Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Autonomic nervous system activity assessement by heart rate variability in experimental bladder outlet obstruction

Aktywność autonomicznego układu nerwowego oceniana metodą zmienności rytmu serca w doświadczalnym modelu blokady podpęcherzowej

Łukasz Dobrek [A, B, C, D, E, F, G], **Agnieszka Baranowska** [B], **Beata Skowron** [B],

Piotr J. Thor [A, G]

Department of Pathophysiology, Jagiellonian University Medical College

Summary

A syndrome with urgency, with or without associated urine incontinence and usually accompanied by higher urinary frequency and nocturia has been named "overactive bladder; OAB". OAB is an entity with complex pathophysiology, involving both myogenic and neurogenic (afferent / efferent bladder innervation) disturbances. OAB symptoms accompany benign prostatic hypertrophy - BPH ("obstructive OAB"). The aim of the study was to estimate the autonomic nervous system activity (ANS) in the experimental bladder outlet obstruction (BOO) which was an animal model of the human BPH. The study was conducted using 30 female rats, divided into two groups: BOO animals (n=15), with surgically induced BOO (by partial ligation of the proximal urethra) and control ones (n=15), which underwent sham procedure (without urethral ligation). Two weeks after the surgery, in both groups, ANS activity was estimated using time- and spectral analysis of the heart rate variability recordings. The bladder overactivity in BOO animals was confirmed using urodynamic recordings and bladder histological assessment, juxtaposed against the results of the control group. The key finding of our study was the development of autonomic disturbances in bladder outlet obstruction (BOO) rats. Our study revealed that BOO animals were characterised by diminished rMSSD and spectral HRV parameters: TP, LF and HF, in comparison with the control group. The normalised nLF and nHF parameters did not differ significantly in both groups, although slight changes in the nLF (increased) and nHF (decreased) were noted in BOO group. The absolute VLF value was almost the same in both studied populations, however, the percentage part of this component in the appropriate HRV spectrum differed considerably in both studied groups. In BOO animals, VLF percentage amounted to about 90%, whereas in control animals this parameter reached only about 53% of the total power spectrum.
Thus, to sum up, our findings suggest autonomic imbalance with decreased global autonomic tension and diminished parasympathetic activity with relatively sympathetic overactivity.

Keywords: bladder outlet obstruction (BOO) • overactive bladder (OAB) • autonomic nervous system (ANS) • heart rate variability (HRV)

Author's address: Łukasz Dobrek MD, PhD, Department of Pathophysiology Jagiellonian University Medical College ul. Czysta 18, 31-121 Kraków; e-mail: lukaszd@mp.pl

Abbreviations: **ANS** – Autonomic Nervous System, **BOO** – Bladder Outlet Obstruction, **HF** – High Frequency (HRV spectrum component at high frequencies), **HR** – Heart Rate, **HRV** - Heart Rate Variability, **ICI** – Inter-contraction Interval (urodynamic parameter), **LF** – Low Frequency (HRV spectrum component at low frequencies), **MVP** – Micturition Voiding Pressure (urodynamic parameter), **nHF** – normalized High Frequency, **nLF** – normalized Low Frequency, **OAB** – Overactive Bladder, **PN** – Peak Number (urodynamic parameter), **PNM** – Peak Number per Minute (urodynamic parameter), **rMSSD** – root Mean Square of the RR intervals Successive Differences, **TP** – Total Power of HRV spectrum, **VLF** – Very Low Frequency (HRV spectrum component at very low frequencies).

Introduction

The main bladder function is to store urine in the filling period, during the continuous process of urine formation by the kidneys and to remove urine during discontinuous voiding process. Bladder functioning implicates cooperation of many regulatory mechanisms, involving afferent pathways sensing bladder filling and distension, central nervous processing of such signals and effector pathways enabling urine storage and voiding. The disturbances of complex mechanisms mentioned above result in voiding dysfunction. It may occur in the form of too frequent micturition with or without inadequate timing, insufficient micturition or involuntary micturition. A syndrome with urgency, with or without associated urine incontinence and usually accompanied by higher urinary frequency and nocturia has been named "overactive bladder; OAB" [24]. Urgency is characterised as a sudden, compelling desire to pass urine and a higher voiding frequency means that according to a patient, he/she voids too often by day; nocturia is defined as a need to wake at night to pass urine [24,26]. The involuntary detrusor contractions during filling period are regarded to be of essential pathophysiological meaning in OAB. The detailed description of bladder physiological aspects and the neurogenic and myogenic theories on OAB pathophysiology have been given in many reviews [1,5,13], including our ones [8,9] and crosses the frames of the present study.

