Microneurography and Venous Occlusion Plethysmography in Heart Failure: Correlation with Prognosis

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Summary
Background: Microneurography and venous occlusion plethysmography can be considered methods of assessment of the sympathetic activity.

Objective: To evaluate the intensity of the sympathetic activity through microneurography and venous occlusion plethysmography in patients with heart failure (HF) and correlate this intensity with prognosis.

Methods: 52 patients with HF (ejection fraction < 45% at the echocardiogram): 12 with FCII and 40 with FCIV. After compensation, the muscular sympathetic nervous activity (MSNA) in the peroneal nerve (microneurography) and the muscular blood flow (MBF) in the forearm were evaluated (venous occlusion plethysmography). After an 18-month follow-up, the patients were divided in 3 groups: 12 with FCII, 19 with FCIV that did not die and 21 with FCIV that died. The intensity of the sympathetic activity was compared in the three different groups.

Results: Patients with FCII presented lower MSNA (p=0.026) and higher MBF (p=0.045) than the ones with FCIV that did not die. The patients with FCIV that died presented higher MSNA (p<0.001) and lower MBF (p=0.002) than the patients with FCIV that did not die. ROC curve: cutoff >53.5 impulses/min for MSNA (S=90.55, E=73.68%) and < 1.81 ml/min/100gr for MBF (S=90.4%, E=73.7%). Kaplan-Meier curve: higher survival with MSNA < 53.5 impulses/min (P<0.001), and/or MBF > 1.81 ml/min/100gr (P<0.001). Logistic regression analysis: the higher the MSNA and the lower the MBF, the higher is the probability of death.

Conclusion: The intensity of the MSNA and the MBF can be considered prognostic markers in advanced HF. (Arq Bras Cardiol 2009;92(1):44-51)

Key words: Heart failure, electromiography, plethysmography, prognosis.

Introduction
Heart failure (HF) is characterized by a generalized activation of the sympathetic nervous system and inhibition of the parasympathetic nervous system. At the start of the myocardial lesion, the adrenergic stimulation occurs as an adaptive response. However, the long-term sustained sympathetic activation leads to the myocardium destruction, sodium retention, peripheral vasoconstriction and disease progression1,2.

There are several methods to evaluate the sympathetic activity. The measurement of plasma noradrenaline levels (the method that is more often employed in clinical practice) and the measurement of the urinary excretion of norepinephrine (currently in disuse) do not supply information on the regional sympathetic activity3-5. The technique that consists in the infusion of tritium-labeled norepinephrine allows the assessment of the regional sympathetic activity (appearance, spillover) (4). Wallin et al showed that the sympathetic activity can be directly quantified in the peripheral nerves (peroneal, brachial or median) through the microneurography technique. These nerves release activity discharges (impulses), which are numerically counted (number of impulses per minute), reflecting the intensity of the muscular sympathetic nervous activity (MSNA)6,7. Some studies have shown that, in patients with HF, the MSNA is higher than in normal individuals; however, these studies did not show any association between this activity and prognosis8-10. He measurement of the muscular blood flow (MBF) through venous occlusion plethysmography can be considered an indirect method of assessing the sympathetic activity. The mechanisms involved in the MBF variation are: endothelial dysfunction, characterized by the impairment of the vasodilating response due to the attenuation of nitric oxide production; activation of neurohormonal factors, represented by the stimulation of the renin-angiotensin system and production of vasodilating hormones; activation of the sympathetic nervous system11.
The aim of this study was to determine the intensity of the sympathetic activity in patients with HF, through microneurography and venous occlusion plethysmography and correlate this intensity with prognosis.

