	6 (5)	2 (3)	4 (8)	0.234	2.67 (0.47–15.21)	0.269
CAV 2 years	19 (17)	8 (13)	11 (22)	0.015	3.37 (1.78–9.67)	0.024
Mortality 2 years	2 (2)	0	2 (4)	0.102	—	—
MACEs	37 (33)	12 (19)	25 (50)	<0.001	4.25 (1.84–9.83)	0.001

ACR, acute cellular rejection; AMR, antibody-mediated rejection; CAV, cardiac allograft vasculopathy; CI, confidence interval; HLA, human leucocyte antigen; MACEs, major adverse cardiovascular events defined as ACR and AMR episodes, CAV development, and mortality during follow-up; OR, odds ratio.

Associations of outcomes

Univariate ORs for outcomes of anti-HLA antibody are shown in *Table 2*. The presence of anti-HLA antibody was associated with 1 year [OR 11.90, 95% confidence interval (CI) 1.43–90.79, P = 0.022] and 2 year CAV incidence (OR 3.37, 95% CI 1.78–9.67, P = 0.024). Due to the absence of AMR episodes as well as 2 year cardiovascular death in HLA− group, the univariate and bivariate analysis between these outcomes and anti-HLA antibody could not be performed. *Table 3* shows the associations of clinical, laboratory, and echocardiographic variables with outcomes. Among clinical and biochemical parameters, time from HTx showed a correlation with the number of ACR episodes at 1 year follow-up (OR 0.83, 95% CI 0.71–0.97, P = 0.017) as well as CMV donor/recipient mismatch with CAV development both at 1 year (OR 4.76, 95% CI 1.17–19.3, P = 0.029) and 2 year follow-up (OR 3.29, 95% CI 1.14–9.52, P = 0.28). Among echocardiographic parameters, DecT E was associated with 1 year AMR (OR 0.97, 95% CI 0.95–1.00, P = 0.021), 1 year CAV (OR 0.96, 95% CI 0.93–0.99, P = 0.004), and 2 year CAV incidence (OR 0.98, 95% CI 0.96–0.99, P = 0.011). Among strain parameters, LV-GLS was significantly associated with 1 year AMR (OR 1.61, 95% CI 1.21–2.14, P = 0.001) and 2 year AMR (OR 1.41, 95% CI 1.08–1.85, P = 0.011). On the other hand, fwRVLS resulted to be predictive of 2 year CAV incidence (OR 1.30, 95% CI 1.07–1.58, P = 0.008). Finally, bivariate analysis between outcomes and statistically significant variables at univariate analysis and anti-HLA antibody was performed, as shown in *Table 4*. CMV donor/recipient mismatch resulted to be an independent predictor of CAV development at 1 year (OR 4.63, 95% CI 1.06–20.23, P = 0.042) and 2 year follow-up (OR 3.17, 95% CI 1.07–9.42, P = 0.038) independently from anti-HLA antibody status. fwRVLS resulted to be a predictor of CAV development at 2 year follow-up independently from anti-HLA antibody status (OR 1.28, 95% CI 1.05–1.56, P = 0.015). On the other hand, DecT E maintained its predictive value of CAV development only at 1 year follow-up (OR 0.97, 95% CI 0.94–1.00, P = 0.049) regardless of the presence of anti-HLA antibodies, losing statistical significance for 2 year CAV (P = 0.057).

Discussion

The main finding of our study is that the presence of anti-HLA antibody in HTx patients is associated with a subtle myocardial dysfunction even in the absence of AMR at endomyocardial biopsy (Figure 2). In particular, among laboratory data, higher values of NT-proBNP and high-sensitivity troponin I were found in patients with anti-HLA antibody, implying the possibility of these two parameters to highlight a certain degree of myocardial injury potentially correlated to HLA status. Among echocardiographic indexes of diastolic function, DecT E resulted to be significantly reduced in patients with anti-HLA antibodies as well as lateral and septal systolic and early diastolic velocities by TDI. Regarding the analysis of myocardial deformation, both LV-GLS and fwRVLS were significantly reduced in the presence of anti-HLA antibodies, which could make them two valuable parameters to assess the possible changes induced by anti-HLA antibody. On the other hand, even though LA strain was reduced in both groups compared with reference values reported for the general population,[15] no significant differences were found between the two groups.

