

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

**Assessment of cardiovascular autonomic regulation in patients
with heart failure**

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**ASSESSMENT OF CARDIOVASCULAR AUTONOMIC REGULATION IN
PATIENTS WITH HEART FAILURE**

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1. List of abbreviations

HF_rEF - heart failure with reduced ejection fraction

HF_{mr}EF - heart failure with mildly reduced ejection fraction

HF_pEF – heart failure with preserved ejection fraction

NYHA Stadium - New York Heart Association classification

NT-proBNP - N - terminal pro - B - type natriuretic peptide

LVEF, EF - left ventricular ejection fraction

RAS – renin-angiotensin system

ACE – angiotensin converting enzyme

SGLT-2 – sodium-glucose cotransporter

MRA – mineralocorticoid receptor antagonist

ARB – angiotensin receptor blockers

MSNA (muscle sympathetic nerve activity) - muscle sympathetic nerve activity

SSNA – activity of skin sympathetic nerve fibers

BRS – baroreflex sensitivity

BRS_{SYMPincidence} – burst incidence based spontaneous baroreflex sensitivity index

HRV (heart rate variability) - heart rate variability

RRI - RR interval

RR mean - mean of RR intervals

RRSD – standard deviation of RR intervals

BMI - body mass index

6MWD – 6-minute walking distance

SLOWB – 6/min breathing, slow controlled breathing, slow patterned breathing

SL – spontaneous breathing

CVLM – caudal ventrolateral group of neurons

RVLM – rostral ventrolateral group of neurons

NTS – nucleus tract solitary

OSA - obstructive sleep apnea

HC – healthy control group

2. Terminology and treatment of heart failure

In many countries, including Hungary, heart failure is one of the leading causes of death. Heart failure is a syndrome with different pathomechanisms, in which patients are classified on the basis of similar clinical presentation and disease progression in parallel directions based on similar mechanisms. Heterogeneous heart failure syndrome can be classified according to several characteristics, such as etiology and functional stage (New York Heart Association [NYHA] classification). The type of heart failure with a severe decrease in ventricular function and contractility (HFrEF - heart failure with reduced ejection fraction; LVEF $\leq 40\%$) has been studied for decades, and we are also paying more and more attention to the form with preserved left ventricular ejection fraction (HFpEF - heart failure with preserved ejection fraction; LVEF $\geq 50\%$). In 2016, the European Society of Cardiology formulated new guidelines for the treatment and diagnosis of heart failure. The heart failure patient group with mildly reduced ejection function (HFmrEF – heart failure with mid-range ejection fraction; in the 2021 ESC guideline: heart failure with mildly reduced ejection fraction; LVEF 41 % - 49 %) was created. HFmrEF is a significant subgroup that includes almost one fifth of patients with heart failure; its epidemiology, etiology, characteristics, treatment, and long-term outcome are currently the subject of intensive research.

One of the connecting links in heterogeneous groups of heart failure is that disease progression is closely related to excessive and prolonged neurohumoral responses, increased sympathetic and RAS activation.

At the time of our studies included in the thesis, the sacubitril-valsartan preparation was not yet generally available in Hungary, and SGLT2 inhibitors were still under investigation, so they were not included in our patients' medications.

3. Autonomic cardiovascular regulation, methods of autonomic test

3.1. The arterial baroreflex

The function of the arterial baroreflex is the key to short-term blood pressure control. Under physiological conditions, the stimulus of the high-pressure baroreceptors located in the aortic arch and the carotid sinus is the vessel wall deformation, which is generated by the change in blood pressure. The afferent axons originating from the receptors reach the brainstem by connecting to the vagus nerve and the glossopharyngeal nerve. While the vessel wall deformation increases during systole, the afferent stimulation from the baroreceptors leads to a

decrease in sympathetic outflow, in diastole the "silence of receptors" leads to an increase in sympathetic outflow. Our pulsatile circulation thus necessarily brings a kind of cyclicity to sympathetic activity. The regulation of parasympathetic outflow is more direct, stimulating impulses from the nucleus tract solitary (NTS) go to the dorsal nucleus of the vagus nerve and the nucleus ambiguus, increasing the activity of the vagus.

3.2. The autonomic nervous system in heart failure

Heart failure is a hyperadrenergic state characterized by abnormally increased sympathetic activity and decreased parasympathetic activity. The elasticity of baroreceptive artery sections is reduced as a result of vessel wall remodeling, thereby reducing the degree of sympathoinhibition associated with systolic dilatation. The sympatho-inhibitory reflex response triggered by low-pressure baroreceptors, such as the receptors of the pulmonary veins, also decreases, and paradoxical sympathetic activation has also been reported. During absolute and relative ischemia, which is often present in this disease, the endogenous humoral factors (bradykinin, adenosine), produced in the heart muscle, stimulate the epicardial sympathetic afferents, thus increasing the functioning of the cardiac sympathetic afferent reflex; sympathetic flow not only to the heart but also to the arteries and kidneys will increase. Enhanced chemoreflexes play a crucial role in the development of the sympathetic overload characteristic of heart failure. Reduced cardiac output, reduced tissue perfusion and oxidative stress continuously stimulate the peripheral chemoreceptors. Sympathetic activity inhibition that is related to breathing may also decrease in severe heart failure. Comorbidities that are present besides heart failure (for example, obesity, hypertension, kidney failure) can increase sympathetic activity by themselves. The obstructive sleep apnea (OSA), as a common comorbidity of heart failure, causes additional oxidative stress to the body.

