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Factors Associated With Sinoatrial Reinnervation After Heart Transplantation

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Background. Factors associated with sympathetic and parasympathetic sinoatrial reinnervation after heart transplantation (HTx) are inadequately studied. **Methods.** Fifty transplant recipients were examined at 7 to 12 wk (index visit), 6, 12, 24, and 36 mo after HTx. Supine rest heart rate variability in the low-frequency (LF) domain (sympathetic and parasympathetic sinoatrial reinnervation) and the high-frequency (HF) domain (parasympathetic sinoatrial reinnervation) were measured repeatedly and related to selected recipient, donor, and perisurgical characteristics. We primarily aimed to identify index visit factors that affect the sinoatrial reinnervation process. Secondarily, we examined overall associations between indices of reinnervation and repeatedly measured recipient characteristics to generate new hypotheses regarding the consequences of reinnervation. **Results.** LF and HF variability increased time dependently. In multivariate modeling, a pretransplant diagnosis of nonischemic cardiomyopathy (P = 0.038) and higher index visit handgrip strength (P = 0.028) predicted improved LF variability. Recipient age, early episodes of rejection, and duration of extracorporeal circulation were not associated with indices of reinnervation. Study average handgrip strength was positively associated with LF and HF variability (respectively, P = 0.005 and P = 0.029), whereas study average C-reactive protein was negatively associated (respectively, P = 0.015 and P = 0.008). **Conclusions.** Indices of both sympathetic and parasympathetic sinoatrial reinnervation increased with time after HTx. A pretransplant diagnosis of nonischemic cardiomyopathy and higher index visit handgrip strength predicted higher indices of mainly sympathetic reinnervation, whereas age, rejection episodes, and duration of extracorporeal circulation had no association. HTx recipients with higher indices of reinnervation had higher average handgrip strength, suggesting a link between reinnervation and improved frailty. The more reinnervated participants had lower average C-reactive protein, suggesting an inhibitory effect of reinnervation on inflammation, possibly through enhanced function of the inflammatory reflex. These potential effects of reinnervation may affect long-term morbidity in HTx patients and should be scrutinized in future research.

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All procedures performed in the present study were in accordance with the ethical standards of the Norwegian National Committee for Ethics in Medical Research and with the 1964 Declaration of Helsinki and its later amendments. This article does not contain any studies with animals performed by any of the authors.

Informed consent was obtained from all individual participants included in the study.

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Heart transplantation (HTx) surgically transects autonomic connections to the heart, leaving the allograft denervated and dependent on intrinsic and hormonal regulatory mechanisms. Increasing evidence supports a time-dependent reinnervation from both the sympathetic[1,2] and parasympathetic[3,4] nervous system. However, the reinnervation process remains incomplete and regionally patchy on long-term follow-up. It affects heart transplant recipients (HTxRs) heterogenously,[3,5,6] and sinoatrial reinnervation only occurs in congruence with ventricular myocardial reinnervation in a portion of patients.[7,8] Reinnervation has not been shown to improve survival,[9] but it improves peak oxygen uptake,[1,10] peak heart rate (HR), and myocardial contractile function in response to exercise,[11] and it has been linked to improved quality of life.[12] Accordingly, knowledge about both the consequences of reinnervation and measures to stimulate the reinnervation process is of interest. This warrants a better understanding of the factors associated with reinnervation, wherein particularly the determinants of sinoatrial reinnervation have been inadequately examined.

We followed 50 HTxRs prospectively for the first 36 mo after surgery. Supine rest heart rate variability (HRV) indices of sympathetic and parasympathetic sinoatrial reinnervation were measured up to 5 times and related to selected recipient, donor, and perisurgical characteristics. Associations were examined in a repeated measures design, which is not previously done to our knowledge. The primary aim was to identify factors at the index visit that significantly affect the sinoatrial reinnervation process. The secondary aim was to examine overall associations between sinoatrial reinnervation and repeatedly measured recipient characteristics, which could generate new hypotheses regarding the consequences of reinnervation.

MATERIALS AND METHODS

Design and Study Population

AccHEART (Autonomic Cardiovascular Control after Heart Transplantation, Clinical Trials ID: NCT01759966) is a prospective cohort study exploring sinoatrial reinnervation after HTx. All HTxRs at the national transplantation center (Oslo University Hospital, Oslo, Norway) between December 2012 and December 2015 were considered for enrollment. Inclusion criteria were (1) age 17 to 70 y and (2) HTx performed during the last 7 to 12 wk. Exclusion criteria were (1) complications resulting in irreversible allograft dysfunction, 2) comorbid chronic medical conditions including diabetes, (3) arrhythmias, (4) multiorgan transplant, and (5) noncompliance to study protocol. Study participants were examined at 7 to 12 wk (index visit) and 6, 12, 24, and 36 mo after HTx. Details of the design have been reported elsewhere.[2,13]

Written informed consent was obtained before enrollment. The study protocol was approved by the Regional Committee for Ethics in Medical Research (2012/1428) and was conducted in accordance with the Declaration of Helsinki.

Autonomic Testing

Participants were instructed to abstain from tobacco and caffeine products for 48 h before attendance and to fast overnight. Adherence to the protocol was assessed by oral questioning. They were maintained on immunosuppressive medications, whereas all other drugs were paused on the morning of testing. Participants were attached to the Task Force Monitor (Model 3040i, CNSystems Medizintechnik, Graz, Austria), a combined hardware and software device for noninvasive real-time recording of cardiovascular variables. Instantaneous HR was obtained from the electrocardiogram signal, and photoplethysmography was used to continuously record arterial blood pressure.[14] The current study uses HRV indices in the frequency domain computed by the Task Force Monitor using an adaptive autoregressive technique.[15] Median values were collected from 240-s epochs at supine rest.