From a prognostic point of view, our results confirmed the role of anti-HLA antibody as a robust prognosticator in HTx, because the majority of the events occurred in patients with anti-HLA antibodies. Furthermore, we highlighted that a significant association between their presence and CAV development was found. Due to the absence of AMR episodes in patients without anti-HLA antibody, it was not possible to assess their predictive value with regard to this outcome, even though a significant difference between the two groups was found. An interesting result was that both DecT E and fwRVLS were predictors of CAV development during 2 year follow-up, independently from the presence of anti-HLA antibodies.

The development of anti-HLA antibodies after HTx has been implicated in allograft injury and dysfunction. In particular, they showed a relevant connection to the onset of rejection episodes and CAV.[16–19] Our study confirmed these associations, showing, in particular, the connection between anti-HLA antibody and CAV development during follow-up.

Table 3 Univariate analysis between clinical, laboratory, and echocardiographic parameters and outcomes

Parameters	AMR 1 year	ACR 1 year	CAV 1 year	AMR 2 years	ACR 2 years	CAV 2 years	Mortality 2 years
Age	1.02 (0.96–1.09) $P = 0.562$	0.99 (0.94–1.03) $P = 0.548$	0.96 (0.91–1.01) $P = 0.101$	0.99 (0.92–1.06) $P = 0.803$	1.01 (0.94–1.09) $P = 0.794$	0.96 (0.94–1.02) $P = 0.219$	0.94 (0.85–1.04) $P = 0.242$
Heart rate (b.p.m.)	1.06 (0.97–1.16) $P = 0.231$	1.03 (0.96–1.11) $P = 0.361$	1.04 (0.96–1.13) $P = 0.367$	1.09 (0.99–1.20) $P = 0.068$	1.1 (0.89–1.36) $P = 0.367$	1.01 (0.94–1.07) $P = 0.841$	1.02 (0.87–1.20) $P = 0.787$
SBP (mmHg)	1.11 (1.01–1.21) $P = 0.052$	1.01 (0.96–1.07) $P = 0.597$	0.97 (0.89–1.06) $P = 0.490$	1.06 (0.98–1.15) $P = 0.126$	1.09 (0.92–1.28) $P = 0.345$	0.95 (0.90–1.00) $P = 0.073$	1.09 (0.92–1.28) $P = 0.345$
DBP (mmHg)	1.17 (1.01–1.37) $P = 0.052$	0.99 (0.93–1.06) $P = 0.727$	0.97 (0.87–1.07) $P = 0.494$	1.02 (0.92–1.13) $P = 0.747$	1.0 (0.81–1.24) $P = 0.991$	0.98 (0.92–1.04) $P = 0.492$	1.0 (0.81–1.23) $P = 0.991$
Time from HTx	0.97 (0.86–1.10) $P = 0.655$	0.83 (0.71–0.97) $P = 0.017$	1.10 (0.98–1.22) $P = 0.109$	0.93 (0.78–1.10) $P = 0.368$	0.90 (0.74–1.10) $P = 0.293$	1.01 (0.93–1.10) $P = 0.865$	0.87 (0.62–1.22) $P = 0.419$
CMV D+/R− mismatch	0.97 (0.18–4.71) $P = 0.917$	1.5 (0.43–5.40) $P = 0.521$	4.76 (1.17–19.3) $P = 0.029$	0.63 (0.07–5.67) $P = 0.682$	—	3.29 (1.14–9.52) $P = 0.28$	—
Arterial hypertension	0.99 (0.19–5.10) $P = 0.988$	1.64 (0.34–7.94) $P = 0.542$	0.53 (0.12–2.31) $P = 0.399$	0.54 (0.99–3.16) $P = 0.497$	1.14 (0.12–10.67) $P = 0.911$	1.07 (0.32–3.59) $P = 0.911$	0.27 (0.02–4.55) $P = 0.366$
CNI	—	0.30 (0.05–1.73) $P = 0.177$	—	—	—	0.49 (0.99–2.76) $P = 0.422$	—
Corticosteroids	2.20 (0.44–11.14) $P = 0.340$	0.94 (0.29–3.11) $P = 0.925$	0.44 (0.11–1.76) $P = 0.246$	—	—	0.78 (0.29–2.14) $P = 0.632$	—
mTORi	1.23 (0.24–6.41) $P = 0.803$	1.32 (0.33–5.28) $P = 0.698$	1.23 (0.24–6.41) $P = 0.803$	—	1.06 (0.11–10.03) $P = 0.958$	0.16 (0.34–3.94) $P = 0.811$	—
MMF	0.72 (0.18–2.86) $P = 0.644$	0.66 (0.21–2.12) $P = 0.483$	0.21 (0.29–5.11) $P = 0.799$	3.13 (0.35–27.73) $P = 0.306$	0.89 (0.14–5.54) $P = 0.897$	0.78 (0.29–2.14) $P = 0.632$	0.59 (0.04–9.67) $P = 0.710$
NT-proBNP	1.00 (1.00–1.00) $P = 0.457$	1.00 (1.00–1.00) $P = 0.925$	1.00 (1.00–1.00) $P = 0.862$	1.00 (0.99–1.00) $P = 0.878$	1.00 (1.00–1.00) $P = 0.862$	1.00 (1.00–1.00) $P = 0.690$	1.00 (0.99–1.01) $P = 0.934$
eGFR	0.98 (0.95–1.01) $P = 0.190$	1.00 (0.98–1.02) $P = 0.758$	1.00 (0.98–1.03) $P = 0.818$	1.00 (0.98–1.03) $P = 0.802$	0.99 (0.97–1.03) $P = 0.923$	1.00 (0.98–1.02) $P = 0.897$	1.00 (0.95–1.05) $P = 0.833$
Hs troponin I	1.00 (1.00–1.01) $P = 0.161$	1.01 (1.00–1.01) $P = 0.127$	1.00 (1.00–1.01) $P = 0.438$	1.01 (1.00–1.01) $P = 0.160$	1.00 (0.99–1.01) $P = 0.987$	1.00 (0.99–1.01) $P = 0.835$	1.01 (1.00–1.01) $P = 0.320$
DecT E	0.97 (0.95–1.00) $P = 0.021$	0.99 (0.97–1.01) $P = 0.237$	0.96 (0.93–0.99) $P = 0.004$	0.99 (0.97–1.02) $P = 0.651$	0.98 (0.95–1.01) $P = 0.196$	0.98 (0.96–0.99) $P = 0.011$	0.99 (0.95–1.04) $P = 0.786$
E/e′ ratio	1.14 (0.86–1.48) $P = 0.309$	0.96 (0.78–1.23) $P = 0.970$	1.07 (0.83–1.40) $P = 0.595$	1.09 (0.80–1.45) $P = 0.575$	1.20 (0.84–1.73) $P = 0.318$	0.93 (0.75–1.14) $P = 0.466$	1.06 (0.62–1.81) $P = 0.821$
Lateral S′ wave	—	0.72 (0.06–8.13) $P = 0.792$	0.56 (0.01–435.08) $P = 0.866$	—	—	0.60 (0.01–27.24) $P = 0.793$	—
Lateral E′ wave	—	—	—	—	—	—	—
Septal S′ wave	—	—	—	—	—	—	—
Septal E′ wave	—	0.75 (0.08–6.79)	—	—	—	2.15 (0.03–143.39)	—