Methods

A total of 40 inpatients admitted at Hospital Auxiliar de Cotoxó of Hospital das Clínicas of the School of Medicine of the University of São Paulo (HC-FMUSP) with a diagnosis of HF FCIV (NYHA) and 12 outpatients from the Outpatient Clinic of Instituto do Coração (The Heart Institute) of HCFMUSP with FCII (NYHA) were prospectively studied. All of the patients presented left ventricular (LV) systolic dysfunction and ejection fraction (EF) < 45% at the echocardiogram. The exclusion criteria were: age < 15 years or > 65 years; current alcoholism; neuropathies of any etiology; valvular diseases; acute coronary failure; symptomatic cardiac arrhythmia; diabetes mellitus; arterial hypertension; renal failure (creatinine > 2.0 mg/dL); chronic or degenerative diseases. Regarding the sample, 30 patients presented a diagnosis of idiopathic dilated cardiomyopathy (7 with FCII, 23 with FCIV), 9 had Chagas cardiomyopathy (1 with FCII, 8 with FCIV), 6 had ischemic cardiomyopathy (4 with FCII and 2 with FCIV), 5 had hypertensive cardiomyopathy with FCIV and 2 had peripartum cardiomyopathy with FCIV (diagnosis attained for more than 1 year). The 40 patients with FCIV were compensated; five patients that did not die and 10 patients that died needed a vasoactive drug (dobutamine) due to the significant low cardiac output. After the clinical stabilization and dobutamine withdrawal, all patients with FCIV received the following medications (individualized doses): captopril - 75 mg/day to 150 mg/day, furosemide - 80 to 160 mg/day, spironolactone - 25 mg/day, hydralazine - 100 to 300 mg/day, dinitrate of isosorbide - 80 to 120 mg/day and 23 patients received digoxin - 0.25 mg/day. None of the patients presented cardiac cachexia. Before the betablocker introduction, the 40 patients with FCIV were submitted to the microneurography and venous occlusion plethysmography. Similarly, the 12 patients with FCII were compensated and received the following medications: captopril - 100 mg/day, spironolactone 25 mg/day, furosemide 40 to 80 mg/day and 7 patients received digoxin 0.25 mg/day. Before the betablocker introduction and approximately 7 days after the clinical compensation, the 12 patients were submitted to microneurography and venous occlusion plethysmography.

The MSNA was assessed through the multimeter recording of the efferent postganglionic pathway, of the muscular nervous fascicle, in the posterior side of the peroneal nerve, immediately inferior to the fibular head. The recordings were obtained through the implantation of a microelectode in the peroneal nerve and a reference microelectrode approximately 1 cm far from the first one. The electrodes were connected to a preamplifier and the nerve signal was fed through a bandpass filter and then routed through an amplitude discriminator to a storage oscilloscope and loudspeaker. For registering and analysis purposes, the filtrated neurogram was fed through a capacitance-resistance integrator to obtain the mean voltage of the neural activity. The MSNA was assessed by counting the number of discharges (impulses) per minute. The MBF was evaluated by venous occlusion plethysmography, immediately after the MSNA. The contralateral non-dominant arm (the one not undergoing the isometric exercise) was elevated above the heart level to guarantee an adequate venous drainage.

A silastic tube filled with mercury, connected to a low-pressure transducer was placed around the forearm, 5 cm from the humeral-radial joint and connected to a plethysmograph-Hokanson 201 AG. A cuff was placed around the wrist and another on the upper part of the arm. The wrist cuff was inflated at a supra-systolic level 1 minute before the measurements were started. At 15-second intervals, the arm cuff was inflated above the venous pressure for a period of 7 to 8 seconds. The increased tension in the silastic tube reflected the increased volume of the forearm, and, consequently, its vasodilation. The basal MBF wave signal was recorded in a polygraph and analyzed every minute, with a mean of 3 recordings per minute. The basal MBF was taken into account in the study (ml/min/100gr).

After the hospital discharge, the patients with FCIV and those with FCII were treated with the conservative therapy for heart failure. The doses of medications were maintained and the betablocker was introduced after the assessment of the sympathetic activity. The patients with FCIV received carvedilol 37.5 to 75 mg/day and the doses were individualized according to the tolerance and efficacy of the response to the betablocker. All of the patients presented a decrease in the heart rate (60 to 65 bpm) as well as in blood pressure. The patients with a diagnosis of Chagas cardiomyopathy received carvedilol, 37.5 mg/day, due to the low tolerance. The patients with FCIV received 50 mg/day. The patients were followed at the Outpatient Clinic of INCOR (The Heart Institute) for a period of 18 months and the occurrence of death was determined through letters or contact by phone. For the purpose of research and evaluation, the patients were divided in three groups: 12 with FCII, 19 with FCIV that did not die and 21 patients with FCIV that died. In the group of patients that died: 12 died due to the heart failure progression, with 8 having a diagnosis of idiopathic dilated cardiomyopathy, 1 with peripartum cardiomyopathy, 1 with hypertensive cardiomyopathy and 2 with Chagas cardiomyopathy. Three patients died due to pulmonary embolism: 1 with a diagnosis of Chagas cardiomyopathy and 2 with idiopathic dilated cardiomyopathy. Six patients died due to sudden death: 3 with a diagnosis of idiopathic dilated cardiomyopathy, 1 with ischemic cardiomyopathy, 1 with Chagas cardiomyopathy and 1 with hypertensive cardiomyopathy.