It is obvious that bladder functioning is under control of many physiological and pathophysiological factors involving local pathologies, such as bladder outlet obstruction (BOO). It is an entity of special clinical meaning in view of the fact that patients with benign prostatic enlargement (benign prostatic hypertrophy; BPH) display OAB symptoms due to BOO – both arising from abnormal voiding (weak stream) as well as irritative symptoms involving impaired bladder storage function (frequency, nocturia, urgency). It must be stressed that BOO surgical relief does not completely abolish OAB symptoms, thus, operated male patients with BPH often still exhibit storage symptoms with resolving voiding disturbances. Such alternation may be a result of the gross anatomical, histological and functional bladder rearrangement [24].

Bladder rebuilding during BOO may be studied in both humans and experimental models. In general, several induced animal models have been developed to study OAB pathophysiology, whereby a relevant pathophysiological condition is experimentally applied to a healthy animal to mimic the human challenge.

Effects similar to BOO in humans are relatively straightforward to replicate in animals by partial obstruction of the urethra development that narrows the urethra immediately or does so gradually as the immature animals grow. The experimental BOO model reveals many structural and functional bladder changes, including total bladder enlargement (that develops over months and years in humans, but over days and weeks in animals), smooth muscle cells hypertrophy of the bladder wall, patchy denervation of detrusor muscle, increased spontaneous myogenic activity with non-micturition contractions [26]. One of the studies to confirm bladder wall rebuilding was Pampinella et al. [25], an experimental work, which demonstrated postnatal structural bladder changes after 7 and 30 days of obstruction in immature, 30-day-old rabbits. In bladder histological specimen assessment they showed acceleration of the fibroblasts conversion to smooth muscle cells and their spatial differentiation and specific arrangement in the serosa. Thus, they demonstrated that BOO leads to the overall bladder weight increase and bladder wall mu-

scurisation. Together with bladder wall rebuilding, bladder autonomic innervation was also observed. Gosling et al. [11] revealed in their histological study a significant reduction in the amount of autonomic nerve supplying detrusor in obstructed patients, thus proving again that functional impairment of the urinary bladder occurs in response to outflow obstruction. These findings have been confirmed by experimental studies. Tammela and Lasanen estimated the effect of distension on both adrenergic [31] and cholinergic [17] innervation in the rat urinary bladder. They demonstrated depletion of catecholamines in the early period after bladder obstruction with almost complete recovery throughout the 21-day study period. Hence, according to Tammela and Lasanen, the primary clinical success of distension therapy for the treatment of OAB may be at least partly due to a reversible disturbance in the function of adrenergic nerves [31]. Moreover, the same researchers showed cholinergic hypoinnervation 7 days after bladder distension, persisting up to 21 days, with an increase of cholinergic nerves in the longer period of the observation. Thus, their findings indicate transient cholinergic innervation damage, which may in turn explain the prolonged voiding difficulties often observed after catheterisation of an overdistended bladder in patients with urinary retention. Similarly to their previous results, they suggested that the short-lasting effect of bladder dilatation therapy used to treat detrusor instability may also be due to the fairly rapid regeneration of cholinergic innervation [19]. Additionally, apart from cholinergic and adrenergic differences in obstructed bladders, alternation of purinergic signalling in this model was also found. The experimental study by Calvert et al. [3] supported earlier findings of impaired cholinergic decrease in the early period after bladder obstruction, but they also demonstrated that bladder purinergic nervous component was at the same time increased. In another experimental study, Lluel et al [18] revealed through a rat model of BOO that in long-lasting obstruction increased basal tone of cholinergic neurotransmission occurs, according to them, due to proliferation of pre-existing cholinergic nerves that become functionally more important than the cholinergic-purinergic nerves which predominate physiologically [18].

To sum up, many clinical and animal studies confirmed abnormal detrusor contractility in BOO, resulting from overall myoelectrical and mechanical disturbed functioning of the bladder smooth muscles (consistently with the general OAB myogenic theory) as well as from impaired bladder innervation and its response to various stimuli. In conclusion, the filling symptoms typically observed in BOO patients or experimental animals could be explained as increased muscarinic or excitatory α-1 adrenergic bladder input or decreased inhibitory β-adrenergic pathways. However, it seems that cholinergic impairment has larger role in BOO pathophysiology as it is more dominant in the bladder [24].