(*Continues*)

Table 3 (continued)

Parameters	AMR 1 year	ACR 1 year	CAV 1 year	AMR 2 years	ACR 2 years	CAV 2 years	Mortality 2 years
		$P = 0.800$				$P = 0.720$	
Tricuspid S' wave	—	—	—	—	—	—	—
TAPSE	0.85 (0.68–1.06) $P = 0.144$	1.09 (0.91–1.30) $P = 0.353$	0.87 (0.70–1.08) $P = 0.211$	0.79 (0.61–1.03) $P = 0.082$	0.86 (0.65–1.14) $P = 0.296$	1.00 (0.86–1.17) $P = 0.984$	0.82 (0.54–1.25) $P = 0.353$
fwRVLS	1.13 (0.93–1.36) $P = 0.234$	1.09 (0.91–1.319) $P = 0.355$	1.24 (0.95–1.62) $P = 0.107$	1.02 (0.82–1.26) $P = 0.871$	0.94 (0.74–1.18) $P = 0.571$	1.30 (1.07–1.58) $P = 0.008$	0.97 (0.68–1.38) $P = 0.851$
LV-GLS	1.61 (1.21–2.14) $P = 0.001$	1.11 (0.91–1.36) $P = 0.300$	1.13 (0.86–1.42) $P = 0.309$	1.41 (1.08–1.85) $P = 0.011$	0.97 (0.70–1.40) $P = 0.936$	1.06 (0.89–1.26) $P = 0.527$	1.14 (0.74–1.75) $P = 0.548$
PALS	0.88 (0.73–1.07) $P = 0.205$	1.00 (0.85–1.17) $P = 0.972$	0.93 (0.77–1.13) $P = 0.468$	1.05 (0.87–1.26) $P = 0.643$	0.90 (0.71–1.15) $P = 0.415$	0.99 (0.87–1.12) $P = 0.855$	1.15 (0.87–1.52) $P = 0.336$