3.3. Methods of autonomic tests

3.3.1. Analysis of RR intervals

Changes in RR intervals can be examined by themselves, as heart rate variability (HRV), or in the context of simultaneous blood pressure changes (as a baroreflex response). The successive RRI changes over time are characterized by the "time domain" HRV parameters. These parameters are reduced in a well-known way in heart failure. In our present studies, we only documented the values of the RRI mean and standard deviation. With the spread of long ECG recordings and Holter monitoring, the examination of HRV has become commonplace.

At the same time, several studies prove that the parameters can also be well-interpreted on short-term recordings of a few minutes.

3.3.2. Measurement of sympathetic activity

There are several methods for examining sympathetic activity, such as the determination of circulating adrenaline and noradrenaline, which require special laboratory conditions, or the noradrenaline spillover technique. The observation of short-term sympathetic activity changes and reflex responses is only possible by examining the muscle sympathetic nerve activity (MSNA), which - as the most important technique used in our research - is described in detail below.

3.3.2.1. Methods of muscle sympathetic nerve activity (MSNA) measurement

The test is most often performed with a puncture of the superficial peroneal nerve. The first step is to localize the nerve, with the help of a blunt-headed stimulator with low-current stimulation through the skin. The right position is indicated by the dorsiflexion and lateral rotation of the hallux/foot. The point can be selected where the response occurs even when stimulated with the smallest voltage. The stimulating electrode with a diameter of 3-5 micrometers is inserted in this point and an uninsulated reference electrode at a distance of a few centimeters. Afterwards, the stimulation is restarted with a low voltage (3-5 V) through the microneedle. The correct position is indicated by the previously described response. If dorsiflexion occurs even at a low voltage (1-2 V), then the stimulation is turned off and thus the sympathetic activity can be registered. The MSNA represents peripheral vasomotor sympathetic activity. During the test, the summed and processed signal of the activity of the sympathetic axons, the envelope peak called burst, is recorded. These typically appear in the recording as a curve with positive direction. MSNA can be characterized by burst frequency, which means the number of bursts appearing per minute. As a sign of close baroreflex control, the appearing of bursts is strongly dependent on blood pressure, and is also closely linked to the cardiac cycle, - and therefore pulse-dependent. For this reason, a normalized value is also defined; the burst incidence, which means the number of bursts appearing during 100 cardiac cycles. The amplitude of the bursts can be determined and also the area under the curve. To determine the area under the curve, we perform a normalization considering the largest amplitude burst of our recording as 100 "arbitrary units" (AU).

3.3.3. Arterial baroreflex sensitivity (=BRS)

Arterial baroreflex responses are reflected in the functioning of both autonomic divisions. It is difficult to measure vessel wall deformation directly, so blood pressure changes are usually interpreted as triggers. The response can be measured in the functioning of the target organ (for example, the sinus node) or in the neural activity that transmits the responses to the target organ. Baroreflex tests can be based on spontaneous blood pressure fluctuations, or also on blood pressure fluctuations induced by vasoactive drugs.

3.3.3.1. Spontaneous cardiovagal BRS

Parasympathetic responses (RRI changes), also called cardiovagal responses, show the closest correlation with changes in systolic blood pressure. The correlation between changes in systolic blood pressure and RR intervals can be described by a sigmoidal curve, with the slope of the linear regression between increasing and decreasing systolic blood pressure values and the subsequent RRI defined on the central linear segment. This slope gives the quantitative value of the cardiovagal baroreflex sensitivity, which is expressed in ms/Hgmm dimension. The cardiovascular BRS determination method used in our own research is based on spontaneous sequences of at least 3 increasing or decreasing systolic blood pressure values and subsequent shortening or prolonging of RR intervals. The spontaneous up-/down BRS is the average of the BRS value of 5 increasing/5 decreasing sequences.

3.3.3.2. Sympathetic baroreflex sensitivity

Sympathetic responses (MSNA changes) show the closest correlation with diastolic blood pressure changes. The sympathetic baroreflex sensitivity expresses the extent of the change in sympathetic burst magnitude or incidence that occurs as a result of a unit change in diastolic pressure. Since not all cardiac cycles have sympathetic bursts, the sympathetic BRS is determined by sorting the recorded diastolic pressures into 3 mm Hg bands and averaging the magnitude of the bursts in each band over all cycles. In our study we have determined the association between diastolic pressure-MSNA burst incidence. The slope of the regression line in the linear section of the correlation plot is the sympathetic" incidence BRS" ($BRS_{SYMIncidence}$); the unit of which is burst/100 cardiac cycles/Hgmm.

3.3.4. Slow patterned breathing (SLOWB)

Slow patterned breathing means breathing with a frequency of 6/minute. As indicated above, heart rate responses are triggered by complex mechanisms, and the response itself at a frequency of 0.1 Hz (6/min) is transmitted to the sinus node predominantly by the

parasympathetic nervous system. The effect of SLOWB on sympathetic vasomotor activity is also important. The lowest sympathetic activity can be measured at the end of inspiration and at the beginning of expiration and the higher MSNA parameters can be registered at the end of expiration and at the beginning of inspiration. The sympathoinhibition that occurs during inspiration is called inspiratory inhibition, the stretch receptors of the lungs play an important role in its creation.