Markers of Sinoatrial Reinnervation

Sympathetic myocardial reinnervation can be examined with several different techniques,[16] whereas methods to investigate sympathetic and parasympathetic sinoatrial reinnervation are few and mainly dependent on the analysis of HRV or HR responses to physical or medically induced stress. HRV in the high-frequency (HF) band (0.15–0.4Hz) is almost exclusively mediated by parasympathetic inputs to the sinoatrial node[17-20] and mainly driven by respiratory activity (the respiratory sinus arrhythmia). Variability in the low-frequency (LF) band (0.04–0.15Hz) can be mediated by both parasympathetic and sympathetic inputs. If there is activity >0.15 Hz, the parasympathetic system must be playing a role. If there is only activity <0.15 Hz, one can infer that the sympathetic system is playing a role. In the current cohort, variability in the LF band increased earlier and more than variability in the HF band.[4] Consequently, the increase in LF variability is a reasonable marker of mainly sympathetic reinnervation, whereas the increase in HF variability marks parasympathetic reinnervation. In statistical analyses, the HRV indices were transformed using the natural logarithm (ln) to approximate the normal distribution better and reduce the effect of outliers.

Explanatory Variables

Medical history was obtained from patient medical records and the national transplantation database. Details regarding right heart catheterization, exercise testing (treadmill, accelerometer, and handgrip isometric strength testing), and laboratory assays have been previously reported.[2,13] Possible explanatory variables were selected on the basis of published associations with reinnervation[9,21,22] or associations with overall survival after HTx.[23] A detailed description of rationales for variable selection is provided in the Supplemental Digital Content (**SDC**, http://links.lww.com/TXD/A584).

We hypothesized that younger female HTxRs with lower body mass index (BMI), higher exercise capacity, higher activity level, a diagnosis of nonischemic cardiomyopathy before HTx, and a shorter duration of heart failure before HTx would demonstrate higher HRV indices. Furthermore, younger donor age, male donor sex, shorter graft ischemic time, shorter time of extracorporeal circulation, and shorter stay in the intensive care unit were hypothesized to enhance indices of reinnervation. We postulated an attenuating effect on the reinnervation process of rejection episodes, occurrence of active cytomegalovirus (CMV) during the first months after surgery, elevated C-reactive protein (CRP), elevated troponin T, and elevated N-terminal pro–brain natriuretic peptide. Finally, we hypothesized that reinnervated individuals would have lower levels of circulating catecholamines.

Immunosuppressive drugs, adrenergic beta-blockers, and other cardiovascular drugs (diuretics, angiotensin-converting enzyme inhibitors, and angiotensin II blockers) have been shown not to significantly affect HRV indices in the current cohort.[4]

Statistics

Statistical analyses were carried out using Stata/SE version 17.0 (StataCorp, TX). Sample size estimations are reported in the Supplemental Digital Content (**SDC**, http://links.lww.com/TXD/A584). Continuous data are presented with mean (SD) or median (interquartile range) depending on the distribution. Categorical data are reported with frequencies. A P value of ≤0.05 was considered statistically significant. The missing data mechanism was considered to be missing at random. Mixed model analysis handles missing data for the dependent variable using maximum likelihood estimation. The fraction of missing explanatory factors at the index visit was low (2%). Accordingly, the mixed models were performed on the original data set. Generalized estimating equations require the data to be missing completely at random, which was considered too strong of an assumption. Therefore, multiple imputation was performed before running these analyses. Details of the imputation model are reported in the Supplemental Digital Content (**SDC**, http://links.lww.com/TXD/A584.

The primary aim of the study was addressed using linear mixed modeling (mixed command in STATA). Measurement dependences (the random effects) were managed with a random intercept and a random slope for time. An unstructured covariance structure and restricted maximum likelihood estimation were selected. Model assumptions were checked by evaluating residual plots.

Univariate analyses were performed with lnLF (mainly sympathetic reinnervation) or lnHF (parasympathetic reinnervation) as the dependent variable, time as a continuous factor, and a single explanatory covariate collected at the index visit. No significant interactions between time and the explanatory variables were identified, and only the main effects were included in the models.

Variable selection for the multivariate models was based on background knowledge, particularly on previously published associations with sinoatrial- and myocardial reinnervations.[9,21,22] As we could not identify literature to support the selection of index visit factors related to lnHF (parasympathetic reinnervation), we abstained from creating such a model. Robustness of the basic conclusions was evaluated by adding and removing variables to/from the multivariate model as suggested by Heinze et al.[24] This is presented in the Supplemental Digital Content.

To address the secondary aim of the study, the use of time-varying explanatory covariates was introduced. Simple regression analysis revealed possible associations between the dependent variables and explanatory covariates measured at later study visits. Accordingly, the time-varying covariates were regarded as endogenous, which might cause the regression coefficients to not have the implied relation in a mixed model setting. Therefore, we regarded the data as a series of cross-sections, and fitted population average marginal models (generalized estimating equations, xtgee command in Stata) with independent covariance structure and robust variance estimation.[25] This form of modeling yields cross-sectional associations and is not suited to evaluate the order of events (causality). Considering the given sample size, 5 of the repeatedly measured recipient characteristics were selected on the basis of clinical relevance and analyzed in multivariate models.