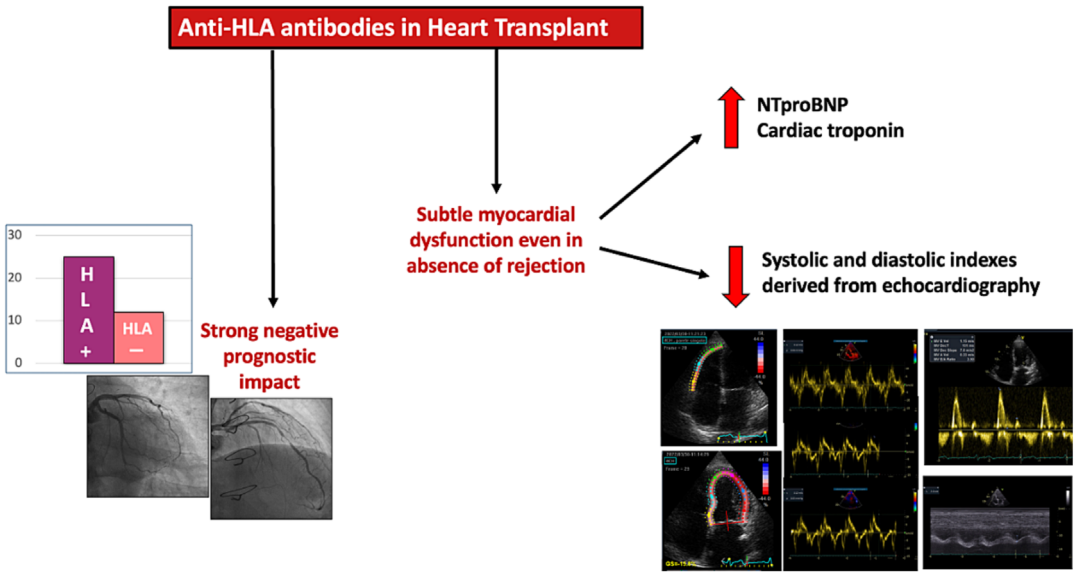
ACR, acute cellular rejection; AMR, antibody-mediated rejection; CAV, cardiac allograft vasculopathy; CMV, cytomegalovirus; CNI, calcineurin inhibitors (tacrolimus/ciclosporin); DBP, diastolic blood pressure; DecT E, deceleration time of E wave; eGFR, estimated glomerular filtration rate with Cockcroft–Gault equation; fwRVLS, free wall right ventricular longitudinal strain; Hs, high-sensitivity; HTx, heart transplantation; LV-GLS, left ventricular global longitudinal strain; MMF, mycophenolate mofetil; mTORi, mammalian target of rapamycin inhibitors (sirolimus/everolimus); NT-proBNP, N-terminal pro-B-type natriuretic peptide; PALS, peak atrial longitudinal strain; SBP, systolic blood pressure; TAPSE, tricuspid annular plane systolic excursion.