Against the background of the reports indicating disturbed bladder autonomic supplying and its warped functioning, the studies estimating global autonomic activity in the course of BOO are scant. Thus, we tried to evaluate indirectly the autonomic nervous system (ANS) functioning in experimental model of bladder outlet obstruction using the heart rate variability (HRV) method. Nowadays, HRV is considered to be one of the best ANS assessment methods (both in experimental and clinical conditions), based on the measurement of the variability of the R-R intervals in [ms] in ECG signal in participants with dominant sinus rhythm [2,30]. There are two main types of HRV analysis: time domain and spectral (frequency) domain analyses. The first one is directly based on the main studied parameter – mean R-R [ms]; also known as mean normal-normal (mNN [ms]) and several statistically derived parameters. In general, the higher increase of R-R intervals variability is demonstrated, the more pronounced heart autonomic control occurs, which also suggests a higher global autonomic tension. The former analysis is associated with so-called HRV spectrum that is a result of ECG signal transformation using special mathematical operating – fast Fourier transformation. In short (usually lasting 5-30 minutes) recordings one can distinguish three main spectral components, expressed in power units [ms*ms] – VLF (at a very low frequency of the HRV spectrum), LF (at a low frequency) and HF (at a high frequency) and total power – TP of the spectrum. There are also normalised LF – nLF and normalised HF – nHF values, expressed in normalised units [n.u.] and derived as a result of referring of the appropriate (LF/HF) component to the total power spectrum diminishing of VLF spectrum. These spectral parameters are regarded to reflect global autonomic tension (TP), common sympathetic and parasympathetic activity (LF) as well as pure parasympathetic (HF, nHF) or sympathetic (nLF) tension. There is no consensus as to the VLF interpretation, as this component is regarded to reflect various, short-term autonomic activities associated with thermoregulatory, endocrine, heart and vasomotor (renin-angiotensin-aldosterone system) responses that may be mediated mostly by sympathetic but also potentially by the parasympathetic ANS branch. Detailed information related to the HRV methodology is contained in the HRV guidelines [21] and some reviews [27].

MATERIALS AND METHODS

Ethics: The study protocol was approved by the First Local Ethic Committee in Cracow (agreement decision 126/2010).

Animals, studied groups and general study plan: The animals were obtained from the central laboratory. Upon arrival at the animal house of Pathophysiology Department, the rats were allowed an acclimatisation period of one week in groups of five per cage. The animals were housed at room temperature, with 12-12 hours day-night cycle, with standard food (Labofeed Kcynia) and water *ad libitum*.

After the acclimatisation period, the animals were randomised into study groups of the animals with surgical-

ly induced proximal bladder outlet obstruction – BOO (mean body weight 243,3±13,5g) and the control group (mean body weight 236,3±6,3g). Each group consisted of 15 female 10-week-old Wistar rats, thus the experiment was carried out using 30 animals. We planned to perform HRV recordings with statistical BOO/control differences analysis, urodynamic bladder functioning estimation and bladders macroscopic and histological assessment in both BOO and control animals.

BOO model: Animals enrolled into the BOO group underwent surgery procedure to induce partial urine outflow obstruction, leading to bladder overactivity. The technique used to obtain a bladder outlet obstruction, both in growing and mature animals, has already been described in the 1980s by some investigators – for example Sibley (1985) [28], Kato et al. (1988) [16] or Harrison et al. (1990) [12] and it is still used with small modifications by many other researchers (e.x. Das et al ; 2002 [6], Kamiyama et al ; 2007 [15], de Jongh et al ; 2007 [7]).

The rats were briefly anesthetised with sodium pentobarbital (Morbital, Biowet, Puławy; 35mg/kg body weight) intraperitoneally. Under general anaesthesia, a 1mm diameter stainless urinary catheter was placed in the urethra. After performing a lower midline laparotomy, the bladder and proximal urethra were exposed and a 4/0 silk ligature was tied around the proximal urethra and the inserted rod. Then the catheter was removed leaving the urethra partially occluded. The laparotomy incision was then closed, two antibiotics (neomycinum – Neomycin spray, oxytetracyclinum – Oxycort spray) were administrated through the surgery wound and the animals were allowed to recover. After 14 days after urethral ligature, HRV recording was taken.

Control group: 15 rats underwent sham surgery in which the urethra was circumferentially dissected but not ligated. The other surgery and post-surgery protocol was similar to BOO animals. The HRV study was also carried out 2 weeks after the sham procedure.