The protocol was approved by the Ethics Committee of Hospital das Clínicas of FMUSP, under number 261/99. The statistical analysis used the t-test of equality of means with the objective of evaluating differences between the MSNA and the MBF in the forearm of the different groups (p values < 0.05). Logistic regression: it estimated the probability of
death through the co-variables MSNA and MBF. Hosner and Lemeshow test: logistic regression adjustment in the probability of death. A ROC curve was adjusted to the values of MSNA and MBF and a cutoff was determined for the two variables. The Kaplan-Meier curve used the two variables: MSNA and MBF.

Results

The microneurography showed that the number of impulses per minute in patients with FCII varied from 26 to 58, with a mean of 38.5. In the patients with FCIV that did not die, it varied from 30 to 76, with a mean of 49.1. In the patients with FCIV that died, it varied from 46 to 94, with a mean of 65 (Table 1). The patients with FCII presented a lower mean MSNA than the ones with FCIV that did not die (p=0.026). The patients with FCIV that died presented a higher MSNA than the ones that did not die (p< 0.001) (Chart 1). The ROC curve showed an area of 0.830 for the patients with FCIV (Chart 2). Combining the values of sensitivity (90.5%) and those of specificity (73.68%), we found a cutoff for MSNA > 53.5 impulses/min (p<0.001) (Chart 3).

The MBF in patients with FCII varied from 1.71 to 3.40 ml/min/100gr, with a mean of 2.53. In patients with FCIV that did not die, it varied from 0.93 to 3.02 ml/min/100gr, with a

<table>
<thead>
<tr>
<th>Patients' Classification</th>
<th>FCII (n=12)</th>
<th>FCIV w/ death (n=19)</th>
<th>FCIV with death (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscular sympathetic nervous activity</td>
<td>Mean</td>
<td>38.5</td>
<td>49.1</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>58</td>
<td>76</td>
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Chart 1 - MSNA in FC IV according to death (Mean comparison test: p < 0.001).

Chart 2 - ROC curve for MSNA in FC IV; Area - 0.830; Cutoff > 53.5 impulses/min.
The MBF of the patients that died varied from 0.61 to 2.31 ml/min/100g, with a mean of 1.49 (Table 2). Patients with FCII presented higher MBF than the ones with FCIV that did not die (p=0.045). Patients with FCIV that died presented a lower mean MBF than the ones that did not die (p=0.002) (Chart 4). The ROC curve showed an area of 0.815 for FCIV (Chart 5). Combining the sensitivity (90.4%) and specificity (73.7%) values, we found a cutoff value for the MBF < 1.81 ml/min/100g. The probability of survival is higher in patients with FCIV that presented MBF > 1.81 ml/min/100g (p <0.001) (Chart 6).

On the other hand, the results can be presented according to the chances of death: a- between two patients with the same MSNA and the MBF differing in only one unit, the chances of death for the one with the higher MBF is approximately 0.027-fold (95%CI=0.001; 0.48) the chance of death of the other individual with a lower MBF. B- between two patients with the same MBF and the MSNA differing in only one unit, the chances of death for the one with the higher MSNA is approximately 1.15-fold (95%CI=1.04;1.27 ) the chances of death of the other individual with lower MSNA.