3.4. Muscle sympathetic nerve activity in heart failure – open questions

3.4.1. Muscle sympathetic nerve activity and characterization of the sympathetic baroreflex in HFmrEF group

It has long been known that sympathetic activation is pathologically elevated in heart failure and physiological inhibitory effects, such as increased blood pressure or inspiration, have low effect. However, the evidence supporting these observations was mostly collected in the most severe HFrEF patients. In the same group, pathological baroreflex control of MSNA was also confirmed. However burst activity associated with pulsatile blood pressure, linked to cardiac cycles, is preserved even in severe heart failure. In severe heart failure, abnormal MSNA has also been proven to be a predictor of mortality. More recent data shows that increased MSNA can also be detected in the HFpEF group. A report published in 2019 comparing MSNA results in heart failure patients with HFrEF, HFmrEF, and HFpEF found that with a worsening heart failure, sympathetic activity gradually increases, and a significant increase can be observed in relation to each other. A similar trend was observed for sympathetic BRS. Despite all this, we still do not have enough information about the HFmrEF patient group in terms of autonomic regulation and sympathetic activity.

3.4.2. Correlation of etiology and sympathetic activity in groups of patients with heart failure with mildly reduced and reduced ejection fraction

The impact of heart failure's underlying disease on sympathetic activity is controversial. Some researcher observed a higher sympathetic activation in patients with ischemic heart failure, but other researchers did not find a similar difference. The HFrEF and HFmrEF patient groups have a high prevalence of ischemic origin. Based on all of this, the etiology may also play an important role in the HFmrEF group and needs clarification.

3.4.3. Technical issues of sympathetic baroreflex calculation in healthy subjects and heart failure groups

The $BRS_{SYMPincidence}$ can be determined in healthy subjects at a high rate, but the methodology for spontaneous sympathetic BRS determination is not standardized. According to a recently reported publication that examined healthy subjects, when calculating sympathetic BRS, the acceptable value of the correlation coefficient for the relationship between burst incidence and diastolic pressure should be increased from the previously used $r \geq 0.5$ to $r \geq 0.7$. The determinability of $BRS_{SYMPincidence}$ in heart failure has not been thoroughly investigated. In our own previous study in patients with heart failure, we found that using the $r \geq 0.5$ correlation coefficient limit, the $BRS_{SYMPincidence}$ could only be determined in half of the patients, it is possible that the determinability rate could be even lower when using the "stricter" $r \geq 0.7$ correlation coefficient value.

An additional problem to standardizing the definition of sympathetic BRS is the rapid, shallow, or periodic breathing that is common in heart failure. Breathing has a strong influence on autonomic activity, so when standardizing the method for determining sympathetic BRS, it may be worthwhile to use 6/min slow patterned breathing.

3.4.4. Questions of calculating cardiovagal baroreflex sensitivity

Cardiovagal BRS abnormalities in heart failure are well documented. It is also known that BRS values in this patient group are often indeterminable due to poor correlations. How SLOWB affects the availability and magnitude of BRS values in heart failure can be a question to be clarified. How SLOWB affects the availability and magnitude of BRS values in heart failure could be a question to be clarified. Cardiovagal BRS values determined in different ways are closely correlated. At the same time, the literature is contradictory on the issue of whether the sympathetic and cardiovagal baroreflex indices are related to each other.

4. Aimes

One of the primary goals of our research was to determine the sympathetic activity in the HFmrEF patient group and to compare the results with the data measured in HFrEF and healthy subjects. We aimed to investigate how sympathetic activity in the HFmrEF and HFrEF groups of heart failure is related to demographic, clinical severity (NYHA stage), laboratory (NT pro-BNP), and noninvasively determined characteristics of patients. We also wanted to

investigate the correlations with the 6-minute walking distance, which is the indicator of the patients' physical performance. No such data have yet been published for the HFmrEF group.

We also investigated how the underlying disease affects sympathetic activity in each group of patients with heart failure.

We further aimed to assess the determinability of cardiovagal and sympathetic baroreflex sensitivity in heart failure patients and healthy subjects and to investigate the effect of different correlation limits ($r \geq 0.5$ vs. $r \geq 0.7$) and slow patterned breathing on the determinability of sympathetic baroreflex sensitivity indices.

5. Patients and methods

5.1. Patients

Our study included heart failure patients (adults; >18 years of age), known to be in stable condition, not requiring hospital admission for at least 3 months, under optimal medication, and cared for at the Outpatient Clinic of the Department of Cardiology and Cardiac Surgery of the University of Debrecen Clinical Centre. Patients with known neuropathies were excluded. In the study, that investigated the role of ejection fraction, our HFrEF - HFmrEF subgroup categories were based on international recommendations. In the study, that investigated methodological issues, we included only those patients who had acceptable recordings; - i.e. 3 minutes extrasystole-free segments during both spontaneous-, and slow breathing. Our age-matched healthy control group was created by involving hospital staff members in the study.