Table 4 Bivariate analysis between outcomes and clinical and echocardiographic parameters corrected for anti-HLA antibody

Echocardiographic parameters	AMR 1 year	ACR 1 year	CAV 1 year	AMR 2 years	ACR 2 years	CAV 2 years	Mortality 2 years
Diabetes	—	(0.28–3.68) $P = 0.991$	0.46 (0.09–2.50) $P = 0.369$	—	—	0.54 (0.16–1.88) $P = 0.333$	—
Dyslipidaemia	—	2.26 (0.57–9.00) $P = 0.247$	0.91 (0.20–4.16) $P = 0.901$	—	—	1.19 (0.39–3.60) $P = 0.759$	0.39 (0.23–6.78) $P = 0.521$
CMV D+/R− mismatch	—	2.23 (0.68–7.35) $P = 0.187$	4.63 (1.06–20.23) $P = 0.042$	—	—	3.17 (1.07–9.42) $P = 0.038$	—
DecT E	—	0.99 (0.97–1.01) $P = 0.432$	0.97 (0.94–1.00) $P = 0.049$	—	0.98 (0.94–1.01) $P = 0.251$	0.98 (0.96–1.00) $P = 0.057$	—

ACR, acute cellular rejection; AMR, antibody-mediated rejection; CAV, cardiac allograft vasculopathy; CMV, cytomegalovirus; DecT E, deceleration time of E wave; HLA, human leucocyte antigen.

Figure 2 Central illustration. The presence of anti-human leucocyte antigen (HLA) antibodies after heart transplantation is associated with a subtle myocardial impairment, best identified by increased values of both N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin as well as reduced values of deceleration time of E wave at transmitral pulsed Doppler as well as reduced values of right and left ventricular longitudinal strain, tricuspid annular plane systolic excursion, and left and right tissue Doppler imaging-derived velocities. Furthermore, our study confirmed the negative impact of anti-HLA antibodies after transplant, in particular with regard to its association with cardiac allograft development.



Anti-HLA antibodies, particularly DSAs, are responsible for the activation of an immune response that leads to the activation of the complement cascade. As a result, C4d complement fragments are one of the main histopathological and immunopathological findings characterizing AMR. In our population, 46% of HLA+ patients showed DSA, with a greater percentage represented by Class II anti-HLA antibodies. Evidence suggests the correlation between Class I DSAs and AMR, whereas Class II DSAs have been associated with CAV progression.[20] Notably, *de novo* DSAs, which develop after HTx, usually carry a worse prognosis regarding AMR onset and CAV development and progression.[20] The correct recognition and diagnosis of AMR as well as its therapy are significantly more challenging compared with ACR, even though subclinical AMR is still a robust prognostic marker, mainly due to its association with CAV.[21] One of the main elements that prompt AMR treatment is myocardial dysfunction, especially in asymptomatic patients.[7] However, based on the reported cases, there is a high variability of whether myocardial dysfunction is present or not, probably due to the heterogeneity in cardiac performance assessment.[22] In this context, recognizing mild cardiac impairment documented by alterations in diastolic function and strain analysis could represent a relevant support in implementing surveillance and prompt therapeutic escalation, avoiding the occurrence of the characteristic histopathological modifications typical of AMR. Our study confirmed the importance of diastolic function in HTx patients, even though its assessment is particularly challenging due to the peculiar physiological characteristics that transplanted heart shows. Furthermore, in this population, the incidence of diastolic dysfunction of both ventricles is relatively common in the early stages after HTx and tends to decrease as time passes.[23] Traditional diastolic parameters such as E/A ratio and E/e' ratio might not properly reflect the different grades of diastolic dysfunction, considering the profound alterations that the left atrium faces after HTx. The results of our study underlined the role of DecT E and TDI-derived velocities as sensible markers of diastolic dysfunction in HTx patients. Another recent study found that DecT E is the main diastolic marker associated with CAV in its early stages.[24] It has to be stressed that in HTx population, some echocardiographic indexes of RV function, such as TAPSE and tricuspid S' velocity, might have a limited value, due to the surgical procedure. In fact, after cardiac surgery, a reduction in RV longitudinal function is generally recorded, which is often seen after HTx and tends to persist during follow-up, though a small but incomplete recovery may occur. Despite this initial impairment, global RV function is generally preserved; therefore, the use of other echocardiographic indexes such as myocardial strain might help in a more complete assessment of its function.