RESULTS

Study Population

Out of 70 eligible HTxRs in the study period, 50 consented to participate. Forty-five patients (90%) were available for follow-up at 6 mo, 47 patients (94%) at 12 mo, 44 patients (88%) at 24 mo, and 40 patients (80%) at 36 mo (Figure 1).

Background characteristics are presented in Table 1, and increases in lnLF and lnHF are presented in Figure 2. At inclusion, the HTxRs had a mean (SD) age of 48.4 (13) y, their BMI was 24.8 (3.8) kg/m^2, and 35 (70%) were male individuals. The most frequent causes of heart failure before HTx were cardiomyopathy (56%) and ischemic heart disease (26%). Participants had heart failure for a median (interquartile range) of 4.5 (8.0) y before HTx. Donors had a mean (SD) age of 36.3 (13.3) y, BMI of 25.5 (5.4) kg/m^2, and 34 (68%) were male individuals. All surgeries were performed using the bicaval technique. Fifteen HTxRs (30%) required mechanical support before transplantation and 11 (22%) required mechanical support after surgery. Mean (SD) values for graft ischemic time was 171 (81) min, for duration of extracorporeal circulation was 123 (35) min, and for average stay in the intensive care unit was 7.0 (3.0) d after surgery. During the study period, 22 (44%) had at least 1 biopsy-verified cellular or humoral rejection, and 13 (26%) had at least 1 CMV DNA >36 IU/mL. The use of pharmaceuticals during the study period is previously reported[4] and listed in **Table S1** (**SDC**, http://links.lww.com/TXD/A584).

Index Visit Factors Affecting Sinoatrial Reinnervation

Time after HTx was positively associated with indices of both sympathetic and parasympathetic sinoatrial reinnervation (Tables 2 and 3). A pretransplant diagnosis of cardiomyopathy was associated with higher lnLF in multivariate modeling, indicating more sympathetic reinnervation in this subgroup. There was no significant association between pretransplant diagnosis and lnHF, indicating no effect on parasympathetic reinnervation.

Higher maximal isometric handgrip strength was associated with higher indices of sympathetic reinnervation in multivariate modeling, whereas peak oxygen consumption at treadmill testing and recipient activity level (measured as steps per day) were not associated in univariate analyses. None of the index visit exercise parameters were associated with parasympathetic reinnervation.

HTxRs with a higher CRP at the index visit had higher lnHF (parasympathetic reinnervation) in univariate analysis, but the association was not significant when adjusting for multiple testing. Furthermore, there was no association between index visit CRP and lnLF.

There were no associations between indices of reinnervation and recipient age, recipient sex, recipient BMI, or duration of heart failure before HTx. Furthermore, there were no associations with early episodes of rejection, active CMV infection in the first months after HTx, index visit markers of myocardial strain or damage, or index visit urine catecholamines to creatinine ratio. None of the examined donor or perisurgical factors were associated with lnLF or lnHF.

Cross-sectional Associations Between Indices of Sinoatrial Reinnervation and Repeatedly Measured Recipient Characteristics

Population average marginal modeling demonstrated that both lnLF (primary marker of sympathetic reinnervation) and lnHF (parasympathetic reinnervation) were positively associated with time after HTx and maximal isometric handgrip strength and negatively associated with urine epinephrine to creatinine ratio and CRP (Table 4). Troponin T and