5.2. Implementation of autonomous tests, data analysis

Our autonomic tests were performed in the morning hours in the autonomic laboratory of our Clinic. The MSNA examination was performed in a resting supine position. After the localization of the right superficial peroneal nerve, the signals were obtained during its microneurographic examination. Sympathetic nerve activity examination was performed with a continuously recorded ECG-, and noninvasive blood pressure (Finapres 2300) signals. In order to determine the respiratory rate, we put a pneumobelt on the waist of our patients, that provides an uncalibrated breathing signal. During our measurements, we recorded 5 minutes of spontaneous breathing, followed by 5 minutes of slow controlled breathing with a breathing frequency of 6/min and a 1:1 inspiration-expiration ratio. The MSNA, ECG, blood pressure, and pneumobelt signals were digitized online with the Windaq program. The recordings were entered into our archives after preliminary quality control (stable baseline, sympathetic burst-

to-noise ratio of 3:1 or higher). The offline analysis was performed using the WinCPRS software package. The WinCPRS program automatically detects R waves of the QRS complexes recorded on the ECG, systolic and diastolic blood pressure values, and sympathetic bursts. WinCPRS software also includes the stroke volume, and the cardiac output determination program based on pulse contour analysis of arterial pressure. The WinCPRS program was used to determine the parameters of heart rate variability in the "time and frequency domain", and the cardiovagal up-BRS and down-BRS values. Sympathetic nerve activity was characterized by determining the burst frequency and burst incidence, the $BRS_{SYMPincidence}$ was determined by considering both the $r \geq 0.5$ and $r \geq 0.7$ correlation limits.

5.3. Additional examinations

In all patients and healthy volunteers included in the study, cardiac echocardiography was performed to determine the left ventricular ejection fraction (Simpson's method) before the measurement of sympathetic nerve activity. Demographic data, resting blood pressure, pulse, and respiratory rate were recorded from our patients and a blood sample was taken for NT-proBNP determination. If a musculoskeletal disease did not prevent it, the 6-minute walking distance (6MWD) was also tested. Gender- and age-adjusted normal ranges for 6MWD were determined based on standard equations. The 6MWD was considered normal if the subject could walk a distance equal to or greater than the expected distance; the result was considered abnormal if the subject could walk less than the expected distance or was unable to perform the test due to heart failure complaints. In order to rule out neuropathy, electroneurography was also performed on the patients.

5.4. Statistical analysis

Statistical analysis of the data was performed using IBM SPSS software (version 26; Chicago, Illinois USA). Kolmogorov-Smirnov test was performed to determine the normality of the distribution. To compare the groups, Mann–Whitney or for parameters not showing normal distribution, Kruskal–Wallis and Fisher's exact nonparametric tests, chi-square (χ^2) test, and analysis of variance were used. Our correlation analyses were performed using Spearman's correlation test. A P value less than 0.05 was considered statistically significant. Correlations between parameters were evaluated by linear regression.

6. Results

6.1. Sympathetic activity in groups of heart failure patients with mildly reduced and reduced ejection fraction

From June 2018 to the end of February 2020, we performed the MSNA examination of a total of 80 patients, of which 56 patients (23 HFrEF and 33 HFmrEF patients) had good-quality, evaluable MSNA recordings. We included 10 healthy subjects in our control group. Both heart failure patient groups had a significantly higher respiratory rate and body mass index compared to healthy controls.

In the group of patients with a mildly reduced ejection fraction, all but one patient belonged to NYHA functional class I or II, as opposed to the HFrEF group, where 61% of patients were NYHA stage III or IV and only belonged 39% to NYHA class I or II ($p = 0.002$).

A total of 11 patients could not complete the 6-minute walk test due to musculoskeletal reasons; 4 of the patients in the HFrEF group had dyspnea at rest, so they did not perform the walking test. 6MWD was abnormal in 23% of patients in the HFmrEF group and in 58% of the HFrEF patient group ($p=NS$).

We also found a significant difference between the NT-proBNP levels of the heart failure patient groups, HFrEF patients had almost ten times higher NT-proBNP levels compared to the HFmrEF patient group ($p<0.001$). Stroke volumen and cardiac output index were also significantly lower in the HFrEF group compared to those with HFmrEF and healthy controls.

The mean RRI is significantly higher among HFmrEF patients compared to healthy controls. The RRSD in the HFrEF group was lower compared to the other two investigated groups, however the difference was not statistically significant..

Systolic and diastolic blood pressure were similar in the 3 groups. The pulse pressure in the HC and HFmrEF groups had similar values (68 ± 12 mmHg and 66 ± 13 mmHg); in the HFrEF group, this parameter was significantly lower (53 ± 17 mm Hg).

The patients' beta-blocker dose was expressed in carvedilol-equivalent doses. In all heart failure patients included in the study, heart rate and sympathetic burst parameters were not statistically different between with beta-blocker-treated, ($n = 45$) and untreated patients ($n = 11$).

Burst frequency of healthy controls was 27 ± 9 burst/min, and the burst incidence was 39 burst/100 cardiac cycles. In HFmrEF patients these values were 41 ± 11 burst/min and 66 ± 17 burst/100 cycles; and in the HFrEF group 58 ± 17 burst/min, and 82 ± 15 burst/100 cardiac cycles

respectively. The difference between the controls and the two heart failure groups, and also between the HFmrEF and HFrEF groups were highly significantly different, ($p < 0.001$ for all comparisons).

BRS_{SYMPincidence} was detectable in all healthy control subjects. In contrast, only 48% of the HFrEF group and 60% of the HFmrEF group had a calculable index. There was no significant difference in BRS_{SYMPincidence} between the two groups of patients, but the measurable value in the HFmrEF and HFrEF groups was also significantly lower compared to the control group.

