

CLINICAL SCIENCE

NOREPINEPHRINE REMAINS INCREASED IN THE SIX-MINUTE WALKING TEST AFTER HEART TRANSPLANTATION

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OBJECTIVE: We sought to evaluate the neurohormonal activity in heart transplant recipients and compare it with that in heart failure patients and healthy subjects during rest and just after a 6-minute walking test.

INTRODUCTION: Despite the improvements in quality of life and survival provided by heart transplantation, the neurohormonal profile is poorly described.

METHODS: Twenty heart transplantation (18 men, 49±11 years and 8.5±3.3 years after transplantation), 11 heart failure (8 men, 43±10 years), and 7 healthy subjects (5 men 39±8 years) were included in this study. Blood samples were collected immediately before and during the last minute of the exercise.

RESULTS: During rest, patients' norepinephrine plasma level (659±225 pg/mL) was higher in heart transplant recipients (463±167 pg/mL) and healthy subjects (512±132), $p<0.05$. Heart transplant recipient's norepinephrine plasma level was not different than that of healthy subjects. Just after the 6-minute walking test, the heart transplant recipient's norepinephrine plasma level (1248±692 pg/mL) was not different from that of heart failure patients (1174±653 pg/mL). Both these groups had a higher level than healthy subjects had (545±95 pg/mL), $p<0.05$.

CONCLUSION: Neurohormonal activity remains increased after the 6-minute walking test after heart transplantation.

KEYWORD: Heart transplantation; Heart failure; Exercise; Norepinephrine; Cardiac rehabilitation; 6-minute walking test.

INTRODUCTION

Heart failure is considered the last stage of heart disease and a significant cause of worldwide mortality and morbidity.^{1,2,3} The end-stage of heart failure, which is marked by a lack of response to medical treatment, disabling symptoms, and repeated hospital stays, is associated with high morbidity and mortality.⁴ Heart transplantation is an acceptable gold standard treatment for select patients in the terminal stages.⁵

The exacerbated neurohormonal activity plays an important role in disease progression and prognosis in heart failure.^{6,7,8,9} This neurohormonal axis has become one of the biggest targets in heart failure interventions.^{10,11,12,13,14,15} It is well known that beta-blockers provide survival improvement and, because of this, have become one of the main drugs for treating heart failure.¹⁶

Despite the significant improvement in quality of life and survival provided by heart transplantation, the neurohormonal profile is not restored to normal values.⁶ The mechanisms involved and the neurohormonal profile after heart transplantation have been poorly described, especially during the 6-minute walking tests that could represent the effort relative to daily activities.¹⁷

The aim of this study was to evaluate the neurohormonal activity in heart transplant recipients and compare it with that in heart failure and healthy subjects during rest and just after the 6-minute walking test.

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MATERIALS AND METHODS

Study population

A total of 20 sedentary heart transplant recipients (18 men, 49±11 years), 11 sedentary heart failure patients (8 men, 43±10 years), and 7 sedentary healthy subjects (5 men 39±8 years) were included in this study. Patient characteristics are shown in Table 1. All heart transplant recipients were in a clinically stable condition 8.5±3.3 years after transplantation. Endomyocardial biopsy did

not show any evidence of tissue rejection during the entire study. Heart transplant recipients and heart failure patients with atrial fibrillation, a pacemaker, and noncardiovascular functional limitations like osteoarthritis were excluded from this study. Heart failure patients whose drug therapy was not optimized were also excluded from this study. Optimization was considered 50 mg/day or more of carvedilol for at least 3 months.¹⁸ Healthy subjects did not have any risk factors for cardiovascular disease or noncardiovascular functional limitations.

This protocol was approved by the Ethics Committee of

Table 1 - Patient characteristics.