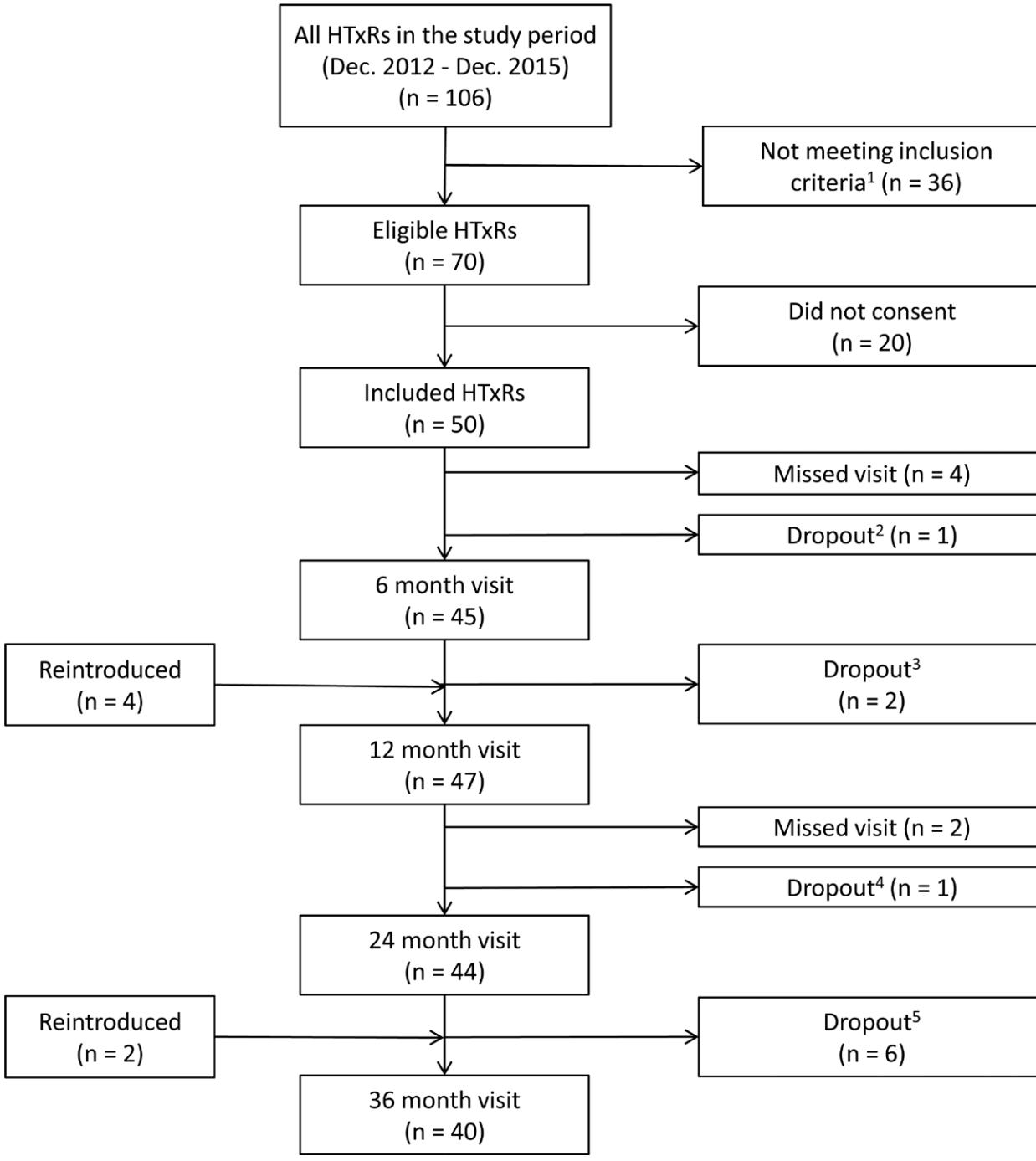

FIGURE 1. Flowchart describing patient inclusion, exclusion, missed visits, and dropouts.[1] Chronic medical condition (n = 14), age younger than 17 y (n = 10), postoperative deaths (n = 10), multiorgan transplant (n = 2).[2] Procedural complication.[3] Cerebral aneurysm, chronic fatigue syndrome.[4] Depression.[5] Rheumatism, depression, cancer, graft failure, death, reason not given. HTxR, heart transplant recipient.

N-terminal pro–brain natriuretic peptide had no significant association with lnLF or lnHF.

DISCUSSION

Our most important findings were that a pretransplant diagnosis of nonischemic cardiomyopathy and higher index visit handgrip strength predicted improved sympathetic reinnervation. Recipient or donor age, perisurgical factors, occurrence of early rejections, and index visit peak oxygen consumption were not associated with indices of reinnervation, which is largely in contrast to previous reports.

Study period average handgrip strength was positively associated with indices of both sympathetic and parasympathetic reinnervation, suggesting a link between reinnervation and improved frailty. Average CRP was inversely associated with indices of reinnervation, suggesting that reinnervation might reduce inflammation, possibly through enhanced function of the inflammatory reflex.

HTxRs with higher indices of reinnervation had lower average urine epinephrine to creatinine ratio, suggesting that reinnervated individuals were less dependent on circulating catecholamines.

TABLE 1.