We examined the correlations between sympathetic activity and clinical/laboratory values for all heart failure patients ($n = 56$). Burst frequency showed a significant inverse relationship with mean RR interval ($R = 0,67$, $p < 0.001$), ejection fraction ($R = 0,67$, $p < 0.001$), stroke volume index ($R = 0,3$, $p = 0.04$), and pulse pressure ($R = 0,36$, $p < 0.01$). We also found a close correlation between burst frequency and NT-proBNP levels, but at the same time, NT-proBNP moved within very wide range and showed a non-normal distribution, so we also examined the correlation after logarithmic transformation, which led us to the same conclusion: higher burst activity is associated with higher NT-proBNP levels. Burst incidence correlated only with ejection fraction and NT-proBNP levels.

Heart failure patients belonging to NYHA III-IV category had significantly higher burst frequency than patients with NYHA I-II (58 ± 19 vs. 46 ± 15 bursts/min; $p = 0.04$).

Among the patients whose 6-minute walk test was abnormal, we found a significantly higher burst frequency than among those who completed or exceeded the expected distance (54 ± 17 vs. 42 ± 13 bursts/min; $p = 0.01$).

6.2. Sympathetic activity in studied patient groups - the role of the etiology

All patients included in the study ($n = 56$) were also grouped according to etiology; we formed two subgroups based on whether the heart failure has ischemic or non-ischemic origin. Of the patients, 22 suffered from heart failure of non-ischemic and 34 from ischemic origin. A significant difference between the ischemic and non-ischemic groups was found only in terms of age (non-ischemic: 55 ± 10 years; ischemic: 63 ± 10 years $p < 0.01$). Burst frequency and burst incidence values of ischemic heart failure (46 ± 16 burst/min and 71 ± 17 burst/100 cycles) was similar to the results of non-ischemic patients (52 ± 17 burst/min and 75 ± 19 burst/100 cycles) ($p = \text{NS}$). We came to a similar conclusion regarding sympathetic activity when we examined the role of etiology separately within the HFrEF and HFmrEF groups.

6.3. Determinability of baroreflex sensitivity in heart failure: the role of slow patterned breathing

For the present analysis, 33 heart failure patients had recordings of adequate length and quality (free of extrasystoles). Our healthy control group still included 10 subjects. The mean ejection fraction of heart failure patients was $37 \pm 10\%$ (17-48%), and the median value of NT-proBNP levels was 337 ng/mL. 27 patients (87 %) received beta blocker, 28 patients MRA, and 32 patients (97 %) ACE inhibitor or ARB therapy. The heart rate of the patients and healthy controls was similar, but the systolic blood pressure of the heart failure subjects was significantly lower compared to the blood pressure of the HC group. Resting respiratory rate, burst frequency, and burst incidence were significantly higher among HF patients.

Slow patterned breathing (SLOWB) induced no significant heart rate changes. The systolic blood pressure of both heart failure group patients and the healthy controls decreased slightly during the 6/min breathing pattern (HF: -5 ± 11 mmHg, HC: -4 ± 7 mmHg; $p = \text{NS}$). The reductions in burst frequency (-5 ± 5 vs -2 ± 3 burst/min; $p < 0.03$) and burst incidence (-9 ± 7 vs -3 ± 4 burst/100 cardiac cycles; $p < 0.003$) were greater in heart failure patients during slow patterned breathing. The burst frequency and burst incidence detected during spontaneous breathing and 6/min patterned breathing in heart failure patients were closely correlated.

During spontaneous breathing, cardiovagal up- and down-BRS values could be determined in a quarter of heart failure patients; their values were similar during SB and SLOWB, however, the determination rate of both cardiovagal BRS indicators increased significantly with the 6/min breathing pattern. Among healthy controls, the up- and down-BRS could be calculated in a very high proportion, and there was no significant change in the determinability of the mentioned parameters due to the effect of SLOWB. Heart failure patients and healthy control subjects did not differ significantly in terms of up- and down-BRS either during spontaneous or slow controlled breathing.

Using a correlation value of $r \geq 0.5$, there was no significant difference between healthy controls and heart failure patients in the BRS determinability of sympathetic incidence. $\text{BRS}_{\text{SYMPincidence}}$ values were significantly higher during spontaneous breathing and not significantly higher during 6/min patterned breathing in healthy controls compared to heart failure patients. Compared to spontaneous breathing, a higher (more negative) $\text{BRS}_{\text{SYMPincidence}}$ value could be determined during slow controlled breathing both among heart failure and

healthy controls, but the difference was not statistically significant in any of the examined groups.

Using a correlation value of $r \geq 0.7$, the determinability of $BRS_{SYMPincidence}$ in healthy controls was unchanged but further decreased in the heart failure group compared with the determinability using a value of $r \geq 0.5$. This reduction resulted in a statistically significant difference between heart failure patients and healthy controls during both spontaneous and 6/min patterned breathing. Using the above correlation value, 6/min patterned breathing significantly increased the value of $BRS_{SYMPincidence}$ among heart failure patients.

In the patients where the $BRS_{SYMPincidence}$ value could be determined with a correlation value of $r \geq 0.7$ too, a significantly lower burst frequency and burst incidence was observed by default, compared to those in whom this value could not be determined. There was also a similar difference between the patients whose BRS values were determinable with $r \geq 0.5$ and $r \geq 0.7$ values, but the difference did not reach the level of significance.

We found no significant correlation between cardiovagal baroreflex indices and $BRS_{SYMPincidence}$ in either the healthy controls or the heart failure group during either spontaneous or 6/min slow patterned breathing.