HRV studies: Under urethane anaesthesia (1200mg/kg body weight; Sigma-Aldrich) ECG recordings were taken during 20-minute rest periods in each studied animal. This anaesthetic agent was chosen having taken into consideration the literature reports which suggested the proportional (up to the applied dose) impact on tonic activity of both, sympathetic and parasympathetic ANS parts and relatively small influence on cardiac reflexes [19,20]. After terminating the ECG registration and extrasinusal extopics elimination, an HRV analysis was performed. Standard time (mNN, SDNN, maxNN, min NN – all in [ms], rMSSD and mean HR [bpm]) and spectral (frequency; TP, VLF, LF, HF – all in [ms*ms] and normalised nLF and nHF in [n.u.]) parameters were calculated. The frequency range for respective spectral components was set as: 0,18<VLF<0,28<LF<0,78<HF<3 and commonly accep-

ted interpretation criteria were admitted, as previously mentioned in the Introduction. Results were presented as mean values ± SD.

Statistical HRV parameters analysis: The statistical assessment of the results obtained in paired studied groups (BOO vs. control) was conveyed after expressing them as LN values using parametric Fischer-Snedecor test with α=0.05. The expression of the spectral parameter values in the form of their natural logarithms was the consequence of the lack of their normal distribution. The H0 hypothesis of equality of analysed parameter variations in two studied populations was verified versus an alternative H1 hypothesis which assumed their inequality (and thus, the existence of statistically significant differences). The results of the statistical HRV parameter analysis are given in the table 1 below.

Table 1. Statistical analysis of obtained HRV parameters

PARAMETER	Groups		Statistical analysis
	BOO	control	
ln mNN	5.15 ± 0.07	5.09 ± 0.06	p=0.076
ln max NN	5.24 ± 0.009	5.24 ± 0.001	p=0.244
ln min NN	5.02 ± 0.11	4.94 ± 0.002	p=0.021
ln SDNN	1.84 ± 0.68	2.12 ± 0.35	p=0.161
ln HR	5.85 ± 0.07	5.91 ± 0.06	p=0.076
ln RMSSD	1.41 ± 1.26	2.42 ± 0.64	p=0.043
ln TP	2.06 ± 1.77	3.46 ± 0.59	p=0.027
ln VLF	1.89 ± 1.84	2.56 ± 1.16	p=0.208
ln LF	-1.11 ± 2.00	1.60 ± 0.47	p=0.002
ln HF	-0.88 ± 1.80	2.18 ± 0.86	p=0.001
ln LF/HF	-0.23 ± 0.86	-0.58 ± 0.68	p=0.213
ln nLF [n.u.]	3.71 ± 0.49	3.54 ± 0.37	p=0.237
ln nHF [n.u.]	3.94 ± 0.37	4.12 ± 0.31	p=0.189

Bladder overactivity assessment: After terminating the HRV registration, still under the conditions of urethane general anaesthesia, 30-minutes-lasting urodynamical recordings were taken to confirm bladder overactivity.

Urethane was used again, after taking into consideration its also relatively small potential for the impairment of the urinary bladder motility, as described previously [19,20]. In an anaesthetised rat, the abdomen was opened through the low midline abdominal incision and the obstructed (BOO group) or sham-operated (control one) urinary bladder was exposed. The polyethylene catheter (external diameter 0.97 mm/internal diameter 0.58 mm, BALT Poland), connected to pressure transducer with analysis hardware (Power Lab; Chart 5 Pro v.5.4.2, ADInstruments) and injection pump (Unipan

340A), was inserted into the dome of the bladder through a small incision. The catheter was fixed in place with silk ligature 4-0. Cathetered bladders were left for 15 minutes to stabilise. To begin with, after system calibration, the baseline recordings were taken at room temperature during continuous saline infusion of 0.046 ml/min for 30 minutes. Selected standard urodynamic parameters were analysed to confirm overreactivity of the bladder contractility: peak number (PN), peak number per minute (PNM), intercontraction interval (ICI [s]) and micturition voiding pressure (MVP [cm H_2O]). During the experiment the animals were kept under a heating lamp to prevent a fall in body temperature.