Heart transplant recipient characteristics

Characteristics	Inclusion	6 mo	12 mo	24 mo	36 mo
Age, y, mean (SD)	48.4 (13.0)	47.9 (12.3)	49.2 (12.9)	50.6 (12.6)	52.1 (12.5)
95% CI	44.7-52.0	44.7-52.1	45.4-53.0	46.8-54.5	48.1-56.1
Male sex, %	35 (70%)	33 (73%)	33 (70%)	31 (71%)	29 (73%)
BMI, kg/m^2, mean (SD)	24.8 (3.8)	25.5 (3.9)	26.4 (4.3)	26.6 (4.3)	27.1 (4.3)
95% CI	23.7-25.9	24.4-26.7	25.1-27.7	25.3-27.9	25.8-28.5
Cause of heart failure, number (%)					
Cardiomyopathy	28 (56%)				
Ischemic heart disease	13 (26%)				
Other[a]	9 (18%)				
Duration of heart failure before HTx, y, median (IQR)	4.5 (8.0)				
95% CI	2.0-7.0				
Mechanical support before HTx, number (%)	15 (30%)				
Left ventricular assist device	6 (12%)				
Intra-aortic balloon pump	9 (18%)				
Extracorporeal membrane oxygenation	2 (4%)				
Rejections, number (%)					
No rejection (normal biopsy)	32 (64%)	43 (96%)	44 (94%)	7 (16%)	1 (3%)
R1	10 (20%)	2 (4%)	3 (6%)	1 (2%)	1 (3%)
R2	7 (14%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Humoral rejection	1 (2%)	0 (0%)	0 (0%)	1 (2%)	1 (3%)
Heart rate, beats/min, mean (SD)	81.6 (10.3)	83.7 (11.8)	83.0 (10.3)	83.1 (13.1)	79.3 (10.0)
95% CI	78.7-84.6	80.2-87.3	79.9-86.1	79.2-87.1	76.1-82.5
VO_2peak, mL/kg/min, mean (SD)	21.1 (5.0)	NA	25.3 (7.2)	NA	24.2 (8.2)
95% CI	19.7-22.6		23.2-27.5		21.5-26.9
Steps per d, number, mean (SD)	5409 (2345)	5996 (2544)	6330 (2705)	6806 (3546)	6016 (3111)
95% CI	4616-6202	5135-6857	5371-7290	5569-8043	4875-7157
Max handgrip strength, Nm, mean (SD)	244 (108)	299 (110)	296 (108)	319 (105)	311 (112)
95% CI	214-275	266-331	265-327	288-350	276-346
C-reactive protein, g/dL, median (IQR)	2.0 (4.4)	1.1 (3.0)	2.0 (3.4)	2.0 (2.3)	3.0 (4.1)
95% CI	1.6-2.7	0.6-2.5	1.4-2.1	0.9-2.2	1.3-4.4
Troponin T, ng/L, median (IQR)	32.0 (33.0)	16.0 (16.0)	14.0 (13.0)	9.0 (13.0)	14.0 (15.0)
95% CI	23.0-39.0	12.0-18.0	10.0-17.0	6.0-13.0	10.0-15.0
NT-pBNP, ng/L, median (IQR)	901 (926)	304 (359)	254 (355)	195 (271)	299 (304)
95% CI	634-1260	254-457	203-389	118-304	178-349
Plasma epinephrine, pmol/L, median (IQR)	577 (405)	636 (603)	464 (538)	498 (493)	355 (204)
95% CI	529-674	515-812	391-728	351-613	313-418
Plasma norepinephrine, pmol/L, median (IQR)	2072 (1844)	2182 (1668)	2288 (1725)	2615 (1325)	2723 (1767)
95% CI	1565-2328	1953-2623	1629-2838	2265-2776	1983-3011
Urine epinephrine to creatinine ratio, nmol/mmol, median (IQR)	1.5 (1.2)	1.5 (1.6)	1.6 (1.6)	1.4 (1.3)	1.6 (2.5)
95% CI	1.2-1.7	1.1-2.3	1.2-2.1	1.1-2.1	1.2-2.4
Urine norepinephrine to creatinine ratio, nmol/mmol, median (IQR)	9.0 (7.2)	9.4 (5.5)	11.4 (8.7)	10.8 (5.0)	13.3 (15.0)
95% CI	8.2-12.0	7.8-10.4	9.1-13.5	9.6-12.4	10.3-20.6

Inclusion was at 7 to 12 wk after HTx. Patients were all operated with the bicaval technique.
[a]Other reasons for HTx: graft failure (8%), congenital heart disease (4%), myocarditis (4%), and valvar heart disease (2%).
BMI, body mass index; CI, confidence interval; HTx, heart transplantation; IQR, interquartile range; NA, not available; NT-pBNP, N-terminal pro–brain natriuretic peptide; VO_2peak, peak oxygen consumption.

A Pretransplant Diagnosis of Nonischemic Cardiomyopathy Predicted Improved Sympathetic Reinnervation

Patients with a pretransplant diagnosis of nonischemic cardiomyopathy had higher indices of mainly sympathetic reinnervation in multivariate modeling. As sympathetic nerve fibers are transected at surgery, reinnervation presupposes the growth of nerve fibers along arterial structures to reach the transplanted heart. It is possible that the diseased, sclerotic arterial vessels found in HTxRs transplanted for ischemic heart disease hamper nerve growth and obstruct reinnervation, yielding a positive association for the remaining HTxRs who had nonischemic cardiomyopathy. The finding is similar to results published by Bengel et al[9] who evaluated myocardial sympathetic reinnervation by measuring C-11 hydroxyephedrine retention, but opposite those by De Marco et al[26] who found less metaiodobenzylguanidine uptake in patients transplanted for idiopathic cardiomyopathy.

Interestingly, in addition to having higher indices of reinnervation, patients transplanted for nonischemic heart failure have less coronary plaque progression than patients transplanted for ischemic cardiomyopathy.[27] Thereby, a potential link between reinnervation and cardiac allograft vasculopathy exists. Estorch et al[28] reported that patients with established angiographic vasculopathy had less metaiodobenzylguanidine uptake, whereas Koskinen et al[29] found no association between graft arteriosclerosis and HRV indices of reinnervation. Future research could scrutinize this possible association