7. DISCUSSION

7.1. Sympathetic activity in groups of heart failure patients with mildly reduced and reduced ejection fraction

The most important finding of our research is that the sympathetic activity (burst frequency and incidence) in the HFmrEF group is significantly, pathologically increased compared to healthy subjects, but does not reach the level measured in the HFrEF group. In the literature, only one report, based on a small number of cases, dealt with the sympathetic activity in patients with a mildly reduced ejection fraction. Our own study confirmed the previous observation in considerably larger patient groups. Based on the $BRS_{SYMPincidence}$ method we also confirmed that the baroreflex control of sympathetic activity was reduced in patients with HFmrEF compared to healthy subjects.

The MSNA burst frequency showed a significant inverse relationship with the length of the average RR intervals. This relationship follows from the close connection of the bursts to the cardiac cycle, - the faster pulse (shorter RRI) enables the occurrence of more potential bursts during a unit of time. When the activity is expressed in burst incidence (normalized), the RR

interval dependence is lost. In the HFrEF group, less effective heart rate control was obviously achieved than in the HFmrEF group, so it may be assumed that the higher burst frequency is related to the higher heart rate. This assumption is refuted by the fact that there was also a significant difference in the burst incidence between the HFmrEF and HFrEF groups, which is explained by factors independent of the heart rate detailed in the introduction. It should be noted that the less successful heart rate control in the HFrEF group cannot be explained by the lower-dose beta-blocker treatment, but by the increased sympathetic activity characteristic of the group, and by the fact that in this group further dose increases were already prevented by the patients' frail state and lower blood pressure values.

In the studied groups, the differences in sympathetic activity were accompanied by the occurrence of abnormal clinical and laboratory parameters. BMI was significantly higher in both patient groups than in volunteers. The reason for this could be the lack of exercise; - associated with the disease, the different eating habits, and the fluid retention that occasionally occurs in the patient. The prognostic significance of NT-proBNP levels in the HFrEF patient group is well known. It seems that the prognostic value of NT-proBNP in HFmrEF is also significant. It is worth remembering that in this group of patients the presence of certain comorbidities (diabetes, obesity, atrial fibrillation) can modify this important laboratory marker. Patients with diabetes and atrial fibrillation were excluded from our present study, so these factors did not influence our results, but the very close, positive correlation between MSNA and NT-proBNP levels was still confirmed.

In our study, we categorized the patient groups based on their ejection fraction, however, the grouping can be based on other functional parameters as well. The role of NYHA functional status is obscured by the fact that in early studies; - owing to the small number of patients functional classes were often combined. A recently published meta-analysis found a significant increase in sympathetic activity in parallel with the deterioration of the functional stage of heart failure (NYHA I-II; and NYHA III-IV). The results of our present study support this observation.

Those patients who completed a shorter distance during the 6-minute walk test, than expected, were mainly members of the HFrEF patient group and had an elevated burst frequency. Examining all heart failure patients, the stroke volume index showed a weak inverse correlation with the burst frequency. The inverse relationship between pulse pressure and burst frequency has clinical significance.

7.2. Correlation of sympathetic activity with etiology in groups of heart failure patients with mildly reduced and reduced ejection fraction

The role of etiology in sympathetic overactivity in the HFmrEF group was unclear until our present study. The prevalence of ischemic etiology is similar in the HFmrEF and HFrEF subgroups; but we do not have clear results about the role of etiology, even for the more-studied HFrEF group. Grassi et al found no significant difference in sympathetic activity between ischemic and non-ischemic HFrEF patients. Different observations were reported by Notarius's et al who found significantly higher sympathetic activity in ischemic heart failure compared to non-ischaemic heart failure. They concluded, that the difference between the two populations could be related to abnormal post myocardial infarction reflex regulation. Both the necrosis-induced interruption of vagal afferents (and loss of their inhibitory effect on sympathetic outflow), and chronic ischemic stimulation of ventricular sympathetic afferent nerves were suggested. Our results from the study of 56 patients who had stable condition regarding the angina confirm the results published by Grassi et al. In separate examination of the subgroups, we were able to extend our findings to the HFmrEF subgroup as well. Further studies are needed to clarify the role of ischemia.

7.3. Methodological issues of calculating sympathetic baroreflex sensitivity, limitations of the test in heart failure

As it is explained in the introduction section, the extent; i.e. the "sensitivity" of sympathetic baroreflex regulation can be derived from MSNA and blood pressure recordings. One might expect that the parameters based on characteristics of the sympathetic burst, such as amplitude, burst area, or total area, (which considers the incidence as well), can describe most precisely the changes in sympathetic activity. In practice the determinability and reproducibility of BRS using the above parameters is limited, even in healthy subjects. The burst amplitude and area are very sensitive to the position of the recording microelectrode. Minimal position changes sometimes result in a complete loss of signal, but at least a change in signal amplitude. Such a change can falsify the baroreflex relationship. Changing of amplitude does not affect the interpretation of the burst incidence, and it can explain why the determination of $BRS_{SYM\text{Pincidence}}$ was more feasible in healthy subjects according to several publications.

Decrease of sympathetic BRS values is well documented in patients with severe heart failure, but limited data is available about the feasibility of the determinations. By general consensus, sympathetic BRS values determined with correlation coefficient $r \geq 0.5$ are accepted

as valid parameters, however by using this cutoff we inevitably include false indices with non-baroreflex mechanism. For this reason, a more stringent correlation cut-off of $r \geq 0.7$ has recently been proposed for healthy subjects. In the present study, we also investigated the correlation limit values of $r \geq 0.5$ and $r \geq 0.7$.