The examples of resting urodynamic recordings obtained in both control (figure 1) and BOO studied animals (figure 2) are given below.
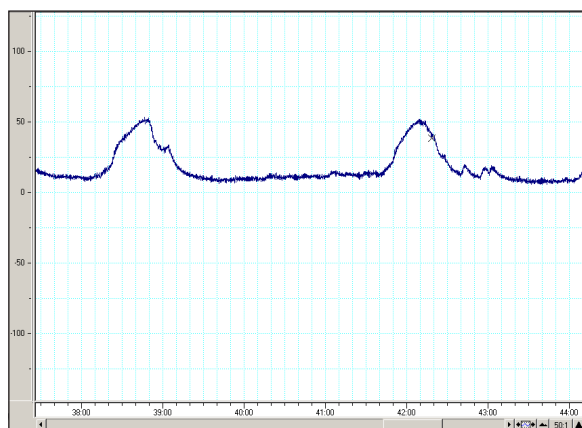

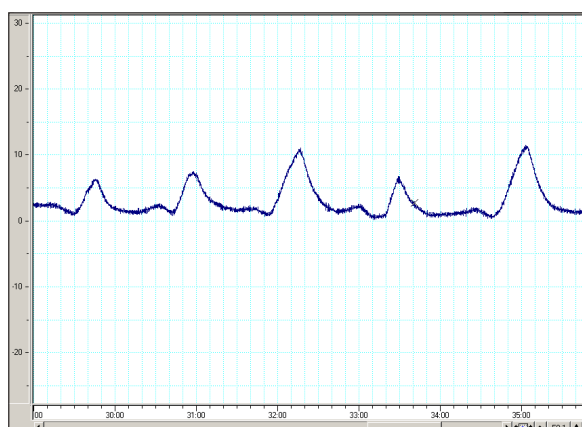**Fig. 1.** The example of urodynamic recordings obtained in control rats

Fig. 2. The example of urodynamic recordings, supporting bladder motor function overactivity, obtained in BOO rats

Taking into consideration the inspection of the visual urodynamical records as well as the calculated urodynamical parameter values, we were able to assure that BOO animals display urodynamical OAB findings. In this group, we observed increased PN, PNM with decreased ICI and MVP, as compared to the control group.

The control rats (group 1) demonstrated normal bladder activity, being an indirect next evidence of bladder overreactivity recorded in BOO animals.

Urinary bladder assessment: In both BOO and in the control group, the bladders were collected once the urodynamic recordings had been taken and a lethal sodium pentobarbital dose (100mg/kg body weight) was administered. In BOO animals, before bladder excision, the ligature around the urethra was carefully removed. In both studied groups, urinary bladders were red, swollen and in most cases covered by abundant serosal petechial suffusions. Histological inflammatory changes with leucocytes infiltration of the bladder wall were demonstrated in BOO animals. The control rats had normal bladders.

Results

HRV time-domain parameters: Most of the obtained time HRV analysis results were insignificant, except min NN and rMSSD. Minimal NN reached higher values in BOO animals (152,64±16,72) and reached lesser values in control ones (139,28±3,67). Contrary to the findings mentioned above, rMSSD reached higher value in control group (13,01±7,35), while it was almost twice lesser (6,92±8,26) in BOO.

When analysing the general trend observed in other time-domain HRV parameters, it should be emphasised that some of them (max NN, mean HR) were almost identical in both studied populations, either increased (SDNN) or decreased (mean NN) in control rats.

HRV spectral-domain parameters: The statistical estimation of calculated spectral HRV analysis parameters indicated differences relating to the non-normalised power components – LF and HF, as well as to the total spectral powers in two compared groups. Generally, the total power spectrum (TP) and its components were lower in BOO animals. A considerable fall of LF (1,31±1,79 vs 5,40±2,54) and HF (0,98±0,96 vs. 11,47±8,89) appeared in the analysis of BOO rats in comparison to those from the control group. These parameters in this group reached only 24% and 8,5%, respectively, with the control group results adopted as reference ones. Contrariwise, normalised (nLF and nHF) spectral parameters displayed similar values without statistical significance, although nLF was higher in the BOO group (45,2±19,67 vs. 36,61±16,04) and nHF in the control one (63,39±16,04 vs. 54,8±16,72).

The absolute VLF value was almost the same in both studied populations (20,87±30,33 in BOO vs. 19,22±15,74 in control). However, the percentage of this component differed considerably in both studied groups. In BOO animals, the VLF percentage amounted to about 90%, whereas in control animals this parameter reached about 53% in relation to the appropriate total power.

The results mentioned above are given in the table 2 below.