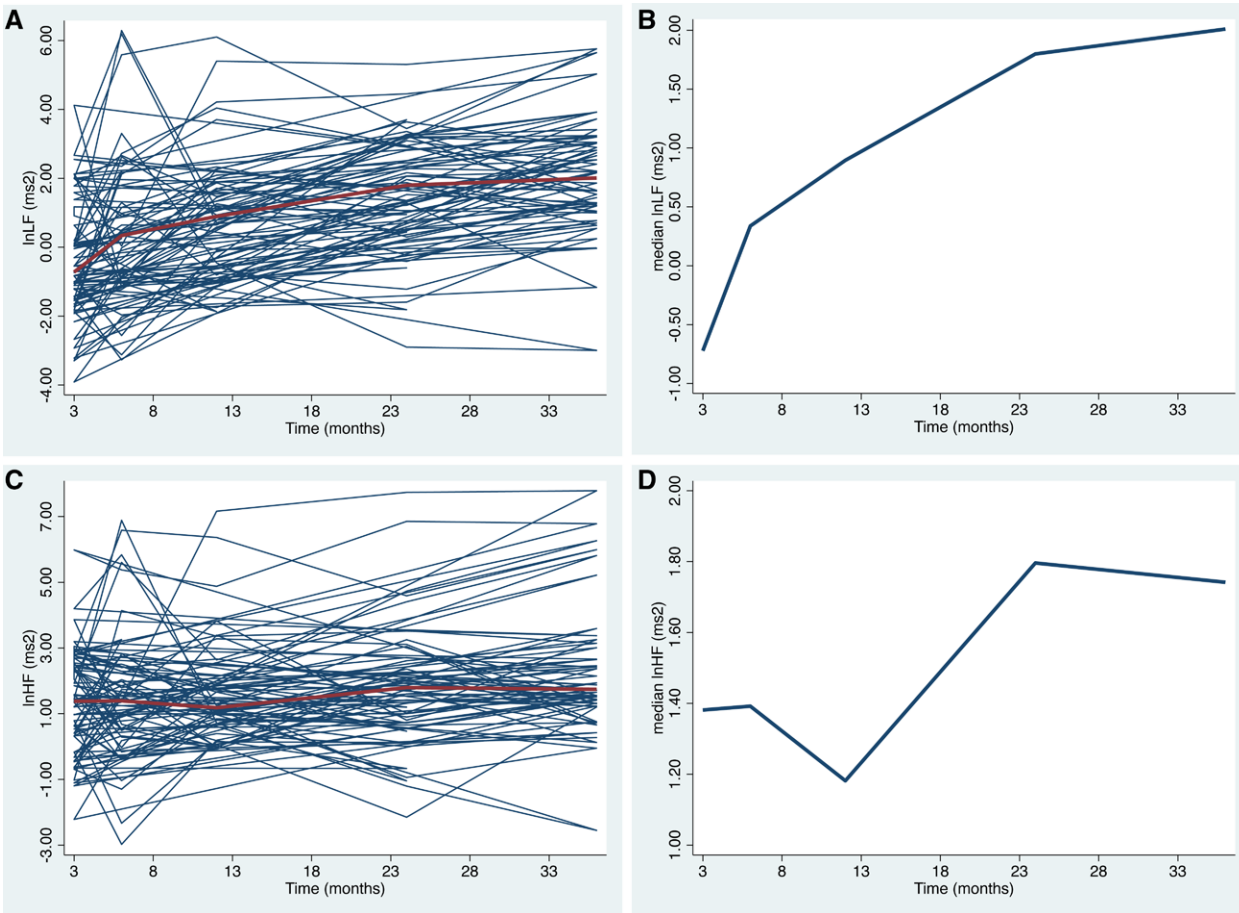

FIGURE 2. Time-dependent increases in lnLF and lnHF during study period. lnLF (primary marker of sympathetic sinoatrial reinnervation) and lnHF (primary marker of parasympathetic reinnervation) increased time dependently during the first 36 mo after heart transplantation. A, lnLF and (C) lnHF are spaghetti plots depicting individual patient trajectories with a red line describing median increase. B, lnLF and (D) lnHF are median increases scaled for better visualization. lnHF, the natural logarithm of power in the high-frequency band (0.15–0.4 Hz); lnLF, the natural logarithm of power in the low-frequency band (0.04–0.15 Hz).

further by combining indices of reinnervation with newer techniques of evaluating allograft vasculopathy, such as intravascular ultrasound or optical coherence tomography.

Factors Not Associated With Sinoatrial Reinnervation

Markers of sinoatrial reinnervation were not associated with recipient age at index visit, donor age, graft ischemic time, time of extracorporeal circulation during surgery, occurrence of early rejections, or index visit peak oxygen consumption at treadmill testing. There are discrepancies in published literature, but these findings are largely in contrast to previous reports.[1,9,21,22] The lack of association between recipient age and reinnervation was particularly surprising. Bengel et al[9] is the only other study identified that primarily analyzed explanatory factors of reinnervation. However, important differences between the studies should be noted. First, Bengel et al used positron emission tomography imaging to study sympathetic ventricular myocardial reinnervation, whereas we used HRV indices to examine sinoatrial reinnervation. It is plausible that, for example, episodes of rejection would affect the ventricular myocardium differently from the sinoatrial node. Furthermore, patients were operated on with the Shumway technique in the Bengel cohort, whereas all of our patients were operated with the bicaval approach. This introduces a systematic difference that likely affects perioperative factors. Similar to Bengel et al,[9] no association was found between reinnervation and recipient sex, donor sex, recipient BMI, or recipient CMV infection.

We have previously published a positive correlation between troponin T and lnLF at 6 mo in the current cohort, hypothesizing a detrimental effect of early sympathetic reinnervation.[2] By using data from all 5 study visits, an association between these variables could not be confirmed. This argues that reinnervation has neither a detrimental nor a protective effect on myocardial cell damage during mid-term follow-up.

Sympathetic Sinoatrial Reinnervation Might Improve Frailty

Handgrip strength is a simple risk-stratifying method shown to be inversely associated with all-cause death, cardiovascular death, and cardiovascular disease in the general population,[30] as well as an independent risk factor for adverse outcomes in heart failure patients.[31] Low handgrip strength is a marker of frailty and global myopathy.[32] In the current cohort, HTxRs with lower index visit handgrip strength (more frail) had lower indices of sympathetic reinnervation (lnLF). Furthermore, higher-average handgrip strength during the study period was associated with increased activity in both branches of the autonomic nervous system. Thus, particularly sympathetic reinnervation was associated with reduced frailty, which has not been previously described.