Table 2 - Muscular blood flow (ml/min/100g) and functional class  

<table>
<thead>
<tr>
<th>Muscular Blood Flow</th>
<th>Patients' Classification</th>
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<tbody>
<tr>
<td></td>
<td>FCII(n=12)</td>
</tr>
<tr>
<td>Mean</td>
<td>2.53</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.6</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.71</td>
</tr>
<tr>
<td>Maximum</td>
<td>3.40</td>
</tr>
</tbody>
</table>

The lower the MBF and the higher the MSNA, the higher is the probability of death. Specifically, when comparing two patients with FCIV, the one with the higher MBF will present the lower probability of death, whereas the one with the higher MSNA will present the higher probability of death.

On the other hand, the results can be presented according to the chances of death: a- between two patients with the same MSNA and the MBF differing in only one unit, the chances of death for the one with the higher MBF is approximately 0.027-fold (95%CI=0.001; 0.48) the chance of death of the other individual with a lower MBF. B- between two patients with the same MBF and the MSNA differing in only one unit, the chances of death for the one with the higher MSNA is approximately 1.15-fold (95%CI=1.04;1.27 ) the chances of death of the other individual with lower MSNA.

**Discussion**

The main results of the study were: 1- patients with advanced heart failure presented higher MSNA and lower MBF in the forearm than patients with FCII. 2- patients with advanced heart failure that died presented higher MSNA and lower MBF than the patients that did not die.

Several studies have shown evidence of the importance of the sympathetic activation in heart failure. Elevated plasma norepinephrine levels are related to the severity and the prognosis in patients with heart failure. Some important studies have evaluated the sympathetic activity with the use of microneurography. Leimbach et al, studying 16 patients with HF (5 FCII, 7 FCIII and 4 FCIV) and 28 normal individuals, showed that patients with HF presented high MSNA (mean of 54 impulses/min) when compared with normal individuals (25 impulses/min). There was a positive correlation between serum levels of norepinephrine and MSNA. Ferguson et al used the same methodology and evaluated 29 patients with...
HF and 10 normal individuals. The patients with HF presented higher MSNA (mean of 54.7 impulses/min) than the control group (mean = 16.7 impulses/min); however, there was no difference between MSNA and functional class (FCII, FCIII and FCIV). There was a positive correlation between serum levels of norepinephrine and MSNA. Our study showed differences between mean MSNA and functional class II and IV. The patients with FCII had lower MSNA than the ones with FCIV that did not die. There was a significant difference between patients with FCIV that died and the ones that did not die. The patients that died presented higher MSNA. These results differ from those by Ferguson et al. and Leimbach et al. that did not show any difference between muscular sympathetic nervous activity and functional classes II and IV. The patients with FCIV in our study presented advanced heart failure, important low cardiac output and 15 of our patients needed a vasoactive drug (dobutamine) during the clinical compensation phase. Although this group of patients was apparently homogeneous regarding the severity and clinical outcome, our study showed differences among the patients. The patients with FCIV that died presented higher MSNA (mean of 65 impulses/min) than the ones that did not die (mean of 49.1 impulses/min). The studies by Ferguson et al. and Leimbach et al., which evaluated patients with FCII, III and IV that were stable and did not have advanced HF, showed that they presented mean MSNA of 54 impulses/min. The ROC curve of our study showed a cutoff value for MSNA > 53.5 impulses/min. It is probable that the sample size and the higher severity of our patients justify these differences.

Two mechanisms have been studied to explain the increase in peripheral muscular tonus: the endothelial dysfunction and the neurohumoral stimulation. The vascular endothelium has been intensively studied in the last years. Among the endothelium-derived vasoactive substances are the vasodilators: nitric oxide, prostacyclin and the endothelium-derived hyperpolarizing factor and the vasoconstrictors: endothelin, angiotensin II and endoperoxides.

The impairment in the production of nitric oxide can reduce the flow-dependent vasodilation and alter the cardiac output through the increase of the post-load. Some studies...
have suggested that the basal production of nitric oxide, would be increased in HF in a compensatory way\textsuperscript{16}; however, direct evidence of production alteration and its importance in the regulation of the basal vascular torticity of HF are still debatable\textsuperscript{17,18}. Yoshida et al\textsuperscript{19} showed that the bioavailability of nitric oxide in patients with HF has an inverse association with the functional class, and is significantly lower than in normal individuals\textsuperscript{19}.