We found that the using of $r \geq 0.7$ correlation value, did not affect the determinability of $BRS_{SYMPincidence}$ in healthy control group. In contrast, determinability of $BRS_{SYMPincidence}$ in heart failure patients in resting position and during spontaneous breathing was only 39%. The indeterminable burst incidence - diastolic pressure correlation could be related to the extremely high burst incidence, - in some cases approaching 100%, which remains on the same elevated level within a wide diastolic pressure range. Drug induced blood pressure fluctuations are significantly higher than spontaneous fluctuations at rest, and it is possible these fluctuations can also reach a diastolic blood pressure range where burst inhibition can already appear. Therefore, our present observations cannot be extended to pharmacological tests.

The group, where $BRS_{SYMPincidence}$ was indeterminable, not only the sympathetic activity was increased, was also characterized by lower EF and higher NT-proBNP values. It can be assumed this is a subgroup with more severe condition. We have not yet data on the prognostic value of the undeterminable sympathetic baroreflex indices; follow-up studies may answer this question.

7.4. The heart rate variability parameters and the cardiovagal baroreflex sensitivity indices during spontaneous breathing at resting condition

The time and frequency domain parameters of HRV are studied for decades. It has been documented, that cardiovagal indices determined by different methods, decrease abnormally in heart failure. A remarkable observation indicated, that abnormally reduced cardiovagal BRS indices (and some HRV parameters) were associated with poor outcomes after acute myocardial infarction. Subsequently, similar observations have been noted regarding the prognostic value of cardiovagal BRS induces in heart failure. Over the past two decades, the treatment of heart failure has changed significantly, new drugs have appeared, and increased attention has been focused on therapy optimization. According to a recently published report, cardiovascular BRS determined by three different methods (one drug, two spontaneous) did not predict 5-year survival.

Our own observation also confirms that cardiovagal BRS values determined by spontaneous methods do not separate patients from healthy controls.

7.5. The role of slow patterned breathing (SLOWB) in calculating baroreflex sensitivity indices in heart failure

Rapid, shallow breathing is common in heart failure, and sometimes periodic breathing can be observed in awake patients. Tachypnoea was common in the patient groups of the present study. SLOWB was used in this study to standardize the breathing pattern. Our results indicated that the determinability of the up-, and down-BRS indices in heart failure patients were significantly increased during 6/min breathing, but their actual values did not change in healthy subjects nor in heart failure patients.

The effects of SLOWB on MSNA in heart failure are well known. Similar to previous reports, in the present study, deep slow breathing reduced burst frequency and burst incidence in heart failure patients and caused a small reduction in sympathetic activity in healthy subjects. As an option to reduce sympathetic activity, the therapeutic use of SLOWB in heart failure may be considered, similar to its use in patients with hypertension.

Our own study has demonstrated that the determinability of $BRS_{SYMPincidence}$, in contrast to cardiovagal BRS, is actually reduced during SLOWB. SLOWB generates a pronounced increase in blood pressure fluctuations compared to normal, or rapid breathing. However, autonomic responses for respiration are not reducible to an arterial baroreflex mechanism. The role of pulmonary stretch receptors and cardiopulmonary baroreceptors is also have important. The vagal responses triggered by the different mechanisms at the beginning of inspiration are directed to the same direction and we acknowledge, that other effects besides the arterial baroreflex "contaminate" the response.

In sympathetic responses, there is no such synergism. Periodic sympathoinhibition caused by deep slow inspiration interferes with the baroreflex mechanism. Although the standardization of breathing is still desirable, for sympathetic BRS tests, a different breathing rate should be chosen instead of the 6/min frequency. The determination of the optimal rate requires further investigation.

8. Summary

Heart failure is currently a worldwide epidemic; one of the leading causes of morbidity and mortality. During the past decades much of our efforts have been dedicated to the management of heart failure with reduced ejection fraction (HFrEF). Remarkable new diagnostic and therapeutic goals have been achieved in this field. More recently other heart failure groups; -

those with mildly reduced (HFmrEF) and preserved ejection fraction (HFpEF) have gained similar attention, however further efforts are required to elucidate all aspects of these entities.

Pathological neurohormonal activations are well known in the HFrEF group, however less data is available regarding the HFmrEF group. The documentation of the degree and pattern of sympathetic activation in this patient group would allow a better understanding of the processes, and could clarify the pathophysiological analogies and connections between heart failure subgroups with different ejection fractions. This would be essential to be known for the prognosis of the heart failure and it could help in the selection of the medical treatment.

Our investigations are aimed for the MSNA characteristics of the HFrEF and the HFmrEF group, while paying particular attention to the MSNA baroreflex regulation.