TABLE 2.

Summary of univariate mixed models exploring factors affecting sinoatrial reinnervation

	lnLF				lnHF			
	Coef.	95% CI		*P*	Coef.	95% CI		*P*
Recipient characteristics								
Sex (male -> female)	–0.254	–1.08	0.573	0.547	–0.379	–1.23	0.469	0.381
Age at inclusion, y	0.023	–0.006	0.052	0.120	0.028	–0.002	0.057	0.067
Dx of cardiomyopathy (other Dx -> cardiomyopathy)	0.854	0.128	1.58	**0.021**	0.495	–0.279	1.27	0.210
Duration of heart failure before HTx, y	0.040	–0.028	0.108	0.250	0.050	–0.020	0.120	0.161
BMI, kg/m^2	0.015	–0.088	0.117	0.779	0.032	–0.073	0.136	0.556
Exercise capacity								
Steps per d[a] (100 steps)	0.003	–0.017	0.023	0.793	–0.002	–0.023	0.019	0.847
VO_2peak, mL/kg/min	0.036	–0.042	0.114	0.368	-0.018	–0.099	0.063	0.667
Max handgrip strength, 10 Nm	0.036	0.001	0.070	**0.046**	0.024	–0.013	0.060	0.206
Laboratory assays								
C-reactive protein, g/dL	0.028	–0.005	0.061	0.094	0.037	0.004	0.070	**0.030**
Troponin T, 10 ng/L	0.030	–0.096	0.155	0.643	0.062	–0.066	0.191	0.343
NT-pBNP, 100 ng/L	0.001	–0.041	0.044	0.953	0.015	–0.029	0.059	0.507
Urine epinephrine to creatinine ratio, nmol/mmol	–0.095	–0.336	0.145	0.437	–0.104	–0.353	0.145	0.413
Urine norepinephrine to creatinine ratio, nmol/mmol	–0.006	–0.035	0.023	0.689	–0.005	–0.035	0.024	0.732
CMV DNA >36 IU/mL (no -> yes)	–0.206	–1.19	0.775	0.681	–0.123	–1.14	0.890	0.812
Cellular or humoral rejection (no -> yes)	–0.126	–0.926	0.674	0.757	0.207	–0.616	1.03	0.622
Donor characteristics								
Age, y	0.016	–0.013	0.045	0.271	0.020	–0.010	0.050	0.190
Sex (male -> female)	–0.135	–0.952	0.681	0.745	-0.065	–0.905	0.775	0.880
Perisurgical characteristics								
Graft ischemic time, min	–0.002	–0.007	0.003	0.386	–0.003	–0.008	0.002	0.220
Extracorporeal circulation, min	–0.006	–0.017	0.004	0.244	–0.006	–0.017	0.005	0.268

Each model had lnLF or lnHF as the dependent variable, time as a continuous factor, and a single explanatory covariate obtained at the index visit. Measurement dependences were handled with a random intercept and a random slope for time.

Index visit was at a mean of 2.5 mo after HTx. lnLF is the primary marker of sympathetic activity, and lnHF is the primary marker of parasympathetic activity. lnLF and lnHF were measured repeatedly throughout the study period, whereas explanatory factors were obtained at the index visit. Time was significant in all models (not shown).

[a]Based on the analysis of 34 patients due to missing data. Bold font marks significance at the 0.05 level. No analysis remained significant after the Bonferroni correction for 19 analyses.

BMI, body mass index; CI, confidence interval; CMV, cytomegalovirus; Coef., coefficient; Dx, diagnosis; HTx, heart transplantation; lnHF, the natural logarithm of power in the high-frequency band (0.15–0.4 Hz); lnLF, the natural logarithm of power in the low-frequency band (0.04–0.15 Hz); NT-pBNP, N-terminal pro–brain natriuretic peptide; VO_2peak, peak oxygen consumption.

TABLE 3.

Multivariate mixed model describing index visit factors associated with lnLF (primary measure of sympathetic sinoatrial reinnervation)

lnLF	Coef.	95% CI		*P*
Time, mo	0.064	0.043	0.084	**<0.001**
Max handgrip strength, 10 Nm	0.040	0.004	0.071	**0.028**
Dx of cardiomyopathy (other Dx ->cardiomyopathy)	0.775	0.044	1.505	**0.038**
Age at inclusion, y	0.027	–0.001	0.054	0.055
Extracorporeal circulation, min	–0.004	–0.015	0.006	0.436
Cellular or humoral rejection (no -> yes)	–0.235	–1.004	0.533	0.548
Constant	–2.151	–4.407	0.104	0.062

Measurement dependences were handled with a random intercept and a random slope for time.

lnLF was measured repeatedly throughout the study period, whereas explanatory factors were obtained at the index visit (mean 2.5 mo after heart transplantation). Bold font marks significance at the 0.05 level.

CI, confidence interval; Coef., coefficient; Dx, diagnosis; lnLF, the natural logarithm of power in the low-frequency band (0.04–0.15 Hz).

It is possible that increased muscle strength enhances sympathetic reinnervation, but it is maybe more reasonable to postulate that the order of events is that reinnervation facilitates higher grip strength. A plausible mechanism is that reinnervation enhances HTxRs' ability to respond to the sympathetic cardiac outflow provided through central command as a response to isometric exercise testing, resulting in an enhanced blood pressure response, which facilitates the isometrically working muscle to create a higher grip strength. It is notable that peak oxygen consumption, another metric of frailty, was not associated with either lnLF or lnHF. However, it is appealing to think that because frailty improves after HTx,[33] improved autonomic control is at least one of the underlying mechanisms.