Global longitudinal strain values of the left ventricle have already been proved to be lower in transplanted hearts, regardless of rejection status.[25] In the early stages after HTx, decreased values of LV-GLS are often seen, even in the presence of preserved LVEF, showing an increasing trend in

the next 6 or 12 months.[11] Moreover, some studies agree that heart graft rejection is associated with a significant reduction of longitudinal strain, while radial and circumferential strains are not affected.[26] Especially in the early stages of rejection, the main histopathological findings are myocardial oedema and fibrosis that precociously affect the longitudinal function of the graft.[10] For this reason, GLS assessment has been recommended by the ASE and the European Association of Cardiovascular Imaging (EACVI) to evaluate HTx recipients for LV dysfunction.[27] Several studies and a recent meta-analysis confirmed the prognostic value of longitudinal myocardial strain of both the right and left ventricles in predicting AMR and ACR, with different cut-off values depending on the investigation.[10,28] As a confirmation of this evidence, we showed a significant correlation between LV-GLS and future AMR development, even though we were not able to test if its predictive value was independent from anti-HLA antibody. Furthermore, DecT E and fwRVLS emerged as independent predictors of CAV development, independently from the sensitization status. Of note, DecT E maintained its independent predictive value only for 1 year CAV while fwRVLS for 2 year CAV incidence. The low incidence of events in our population might account for these differences. Finally, despite the increasing role of LA myocardial deformation in several cardiovascular diseases, its utility in HTx patients might be limited. In fact, regardless of the surgical technique, the physiology of the left atrium is profoundly altered by HTx; therefore, it is not surprising that several studies attested that not only the left atrium is significantly larger[29] but also its function is impaired, as attested by markedly reduced LA strain values in HTx patients.[30] Our results confirm this finding because both groups of HTx patients showed reduced values of PALS compared with a healthy population, even though no significant difference was found with regard to the presence of circulating anti-HLA antibodies.

Because the development of anti-HLA antibody after HTx is correlated with a mild myocardial dysfunction, which seems to be best detected by NT-proBNP and high-sensitivity troponin I, biventricular longitudinal strain, and diastolic indexes such as early diastolic TDI-derived velocities and DecT E, it is essential to assess these parameters in HTx patients, especially in those with anti-HLA antibodies. Furthermore, these echocardiographic indexes might contribute to a more standardized definition of myocardial dysfunction, even more in the presence of AMR. In doing so, recognizing mild cardiac impairment by alterations in diastolic function, fwRVLS and LV-GLS could represent incentive to increase clinical surveillance and eventually prompt therapeutic escalation. Starting from the results of our study, it would be interesting to investigate whether a more aggressive immunosuppressive regimen or the use of the available desensitization strategies in patients with anti-HLA antibody could convey a benefit in terms of survival and complication rates.

The first limitation of the study was that it was conducted only in one transplant centre. While the total number of patients is substantial for a study based on an HTx population, the total number of patients with anti-HLA antibodies is relatively small, which might limit generalizability and, at the same time, limit a further analysis of anti-HLA antibody subclasses. In addition to that, the time frame for patient inclusion was very broad (3–12 years after transplant), which might have affected the results of our study. In fact, we believe that it would be useful to design a prospective study in which patients are enrolled right after HTx, limiting the variability of the distance from HTx within the population study and even though the echocardiographic examination during the first 3–6 months after HTx might be impaired to modifications related to the transplant. Another aspect that is not analysed in our study is if myocardial impairment potentially associated with *de novo* anti-HLA antibodies is evident from the first detection of these antibodies or takes more time to occur.

Furthermore, the follow-up was relatively short, thus limiting the number of events. In addition to that, CAV was diagnosed only with coronary angiography, without intracoronary imaging. Finally, regarding the echocardiographic assessment, both LV multi-layer strain and LV circumferential as well as radial strain were not assessed in this study, focusing only on LV-GLS.

In conclusion, the presence of circulating anti-HLA antibodies is associated with a mild cardiac dysfunction, even in the absence of AMR. Higher values of NT-proBNP and high-sensitivity cardiac troponin could be the expression of this impairment. Regarding echocardiographic parameters, reduced values of DecT E, LV-GLS, and, in particular, indexes of RV systolic function, such as TAPSE, tricuspid S' wave, and fwRVLS, resulted to be the best parameters able to detect myocardial alterations induced by anti-HLA antibodies. Furthermore, the presence of anti-HLA antibody is associated with a worse outcome, especially in terms of CAV development. Finally, DecT E and fwRVLS resulted to be associated with a higher incidence of CAV during follow-up, independently of anti-HLA antibody.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

No funding was received for this study.

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