Etiology:	Number of patients (%), dose (mg/day)	
	Heart Failure	Heart Transplantation
Ischemic	35%	16%
Nonischemic	65%	84%
NYHA functional class:		
I	60%	---
II	40%	---
Left ventricle ejection fraction	25±11%	---
Current medications:		
Diuretics		
Furosemide	60%, 52±30mg/day	25%, 33±11 mg/day
Hydrochlorothiazide	50%, 43±24md/day	25%, 26±2md/day
Angiotensin-converting enzyme inhibitors		
Enalapril	70%, 38±6mg/day	50%16±5mg/day
Captopril	15%, 93±37mg/day	---
Losartan	15%, 75±29mg/day	10%, 50±0mg/day
Carvedilol	100%, 61±30mg/day	---
Spirolactone	35%, 25±0mg/day	---
Digoxin	45%, 0.25±0mg/day	---
Isosorbide 5-mononitrate	15%, 56±35mg/day	---
Immunosuppressive drugs		
Corticosteroids (prednisone)	-----	57%, 4.5±1mg/day
Antiproliferative agents		
Azathioprine	-----	21%, 75±35mg/day
Mycophenolate mofetil	-----	71%, 744±488mg/day
TOR inhibitors		
Tacrolimus	-----	7%, 8±0mg/day
Sirolimus	-----	7%, 2±0mg/day
Calcineurin inhibitors (Cyclosporine)	-----	71%, 161±57mg/day
Calcium channel blocker (Diltiazem)	-----	78% 78±54mg/day
Hydralazine	-----	14% 25±0mg/day
Clonidim	-----	28% 0.1±0.1mg/day
Atorvastatin	-----	7% 20±0mg/day
Ezetimibe	-----	7% 10±0mg/day

the Heart Institute, InCor HCFMUSP. All patients provided informed consent prior to participation.

Six-minute walking test and blood norepinephrine

All patients were asked to refrain from both strenuous physical activity and the consumption of any stimulants (eg, coffee, tobacco, alcohol) 24 hours prior to the 6CWT. The patients' last meal was ingested at least 2 hours before the start of the test. Before starting the 6CWT and blood sample collection, the patients rested for 15 minutes on a chair. The 6CWT using the Borg scale was performed on a treadmill with zero inclination and patient-controlled speed in a temperature-controlled room (21–23°C) in the afternoon (between 13:00 hours and 15:00 hours). All patients were advised to keep walking during the test at a pace between “relatively easy and slightly tiring” (between 11 and 13 on the Borg scale). The distance walked was recorded by the treadmill microprocessor (Series 2000, Marquette Electronics, Milwaukee, WI, USA). Encouragement was standardized with phrases like “if you can walk faster, increase the speed”, “you are doing very well,” and “if it is tiring, you can reduce the speed.” Blood pressure was measured at rest and at the sixth minute by the auscultation method. The electrocardiography (Max 1, Marquette Electronics), ventilatory and gas exchange variables were continuously evaluated breath-by-breath by a computerized system (Vmax 229 model, SensorMedics, Yorba Linda, CA, USA) during the entire test but only collected at rest (stand position) and just after the 6-minute walking test. The 6-minute walking test has been shown to reflect submaximal effort in heart failure patients¹⁹ and to reproduce the daily activities.²⁰ Blood samples were collected immediately before the 6-minute walking test with patients in the upright position and just after the exercise by the antecubital vein. The norepinephrine assay was performed as previously reported.²¹

Current medication intake

All heart failure patients were receiving carvedilol associated with an ACE inhibitor or losartan. Medication profile is shown in Table 1. Patients took carvedilol, angiotensin-converting enzyme inhibitors, losartan, and Isosorbide 5-mononitrate twice per day, one half of the daily dose in the morning (9:00 A.M.) and the other half at night (9:00 P.M.). Diuretics, digoxin, and spironolactone were taken in the morning (9:00 A.M.). All heart transplant recipients were receiving immunosuppressive therapy two times per day, one half of the daily dose in the morning and the other half at night. Antihypertensive drugs were normally taken in the morning.

Statistical analysis

The descriptive analysis is presented as the mean and standard deviation. To compare the norepinephrine levels between the 3 groups, we used the ANOVA with the post-hoc Tukey test.

Data were analyzed using the *Statistical Package for Social Sciences* for Windows, 11.5 (SPSS Inc, Chicago, IL). Statistical significance was set at $p < 0.05$.