Table 2. Analyzed HRV time- and frequency (spectral) parameters

TIME DOMAIN PARAMETERS	Groups		Statistical significance (for ln values)
	BOO	control	
mean NN [ms]	173.03 ± 11.88	163.41 ± 10.27	NS
SDNN	7.43 ± 3.96	8.70 ± 2.73	NS
max NN [ms]	188.34 ± 11.71	188.76 ± 8.28	NS
min NN [ms]	152.64 ± 16.72	139.28 ± 3.67	*
mean HR [bpm]	348.29 ± 25.26	368.32 ± 22.55	NS
rMSSD	6.92 ± 8.26	13.01 ± 7.35	*

SPECTRAL DOMAIN PARAMETERS	Groups		Statistical analysis (for ln values)
	BOO	control	
TP [ms2]	23.16 ± 32.56	36.09 ± 17.83	*
VLF [ms2]	20.87 ± 30.33	19.22 ± 15.74	NS
LF [ms2]	1.31 ± 1.79	5.40 ± 2.54	**
HF [ms2]	0.98 ± 0.96	11.47 ± 8.89	**
LF/HF	1.06 ± 0.75	0.70 ± 0.63	NS
nLF [n.u.]	45.2 ± 19.67	36.61 ± 16.04	NS
nHF [n.u.]	54.8 ± 16.72	63.39 ± 16.04	NS

Legends:
* - statistical significance with p<0.05
** - statistical significance with p<0.01

Discussion

The key finding of our work was the development of autonomic disturbances in bladder outlet obstruction rats (BOO). Our study revealed that BOO animals were characterised by diminished parameters such as: rMSSD and TP, LF and HF, when compared to those from the control group. A common agreement exists that rMSSD and HF are strongly dependent on parasympathetic drive, while LF reflects both sympathetic and parasympathetic activity. Total power (TP) represents global autonomic tension. [12,14] Thus, our findings may suggest decreasing parasympathetic activity that is responsible for both rMSSD and HF fall while also contributing to LF and TP decrease. The next, indirect evidence, confirming parasympathetic withdrawal in BOO rats, is the trend in normalised spectral parameters, namely, slight changes in the nLF (increased) and nHF (decreased), although these differences were not statistically significant. Thus, to sum up, our findings suggest autonomic imbalance with diminished parasympathetic activity and decreased global autonomic tension.

A reduction of HRV was primarily observed mostly in cardiological diseases - it has been reported in patients with myocardial infarction, chronic heart failure, and in those after cardiac transplantation. Similarly, the HRV decrease was also described in patients with diabetes being a marker of the developing autonomic neuropathy [21,30].

In the field of urology, the ANS assessment and HRV studies are scant. In one study, Choi et al [4] defined autonomic status in OAB patients. They showed both SDNN and rMSSD decrease through time domain HRV analysis. Also, spectral parameters: TP, VLF and HF were found to be diminished, with no significant change in LF and LF/HF, compared to control subjects. Thus, Choi et al. [4] demonstrated that female OAB patients, exhibited both time and spectral lowered HRV parameters (with the exception of LF and LF/HF) which served as evidence of autonomic imbalance in this population. In another study by Juszczak et al [14], performing autonomic Ewing's battery tests revealed an overstimulation of the sympathetic tension in male OAB patients with benign prostatic hyperplasia (BPH) in resting conditions. Moreover, they demonstrated impaired parasympathetic response to stimulation, demonstrating mixed autonomic disturbances in BPH [14].

It is difficult to compare clinical and experimental results, however, our findings are generally consistent with the results quoted above. Again, we have revealed the diminished values of main HRV spectral parameters in BOO model

that indicate a parasympathetic withdrawal and decrease global autonomic activity.

Moreover, in our opinion, the VLF percentage in the global power spectrum is also worth taking into consideration. In BOO, a relative percentage increase of VLF, comparing to other spectrum components, was observed. However, it is difficult to interchangeably discuss, whether more heightened sympathetic or parasympathetic activity contributes to observed VLF changes, due to still unknown VLF background which warrants further elucidation. According to reference books, the VLF component is regarded to reflect circadian and neuroendocrine rhythms, thermoregulatory processes, the renin-angiotensin system activity, as well as hemodynamic feedback delays. Regardless of the VLF origin mechanisms, a great interest in this component exists, combined with the observations that slow rhythms (VLF and even ultra low frequencies - ULF) are predictors of severe cardiac complications, including cardiac death [2,21]. Thus, clear VLF meaning is still unknown, although most HRV authorities recommend considering this component as a marker of sympathetic activity. Accordingly, our VLF findings, observed in BOO rats, may result from sympathetic overactivity together with parasympathetic withdrawal, as it has already been stated above.

Again, these findings are consistent with other clinical reports. Mc Vary et al. [22] and previously Meigs et al. [23] found that BPH patients are more likely to have increased sympathetic activity and revealed a relationship between BPH development and sympathetic overactivity. These authors hypothesised that ANS disturbances may have a casual role in the symptomatic development and progression of BPH.