New observations:

1. We have documented in a large patient population that the MSNA activity in the HFmrEF group is significantly higher than in the healthy controls, but also significantly lower than measured in the HFrEF groups. We also demonstrated that the sympathetic baroreflex index ($BRS_{SYMP-incidence}$) has a lower value than in healthy controls, but larger than what was found in the HFrEF group. We have found a significant correlation between burst frequency and NTproBNP level. The burst frequency showed a significant inverse correlation with the ejection fraction, the RR interval, the pulse pressure and with the stroke volume index.
2. We have confirmed that the etiology of heart failure does not influence the level of sympathetic activity in the HFrEF group. Our study is the first to document the same relationship in the HFmrEF subgroup.
3. We have demonstrated that the determinability of the $BRS_{SYMP incidence}$ in heart failure patients is limited. The undeterminable $BRS_{SYMP incidence}$ is correlated with the higher values of the resting MSNA burst frequency and the burst incident. Thus, the undeterminable $BRS_{SYMP incidence}$ in heart failure patients could be associated with more advanced disease, however the prognostic value of this sign is not yet known.
4. We have demonstrated that the determinability of the cardiovagal BRS values in the heart failure group is limited, and these parameters cannot differentiate the heart failure patients from the healthy controls.
5. The 6/min slow patterned breathing caused a decline in MSNA burst frequency and burst incidence in both studied groups, however the degree of changes was significantly

higher in heart failure patients than in healthy controls. The slow patterned breathing increased the determinability of the cardiovagal BRS indices in the heart failure group, but their discriminative capabilities were not increased. Slow patterned breathing on the other hand decreased the determinability of the BRS SYMP incidence .

9. Publications



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PhD Publication List

Candidate: Réka Urbancsek

Doctoral School: Kálmán Laki Doctoral School

List of publications related to the dissertation

1. **Urbancsek, R.**, Csanádi, Z., Forgács, I. N., Papp, T. B., Boczán, J., Barta, J., Jenei, C., Nagy, L., Rudas, L.: The Feasibility of Baroreflex Sensitivity Measurements in Heart Failure Subjects: the Role of Slow-patterned Breathing.
Clin. Physiol. Funct. Imaging. 42 (2), 260-268, 2022.
DOI: <http://dx.doi.org/10.1111/cpf.12755>
IF: 2.121 (2021)
2. **Urbancsek, R.**, Csanádi, Z., Forgács, I. N., Papp, T. B., Boczán, J., Barta, J., Jenei, C., Nagy, L., Rudas, L.: Sympathetic activation in heart failure with reduced and mildly reduced ejection fraction: the role of aetiology.
ESC Heart Failure. 8 (6), 5112-5120, 2021.
DOI: <http://dx.doi.org/10.1002/ehf2.13580>
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List of other publications

3. **Urbancsek, R.**, Forgács, I. N., Papp, T. B., Boczán, J., Barta, J., Édes, I., Csanádi, Z., Rudas, L.:
Cardiovagalis és szimpatikus artériás baroreflex szabályozásának vizsgálata
szívelégtelenségben.
Orv. hetil. 162 (3), 91-98, 2021.
DOI: <http://dx.doi.org/10.1556/650.2021.31962>
IF: 0.707
4. **Urbancsek, R.**, Forgács, I. N., Papp, T. B., Boczán, J., Barta, J., Édes, I., Csanádi, Z., Rudas, L.:
Az izom szimpatikus idegaktivitás vizsgálatának elméleti alapjai és története.
Orv. hetil. 161 (29), 1190-1199, 2020.
DOI: <http://dx.doi.org/10.1556/650.2020.31780>
IF: 0.54
5. Hajas, O., Bagoly, Z., Tóth, N. K., **Urbancsek, R.**, Kiss, A., Kovács, K. B., Sarkady, F., Nagy, A.
C., Oláh, A., Nagy, L., Clemens, M., Csiba, L., Csanádi, Z.: Intracardiac Fibrinolysis and
Endothelium Activation Related to Atrial Fibrillation Ablation with Different Techniques.
Cardiol Res Pract. 2020, 1-8, 2020.
DOI: <http://dx.doi.org/10.1155/2020/1570483>
IF: 1.866
6. Jenei, C., Nagy, L., **Urbancsek, R.**, Czuriga, D., Csanádi, Z.: Three-Dimensional
Echocardiographic Method for the Visualization and Assessment of Specific Parameters of
the Pulmonary Veins.
J. Vis. Exp. 164, 1-13, 2020.
DOI: <http://dx.doi.org/10.3791/61215>
IF: 1.355
7. Bagoly, Z., Hajas, O., **Urbancsek, R.**, Kiss, A., Fiak, E., Sarkady, F., Tóth, N. K., Orbán-Kálmándi,
R. A., Kovács, K. B., Nagy, L., Nagy, A. C., Kappelmayer, J., Csiba, L., Csanádi, Z.:
Uninterrupted Dabigatran Administration Provides Greater Inhibition against Intracardiac
Activation of Hemostasis as Compared to Vitamin K Antagonists during Cryoballoon Catheter
Ablation of Atrial Fibrillation.
J Clin Med. 9 (9), 1-13, 2020.
DOI: <http://dx.doi.org/10.3390/jcm9093050>
IF: 4.241
8. Szirá, K., Soltész, B., Hajas, O., **Urbancsek, R.**, Nagy-Baló, E., Penyige, A., Csanádi, Z., Nagy,
B.: PITX2 and NEURL1 SNP polymorphisms in Hungarian atrial fibrillation patients
determined by quantitative real-time PCR and melting curve analysis.
J. Biotechnol. 299, 44-49, 2019.
DOI: <http://dx.doi.org/10.1016/j.jbiotec.2019.04.022>
IF: 3.503





9. Soltész, B., **Urbancsek, R.**, Pös, O., Hajas, O., Forgács, I. N., Szilágyi, E., Nagy-Baló, E., Szemes, T., Csanádi, Z., Nagy, B.: Quantification of peripheral whole blood, cell-free plasma and exosome encapsulated mitochondrial DNA copy numbers in patients with atrial fibrillation.
J. Biotechnol. 299, 66-71, 2019.
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