RESULTS

During rest, norepinephrine plasma level was higher in heart transplant recipients and healthy subjects. Heart transplant recipients' norepinephrine plasma level was not different than that of healthy subjects. Just after the 6-minute walking test, heart transplant recipients' norepinephrine plasma level was not different than that of heart failure patients and both these groups had a higher level of norepinephrine than did healthy subjects. Exercise capacity and hemodynamic data are listed in table 2.

DISCUSSION

The main finding of this study is that norepinephrine remained increased just after the 6-minute walking test in heart transplant recipients. This is the first report of the norepinephrine profile during a 6-minute walking test in heart transplant recipients.

It is known that daily activities are not performed at maximal effort. That it's why we studied the neurohormonal profile in a 6-minute walking test. This way, we could access the norepinephrine in a more functional status.

The intensity of the 6-minute walking test, between 11 and 13 on the Borg scale, was similar for all groups, which are expected to spend about the same metabolic or VO_2 , regardless of physical status. The same distance covered by the groups could be explained by their sedentary characteristic. Moreover, they were all clinically stable outpatients with optimized medication.²²

Pérez-Villa et al⁶ studied the neurohormonal profile of 37 heart failure patients on the waiting list for heart transplantation before and 1, 4, 9, and 12 months after heart transplantation. The authors concluded that the neurohormonal activation did not normalize after heart transplantation. In this study, plasma norepinephrine level was only recorded before and 1 month after heart transplantation, showing a tendency to decrease. In our study, the heart failure group had a higher level of resting norepinephrine compared with heart transplant recipients and healthy subjects. The norepinephrine level

Table 2 - Differences between patients.

Variable	NL (n=7)	HF (n=11)	HT (n=20)
Resting heart rate (bpm)	80±10	72±5	104±14*‡
6WT Heart rate (bpm)	97±15	95±12	122±21*‡
Resting Norepinephrine (pg/mL)	512±132	659±225*†	463±167
6WT Norepinephrine (pg/mL)	545±95	1174±653*	1248±692*
Resting SBP (mm Hg)	121±12	111±12	140±24*‡
6WT SBP (mm Hg)	142±15‡	123±19	152±24‡
Resting DBP (mm Hg)	69±11	61±12	83±16*‡
6WT DBP (mm Hg)	74±9‡	61±10	82±16‡
6WT VO ₂ (mL O ₂ /L/min)	12.6±4.1	12.3±4.8	14.1±2.2
VE/VCO ₂ slope	20±3	25±5*	33±6*‡
Distance (meters)	386±96	322±48	354±64

HT= Heart transplanted; HF= Heart failure; NL= Normals; 6WT= Six-minute walking test; SBP= Systolic blood pressure; DBP= Diastolic blood pressure; VO₂= Oxygen Consumption; *p<0.05 different to NL; †p<0.05 different to HT; ‡p<0.05 different to HF.

of the heart transplant recipients did not differ from that of healthy subjects, probably by the partial reinnervation that occurred during the 8.5 years. Heart function after transplantation depends on the circulating norepinephrines. The progressive heart reinnervation through the years could have progressively decreased the necessity for norepinephrine plasma levels in heart transplant recipients.

The study by Ferretti et al⁸ evaluated the neurohormonal profile of 17 heart transplant recipients and 9 healthy subjects at rest and at maximal exercise. The resting norepinephrine plasma level was higher in heart transplant recipients than in healthy subjects. In our study, the resting norepinephrine did not differ between heart transplant recipients and healthy subjects. The time of heart transplantation, ie, different stages of reinnervation, between our heart transplant recipients and the ones in the study by Ferretti et al was different (8.5 versus 3.4 years). This difference could explain these divergent results. During

exercise, the norepinephrine plasma level was also higher in heart transplant recipients compared with that in healthy subjects. This is in accordance with our results, despite the fact that our heart transplant recipients had performed a 6-minute walking test.

Study limitation

This study was limited by the study design (cross-sectional), small study population, and the use of a single method of neurohormonal evaluation.

CONCLUSION

The norepinephrine remained increased just after the 6-minute walking test in heart transplant recipients. The consequences of these data should be better investigated in heart transplant recipients.

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