On the other hand, however, there are also premises suggesting that VLF is to be regarded as the marker of parasympathetic stimulation. According to Taylor et al [32], VLF depends primarily on the presence of parasympathetic outflow. They showed that atropine almost abolishes VLF power and other spectral components, suggesting that HRV, also in the VLF band, is driven by parasympathetic modulation. Soares et al [29] demonstrated in experimental study that pyridostigmine (a reversible cholinoesterase inhibitor) stimulation produced strongly emphasised VLF increase [7]. Eckberg and Kuusela [10] studied rhythmical variation in arterial pressure, depending on vagal baroreflex activity. The-

ir analysis indicated that baroreflex sensitivity fluctuations (expressed as a percentages of the total power) concentrated more in very low frequencies. They also revealed that the periodicity of very low frequency baroreflex sensitivity fluctuations was not influenced significantly by upright tilt test that is considered to be mediated by the sympathetic drive. Thus, according to Eckberg and Kuusela [10], human vagal baroreflex activity is reflected mostly at very low frequencies.

Therefore, the prognostic value of VLF oscillations may derive from the fundamental importance of parasympathetic mechanisms. From this point of view, our VLF findings would be contrary to the previously described TP, LF and HF results interpretation. Together with the decrease of the HF and nHF parameters, reflecting diminished parasympathetic drive, we would expect the simultaneous VLF decrease. Hence, the relatively high VLF percentage observed in the BOO group, may be rather due to possibly sympathetic predominance.

In conclusion, we revealed, that short-term animal model of partial bladder obstruction, is associated with ANS abnormalities, including parasympathetic withdrawal and relative sympathetic predominance. It is questionable, if these findings may be directly extrapolated to clinical conditions. There are limitations in experimental studies interpretation in relation to clinical deduction. Ideally, animal model should reproduce all the facets of the human condition but it is inconceivable that any animal model will replicate all the symptoms, mechanisms and consequences of a disease. A model is usually only relevant for a limited number of aspects of the human conditions and it is rather a tool tailored to answer a particular experimental hypothesis. Additionally, research into bladder overactivity is also hampered by the lack of a bladder or serum specific biomarker that would reflect the disease process [26]. Thus, taking into account these limitation aspects and given the likely multifactoral OAB aethiology, it seems that our study should be followed by clinical research to confirm if similar ANS abnormalities are observed in BOO patients as those observed by us in animals.

Acknowlegements

References

[1] Andersson K.E., Pehrson R.: CNS involvement in overactive bladder. Pathophysiology and opportunities for pharmacological intervention. Drugs, 2003; 63: 2595-2611

[2] Berntson G.G., Bigger J.T., Eckberg D.L., Grossman P., Kaufmann P.G., Malik M., Nagaraja H.N., Porges S.W., Saul J.P., Stone P.H., van der Molen M.W.: Heart rate variability: origins, methods and interpretive caveats. Psychophysiology, 1997; 34: 623-648

[3] Calvert R.C., Thompson C.S., Khan M.A., Mikhailidis D.P., Morgan R.J., Burnstock G.: Alternations in cholinergic and purinergic signaling in a model of the obstructed bladder. J. Urol., 2001; 166: 1530-1533

[4] Choi J.B., Cha M.H., Seo K.S., Ahn B.C., Kim B.T., Kim Y.S.: Analysis of heart rate variability in patients with overactive bladder. Continence, 2005; 1: 9-13

[5] Chu F.M., Dmochowski R.: Pathophysiology of overactive bladder. Am. J. Med., 2006; 119: 3S-8S

[6] Das A.K., Leggett R.E., Whitbeck C., Eagen G., Levin R.M.: Effect of doxazosin on rat urinary bladder function after partial outlet obstruction. Neurol. Urodyn., 2002; 21: 160-166

[7] de Jongh R., van Koeveringe G.A., van Kerrebroeck P.E., Markerink-van Ittersum M., de Vente J., Gillespie J.I.: Damage to the blad-

der neck alters autonomic activity and its sensitivity to cholinergic agonists. BJU International, 2007; 100: 919-929

[8] Dobrek Ł., Juszczak K., Wyczółkowski M., Thor P.: Current management and future perspectives of overactive bladder (OAB) pharmacotherapy. Acta Pol. Pharm. Drug Res., 2011; 6: 807-821

[9] Dobrek Ł., Juszczak K., Wyczółkowski M., Thor P.: Overactive bladder (OAB) – current definition and basic pathophysiological concepts. Adv. Clin. Exp. Med., 2011; 20: 119-129