Kubo et al\textsuperscript{20} showed an attenuation of the endothelium-dependent vasodilation in the peripheral circulation in patients with heart failure after the administration of acetylcholine (endothelium-dependent vasodilator) and sodium nitroprussiate (endothelium-independent vasodilator)\textsuperscript{20}. However, Negrao et al\textsuperscript{21}, using mental stress and infusion of L-arginine, acetylcholine and sodium nitroprussiate, showed that the impairment of the endothelium-dependent vasodilation is not the main cause of peripheral vasoconstriction in HF\textsuperscript{21}. Nakamura et al\textsuperscript{22}, studying 46 patients with HF and using the infusion of acetylcholine and sodium nitroprussiate, showed that the patients that presented impairment of the endothelium-independent peripheral vascular response at a higher degree than the endothelium-dependent response, were more frequently admitted at the hospitals due to the worsening of symptoms of HF. These results suggest that alterations in the smooth muscle of the vessel walls and/or their structure have an important role in the disease worsening and evolution and that other factors, in addition to the endothelial dysfunction, participate in the maintenance of the increased peripheral vascular tonus\textsuperscript{22}. The renin-angiotensin system also contributes significantly to the increase of systemic vascular resistance. Several studies have shown the benefit of using angiotensin-converting enzyme inhibitor (ACEI) in the treatment of these patients\textsuperscript{23-25}.

The aforementioned studies have shown evidence that the endothelial dysfunction has an effective participation in the maintenance of elevated peripheral resistance during the disease evolution; however, at the advanced stages, the endothelial dysfunction is probably not the main cause of the increase in the peripheral vascular tonus in patients with HF. Our studies showed significant differences between MBF and functional class II and IV, confirming the results by Negrao et al\textsuperscript{21} that demonstrated a mean MBF of 2.5 ml/min/100gr in patients with FC II and 1.9 ml/min/100grs in those with FC IV\textsuperscript{10}. However, our study showed differences among patients with FC IV. The ones that did not die presented a mean MBF of 2.06 ml/min/100gr and the ones who died, 1.49 ml/min/100gr. It is likely that the differences found among the patients with FC IV in our study are due to a higher severity of our patients, a specific

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**Table 3 - Estimated probabilities of death**

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<tr>
<th>Probability of death</th>
<th>Muscular sympathetic nervous activity</th>
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<tr>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Muscular blood flow</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.91</td>
</tr>
<tr>
<td>1.5</td>
<td>0.21</td>
</tr>
<tr>
<td>2.5</td>
<td>0.01</td>
</tr>
<tr>
<td>3.5</td>
<td>~0</td>
</tr>
</tbody>
</table>

**Chart 6 - Survival curve for muscular blood flow (FC IV).**
group that presented advanced HF. The ROC curve showed that patients with MSNA > 53.5 impulses per minute or MBF < 1.81 ml/min/100g presented a higher probability of death. The logistic regression analysis showed that the higher the MSNA and the lower the MBF in the forearm, the higher the probability of death. These results allow us to infer that, the accentuated peripheral vasoconstriction, found in patients with advanced HF, have as the probable main cause the increase in the sympathetic activity. The significant difference in the sympathetic activity between the patients with FCIV that died and those that did not die also allows us to infer that the sympathetic activity recorded by the microneurography and by the quantification of the muscular blood flow in the forearm can be considered a prognostic marker in patients with advanced HF.

Study limitations

Our study evaluated patients with advanced HF that needed to be hospitalized for clinical compensation. This was a specific group of patients that presented important low cardiac output, a short and mid-term reserved prognosis and most had a heart transplant indication. The patients were submitted to the assessment of the sympathetic activity before the introduction of the betablocker, as we considered that we would have varied responses of the sympathetic activity with the use of the betablocker, a drug that is known to reduce the sympathetic activity and would probably limit the assessment. All the aforementioned studies and that were compared to our study evaluated the sympathetic activity without the betablocker use. The posterior optimized introduction of the betablocker in all patients and the significant correlation found between death and sympathetic activity allow us to infer that, probably, in the patients with advanced HF, the optimization of the classical treatment for HF: diuretics and digitalis, according to the necessity, ACEI, spironolactone and betablockers, can change the prognosis of the disease.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

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Study Association

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