Parasympathetic Reinnervation Might Enhance the Inflammatory Reflex

The inflammatory reflex describes real-time modulation of inflammatory responses from the autonomic nervous system. Efferent parasympathetic activity in the vagus nerve leads to cholinergic-mediated inhibition of inflammatory cytokines produced in the heart, liver, spleen, and GI tract.[34,35] In the

TABLE 4.

Multivariate generalized estimating equations exploring associations between lnLF (mainly sympathetic reinnervation) or lnHF (parasympathetic reinnervation) and repeatedly measured recipient characteristics

	Coef.	95% CI		*P*
Mainly sympathetic reinnervation (lnLF)				
Time, mo	0.056	0.036	0.077	**<0.001**
Urine epinephrine to creatinine ratio, nmol/mmol	–0.064	–0.104	–0.023	**0.003**
Max handgrip strength, per 10 Nm	0.036	0.011	0.061	**0.005**
C-reactive protein, g/dL	–0.025	–0.044	–0.005	**0.015**
NT-pBNP, 100 ng/L	0.020	–0.031	0.070	0.440
Troponin T, 10 ng/L	0.032	–0.145	0.210	0.720
Constant	–1.01	–2.08	0.055	0.063
Parasympathetic reinnervation (lnHF)				
Time, mo	0.029	0.008	0.051	**0.008**
Urine epinephrine to creatinine ratio, nmol/mmol	–0.078	–0.115	–0.042	**<0.001**
Max handgrip strength, per 10 Nm	0.028	0.003	0.054	**0.029**
C-reactive protein, g/dL	–0.027	–0.047	–0.007	**0.008**
NT-pBNP, 100 ng/L	0.042	–0.007	0.090	0.093
Troponin T, 10 ng/L	0.103	–0.072	0.279	0.249
Constant	0.228	–0.926	1.38	0.698

An independent covariance structure and robust variance estimation were selected. Bold font marks significance at the 0.05 level.
Variables were measured repeatedly up to 5 times during the study period. Analyses were performed after multiple imputation.
CI, confidence interval; Coef., coefficient; lnHF, the natural logarithm of power in the high-frequency band (0.15–0.4 Hz); lnLF, the natural logarithm of power in the low-frequency band (0.04–0.15 Hz); NT-pBNP, N-terminal pro–brain natriuretic peptide.

current cohort, average CRP levels during the study period were inversely associated with activity in both autonomic branches. This might suggest that sinoatrial reinnervation reduces inflammation, possibly through enhanced function of the inflammatory reflex. The results are in line with findings in presumed healthy individuals[36] but not previously described in HTxRs. To speculate regarding clinical relevance, the finding provides a theoretical construct of how parasympathetic reinnervation might lower the incidence of cardiac allograft vasculopathy, which is considered an inflammatory disease.

Reinnervated HTxRs Are Less Dependent on Circulating Epinephrine

Most previous publications report resting plasma norepinephrine and epinephrine at similar levels in HTxRs early and late after transplantation.[16] In contrast, we found that resting plasma norepinephrine increased, whereas plasma epinephrine decreased with time after HTx. This finding is in line with Goncalvesova et al[37] who observed a time-dependent decrease in mRNA levels of phenylethanolamine *N*-methyltransferase after HTx. Phenylethanolamine *N*-methyltransferase is the enzyme catalyzing the conversion of norepinephrine to epinephrine in the last step of epinephrine synthesis. This is evidence of sympathetic reinnervation with increased norepinephrine release from sympathetic neurons, resulting in less dependence on synthesized circulating epinephrine. Underlining the same argument, there was a negative association between the study average urine epinephrine to creatinine ratio and HRV indices of reinnervation. Patients with more autonomic input to the sinoatrial node had less catecholamines in their morning spot urine.

Strengths and Limitations

A major strength of the current study is that data were collected prospectively from a national cohort and analyzed in a repeated measures design. This yields high statistical power for the given sample size. Study participants had similar background characteristics as nonparticipants,[13] suggesting good generalizability.

An important limitation of population average marginal modeling should be noted. The repeatedly measured data were regarded as a series of cross-sections, making interpretations of the order of events speculative. Furthermore, although generally well established, the physiological correlates of HF and LF variability remain under some debate[38] and spectral estimates of HRV have been shown to only have moderate reproducibility.[39]

CONCLUSIONS

Indices of both sympathetic and parasympathetic sinoatrial reinnervation increased with time after HTx. A pretransplant diagnosis of nonischemic cardiomyopathy and higher isometric handgrip strength at the index visit predicted improved indices of sympathetic reinnervation. Recipient or donor age, perisurgical factors, occurrence of early rejections, and index visit peak oxygen consumption were not associated with indices of reinnervation, which is largely in contrast to previous reports.

Participants' average handgrip strength during the study period was positively associated with indices of both sympathetic and parasympathetic reinnervation, which suggests a link between reinnervation and improved frailty. Average CRP was inversely associated with indices of reinnervation, suggesting an inhibitory effect of reinnervation on inflammation, possibly through enhanced function of the inflammatory reflex. These potential effects of sinoatrial reinnervation may affect long-term morbidity in HTxRs and should be scrutinized in future research.

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