[10] Eckberg D.L., Kuusela T.A.: Human vagal baroreflex sensitivity fluctuates widely and rhythmically at very low frequencies. J. Physiol., 2005; 567. 1011-1019

[11] Gosling J.A., Gilpin S.A., Dixon J.S., Gilpin C.J.: Decrease in the autonomic innervation of human detrusor muscle in outflow obstruction. J. Urol., 1986; 136: 501-504

[12] Harrison S.C., Ferguson D.R., Doyle P.T.: Effect of bladder outflow obstruction on the innervation of the rabbit urinary bladder. Br. J. Urol., 1990; 66: 372-379

[13] Hashim H., Abrams P.: Overactive bladder: an update. Curr. Opin. Urol., 2007; 17: 231-236

[14] Juszczak K., Mazur M., Wyczółkowski M., Filipek M., Thor P.J.: Autonomic nervous system activity in patients with lower urinary tract symptoms secondary to benign prostatic hyperplasia estimated by heart rate variability. Open Urol. Nephrol. J., 2008; 1: 44-49

[15] Kamiyama Y., Muto S., Masuda H., Ide H., Ishizuka N., Saito K., Horie S.: Inhibitory effects of nicorandil, a K ATP channel opener and a nitric oxide donor, on overactive bladder in animal models. BJU Int., 2008; 101: 360-365

[16] Kato K., Wein A.J., Kitada S., Haugaard N., Levin R.M.: The functional effect of mild outlet obstruction on the rabbit urinary bladder. J. Urol., 1988; 140: 880-884

[17] Lasanen L.T., Tammela T.L., Kallioinen M., Waris T.: Effect of acute distension on cholinergic innervation of the rat urinary bladder. Urol. Res., 1992; 20: 59-62

[18] Lluel P., Barras M., Palea S.: Cholinergic and purinergic contribution to the micturition reflex in conscious rats with long-term bladder outlet obstruction. Neurol. Urodyn., 2002; 21: 142-153

[19] Maggi C.A., Meli A.: Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 1: general considerations. Experientia, 1986; 42: 109-114

[20] Maggi C.A., Meli A.: Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 2: cardiovascular system. Experientia, 1986; 42: 292-297

[21] Malik M. (ed.): Guidelines. Heart rate variability. Standards of measurements, physiological interpretations and clinical use. Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology. Eur. Heart J., 1996; 17: 354-381

[22] Mc Vary K.T., Rademaker A., Lloyd G.L., Gann P.: Autonomic nervous system overactivity in men with lower urinary tract symptoms secondary to benign prostatic hyperplasia. J. Urol., 2005; 174: 1327-1433

[23] Meigs J.B., Mohr B., Barry M.J., Mc Naughton Collins M., Mc Kinlay J.B.: Risk factors for clinical benign prostatic hyperplasia in a community-based population of healthy aging men. J. Clin. Epidemiol., 2001; 54: 935-944

[24] Michel M.C., Barandrecht M.M.: Physiological and pathological regulation of the autonomic control of urinary bladder contractility. Pharmacol. Ther., 2008; 117: 297-312

[25] Pampinella F., Roelofs M., Castelluci E., Passerini-Glazel G., Pagano F., Sartore S.: Time dependent remodeling of the bladder wall in growing rabbits after partial outlet obstruction. J. Urol., 1987; 157: 677-682

[26] Parsons B.A., Drake M.J.: Animal models in overactive bladder research. In: Handbook of experimental pharmacology, ed: Andersson K.E., Michel M.C., Springer-Verlag Berlin Heidelberg 2011, 15-43

[27] Rajendra Acharya U., Paul Joseph K., Kannathal N., Lim C.M., Suri J.S.: Heart rate variability: a review. Med. Biol. Eng. Comput., 2006; 44: 1031-1051

[28] Sibley G.N.: An experimental model of detrusor instability in the obstructed pig. Br. J. Urol., 1985; 57: 292-298

[29] Soares P.P., da Nobrega A.C., Ushizima M.R., Irigoyen M.C.: Cholinergic stimulation with pyridostigmine increases heart rate variability and baroreflex sensivity in rats. Auton. Neurosci., 2004; 113: 24-31

[30] Sztajzel J.: Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system. Swiss. Med. Wkly., 2004; 134: 514-522

[31] Tammela T., Lasanen L., Waris T.: Effect of distension on adrenergic innervation of the rat urinary bladder. Urol. Res., 1990; 18: 345-358

[32] Taylor J.A., Carr D.L., Myers C.W., Eckberg D.L.: Mechanisms underlying very low frequency RR-interval oscillations in humans. Circulation, 1998; 98: 547-555